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THIRD EUROPEAN MARINE SCIENCE AND TECHNOLOGY CONFERENCE

Lisbon, 23-27 May 1998

PROJECT SYNOPSES
Volume III:
Generic Technologies

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European Commission

THIRD EUROPEAN MARINE SCIENCE AND TECHNOLOGY CONFERENCE

Lisbon, 23-27 May 1998

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PREFACE

In 1993 and 1995, MAST, the Marine Science and Technology Programme of the European Union, managed by Directorate General XII (Science, Research and Development) of the European Commission, and EUROMAR, a marine technology "umbrella" within the framework of the EUREKA initiative, organised the first 2 sessions of the so-called "MAST Days and EUROMAR Market". It would have been unthinkable not to plan a third edition in Lisbon on the occasion of the International Year of the Oceans. By and large, public awareness of the oceans as a major controlling factor of mankind's future seems to be growing, but there remains much to be done for promoting marine research and international co-ordination on this matter. The time has come to present a more complete view of research efforts carried out and co-ordinated on a European scale. Therefore, other actors have joined MAST and EUROMAR in organising the Lisbon conference: Directorate General XIV (Fisheries) of the European Commission, the Environment and Climate Programme in DG XII, and the secretariat of the European Marine and Polar Science (EMaPS) Boards. Hence the change of denomination to: Third "European Marine Science and Technology Conference"[1].

MAST dates back to 1989. The present programme, MAST-III, will terminate at the end of this year; its budget was 244 million ECU, a five-fold increase compared to MAST-I. The ultimate objective of research is to understand the functioning of marine systems around Europe, both in shelf and deep seas, and thus to help establishing the scientific and technical basis for their exploitation, management and protection.

Fisheries and aquaculture research was initiated at European Community level in 1988 and has taken since then several denominations: FAR 1988-92, AIR 1990-94, and now FAIR 1994-98. The programmes concentrated on the promotion of research in support of the Common Fisheries Policy, dealing with fisheries management, aquaculture, product development and, more recently, the interactions between fisheries, aquaculture and the marine environment.

The Environment research programmes of the 1970's and 80's already addressed issues of marine pollution and ecotoxicology. These topics have remained on the agenda of successor programmes: STEP (1989-92), Environment (1990-94) and the current Environment and Climate Programme (1994-98). Due to an obvious risk of overlap with MAST in the coastal zone, both programmes are co-operating on the management of projects on coastal ecosystems, in conformity with the so-called ELOISE science plan. Finally, the Environment and Climate Programme also supports some marine research, especially in sub-arctic seas, through climate-oriented projects.

By contrast with the EU programmes outlined above, EUROMAR is industry-led. Launched in 1986, it deals with the development, application and successful exploitation of Europe's advanced marine technology and tends to operate closer to the market than MAST. In recent years, it has become apparent that the scheme needs some adaptation to changing conditions in Europe and in the world. EUROMAR looks set to evolve into a form of network of manufacturers, research institutes and end-users.

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1 A really complete overview of marine and maritime research supported by the EU should include activities on renewable energies and industrial and material technologies managed by DG XII, as well as marine related activities in DG XVII (ENERGY), DG VII (TRANSPORT) and the Joint Research Centre (JRC) of the European Commission.

The European Marine and Polar Science (EMaPS) Boards were established in 1995 under the auspices of the European Science Foundation (ESF), and are served by a permanent secretariat hosted by ESF in Strasbourg. The boards are intended to improve co-ordination between European organisations in marine and polar science and seek to develop scientific strategies. The boards and the secretariat are also active in facilitating the implementation of scientific challenges of the future. In many ways, the roles of EMaPS and of the Commission can be seen as complementary and it is therefore natural that both bodies should co-operate closely.

As this preface is being written, the procedure leading to the adoption of the EU Fifth Framework Programme for the period 1998-2002 is under way. EU research ministers have agreed on the structure of FP5: it departs radically from that of its predecessors. In the place of the current so-called "specific" programmes, such as MAST, FAIR, Environment and Climate, we have a structure dominated by "key actions", e.g., for what relates to marine research: "global change, climate and biodiversity", "sustainable marine ecosystems", "sustainable agriculture, fisheries and forestry", "land transport and marine technologies". The novelty of the approach seems obvious, not least because it focuses on problems instead of processes, and stresses the need that the scientific community address socio-economic issues in preparing projects and implementing research.

This conference must therefore serve a dual purpose: to review the past decade of European marine research programmes, and to pave the way for future developments in this domain. All colleagues from the Commission, EMaPS and EUROMAR have to be thanked who joined their efforts in preparing such an outstanding blend of scientific sessions and discussion meetings. While it is left to the conference proceedings to capture the flavour of the discussions held at Lisbon, the project synopses presented here record the state of integration reached in recent years by the community of marine scientists in Europe.

Brussels, in March 1998

Jean Boissonnas
Marine Science and Technology

Willem Brugge
Fisheries and Aquaculture

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III.1.1. Non-disturbing techniques

TITLE: Broadband acoustic scattering signatures of fish and zooplankton (project BASS)

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BROADBAND ACOUSTIC SCATTERING SIGNATURES OF FISH AND ZOOPLANKTON (BASS)

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SUMMARY

The three-year, shared-cost project BASS, begun on 1 February 1996, aims to develop, test, and apply a set of seven octave-bandwidth transducers spanning the frequency range 25 kHz - 3.2 MHz to fish and zooplankton. Interim progress is reviewed through description of the following activities: development of the seven transducers and associated transmitting and receiving electronics; measurement of animal morphology, mass density, and sound speed; applications of the finite-element boundary-element (FEBE) model to transducer design and to modelling acoustic scattering; preparation of facilities in Bergen for testing the new system; and planning of two sea trials for 1998, when attempts will be made to classify scatterers on the basis of their measured backscattered spectra as interpreted with the FEBE model. Measures taken to ensure dissemination and exploitation of results are summarized.

1. INTRODUCTION

Interest in observing fish and zooplankton in the sea ranges from the purely operational to the ostensibly purely scientific. For example, fish may be observed in order to determine their spatial distribution and abundance for regulation of fishing activity. Individual zooplankton may be observed in relation to their immediate environment, including predators and prey, to learn what influences their behaviour, without any other particular objective in mind.

In both of the mentioned examples, in addition to others involving the observation of distribution, abundance, or behaviour, acoustics is a major modality. Principal advantages spring from its remote, non-invasive character; fine resolution within a relatively large sampling volume; capacity for rapid, synoptic surveying; visualizing power; and quantitative nature. The chief disadvantage is that the modality, when unassisted by physical capture or optical registration, is literally blind. However, even when supported by other means of observation, acoustics may sense the presence of fish or zooplankton that otherwise elude detection.

It is to exploit this remote sensing potential that intrinsically acoustical schemes of classification have been proposed. In the present case, this involves developing a genuinely broadband system of sufficient bandwidth for distinguishing biological scatterers ranging in size from large commercial fish, of the order of 1-m length, to mesozooplankton, 0.2 - 2 mm in equivalent spherical diameter.

The particular proposal made to the third Marine Science and Technology Programme of the European Union in 1995 was, first, to develop and construct a seven octave-bandwidth transducer system spanning the frequency range 25 kHz - 3.2 MHz. The nominal wavelengths, which are suggestive of resolving power, are 6 cm - 0.5 mm, respectively.

In order to use the system, and be able to interpret the results of measurement, collateral information is required on the kinds of scatterers that may be encountered in the water column. There can be no general solution to this problem, but some of the dominant, widely distributed species in the northeast Atlantic Ocean, can be examined. Thus it was proposed that the following scatterers be considered: swimbladder-bearing fish, including cod (*Gadus morhua*) and herring (*Clupea harengus*), the euphausiid *Meganyctiphanes norvegica*, and the copepod *Calanus finmarchicus*. Data are to be collected on animal morphometry, including internal structures and other physical properties, for use in modelling computations. The results of these computations are to be compared against measurements made on live, free-swimming, if encaged, animals in a test facility. Sea trials are also to be conducted with the new transducer system, following its calibration.

It is the present aim to describe this ambitious programme of research and technology development. A number of tasks are being performed in parallel; others are sequential, depending on the availability of the new instrument, for example, before calibrations or other measurements can be performed. Consequently, progress in the original work consists of a mix of concrete accomplishments and unexecuted plans.

The particular organisation adopted here is the following. Development of the heart of the BASS-system, the transducer array, is described. Design and development of the associated, controlling electronics is then reviewed. Performance of animal morphometry and measurement of physical properties is outlined. The finite-element boundary-element (FEBE) model is described, as are applications of this to transducer design and computation of scattering by animals. Development of test facilities for calibration and acoustic measurement of live animals is also reviewed. Plans for two sea trials in the third project year are sketched. Actions taken to ensure the dissemination and exploitation of results are summarized in conclusion.

2. TRANSDUCER DEVELOPMENT

The first transducers developed by RESON A/S in the project covered the frequency band from 400 to 3200 kHz. One of the design tools for these was the mentioned finite-element boundary-element (FEBE) model. For the lower-frequency transducers, a simpler, finite-element (FE) model was used as a primary design tool.

In the following, the transducers are described in order of increasing frequency.

2.1 Transducer for the frequency band 25-40 kHz

The basic element is a two-mode-resonance, broadband, high-power transducer of tonpilz design, shown in Fig. 1. Seven identical elements are assembled in a planar circular array, also shown in Fig. 1. Because of the order of development, this transducer needed to cover only the restricted frequency band 25-40 kHz, rather than the nominal octave band 25-50 kHz. The transmitting sensitivity function is nearly flat-topped, but falls by 6 dB from the peak to the value at 40 kHz. The approximate beamwidth at 30 kHz is 22.4 deg.

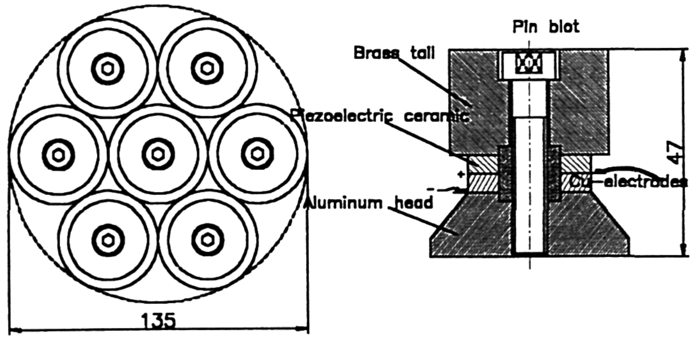

Fig. 1 Sketch of a tonpilz transducer element and its configuration in a planar circular array forming the transducer, 25-40 kHz. Dimensions in millimeters.

2.2 Transducer for the frequency band 40-100 kHz

The basic element is horn-shaped, with cylindrical piezoelectric ceramic drive without attached backing or tail mass, shown in Fig. 2. It has three resonances: at 45 kHz due to thickness mode, 70 kHz due to flapping mode, and 105 kHz due to the quarter-wavelength front-matching layer. The transmitting response extends over the frequency band 40-100 kHz, with variations mostly confined within a 3-dB range. Thirteen identical elements are assembled in a planar star-shaped array, as shown in Fig. 2. The beamwidth varies from 16.6 deg at 45 kHz to 7.3 deg at 100 kHz.

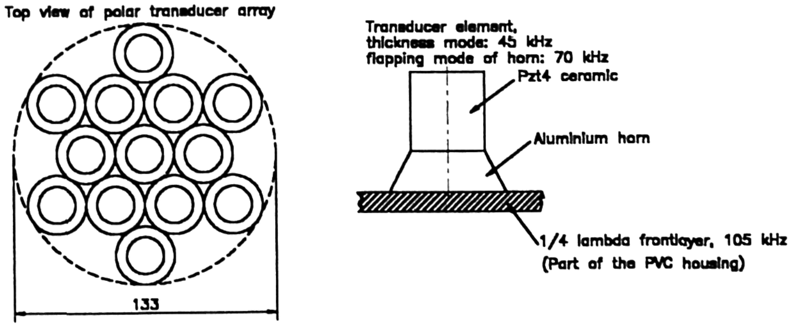

Fig. 2. Sketch of a horn element and its configuration in a planar array defining the transducer, 40-100 kHz. Dimensions in millimeters.

2.3 Transducer for the frequency band 100-200 kHz

This transducer resembles that described in Section 2.2. Thus the basic element is horn-shaped, with three resonance modes: at 100 kHz due to thickness mode, 150 kHz due to flapping mode, and 200 kHz due to the quarter-wavelength front-matching layer. The transducer is composed of 13 identical elements arranged in a star-shaped pattern, as in Fig. 2. The beamwidth varies from 13.6 deg at 100 kHz to 6.8 deg at 200 kHz.

2.4 Transducer for the frequency band 200-400 kHz

Only a single element is used. This is based on a so-called 1-3 composite piezoelectric drive with disk-like shape, shown in Fig. 3. The beamwidth ranges from 15.3 deg at 200 kHz to 7.6 deg at 400 kHz.

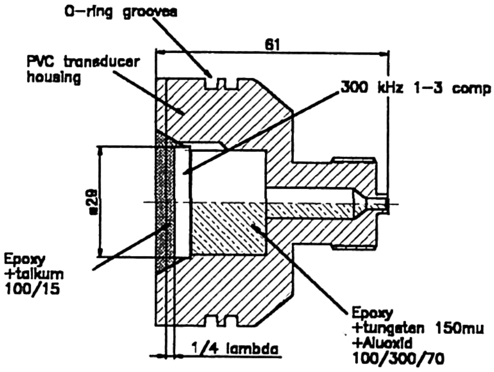

Fig. 3. Sketch of a 1-3 composite transducer, 200-400 kHz. Dimensions in millimeters.

2.5 Transducer for the frequency band 400-800 kHz

The basic design is that of a thin disk with two matching front layers and a solid impedance-matched backing. Each of the front layers is approximately one-quarter wavelength thick at 500 kHz. An improved design is shown in Fig. 4. The beamwidth is 8.1 deg at 500 kHz.

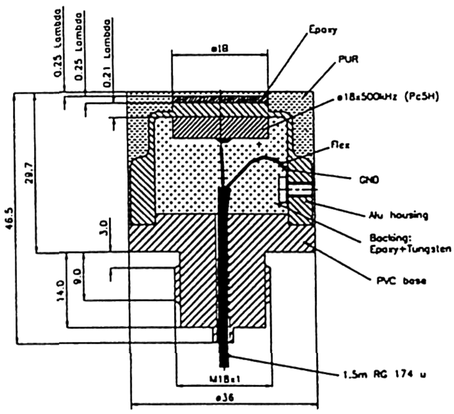

Fig. 4. Sketch of a thin-disk transducer with two matching front layers and solid backing, 400-800 kHz. Dimensions in millimeters.

2.6 Transducer for the frequency band 800-1600 kHz

The transducer is composed of a solid-backed thin disk with two matching front layers. The outermost layer is three-quarter wavelength thick at 1100 kHz, while the inner layer is one-quarter wavelength thick at the same frequency. The beamwidth at 1100 kHz is 7.2 deg.

2.7 Transducer for the frequency band, 1600 -3200 kHz

This transducer is composed of a solid-backed thin disk with two matching front layers, each with three-quarter wavelength thickness at 2000 kHz. The beamwidth at the same frequency is 2.3 deg.

2.8 Sensitivity functions

Transmitting and receiving sensitivities have been measured, by RESON, as functions of frequency. Only the transmitting sensitivity functions for the frequency bands from 100 kHz to about 2500 kHz are shown here, in Fig. 5. The transmitting sensitivity functions for the frequency bands from 25 to 100 kHz are described above in Sections 2.1 and 2.2. The receiving sensitivity function is roughly comparable, if measured in different units.

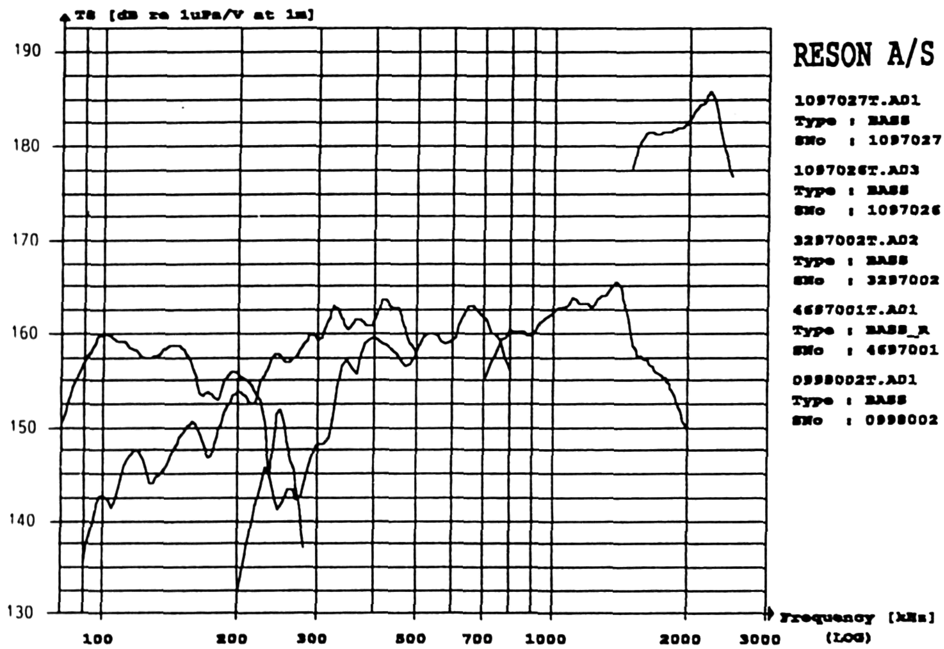

Fig. 5. Transmitting sensitivity from 100 to about 2500 kHz.

3. TRANSMITTING AND RECEIVING ELECTRONICS

In order to control the transducer array operation, the Acoustics and Sonar Group (ASG) at the University of Birmingham is designing and building the required electronics. Elements of this are shown in a block diagram in Fig. 6. In fact, since there are seven transducers covering different bands, as many electronic designs are necessary, each covering the respective nominal octave bandwidth. The electrical impedance of the transducers varies with frequency, thus requiring broadband matching networks to transform the transducer impedance to the standard fifty-ohm resistive termination.

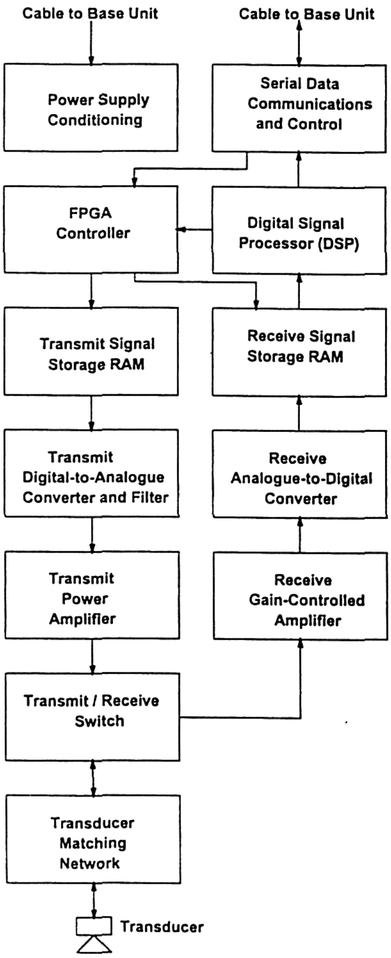

Fig. 6. Block diagram of a single set of transmitting and receiving electronics.

A transmit-receive switch is used to route the transmit signal from the transmit power amplifier to the transducer, and the echo signal to the receive gain-controlled amplifier. The transmit signal is generated from a digital replica stored in random access memory (RAM) connected to a digital-to-analogue converter. This technique is convenient for modifying the signal duration and modulation technique from the base unit, a personal computer (PC) mounted onboard the vessel platform rather than on the transducer-bearing sonde. Control of the transmission circuitry is effected through a field-programmable gate array (FPGA), which is a very large-scale solid-state device incorporating many thousands of digital gates.

During reception, the received signal is amplified by a variable-gain amplifier and quantised by a 12-bit analogue-to-digital converter. The receiver gain can be adjusted in coarse but calibrated steps, allowing the received echo signal to be digitised from widely differing operating target ranges. The digitised received signal is stored in RAM, with the possibility of transfer to the base unit or direct processing by the digital signal processor (DSP).

The seven units, each with the generic elements shown in Fig. 6, are linked together using a time-division multiplexed (TDM) serial link to an intermediate processor. This will also serve as the link to the base unit via the connecting cable.

The underwater electronics unit is powered by a medium-voltage DC supply operating in the nominal range 40-100 V. The supply can be transformed to the voltages required by the power amplifiers and digital systems by means of switched-mode DC-DC converters.

The electronics unit is being constructed on a modular, slot-together basis. Space has been allocated for eight frequency bands. Each module is fabricated from a solid aluminium block to minimise electromagnetic compatibility (EMC) issues. The functions of the six modules are listed in order from the sub-con transducer connector to the sonde cable connector.

Module 1: Broadband matching network This matches the impedance of the transducers to that of a standard fifty-ohm resistive termination.

Module 2: Transmit-receive switch and transmit power amplifier The transmit signal is amplified and the TX/RX switch is effected with very low distortion levels.

Module 3: Receiver amplifiers These amplify the received signals with variable gain.

Module 4: DSP unit Arbitrary transmit waveforms can be entered and generated on demand. The received signal is digitised and its spectrum analysed.

Module 5: Serial communication cable interface This ensures the connection to the main cable of as many as thirty two channels represented by Modules 1-4.

Module 6: Power supply conditioning A medium-voltage, unregulated DC supply in the range 40-100 V is used to produce the required supplies for the rest of the system.

4. ANIMAL MORPHOMETRY AND PHYSICAL PROPERTIES

For swimbladder-bearing fish, the swimbladder is the predominant scattering organ. At ordinary ultrasonic frequencies, at least, it is sufficient to represent such a fish by the swimbladder form. This has been done in the past for a number of species, including saithe (*Pollachius virens*) and pollack (*Pollachius pollachius*) (Foote and Ona 1985), among others. Triangulations of the swimbladder form of two saithe and thirteen pollack have been communicated by IMR to ASG for computation of scattering responses by means of the FEBE model, described briefly below in Section 5. Comparisons will be facilitated by the availability both of measurements of the target strength functions of tilt angle at each of four single frequencies, 38, 49, 70, and 120 kHz, and computations of the same by the Kirchhoff approximation (Foote 1983).

Characterising zooplankton without gas inclusions is a much more complicated matter. Both external form, internal structures, and corresponding mass densities and elastic constants are required for use in modelling the acoustic scattering properties.

In the case of euphausiids, there is little systematic knowledge of form. Both mass density and sound speed, which is related to the bulk modulus of elasticity and the compressibility, have been measured for a number of species, but it is appreciated that the properties vary seasonally and probably with individual specimen too. For the particular, subject euphausiid, *Meganyctiphanes norvegica*, external geometric cross sections have been observed in both dorsal and lateral aspects by means of videomicroscopy in the laboratory. The operations of digitisation of the geometric cross sections and measurement of mass density and sound speed are to be completed or undertaken in the third year of the project.

Morphometry of the copepod *Calanus finmarchicus* has been performed systematically, if at different seasons. A range of copepodite stages has been observed, principally CIII-CVI. Examples of geometric cross sections are shown in Fig. 7 for a CIV specimen. Both the cephalothorax and oil sac have been digitised. Attempts have been made to measure the mass density by means of a density-gradient column, but so far these have been inconclusive. A careful reading of the literature indicates that such a measurement remains an outstanding scientific problem. Measurements have been made of the sound speed in bulk, in a large assemblage of specimens held in an inverted T-shaped tube according to the method described in Foote (1990). Some preliminary results are presented in Foote et al. (1996).

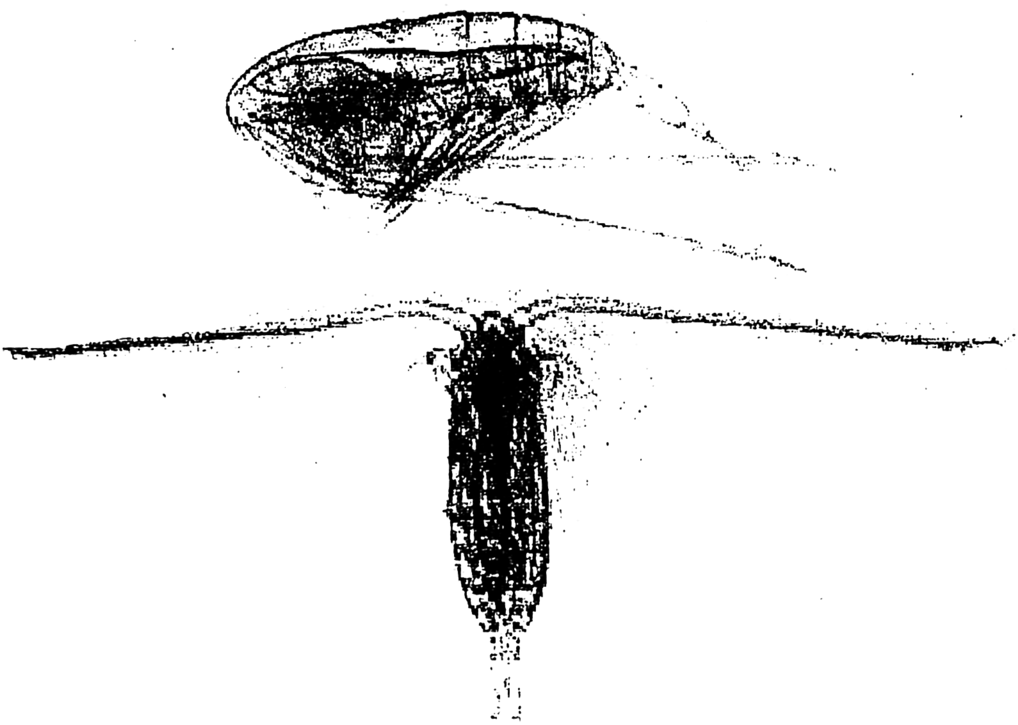

Fig. 7. Lateral and dorsal views of a copepod in stage CIV, with cephalothorax length 1.82 mm.

5. FINITE-ELEMENT BOUNDARY-ELEMENT (FEBE) MODEL

The FEBE model unites two distinct acoustic methods (Francis et al. 1996). The finite-element (FE) method (Zienkiewicz and Taylor 1989) describes the vibration of three-dimensional structures. These are represented by finite-sized elements, which *in toto* describe the structure. A quadratic isoparametric formulation is used: variables over an element are determined from the values at the nodes by quadratic interpolation. Structural damping is incorporated through the use of complex variables, with imaginary components in the material properties.

The boundary-element (BE) method (Francis 1993) describes the acoustic effect of a vibrating surface, hence both radiation and scattering. It solves the wave equation as expressed in the Helmholtz integral form. Since only the pressure and velocity fields on the element surface are required, it is sufficient to describe the transducer or passive body by a surface grid or mesh. A technical detail of some importance is the addition of the gradient field to the field itself in the model, which avoids the problem of numerical divergence at certain frequencies. As with the FE method, a quadratic isoparametric formulation is used.

Combination of the FE and BE methods occurs at the bounding surfaces of the structure, where the coupling is direct (Mathews 1986). Since the elements are common, internal structural vibrations are linked to the external acoustic field, as indeed they are in most radiation and scattering problems.

To verify the model, a number of computations have been performed with FEBE for solid elastic bodies and other special shapes for which solutions are known. In addition, prolate elastic spheroids are being machined for laboratory measurement in order to provide additional validating data.

The FEBE model has had and has a number of applications in the project. As already mentioned in Section 2, it was instrumental in the design of the three highest-frequency transducers, described in Sections 2.5-2.7. When configured for modelling piezoelectric transducers, the FEBE model is realised in software known as PHOEBE.

The FEBE model is presently being used to compute target strength functions of tilt angle for swimbladder-bearing fish. These will be compared with reference measurements and Kirchhoff-model computations, mentioned in Section 4.

Modelling of acoustic scattering by copepods is also being undertaken by means of FEBE. This is indeed a challenge, for the composite physical properties of the animal resemble those of sea water, and echoes arise mainly from internal heterogeneities, that is, differences in internal properties. The animal is being modelled by an oil sac embedded within the cephalothorax, both fluid-like structures, where the geometric cross sections have been determined in dorsal and lateral aspects by videomicroscopy. Examples of wireframe models derived by a mesh-generation algorithm, which are required for exercise of FEBE, are presented in Fig. 8. The mass density is being determined by reference to the literature on the density of lipids in the oil sac, and assumption of neutral buoyancy. Bulk measurements of sound speed are also being applied to individuals.

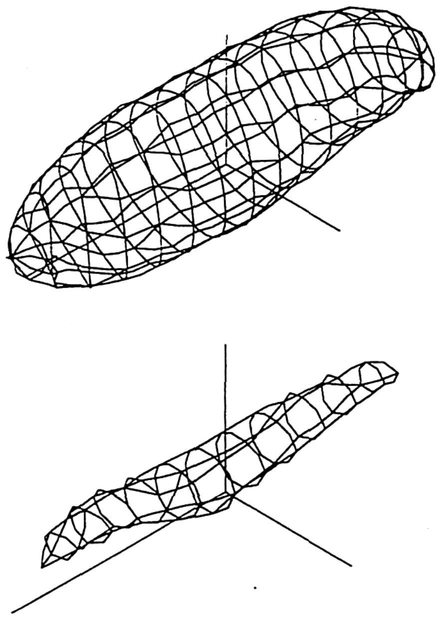

Fig. 8. Wireframe models of cephalothorax and oil sac of a copepod in stage CVIf, with cephalothorax length of 2.74 mm.

6. TEST FACILITIES

Several test facilities are available at IMR and at the Bergen Large Scale Facility (LSF) for Marine Pelagic Food Chain Research. In particular, a tank connected with the Bergen Aquarium has been used to calibrate high-frequency acoustic transducers, including that of the SIMRAD EK500/714-kHz echo sounder. The applied method has been that of standard-target calibration recommended by the International Council for the Exploration of the Sea (ICES) (Foote et al. 1987), using a 10.3-mm-diameter sphere of tungsten carbide with 6% cobalt binder.

A second test facility has been developed at the Bergen LSF at Espegrend. In particular, mesocosms have been developed since autumn 1996 for the purpose of controlled acoustic measurement on small animals (Knutsen and Foote 1997). A mesocosm is defined here as a cylindrical enclosure in the sea, open at the surface but otherwise impermeable. In particular, mesocosms of diameter 2 m and 4 m have been established, with similar 9-m depth, illustrated in Fig. 9. In the absence of project transducers and electronics, a rig consisting of three SIMRAD resonant transducers with center frequencies at 120, 200, and 714 kHz have been assembled, also shown in Fig. 9. These were connected to the SIMRAD EK500 echo sounder (Bodholt et al. 1989). By moving the rig vertically in the water column, the contents of the enclosures could be imaged. By means of echo integration (MacLennan 1990), realised in the Bergen Echo Integrator (BEI) (Foote et al. 1991), the scatterers could be quantified and vertical profiles derived.

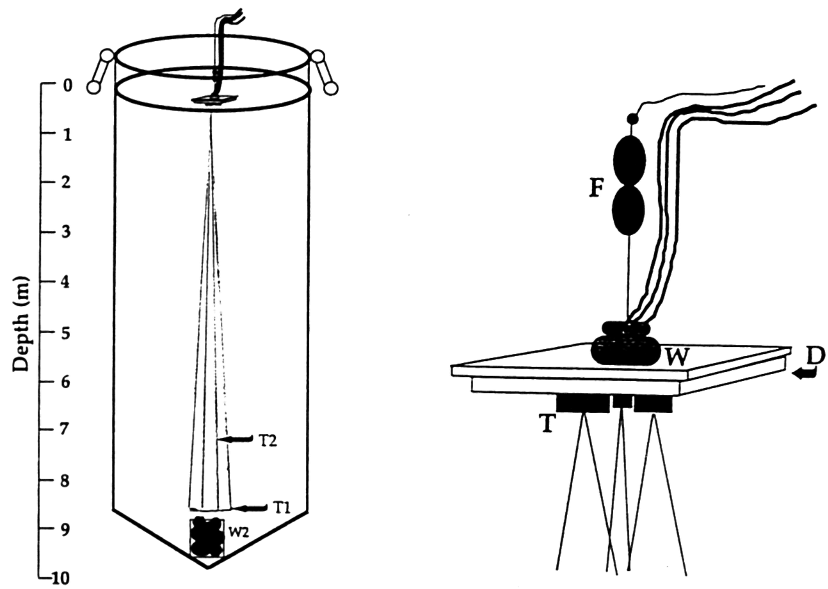

Fig. 9. Schematic diagrams of a mesocosm and transducer rig. The central parts of the beams at 200 and 714 kHz are indicated by T1 and T2, respectively, and stabilizing weights by W2. On the transducer rig, D denotes damping material, W weights, and F floats for buoyancy.

In the most recent measurement series, performed at the Bergen LSF in autumn 1997, a 4-m-diameter mesocosm was established. Acoustic backscattering measurements were made first after filling the enclosure with filtered sea water, then after the addition of a zooplankton assemblage consisting mainly of *Calanus finmarchicus*. Euphausiids were then introduced, and the measurements were repeated. Small fish were subsequently introduced, and the measurements again repeated. Pump samples were taken at the different depths for comparison with the backscattering profiles. Collateral measurements of animal morphometry and sound speed were performed concurrently in a nearby shore-based laboratory. Representative echograms are shown in Fig. 10 for ensembles composed mainly of copepods and of copepods and euphausiids at both 200 and 714 kHz.

Similar measurement series are planned for the BASS transducer system in the third project year. If possible, validating measurements will be made with an optical plankton counter (Herman 1992).

An auxiliary activity, in preparation for use of the new system, is the design of precision elastic spheres for calibration. This consists of specification of the diameters of spheres composed of tungsten carbide with 6% cobalt binder. Both IMR and ASG have performed computations of target strength spectra for 10- and 20-mm-diameter spheres, with excellent agreement for wavenumber-radius products of the order of 80. Independent experimental work on actual spheres of the same diameters, procured at Spheric Engineering Ltd. in the United Kingdom, is being performed at the University of Bergen, Department of Physics.

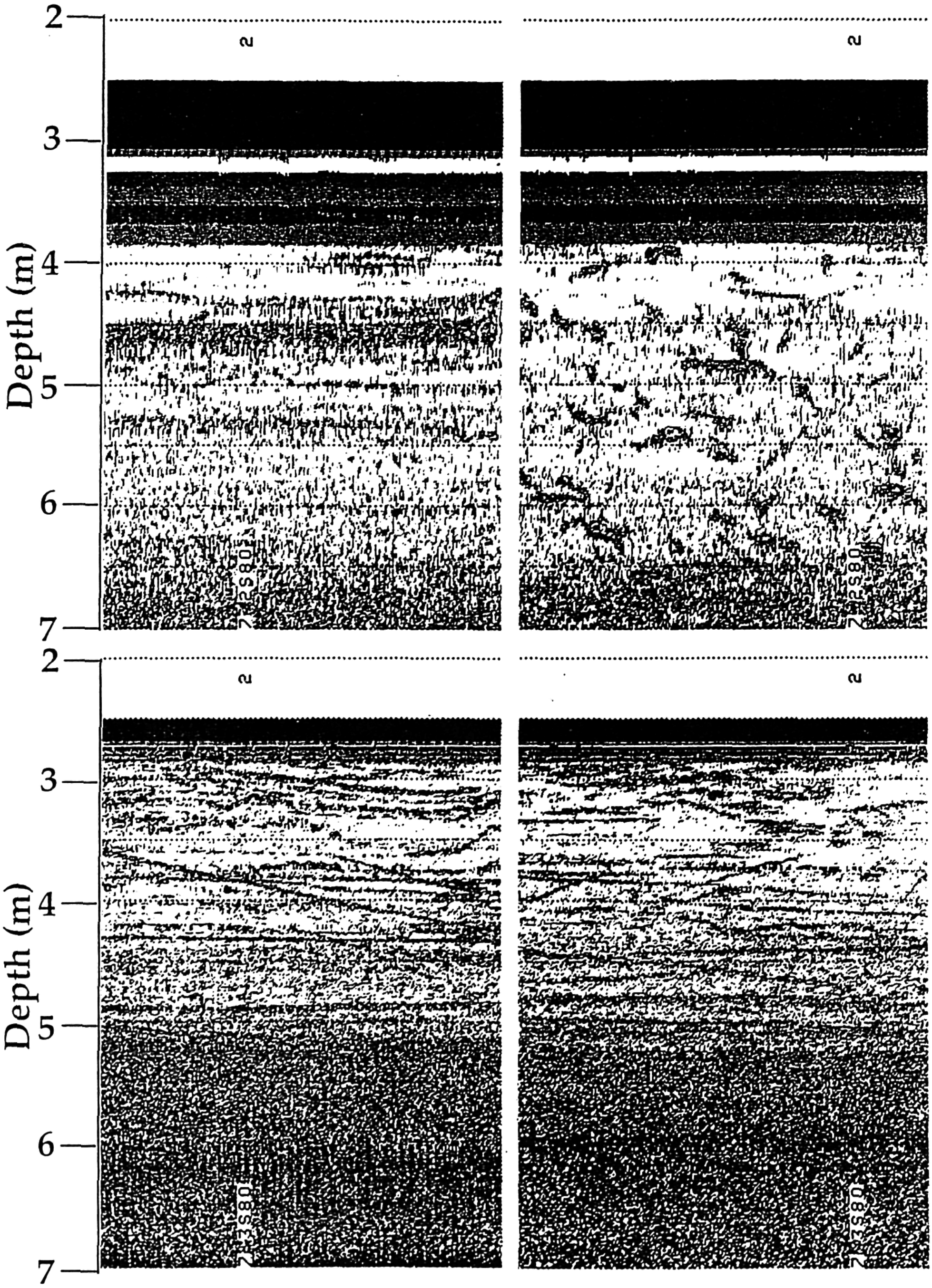

Fig. 10. Echograms collected at 200 kHz, top row, and 714 kHz, bottom row, with the transducer surfaces at 2.5-m depth in the 4-m-diameter mesocosm. The echograms on the left were recorded simultaneously on 10 November 1997 following introduction of copepods, while the echograms on the right were recorded simultaneously on 12 November 1997 after introduction of euphausiids, hence with both copepods and euphausiids present.

7. SEA TRIALS

Two sea trials are planned for the third project year. In both, R/V "Johan Hjort" will be used. This is a 2000-BRT, stern-trawling, specially built research vessel. Its forward winch has already been modified for mounting the 500-m cable that will bear the new BASS system when used as a sonde, providing both power and communication link.

In the first sea trial, 27 April - 3 May 1998, measurements can be performed on a layer of zooplankton in Storfjord at about 200-300 m depth or in the open sea to the west of Ålesund at latitude 62.5 deg. N. It is likely that a spring bloom will be in progress.

In the second sea trial, 9-18 October 1998, measurements will be performed in northern Norway in Vestfjord or in nearby Tysfjord or Ofotfjord. A major object of investigation will be Norwegian spring-spawning herring, returning to its wintering area after summer grazing in the Norwegian Sea. Zooplankton will be a secondary object of investigation.

An attempt will be made to classify scatterers on the basis of their measured spectra, as interpreted by means of the FEBE model using data on animal properties. These auxiliary data may be collected in advance or during the cruise itself.

In conjunction with both trials, supporting measurements will be made with traditional sampling gear, including pelagic trawl, MOCNESS, and conventional scientific echo sounders with operating frequencies of 18, 38, 120, and 200 kHz. As with the mesocosm work, profiling measurements may also be made with an optical plankton counter.

8. DISSEMINATION AND EXPLOITATION OF RESULTS

Specific measures have been undertaken to ensure dissemination of results. Some preliminary results have already been published, others are to be presented at international meetings in 1998, and other publications are planned, pending completion of the design and development work and successful use of the system at sea.

The manufacturing partner RESON has begun exploiting new knowledge and techniques from the first year of the project. Knowledge acquired in the design and fabrication of the three octave-bandwidth transducers spanning the range 400-3200 kHz has already been exploited in a number of products. The three lowest-frequency transducers, covering the range 25-200 kHz, have defined new products, with respective RESON transducer model numbers TC2125, TC2116, and TC2130.

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REFERENCES

Bodholt, H., Nes, H., and Solli, H. 1989. A new echo-sounder system. Proc. IOA, 11(3): 123-130.

Foote, K. G. 1983. Linearity of fisheries acoustics, with addition theorems. J. Acoust. Soc. Am., 73: 1932-1940.

Foote, K. G. 1990. Speed of sound in *Euphausia superba*. J. Acoust. Soc. Am., 87: 1405-1408.

Foote, K. G., and Ona, E. 1985. Swimbladder cross sections and acoustic target strengths of 13 pollack and 2 saithe. FiskDir. Skr. Ser. HavUnders., 18: 1-57.

Foote, K. G., Knudsen, H. P., Vestnes, G., MacLennan, D. N., and Simmonds, E. J. 1987. Calibration of acoustic instruments for fish density estimation: a practical guide. ICES Coop. Res. Rep., 144. 69 pp.

Foote, K. G., Knudsen, H. P., Korneliussen, R. J., Nordbø, P. E., and Røang, K. 1991. Postprocessing system for echo sounder data. J. Acoust. Soc. Am., 90: 37-47.

Foote, K. G., Knutsen, T., Baekkevold, A. E., Dalpadado, P., and Johannessen, S. E. 1996. Initial, collateral measurements of some properties of *Calanus finmarchicus*. ICES C.M. 1996/L:21. 23 pp.

Francis, D. T. I. 1993. A gradient formulation of the Helmholtz integral equation for acoustic radiation and scattering. J. Acoust. Soc. Am., 93: 1700-1709.

Francis, D. T. I., Oswin, J. R., and Macey, P. C. 1996. Comparing FE/BE models with measurement: flextensional transducers. Proc. IOA, 18(10): 31-40.

Herman, A. W. 1992. Design and calibration of a new optical plankton counter capable of sizing small zooplankton. Deep-Sea Res., 39: 395-415.

Knutsen, T., and Foote, K. G. 1997. Experiences in making acoustic measurements in mesocosms with *Calanus finmarchicus*. Institute of Marine Research project report, 12 October 1997. 41 pp.

MacLennan, D. N. 1990. Acoustical measurement of fish abundance. J. Acoust. Soc. Am., 87: 1-15.

Mathews, I. C. 1986. Numerical techniques for three-dimensional steady-state fluid-structure interaction. J. Acoust. Soc. Am., 79: 1317-1325.

Zienkiewicz, O. C., and Taylor, R. L. 1989. The finite element method. Vol. 1. McGraw-Hill, London.

TITLE: Automated Identification and Characterisation of Microbial Populations (Project **AIMS**)

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AUTOMATED IDENTIFICATION AND CHARACTERISATION OF MICROBIAL POPULATIONS (PROJECT AIMS)

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SUMMARY

The AIMS project is developing flow cytometry technology for identification of microbial cell populations and determination of their cellular characteristics. This involves developing new approaches for using neural nets to identify cell populations and deriving and verifying algorithms for assessing the chemical, optical and geometric characteristics of these populations. The products of AIMS will be calibrated data, protocols, algorithms and software designed to turn flow cytometric observations into a data matrix of the abundance and cellular characteristics of identifiable populations. The AIMS project started 1 October 1997 and runs for 36 months.

1. INTRODUCTION

Understanding the population dynamics of planktonic microbial communities is central to many contemporary concerns in marine science. Such concerns include fuelling the food webs that lead to fisheries, assessing the responses of coastal waters to pollution and determining the role of the ocean in sequestering anthropogenically produced CO_2. Marine microbial communities are taxonomically and functionally diverse, comprising phytoplankton, bacteria and protozoa. Moreover, marine plankton exhibit a wide range of scales of variability both in space and time. This variability can only be explained by a sophisticated knowledge of the interactions of different biological and physical processes. A prerequisite for obtaining this knowledge is the ability to sample the physical, chemical and biological properties of marine waters on appropriate time and space scales. Not surprisingly, progress in continuous assessment of ocean water quality variables was first achieved for physical variables including temperature and salinity. Nutrients and trace elements have also proven to be amenable to automated analysis, and rapid progress has recently been made in automated analysis of trace elements in seawater. Some biological variables such as chlorophyll fluorescence can be measured continuously. However, assessment of changes in the population structure of marine microbial populations remains extremely time consuming.

A methodological approach that permits the precise, rapid, repetitive description of the population structure of primary producers, bacterioplankton and zooplankton is highly relevant to interdisciplinary studies. Analytical flow cytometry provides a means of obtaining descriptions of the structure of microbial communities based on optically defined populations. Such descriptions facilitate attempts to relate microbial activity to ocean colour, and assimilation of data on microbial populations into coupled physical/biological models of ocean geochemistry. However, exploiting flow cytometry for examining marine microbial processes is still limited by the time required for data analysis.

The fundamental requirement for automated identification and characterisation procedures in marine science was acknowledged in a 1996 MAST-III workshop (EC contract MAS3-ct95-63-81; Culverhouse, Williams and Reguera). The workshop recommendations stressed the need for improved automated analysis of marine organisms and development of flow cytometric techniques for analysis of phytoplankton. The EurOPA flow cytometer was produced by a previous MAST project to provide a flexible tool for examining phytoplankton populations. More recently, the Cytobuoy flow cytometer has been developed with MAST III funding to provide a platform for autonomous collection of data on population sizes and characteristics of marine phytoplankton. The AIMS project will complement the hardware developed with MAST funding by providing software to automate data reduction, by providing new data on the inherent properties of marine microbes that contribute to flow cytometric signatures and by further developing molecular markers for identification and characterisation of marine microbes.

2. THE SCOPE OF AIMS

The AIMS project is developing and testing automated techniques for identifying and characterising phytoplankton and bacterial populations using analytical flow cytometry (AFC). Identification involves using objective procedures to differentiate amongst populations of phytoplankton and bacteria within complex natural microbial communities and classifying a community of heterogeneous organisms into its component populations. Identification is based on optical properties of single cells measured by analytical flow cytometry (AFC) and will be verified using ribosomal RNA (rRNA) probes with varying levels of taxonomic discrimination. Characterisation involves determining cell abundance together with the intrinsic optical and chemical properties of these cells. These intrinsic properties include cell size, cell light scattering cross-section, cell carbon content, and additionally for phytoplankton, the light absorption cross-section and cell chlorophyll *a* content.

The objectives of the project are as follows.

- To develop and apply automated identification techniques for identifying phytoplankton populations based on optical properties measured by analytical flow cytometry.
- To develop and apply techniques for analysing bacteria populations based upon optical properties measured by analytical flow cytometry.
- To test the veracity of the automated optical identification using ribosomal RNA (rRNA) probes.
- To provide a calibrated data set of flow cytometric determinations of cell size and light scattering in conjunction with conventional measurements of cell size, light scattering and cell carbon content.
- To provide a calibrated data set of flow cytometric measurements of laser stimulated red fluorescence together with measurements of light absorption and chlorophyll *a* content.
- To develop algorithms for measuring intrinsic optical properties and cell carbon and chlorophyll contents using data collected by AFC.
- To compare molecular measurements of rRNA content with direct measurements of cell carbon content, and to develop appropriate conversion factors relating rRNA and organic carbon.

- To demonstrate the utility of the approach and the applicability of the software by measuring the carbon and chlorophyll contents of populations of the dominant phytoplankton and bacterioplankton populations in a range of water masses using calibrated flow cytometric and molecular techniques.

3. METHODOLOGY

ANALYTICAL FLOW CYTOMETRY (AFC)

AFC is a technique for characterising, quantifying and sorting cells based upon their optical properties. Analysis is achieved by passing cells singly across a focused laser beam at rates which can exceed 1,000 per second. The cells scatter light and those that contain a fluorochrome such as chlorophyll, also fluoresce. The light scatter can yield cellular indices of size and refractive index while the fluorescence can be related to the cellular fluorochrome content. Originally designed for biomedical use, flow cytometry has been applied to analysing phytoplankton and bacteria in seawater using a variety of protocols that are still under-developed (Burkill and Mantoura, 1990). AFC has been used to identify and characterise microbial populations within mixed natural assemblages. However, identification has often been limited to a few (typically 3 or 4) populations, and characterisation has often been qualitative rather than quantitative (Olsen *et al.*, 1990; Li *et al.*, 1992; 1993).

ARTIFICIAL NEURAL NETWORKS (ANNs)

Flow cytometry offers considerable potential for the rapid, accurate and precise analysis of phytoplankton and bacterial populations in seawater (Jonker *et al.*, 1995). The vast quantities of multivariate data generated by flow cytometers provide a considerable challenge for data analysis, and previous work has shown that artificial neural networks (ANNs) are well suited to the task (Boddy *et al.*, 1994). Comparison of a range of ANN paradigms and non-neural techniques revealed that radial basis function ANNs are most suitable for the task (Wilkins *et al.*, 1994, 1996). They can be trained relatively rapidly, and they make identifications in real time.

The major difficulty in extending the ANN methodology from laboratory pure cultures to mixed populations in oceans is that the number of possible classes is unknown (i.e., the problem is unbounded). It is essential to be able to acknowledge as unknown cells from classes upon which the network has not been trained. This case is common to biological and medical problems though not to other classification problems and has not been extensively investigated. Radial basis function (RBF) ANNs can provide a solution to this problem (Morris and Boddy, 1996); this will be validated and limitations determined in this study using mixed cultures and natural samples.

Further, different parts of the ocean and the same area at different times will have different species compositions. It is therefore necessary to be able to customise networks. For traditional neural networks this usually requires retraining of the network from scratch which is time consuming. Because retraining networks by non-specialists should be avoided as far as possible, a solution is to employ single species networks, each of which discriminates a single taxon from all others. Numerous individual networks will be implemented during the project and the facility to train a net for a new taxon will be made available. Training these single nets is a simple task that can be automated. Appropriate single species networks will then be combined to provide identifications. An ideal identification system should be able to make decisions about which networks should be employed. Further, identification of taxa may be less important than identification and quantification of functional groups of organisms. Tied to this, discriminating populations of phytoplankton in field samples without prior training of ANNs with laboratory cultures would often be of benefit. To these ends, clustering approaches (ANNs employing unsupervised learning) will be developed both as input stages to supervised networks and as a means to forming groupings more appropriate to flow cytometric analysis.

BIO-OPTICAL PROPERTIES OF MICROORGANISMS

The light scattering and absorption properties of a microbe are directly related to the size, shape and refractive index of the cell (Spinrad and Brown, 1986; Morel, 1991). The refractive index of the cell is determined by its chemical composition (Barer and Joseph, 1954). Specifically, the real part of the refractive index, which is important to light scattering, is related to the intracellular organic carbon concentration. In phytoplankton, the imaginary part of the refractive index, which is important to light absorption, is related to the intracellular pigment concentration. The relationships between intracellular carbon concentration and the real part of the refractive index and between intracellular pigment concentration and the imaginary part of the refractive index provide the basis for the optical determination of phytoplankton carbon and chlorophyll *a* contents. However, the real and imaginary parts of the refractive index of cells are not directly observable. The most readily observable quantities are the light scattering and absorption coefficients of cells in suspension. The scattering and absorption coefficients depend, in a complicated manner, on cell size and shape, the real and imaginary parts of the refractive index, and the distributions of these attributes amongst a population of similar but not identical cells (Morel and Bricaud, 1981; Morel 1991). Optical parameters will be determined from cell size, light scattering and light absorption. Comparison between theory and experiment will be made using full Mie programs. The imaginary part of the refractive index (n') will be calculated and values for n', together with the size distributions will be used in the Mie calculations to determine the real part (n) of the refractive index.

Flow cytometry provides a technique for obtaining measurements of the size and amount of light scattered by individual cells. The time of flight of a particle through the measurement beam is related to a length scale, and the amount of forward and side scattering of light by individual cells is related to the scattering of light by the cell population in suspension. The red fluorescence arising from the stimulation of chlorophyll *a* (and associated pigments) as a cell passes through the measurement beam is related to cell chlorophyll content (Graziano *et al.*, 1996). Thus, flow cytometry can, in principle, allow the determination of the properties necessary to estimate cell size, cell carbon content and cell chlorophyll *a* content.

Simultaneous measurements of biomass, pigment content, light absorption, light scattering, cell size will be compared with flow cytometric measurements of forward scattering, right angle scattering, time of flight and fluorescence to develop algorithms for estimating pigment and carbon contents of microbial cells from flow cytometric observables. A number of different analytical flow cytometers will be employed in this research because differences in optical geometry can lead to wide variations in scattering signals and because the end product software will be designed for use with multiple flow cytometer configurations. Although the instrumentation for laboratory and field research has not been finalised at the time of writing, measurements will be made using commercially available (Becton Dickinson FacScan and FacSort) and custom built (CytoBuoy) flow cytometers

SOFTWARE

The software developed during the AIMS project will focus on two main items. The first is the integration of artificial neural nets in a software package for flow cytometry. The second is the integration of algorithms that convert raw data from flow cytometers into inherent properties of cells, like cell size, volume and pigment content. A short description of these two follows:

Artificial neural nets. In order to have a flexible system of using the analysis power of artificial neural nets for flow cytometry, this technique is being integrated directly in a software package for flow cytometry. This allows for a combination of classification with neural nets with standard graphical representation of data using dot plots and histograms such that (semi-)real time classification using ANN of flow cytometric data should be possible. The software will be able to train neural nets on different species or subpopulations. Trained neural nets will be produced for different sets of species and also combinations of nets are foreseen. This should facilitate combining existing nets with newly trained nets on additional species. The results of neural net classification will be used to produce sample information, but also to graphically present the classification in dot plots and histograms to allow a visual assessment of the quality of the nets and possible changes in optical properties of cells.

Algorithms for inherent properties of cells. The software of the AIMS project will have features for flexible integration of algorithms that translate raw data from flow cytometers into inherent properties of cells. The user of the software will be able to flexibly derive extra parameters like cell length and volume from parameters like scatter, fluorescence and time of flight. The software also allows for integration of more or less fixed and calibrated parameters as soon as they come available.

MOLECULAR PROBES OF TAXONOMIC AFFILIATION

Ribosomal RNA sequence comparisons, particularly those involving the small subunit rRNA (18S rRNA in eukaryotes; 16S rRNA in prokaryotes), are commonly used in the study of evolutionary relationships between species. Ribosomal RNA genes contain regions of considerable sequence diversity (variable regions) but also contain regions that are evolutionarily conserved. These regions lend themselves to both the design of universal primers for amplification of rRNA sequences using polymerase chain reaction (PCR), and to the construction of family-, genus- and species-specific oligonucleotide probes using the sequence data obtained from the PCR amplification products. Whilst these attributes are also true of a number of evolutionarily conserved protein-encoding genes, the high copy of rRNA molecules (up to 10^5 per cell) leads to vastly increased sensitivity and enables single cell analysis to be consistently achieved. Initially radiolabelled probes were used for identification of single cells (Giovannoni *et al.*, 1988), but more recently fluorescently labelled oligonucleotides have been combined with flow cytometry for the investigation of the taxonomy and distribution of marine organisms, including phytoplankton (Simon *et al.*, 1995; Knauber *et al.*, 1996).

RNA content can be measured by either biochemical methods or hybridisation methods. Biochemical methods utilise colourimetric reactions or nucleic acid specific fluorochromes such as ethidium bromide. Hybridisation methods are based on the binding of specific nucleotide sequences on a labelled probe to the complementary sequences on the target RNA molecule. Hybridisation methods allow rRNA to be readily quantified in the presence of messenger RNA (mRNA) and transmitter (tRNA). The probes carry a fluorescent or radioactive label, or alternatively carry a target for secondary detection. Hybridisation methods capitalise on the evolutionarily conservative nature of rRNA which provides the ability to selectively target locations on the rRNA molecule whose nucleotide sequences are specific to a particular taxonomic group of interest. Differences in the extent of evolutionary conservation between segments of different rRNA molecules allow design of taxonomically broad or taxonomically narrow probes. Hybridisation methods can be used with bulk samples of extracted nucleic acids or with intact cells (*in situ* hybridisation). *In situ* hybridisation techniques have been employed to study the rRNA content of bacterioplankton (Kemp, 1995). It is natural to extend this approach to the examination of the rRNA content of individual phytoplankton cells (Simon *et al.*, 1995).

WEB SITE FOR PUBLICATION OF RESULTS AND TOOL FOR FUTURE ANALYSIS

An integrated and hypertext based data archive will be developed such that links can easily be made between data from flow cytometry, molecular biology and the optical and chemical characterisation of samples. A reference guide will be developed that consists of the procedures, as developed during this project, the data bases of flow cytometric data of individual species and field samples, algorithms for translation of flow cytometric parameters to inherent optical and chemical properties and the software for analysis of flow cytometric data using artificial neural net analysis. Background information will be included in the database. Keys to this data base will include species, origin of the sample, date and time of sampling and the various analyses. Links will be made to the flow cytometric data files with the raw data. The processed flow cytometric data will be included in the data base. Links will also be made with external data bases summarising genetic information in order to facilitate commercialisation of the results. This should result in a 'multi-media' product that will be available to future users of flow cytometry for analysis of phytoplankton. The progress of the AIMS project can be seen at the AIMS-site: http:\\www.flowyctometry.org.

4. WORK IN PROGRESS

The AIMS project started on 1 October 1997. Experimental work is currently being undertaken to provide a data matrix for developing algorithms for calculating intrinsic intrinsic optical properties and cell carbon and chlorophyll contents from flow cytometric variables. The utility of various analytical neural nets to identify phytoplankton microbial populations is being assessed, and software is being developed to allow identification and characterisation procedures to be implemented in near real time. The utility of the approach and the applicability of the software will be tested with laboratory populations in 1998, natural populations of marine microbes in a mesocosm experiment in 1999 and a research cruise in 2000.

REFERENCES

Barer, R. and Joseph, S., 1954. Refractometry of living cells. Part 1. Basic principles. Q. Journal of Microscopical Science, **95**, 399-423.

Boddy, L., Morris, C.W., Wilkins, M.F., Tarran, G. and Burkill, P. H., 1994. Neural network analysis of flow cytometry data for 40 marine phytoplankton species. Cytometry, **15**, 283-293.

Burkill, P.H. and Mantoura, R.F.C. 1990. The rapid analysis of single marine cells by flow cytometry. Philosophical Transactions of the Royal Society A, **333**, 99-112.

Culverhouse, P., Williams, B. and Reguera, B., 1996. Workshop on automatic categorisation of marine biological material for ecosystem research and monitoring, 1996. University of Plymouth. EC contract MAS3-ct95-63-81.

Giovannoni, S.J., Delong, E.F., Olsen, G.J. and Pace, N.R., 1988. Phylogenetic group-specific oligodeoxynucleotide probes for identification of single microbial cells. J. Bacteriology, **170**, (2) 720-726.

Graziano, L.M., Geider, R.J., Li, W.KW. and Olaizola, M., 1996. Nitrogen limitation of North Atlantic phytoplankton: analysis of physiological condition in nutrient enrichment experiments. Aquatic Microbial Ecology, **11**, 53-64.

Jonker, R.R., Meulmans, J.T., Dubelaar, G.B.J., Wilkins, M.F. & Ringelberg, J., 1995. Flow cytometry: a powerful tool in analysis of biomass distributions of phytoplankton. In: Mur, L. R. *et al.* (eds.) IAWQ SIL Conference on Selection Mechanisms controlling biomass distributions. **32**, 177-182.

Kemp, P. F., 1995. Can we estimate bacteria; growth rates from ribosomal RNA content? pp 279-302 in Joint, I. (ed.) Molecular Ecology of Aquatic Microbes. Springer-Verlag, Berlin/New York.

Knauber, D.C., Berry, E.S. and Fawley, M.W., 1996. Ribosomal RNA-based oligonucleotide probes to identify marine green ultraphytoplankton, J. Euk. Microbiol., **43**, (2) 89-94.

Li, W. K. W., Dickie, P.M., Irwin, B.D. and Wood, A.M., 1992. Biomass of bacteria, cyanobacteria, prochlorophytes and photosynthetic eukaryotes in the Sargasso Sea. Deep Sea Research, **39**, 501-519.

Li, W.K.W., Zohary, T., Yacobi, Y. Z. and Wood, A. M. 1993. Ultraphytoplankton in the eastern Mediterranean Sea: towards deriving phytoplankton biomass from flow cytometric measurements of abundance, fluorescence and light scatter. Marine Ecology Progress Series, **102**, 79-87.

Morel, A. 1991. Optics of marine particles and marine optics. pp 141-188 in Demers, S. (ed.) Particle analysis in Oceanography NATO ASI Series, Vol. G 27. Springer-Verlag, Berlin.

Morel, A. and Bricaud, A., 1986. Inherent properties of algal cells including picoplankton: theoretical and experimental results. Canadian Bulletin of Fisheries and Aquatic Science, **214**, 521-559.

Morris, C.W. and Boddy, L., 1996. Classification as unknown by RBF networks: discriminating phytoplankton taxa from flow cytometry data. In: Dagli, C. H. *et al.* (eds.) Intelligent Engineering Systems through Artificial Neural Networks vol. 6. ASME Press.

Olsen, R.J., Chisholm, S.W., Zettler, E.R. and Armburst, E.V., 1990. Pigments, size and distribution of *Synechococcus* in the North Atlantic and Pacific Oceans. Limnology and Oceanography, **35**, 45-58.

Simon, N., LeBot, N., Marie, D., Partensky, F. and Vaulot, D., 1995. Fluorescent in situ hybridisation with rRNA-targeted oligonucleotide probes to identify small phytoplankton by flow cytometry. Applied and Environmental Microbiology, **61**, 2506-2513.

Spinrad, R.W. and Brown, J.F., 1986. Relative real refractive index of marine microorganisms: a technique for flow cytometric estimation. Applied Optics, **25**, 1930-1935.

Wilkins, M. F., Morris, C.W. and Boddy, L., 1994. A comparison of radial basis function and back propagation neural networks for identification of marine phytoplankton from multivariate flow cytometry data. CABIOS, **10**, 285-294.

Wilkins, M.F., Boddy, L., Morris, C.W. and Jonker, R.R., 1996. A comparison of some neural and non-neural methods for identification of phytoplankton from flow cytometry data. CABIOS, **12**, 9-18.

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1. INTRODUCTION

During the last few years, some important research activities relating to bottom sediments in a marine environment have been developed. The fundamental scientific research, however, has been stimulated by new questions, which arise from the pollution of the environment and from large infrastructure constructions, which were erected on the continental shelf and in estuaries of industrialised countries or which are still under development. Concern about pollution of the European seas and estuaries, together with large civil engineering constructions in many European harbours, are the principal agents for a more profound knowledge of the marine environment.

The characteristics of the upper few metres of the sea bed in terms of structure and type of sediments are furthermore of particular interest to industry and sciences for applications such as:

* Laying of cables and pipes;
* Foundations for off-shore structures;
* Investigating soil and slope stability;
* Sediment migration studies;
* Object detection;
* Improving the accuracy of propagation modelling;
* Relating sub-bottom properties to biological activity.

The methodology of investigating the sea floor has changed drastically in the various disciplines involved in a marine study. This holds for sedimentology and stratigraphy in the discipline of marine geology, but also for biology, geochemistry, hydrography etc. The availability of data has also increased enormously. An important aspect of these investigations is that they require more or less an a priori knowledge of the sediments of the subbottom.

In some cases, the sea bed could be investigated by taking samples or cores on-site, but these methods provide significantly less information than a continuous measurement. An increasingly environmentally conscious community would prefer a non-invasive method to reduce the impact on the sea bed environment and, hence its fauna and flora. An acoustically based remote sensing method for determining sediment properties, using equipment either on a research vessel or a near-surface tow fish, would provide such detailed information, and is likely to be less time consuming than a method requiring equipment deployed on the bottom.

Acoustic time domain reflectometry is one of the most important investigation techniques to perform remote sensing of the sea bed. It can be configured to measure depth (echo sounding to obtain the bathymetry), to map the bottom morphology (side scan sonar) and to study the upper sub-bottom layers (sub-bottom profiling) or the deeper layers of the sea bottom (seismic investigations). The distinction between the seismic and sub-bottom profiling techniques is important. The first yields information about the geological structure of the ocean bottom, although with limited vertical resolution, whereas the latter becomes extremely useful in studies of the water- sediment interface and of superficial layers, which are in the few meters depth and which contain the youngest deposits. These deposits are important in environmental problems and for sustainable management.

Therefore, an appropriate systematism needs to be developed for inventory purposes (mapping) and for the accurate location of the various sediment types, but also for the gathering of information of the layers' thickness and stratigraphic features. This all contributes to enhance knowledge of the sediments' origin and the dislocations and movements induced by tidal currents and waves, the discharge of rivers (in the case of estuaries, deltas), storm conditions, etc.

Commercially available acoustic measurement instruments can generally only provide information about the thickness of sediment layers. The intrinsic sediment properties can not up to now be defined, unless sampling or coring is foreseen. However, parameters such as the sand-silt-clay ratio, density and porosity are of the greatest importance in correlating results with other research disciplines and for practical marine engineering.

Some attempts were made to propose techniques of classifying the sea bed by acoustic means. These attempts, however, were based on empirical considerations and laws and could produce serious errors and ambiguities. In this project, fundamental scientific research is combined with applied engineering to obtain functional relations between estimated quantitative acoustic parameters and sediment properties. This leads, furthermore, to versatile, high resolution, general purpose acoustic remote sensing instrumentation. The instruments developed in the SIGMA project provide continuous measurements to characterise ocean sub-sea sediments by the means of a system towed below the water surface.

2. PROJECT OBJECTIVES

2.1. SUMMARY

The aim of the SIGMA project is to investigate the relations between the acoustic parameters of marine sediments (reflection factor, sound velocity, attenuation, dispersion etc.) and their geophysical and geotechnical properties (sediment type, grain size distribution, cohesion, gas content, etc.).

The acoustic parameters of the sediments are estimated from wide frequency band measurements, using a steerable parametric array combined with a towed array of receivers. Such a source allows spot measurements on the sediments and allows to estimate with high accuracy their acoustic properties.

The aim will be achieved by means of a multidisciplinary approach:

- acoustic modelling
- development of inverse procedures
- calibration
- generation of optimal test signals
- system identification
- validation by tank experiments
- development of specific instruments
- sea trials
- ground truthing
- sea data processing and archiving

2.2. DESCRIPTION OF THE OBJECTIVES

The overall aim of the SIGMA project is to determine the characteristics (i.e. fine scale structure and sediment properties) of the sea bed using remote sensing technologies based on the reflection of acoustic pulses. The objectives to achieve this goal are:

1) To treat the marine environment as a complex system to be identified. A MIMO (Multiple Input Multiple Output) SI (System Identification) scheme will be used to determine the sea bed characteristics.

2) To stimulate the marine system, active acoustic sources will be used. High power acoustic signals (Multiple Inputs) will be generated using a steerable parametric array and a vertical parametric down looking array, mounted in a tow fish. This allows the transmission of relatively high power acoustic signals into a spot on the sea bed at a predetermined angle. Complementary, an high resolution shear wave seismic chirp source will be used as well.

3) To observe the response of the marine system the scattered and reflected acoustic signals from the sea bed and from sub-bottom layers will be detected by means of a towed hydrophone array (Multiple Outputs). To compensate for the motion of the tow fish (acoustic transmitter) a real time electronically stabilised system will be developed, while for the compensation of the movement of the towed array a special purpose acoustic device will be developed as well.

4) Theoretical and numerical accurate acoustic wave propagation models as well as inverse procedures will be investigated. The models and procedures will be generalised to include realistic cases like rough and oblique interfaces, hybrid velocity profiles (continuously layered and stepped variations), inhomogeneities and inclusions. Direct inversion computational methods and parameter estimators, such as an MLE (Maximum Likelihood Estimator), will be developed and analysed taking into account instrumental factors such as noise and the transmitter and receiver characteristics, which will be obtained by extensive calibration measurement procedures.

5) The theoretical models and inverse procedures will be validated based on the design of measurement procedures adapted to the wave propagation models. The validation will first be performed based on tank experiments (laboratory scale), before being applied to data from sea experiments (real-life). Ground truthing will be obtained using conventional equipment and improved geophysical and geotechnical instruments such as high resolution seismics and a geotechnical module.

6) Data analysis will be performed in order to investigate, in detail, the functional relations between the estimated acoustic parameters and sedimentological parameters obtained by ground truth in the sea experiments or from tank experiments, where the simulated sea bed is composed of calibrated sediments.

7) The data obtained during the sea experiments will be compiled in a GIS (Geographical Information System). The surveys, conducted in selected representative test areas will, therefore, lead to the constitution of a well controlled data base on bottom characteristics. This data base will be available on a CD-ROM and the small test areas may be used as benchmark for future instrument calibration.

3.0 PROJECT METHODOLOGY

In this project, the estimation problem is formulated in the framework of system identification. The noise on input as well as output data will be taken into account, since, beside direct inversion schemes, a Maximum Likelihood approach will be used. This approach allows the incorporation of the calibration procedure, as well as the model validation, since model errors can be detected and corrected easily.

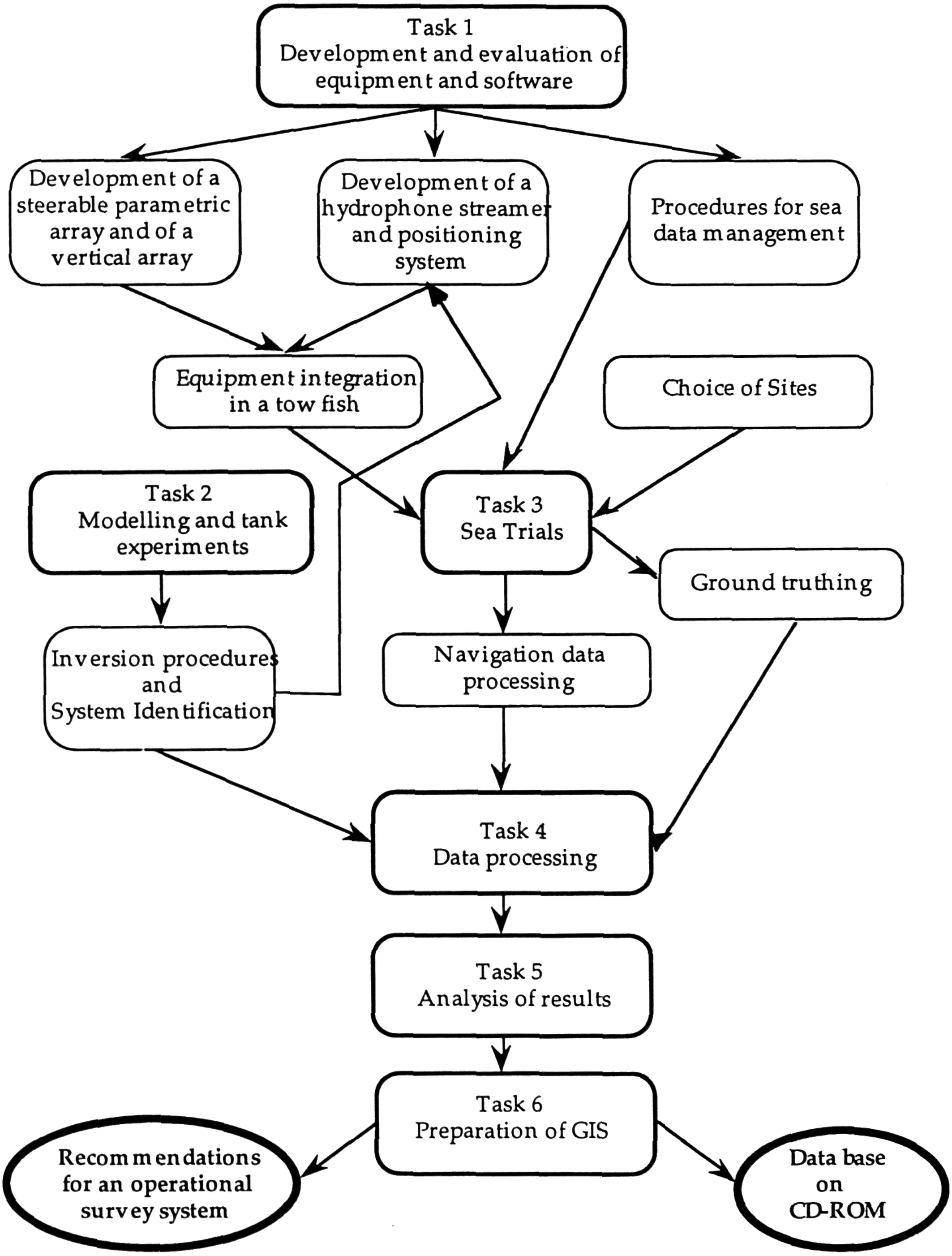
A key issue of this project requires the use of a parametric array for sea surveys during rough surface conditions, for example sea-state 5-6. In particular, automatic beam stabilisation will be addressed in detail in this project, so that the array can be programmed to transmit vertically downwards, through a repeated range of angles or at some preferential angle irrespective of the pitch of the towed fish.

In this project, it is aimed to demonstrate that the identification of sediment parameters can be performed using underwater acoustics, because the identification of sediment parameters fits into a global system identification approach. First acoustic parameters are estimated using robust estimators and then, using tank experiments and extensive ground truthing, functional relations between sedimentological and acoustic parameters are inferred.

The way to sense the marine environment acoustically in the project, has advantages over the classical echo sounding procedures, where the angle of incidence during sea trials remains difficult to control. Therefore, two arrays are foreseen. A vertical down looking one (monostatic) and a second one (bistatic), which allows enhanced control of the zone to be insonified during the trials. It is envisaged that the second one will act like a looking glass, which can be moved over the sea bed, whereas the first one is used to deliver raw data representing the sea bed condition, such as the bathymetry and estimates of the sedimentation layers (rough measurements of the number of layers, thickness etc.). This is used as a priori information in the system identification tasks to ensure fast convergence and minimisation of the modelling errors.

4. TASK STRUCTURE OF THE PROJECT

In order to reach the objectives of the project, the Sub-tasks are defined as presented in the general scheme of Fig. 2.1. Four major areas are covered: underwater acoustics, marine geology, measurement science and data processing. Table 2.2 summarises the Tasks and Sub-tasks and indicates the role of the partners.


Task 1
Development and evaluation of equipment and software
Development of a steerable parametric array and of a vertical array
Development of a hydrophone streamer and positioning system
Procedures for sea data management
Equipment integration in a tow fish
Choice of Sites
Task 2
Modelling and tank experiments
Task 3
Sea Trials
Ground truthing
Inversion procedures and System Identification
Navigation data processing
Task 4
Data processing
Task 5
Analysis of results
Task 6
Preparation of GIS
Recommendations for an operational survey system
Data base on CD-ROM

TITLE: Transmission of Electromagnetic Waves Through Sea Water for Imaging, Parameter Measuring and Communications

(Project DEBYE)

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Transmission of Electromagnetic Waves Through Sea Water for Imaging, Parameter Measuring and Communications

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Liverpool L69 3GJ

1. Summary

This project concerns the propagation of electromagnetic (EM) waves through sea water (clear, murky and polluted) for frequencies in the range 10MHz to 30GHz.

The propagation characteristics depend upon a combination of the conductivity (σ) and dielectric (ε) properties of the various conditions of sea water. Theoretically the spatial and temporal variations of the electric E and magnetic H fields are obtained from Maxwell's equations with the dielectric properties being given by Debye's equation. Experimentally these solutions can be examined in the laboratory using resonance cavities filled with simulated sea water with various quantities of included sand and pollutants.

Initially measurements of the attenuation coefficient (α) and the velocity (v) of the E (or H) field waves may be obtained from which σ and $\varepsilon(=\varepsilon^{1} + j\varepsilon^{11})$ will be obtained as a function of frequency. Precision measurements will be obtained by using "state of the art" spectrum analysers and data logging systems thus allowing accurate measurements of both σ and ε to be obtained for comparison with the relaxation theory. The cavity technique can also be readily used in both a dock and a coastal water environment. Hence the variations of σ and ε with frequency may be compared for conditions of clear, murky and polluted sea water.

The results of this investigation will be aimed at two important offshore applications. The first application is as a pollution monitor. The second application is to allow through water propagation between a transmitting aerial and a receiving aerial for a distance of the order of 50m and a propagation frequency of about 10MHz. This will confirm previous but scarce results, obtained about two decades ago, which showed that such transmission was possible. This new investigation will benefit from the modern equipment now available from satellite and mobile radio research which allow signal attenuation up to -200dB to be successfully recovered and interpreted.

2. Introduction

The nature of the ocean environment and its vast size have necessitated the development of sophisticated equipment and techniques. To facilitate scientific exploration a wide variety of systems and vehicles have been developed to operate either within the shallow continental shelf region or in deep oceans. For successful operation knowledge is required of the electromagnetic transmission properties of water over all distances both short and long. This information is required for such activities as:-

- Sensor Systems
- Obstacle Detection
- Imaging
- Guidance

- Position Fixing
- Measurement of Speed
- Communication of Data/Voice
- Remote Control

Present systems involve the transmission of information in the form of light, sound or electric and magnetic fields. However each of these techniques have advantages and limitations. Optical systems are generally limited to extremely short distances because of backscatter and absorption. In the Irish Sea, for example, visibility is often zero or at best 1 - 2m due to suspended matter in the water. Acoustic systems are the most versatile and widely used technique. Both optical and acoustic systems, however, are unable to penetrate behind an object and suffer from shadow zones. In shallow water, the use of acoustic techniques can be severely affected by multi-path propagation in water due to reflection and refraction. The comparatively slow speed of acoustic propagation in water, of the order of 1500m/s, is a limiting factor in terms of transmission data rates. Where optical systems fail because of suspended matter, and acoustic systems because of high ambient noise levels, methods using electric and magnetic fields may offer an effective alternative for use over short distances.

Maxwell's equations predict the propagation of electromagnetic waves travelling in sea water. A linearly polarised plane electromagnetic wave travelling in the z direction may be described in terms of the electric field strength E_x and the magnetic field strength H_y with,

$$E_x = E_o \exp. (jwt - \gamma z), \qquad H_y = H_o \exp. (jwt - \gamma z)$$

The propagation constant (γ) is expressed in terms of the permittivity (ε), permeability (μ) and conductivity (σ) by

$$\gamma = jw\sqrt{\varepsilon\mu - j\frac{\sigma\mu}{w}} = \alpha + j\beta$$

where α is the attenuation factor, β is the phase factor and $w = 2\pi f$ is the angular frequency.

The term $\varepsilon\mu$ arises from the displacement current and the term $\sigma\mu/w$ from conduction current. It is convenient to consider the solutions for the conduction band ($\sigma/w > \varepsilon$). and the dielectric band ($\varepsilon > \sigma/w$). For sea water the condition $\varepsilon = \sigma/w$ occurs for f = 1 GHz.

Investigations of the parameters σ and $\varepsilon(=\varepsilon^1 - j\varepsilon^{11})$ over the full EM frequency spectrum have been obtained in electrolytic solutions by using a wide variety of experimental techniques. The more popular techniques involve the use of coaxial transmission lines and waveguide cavities. If the sea water is in direct contact with the walls of the line or cavity then the conduction band losses apply. For example [1], a coaxial transmission line filled with sea water gave an attenuation coefficient α = 300dB/m for f = 67MHz which is in good agreement with the conduction band solution as shown in figure 1.1. (This result is shown by the symbol □). In experiments where the sea water is placed in an insulating container [2] within the waveguide, the conduction losses are extremely small and the dielectric solution applies. (These results are shown by the symbol o). Hence combining these two experimental techniques will allow the simultaneous evaluation of a range parameters for sea water such as σ, ε^1 and ε^{11} and the wave velocities v_σ and v_ε. Such an investigation will allow a detailed documentation of the properties of coastal sea water (clear, murky and polluted) to be investigated for a wide range of temperatures, depths and frequencies. Because of the longer wavelength of the EM waves, to be used in this study, it is anticipated that the effect of murky water or pollutants will be less severe than for the optical systems.

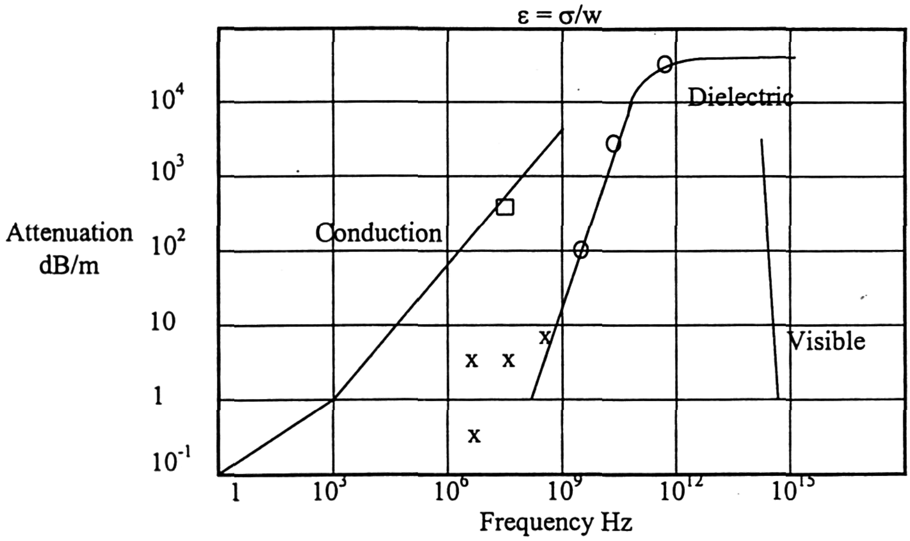

Figure 1.1 The EM Wave Propagation in Sea Water

The electrical permittivity ($\varepsilon = \varepsilon' - j\varepsilon''$) of water is very sensitive to temperature, frequency and conductivity. At 3GHz frequency, water has a dielectric constant (ε') of 80 at 1.5°C and 52 at 95°C. whilst Tan δ (= $\varepsilon''/\varepsilon'$) varies over the same temperature range from 0.31 to 0.047. In addition the dielectric properties of water are greatly modified by the presence of salts.
At low frequencies sea water has a dc conductivity σ = 4S/m which corresponds to 0.4 gram mole/litre of NaCl. Experimentally the dc conductivity (σ) can be isolated from the ac conductivity (ε^{11}). A theoretical explanation of the frequency variation of both ε' and $j\varepsilon''$ has been given by Debye [2] as follows

$$\varepsilon = \varepsilon^{1} - j\varepsilon^{11}$$ Dielectric Permittivity

$$\varepsilon^{1} = \varepsilon_{\infty} + \frac{\varepsilon_{S} - \varepsilon_{\infty}}{1 + j\omega\tau},$$

where τ = Relaxation Time

By substitution we have

$$\varepsilon^{1} = \varepsilon_{\infty} + \frac{\varepsilon_{S} - \varepsilon_{\infty}}{(1 + \omega^{2}\tau^{2})}$$ Dispersion

$$\varepsilon^{11} = (\varepsilon_{S} - \varepsilon_{\infty})\frac{\omega\tau}{1 + \omega^{2}\tau^{2}}$$ Conductivity

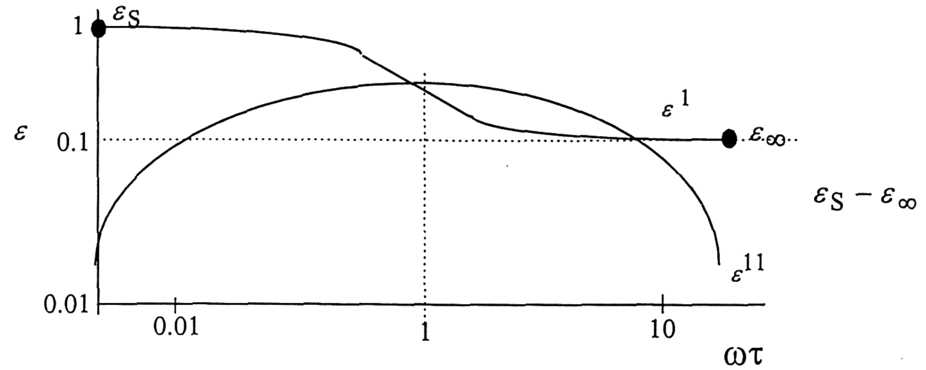

Figure 1.2 The Dielectric Coefficient for Water

Three coefficients can be deduced by experimentation, namely ε_S and ε_∞ and τ. A more detailed equation by Cole and Cole [2] has also been used in the analysis of the experimental data.

One important aspect of this detailed study would be an assessment of the possibility of short range propagation of EM waves through sea water for imaging, positioning and communications activities. The ability to communicate over a distance of 50m by using EM waves would have major scientific and commercial benefits for subsea activities.

A literature survey of EM wave propagation has shown that such measurements are scarce, but such limited investigations have shown that short range propagation between a transmitter and a receiver is possible. (These have been shown by the symbol x in figure 1.1). Propagation in sea water at 7MHz [3] has produced a transmission distance of 460m at a depth of 76m. Measurements at 150MHz [3] and 14MHz [4] have measured propagation over a distance of 30m with the attenuation coefficient (α) of 2.6dB/m respectively. Previous measurements by Liverpool have shown propagation down a waveguide (WG9A) immersed in sea water for a frequency of 500MHz, which is much higher than the cut-off frequency, is possible with an attenuation of 8dB/m with the propagation having a Gaussian type mode. All these results have shown that when EM waves are propagated through sea water, the attenuation is reasonable and as expected from the dielectric theory. However, the launching of the wave into the water using conventional metal aerials occurs within the conduction band theory and may result in a loss as high as -50dB for a 14MHz frequency [4]. This loss is expected to decrease with increasing frequency. Theoretical considerations indicate that the conductivity and hence the aerial loss will decrease with increasing frequency [5]. This is a well known relaxation effect found in plasma physics and is also expected to occur in electrolytic solutions [2]. There is a relaxation time before the conductivity losses can occur and this is expected to occur at UHF frequencies and above. As the frequency decreases the cloud of positive ions around the negative ion reaches equilibrium state and the conduction losses are able to build up to the low frequency value. In addition, for frequencies in the range 30MHz to 3GHz, low loss parabolic aerials can be designed having good directional capabilities and this will improve the transmission of the EM signal between the aerials.

To summarise it is unlikely that wave transmission can be obtained over long distances but short range transmission up to 50m should be possible at a frequency of about 10MHz provided sufficient transmitter power is available to compensate for both water attenuation and aerial losses. Modern communication systems can operate with a total system loss of

-200dB. Thus allowing for total loss of -40dB by both the transmitter and the receiver and a -2dB/m loss in the sea water, a range up to 80m may be anticipated.

3. Objectives

The project aims at undertaking the following objectives:-

1. To investigate the propagation of EM waves within sea water (simulated, clear and murky) and to measure the velocity (v) and attenuation coefficients (α) within the high frequency range (10MHz to 26.5GHz).
2. To model the propagation of EM waves through sea water, within the conduction and dielectric bands, by using Maxwell's equation and to ascertain the frequency dependency of ε^{1} and ε^{11} and σ and their theoretical explanation using relaxation theory (Debye).
3. To investigate the suitability of using EM waves for sub-sea activities such as water condition monitoring, imaging, communication, positioning and locating objects and to specify the preferred frequencies and mode of operation.

4. Workplan

The main activity of the research is to measure the propagation of EM waves through sea water over the frequency range 10MHz to 26.5GHz with special preference being given to the frequency range 10MHz to 3GHz because of the scarcity of experimental information. The parameters to be measured are the EM wave velocity (v m/s) and the EM wave attenuation coefficient (α dB/m). A laboratory EM cavity, will initially be used for undertaking this investigation. Measurements will firstly be undertaken within the laboratory using a rectangular cavity and filled with simulated sea water followed by further measurements in sea water within a dock complex. These experimental results will be rigorously compared with EM wave theory for both the conduction zone and for the dielectric zone in order to understand the transition between the zones and to provide satisfactory models for EM wave propagation. A second series of experiments will be undertaken using transmitting and receiving aerials within both a pool and dock complex.

Based upon the results, two application activities will be undertaken namely:-

- A selection of the best operational frequency for short range transmission between 30m and 50m of the EM wave for use with imaging, communication, positioning and object tracking systems. A demonstrator system will be produced.
- The best operational frequency range for using the EM cavity to measure such parameters as σ, ε^{I}, ε^{II}, v_σ and v_ε and thereby investigate the effect of pollutants and turbidity around an outfall from sewerage and industrial waste water. A demonstrator system will be produced.

5. Experimental Arrangement

The experimental apparatus is shown in figure 5.1. It consists of an HP Spectrum Analyser operating over the frequency range from 10kHz to 2.9GHz. It uses an HP Tracking Generator to automatically sweep through this frequency range and the recorded output signals may be produced as a hard copy by using an HP deskjet Plotter. The signals are transmitted by using a simple loop aerial down a choice of several rectangular and cylindrical waveguides ranging in length from 0.3m to 2.8m. Each waveguide has been totally filled with the water mixtures.

Each of the guides could be operated in either air, distilled water, tap water and "salted" water with various concentrations of sea water simulant. The salt water solution with 0.4 gm mole/litre concentration has an equivalent electrical conductivity to that for sea water (4S/m). Results have been recorded up to 1 gm mole/litre concentration i.e. 2.5 times the concentration expected in sea water. Sea water simulant, provided by DRA, has also been investigated.

Some trials have also been undertaken using loop aerials unmatched to the input or output electronics which had been designed for 50Ω terminations. However the loop aerials were coated in a thin layer of insulation to prevent electrical conduction and corrosion contact with the water.

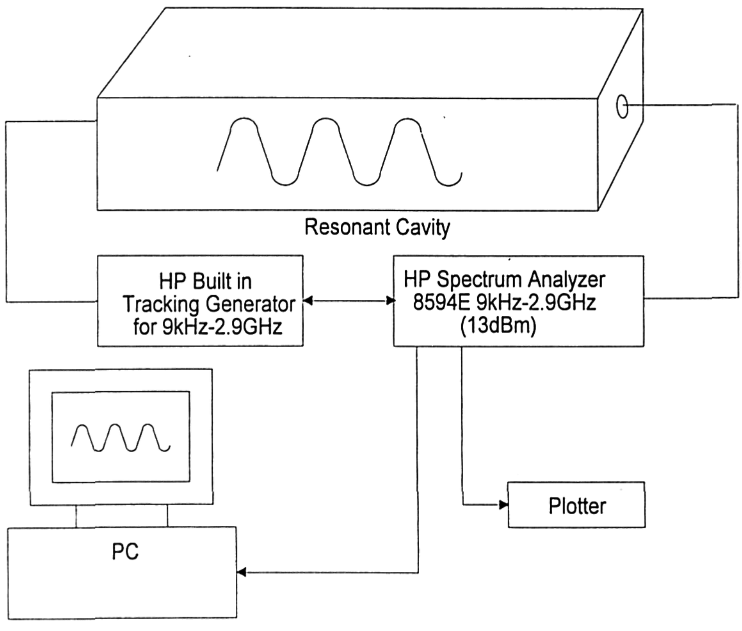

Figure 5.1 The Laboratory Experimental Apparatus

Preliminary Experiments

In this experiment a cylindrical cavity of 159mm long by 190mm diameter is filled with water. Figure 5.2 shows the range of the frequencies whose range varies from 50 MHz to 550MHz. This low frequency is because the dielectric constant of water is of the order of 81. There are a series of clearly defined resonances whose amplitude decays at the higher frequencies.

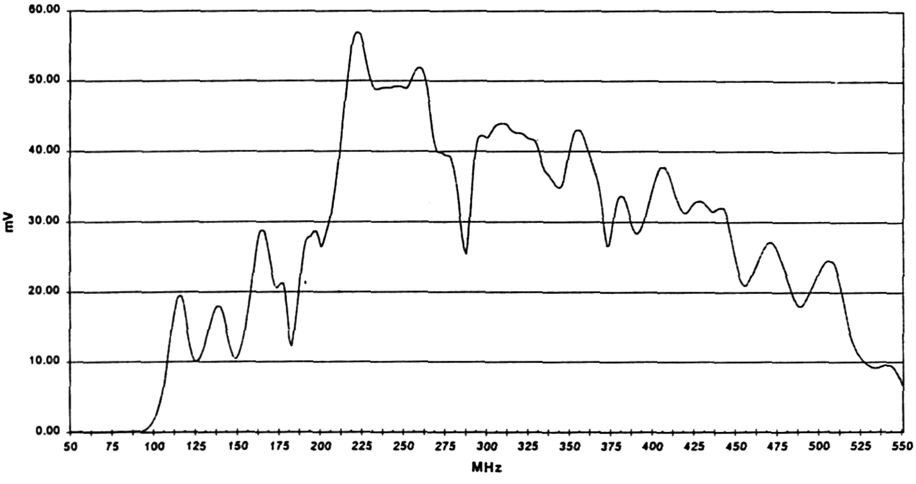

Figure 5.2: The full spectrum of the cavity when it is filled with water.

6. Task Structure of the Project

Using such cavities the programme of research will be undertaken by carrying out seven tasks. These are as follows

TASK 1. The EM cavity measuring and data logging system.

A resonant cavity will be designed and constructed to operate in the frequency range 10MHz to 3GHz. Alternative designs will be used to examine the region 3GHz to 26.5GHz where the microwaves are expected to be strongly absorbed by the water. Corrosion resistant cavity designs in sea water will be established

TASK 2. Theoretical considerations of EM wave propagation in sea water.

Solutions to Maxwell's equations will be obtained for the cavity for comparison with the experimental results. The theoretical solutions will provide guidance to finding suitable frequency ranges which will allow accurate and informative information to be provided. The effects introduced by the range of conductivity and temperature values found within sea water will be specified.

TASK 3. Laboratory measurements of EM wave velocity and attenuation in sea water and their theoretical interpretation.

Measurements will be taken over the frequency range 10MHz to 26.5GHz from which the attenuation coefficient and the wave velocity will be evaluated. The values for both α and v will be compared with the theoretical values obtained from the models of Debye and Cole-Cole with reference if necessary to plasma theory.

TASK 4. Measurements within a dock complex (for both clear and murky water conditions).

A portable EM cavity will be used within a dock environment to investigate the results obtained under tidal and turbulent conditions. Such sensors will also be placed near to outfalls to examine the pollutants occurring from land and river drainage

TASK 5. A short range EM wave transmission system (for imaging, communication, position and object location).

To undertake the design and construction of aerial design and the corresponding electronics for low signal recovery. The signal attenuation with the distance between the propagating and receiving aerials will be measured as a function of signal frequency. The measurements will be made mainly at the low signal frequencies (~10MHz).

TASK 6. A pollution monitoring system (around sewerage outfalls).

The EM cavity will be used to take measurements in both clear and polluted water. The spectral response, upto 26.5 GHz, will be explored to look for identifiable changes in the resonance spectrum. A chemical analysis of the pollutants will be undertaken and further trials will be undertaken, in the laboratory, to investigate in detail the polluting chemicals.

TASK 7. Data management.

The data will be documented to an agreed standard for each activity. The Internet will be used for data collection and information exchange. A WWW page will be used to publicise and provide information concerning many aspects of the Debye project as it develops

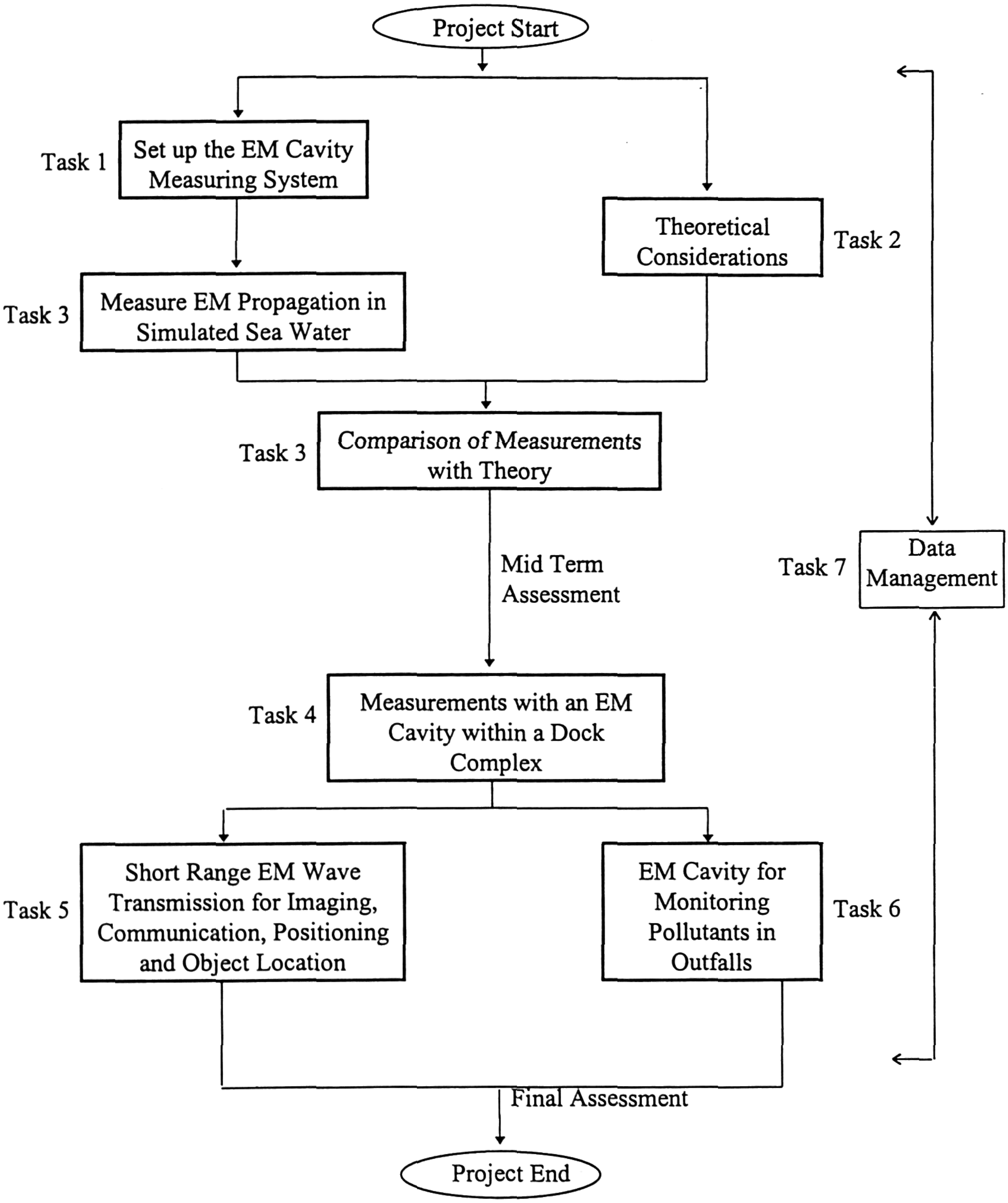

Figure 5.2 The Project Flow Diagram

7. Exploitation

The results of this project could have a major impact on sub-sea sensing. The ability to transmit long wave EM signals through water will allow a series of new IMR sensors to be produced for such activities as imaging, vehicle positioning and navigation and vehicle/base station communication. The advantages of EM waves is their high band width which will allow video images to be transmitted in real time which is not presently possible by using ultrasonic systems.

The pollution monitoring sensor having the capability of operating in both the conduction band and the dielectric band over a wide frequency range will undoubtedly have a technical advantage over the present low frequency conduction band system. The cavity technique will allow σ, ε^{1} and ε^{11} to be measured. Increased sea water conductivity should effect σ, ε^{1} and ε^{11} whilst increased turbidity should mainly effect ε^{11} . The cavity can be miniaturised and used with custom electronics to enable it to be carried by an ROV or placed on a buoy.

The ability to produce an EM wave communication system will have a major impact on subsea sensors and will have particular applications for positioning and navigation of ROV's within structures and also providing communications between the base station (buoy or benthic station) and the ROV. The EM wave bandwidth will be sufficient to transmit real time video images from the on-board ROV cameras to the base station.

8. References

1) D. Scott and G. S. Smith., "Measurement Techniques for Antennas in Dissipative Media"., IEEE Trans. on Antennas and Propagation, 1973, 4, 499-506.

2) J. D. Hasted., "Aqueous Dielectrics"., 1973, Chapman and Hall, SBN: 412 098008.

3) S. Bogie, "Conduction and Magnetic Signalling in the Sea"., The Radio and Electronic Engineer, 1972, 42, 447-452.

4) A. Siegel and R. W. P. King, "Electromagnetic Propagation Between Antennas Submerged in the Ocean"., IEEE Trans. on Antennas and Propagation, 1973, 4, 507-513.

5) E. Thomas, "Electrical Properties of Sea water (including conductivity relaxation)", 1984, 39pp, John Hopkins University (USA), Department of Submarine Technology. (AD-A151 382/9/GAR).

TITLE: **Improved MIcrostructure measurement TEChnologies for marine near surface flux studies (MITEC)**

CONTRACT NO: MAS3-CT97-0114

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Improved MIcrostructure measurement TEChnologies for marine near surface flux studies (MITEC)

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Summary

MITEC aims to design, develop and construct an advanced, efficient, integrated system for measuring, processing and evaluating oceanographic parameters - stratification, mixing, fluorescence, near surface microstructure and others -, that are essential to determine near surface fluxes which are required for a correct description of physical and biological processes in the surface boundary layer of natural waters. Application studies will be performed to demonstrate the potential of the developed system. The development of a methodology to investigate the influence of small-scale physical processes on the dynamics of marine ecosystems, and the responses of key organisms to varying oceanographic regimes, will be specifically addressed. In order to achieve this goal within the framework of MITEC the following activities are planned:

⇒ Development, adaptation and assessment of methodologies for performing the near surface microstructure measurements and to evaluate the data.
⇒ Development and construction of a free rising system to measure the near surface microstructure of density and current fields with a resolution in the millimetre-range, and to measure bio-optical properties. The data acquisition and basic evaluation software will be integrated within the system.
⇒ Laboratory and field measurements as well as application studies to assess the performance of the developed system and to demonstrate its application potential.

The final deliverable will be a complete end-to-end system for the assessment of important physical and biological parameters in the surface boundary layer, from gathering the data to the evaluation of surface boundary layer processes. The resulting prototype is intended to be the base for an industrial-commercial product of potential interest to a world-wide community involved in research and management of the marine environment.

Note: *During the time this report was written (January 1998) MITEC was still not in its working phase, so it is written in a style of describing work intended to be performed in near future.*

1. Introduction

The upper layer of the ocean, the so-called Surface Boundary Layer (SBL), is characterised by a highly dynamic behaviour with cycling between stable and unstable density stratification, depending on the relation between the turbulent momentum fluxes at the sea-atmosphere interface on the one hand and the corresponding buoyancy fluxes on the other. Vertical transport of momentum, heat and matter, and as a consequence all physical, chemical and biological processes in the SBL, are determined by this dynamic behaviour, which as a result affects climate and environment. Considering for instance the pelagic ecosystem, vertical transport causes nutrient pulses into the euphotic layer and sustains 'new' production of pelagic primary producers. In addition, small-scale turbulence has a pronounced effect on nutrient uptake, growth, strophic couplings and allelopathy of the smallest plankton organisms (see reviews e.g. by Thomas and Gibson 1990, Kiörboe 1993).

There are many unresolved problems in the experimental coverage as well as in the theoretical description and parameterisation of these turbulent microstructures. The same is therefore true for the related transport processes. There is no generally accepted parameterisation of the turbulent energy dissipation in terms of wind stress or heat flux in the near surface layer (Anis and Moum, 1995, Caldwell and Moum, 1995). Progress in this matter is necessary for investigations of any marine problem requiring knowledge of near surface fluxes at global, regional or local scale.

2. Objectives for MITEC

Exchange processes at the marine surface boundary are of a complex nature, comprising turbulent structures at different spatial and temporal scales. To improve our understanding of these processes to the point where they can be modelled or parameterised, it is essential to devise the means to acquire *reliable* data on the structures and interactions within the near surface layer. Small-scale turbulence in the surface boundary layer is relevant to many important physical and bio-geochemical processes in the aquatic environment, governed by mass, momentum and energy fluxes through the water-atmosphere interface. Consequently, these small scale processes are of important ecological relevance for aquatic systems in general and the marine surface boundary layer in particular. In addition, near surface transport processes represent a transfer function between the processes in the bulk of the water and their fingerprint at the sea surface - and this knowledge is essential for the correct interpretation of a satellite sensor's record of surface conditions.

Consequently the following two overall objectives were chosen for MITEC:

1. **to design, develop and construct an advanced integrated system for measuring, processing and evaluating oceanographic parameters - stratification, mixing, fluorescence, near surface microstructure and others -, that are essential to determine near surface fluxes which are required for a correct description of physical and biological processes in the surface boundary layer.**
2. **to perform application studies in order to demonstrate the potential of the developed system. This includes specifically the development of a methodology to investigate the influence of small-scale physical processes on the dynamics of marine ecosystems, and the responses of key organisms to varying oceanographic regimes.**

The development within MITEC will include new sensors and probes, as well as the best methodology for measurement and data processing. The envisaged system will be extensively

tested and the results of these tests will lead to further improvements. Within MITEC the capability and potential of the integrated measurement and data evaluation system will be demonstrated to obtain detailed information on the near-surface flux processes in the water.
The development of a methodology to investigate the influence of small-scale physical processes on the dynamics of marine ecosystems, and the responses of key organisms to varying oceanographic regimes, will be specifically addressed.
In MITEC close links between industry (SME's) and academic research will be established in order to improve technology transfer and ensure effective industrial exploitation. Further, a significant know-how transfer between the partners from different countries will occur.
The system development will progress within a close co-operation of the developers and producers on one side and typical users on the other, in order to ensure that the final product will meet their needs. This close co-operation will also allow the development to be carried out in two steps, so that the measuring system may be redefined following the first tests, should specific new user requirements arise.

The objectives will be met in 5 phases as follows:

- Development, adaptation and qualification of unified standard algorithms prescribing, how to carry out the measurements and evaluate the measured data.
- Development of a free rising microstructure profiler aimed at measuring the near surface microstructure of density, current fluctuations and current shear (turbulence) with the highest possible resolution. Other sensors providing information on bio-optical and standard oceanographic parameters will be integrated.
- Development, testing and qualification of software for data acquisition, calculation of basic turbulence parameters and data visualisation, specially designed for near surface uprising measurements. This software will have to work routinely under field conditions.
- Laboratory and field measurements with the prototype to evaluate performance and to define the final product. Application studies to demonstrate the potential of the developed measuring system and data evaluation, for investigating near-surface flux processes in the water and the influence of small-scale physical processes on the dynamics of marine ecosystems.
- The final definition of a complete end-to-end methodology for microstructure measurements, flux estimations and description of interactions between small scale turbulence and biology in the surface boundary layer.

The final deliverable will be a complete methodology for the assessment of important physical and biological processes in the surface boundary layer, from gathering the data to the evaluation of surface boundary layer parameters.

3. State of the Art

3.1 Instrumentation

Near surface shear microstructure measurements in the ocean were initiated with the use of free falling probes (Oakey and Elliott, 1982). Although this technique revealed important insights from relatively thick SBL's (Shay and Gregg, 1986), the most important near surface layer could not be studied due to profiler and ship disturbances at the very top of the water

column. An improved experimental set-up was established which used guided or quasi free rising profilers, equipped not with shear but with temperature microstructure sensors to probe the temperature small-scale fluctuations in the near surface zone (see for instance Thorpe, 1978, Soloviev and Vershinski, 1982, Carter and Imberger 1986, Wüest et al. 1996). Near surface small-scale turbulence measurements, using a quasi free rising profiler are also reported by Soloviev et al, 1988. Due to the deployment mode, these measurements only covered the uppermost 10 meters of the SBL, obviously missing an important part of it, especially with respect to convective processes, that can penetrate down to about 100 meters. The first reliable near surface small-scale current shear measurements using an airfoil shear sensor mounted on a tethered uprising profiler were performed by Anis and Moum (1992, 1995). Due to the unsatisfactory deployment method which was applied, only a very limited data set was recorded. Furthermore the cable connected directly to the profiler produced profiler vibrations, especially when strong currents were present.
An improved profiler and deployment technique, using a quasi free rising mode developed on the experience gained previously, was applied by Stips 1995. However, this technique which allowed reliable near surface small-scale current shear estimates under the special conditions of the Baltic Sea, is not applicable for measurements in the deep sea and only with restrictions in coastal regions with strong currents or tides.

Until now microscale density has been calculated from measurements of electrical conductivity and temperature microstructure with separated sensors at a horizontal distance of several centimetres. However, as it is known from horizontal coherence investigations within microstructure patches (Prandke and Stips 1992), only scales smaller than 1 cm show coherent structures in temperature profiles. For salinity this scale is even smaller. It can therefore be concluded that so far no reliable microscale density measurements have been performed under marine conditions.

Another important problem revealed during the above mentioned measurements (Anis and Moum 1995, Stips 1995) concerns the exact determination of the point where the sensors hit the sea surface. The temperature gradient alone is often not accurate enough to indicate the precise water surface. Even if the sensor has a response time of 7 ms (about 4 mm spatial distance, assuming a nominal rising speed of 0.5 m/s) the resulting uncertainty of the vertical position will not be better than about 1 cm. This is compounded by the fact that the sensor penetrating the water surface is covered by a thin water film and loses heat by evaporation.

Shear probe calibration is performed at a limited number of laboratories in the world. Due to the probes characteristics no calibration has been performed under field conditions. To ensure higher accuracy it would be necessary to introduce a regular calibration scheme.

3.2. Turbulence estimates and data processing

The energy dissipation is the most important parameter to be calculated from microstructure measurements. In principle, a generally accepted method exists for the calculation of the energy dissipation rate from current shear measurements (Gregg, 1995). The concept consists basically of the following three steps: Transferring the measured voltage signal to physical shear, calculating the shear spectra, and integrating the spectra. The spectra are estimated over fixed depth bins (i.e. 1 m) and the integration process is controlled by noise-determined cut-off criteria. The drawbacks of this method are the time consuming procedure and the fixed vertical integration limits. The adaptation of vertical integration limits to the actual patch ranges is however import as it will provide true estimations for the dissipation in single turbulence patches. Since turbulence generally has an intermittent nature, single-patch analysis is necessary for the correct assessment of local turbulence.

Single-patch analysis is not completely new as it has been used for temperature microstructure profiles (Carter and Imberger, 1986). There, to detect the turbulent mixing sections, an algorithm based on a stationary turbulence criteria was employed (Imberger and Ivey, 1991). This algorithm allowed the vertical temperature microstructure profiles to be divided into turbulent and non-turbulent segments. Power spectra were calculated for both types of segment, allowing the dissipation of turbulent kinetic energy to be determined by fitting the measured spectra to Bachelor's model spectrum (Gibson and Schwartz, 1963; Dillon and Caldwell, 1980).

For improvement towards a single-patch analysis for shear data processing, Yamazaki and Lueck 1990, proposed the computation of "instantaneous dissipation rates", a method, which partially overcomes the fixed vertical integration limit but has only limited control over the spectral bandwidth. Obviously, by this technique, drifts, high-frequency noise and vibrational noise cannot be eliminated.

The calculation of the dissipation rate through the estimation of the "cut off" wave number of the Batchelor-spectrum was introduced by Dillon and Caldwell 1980, when high resolution temperature sensors, which could resolve the high frequency part of the fluctuation spectra became available. Problems often arise if this method is applied to the SBL, a quasi homogeneous boundary layer, where the sensor sensitivity and resolution are not high enough. Oakey 1982 demonstrated indeed, that the determination of the energy dissipation rate in the SBL from temperature microstructure data measured with presently available sensors is questionable.

3.3. Boundary layer processes

Mixing and turbulent fluxes in the SBL are driven by wind and surface heat fluxes. In the last decade, measurements in the marine and limnetic surface layers allowed zero-order parameterisation as a function of wind (Lombardo and Gregg, 1989), and as a function of heat (buoyancy fluxes) (Shay and Gregg, 1986) to be achieved for steady conditions.

However, as mentioned in the objectives, most data do not cover the uppermost layer, which is very sensitive to waves and their interactions with surface processes. Therefore the focus of the most recent observations has been moved to the uppermost few meters, however without conclusive findings. There is no generally accepted parameterisation of the turbulent energy dissipation in terms of wind stress or heat flux (Anis and Moum, 1995). There are several indications of an enhanced dissipation rate below the sea surface, compared to the usually assumed "law of the wall" (Agrawal et al, 1992, Terray et al. 1996 and Drennan et al, 1996), but the scientific discussions about this remain the subject of much debate. In this context the role of the waves for mixing in the uppermost zone is an important item (Caldwell and Moum 1995).

Another key issue in today's SBL turbulence investigations is the parameterisation of mixing under non-steady conditions. In the past, for derivations of scaling laws and similarity theory, usually homogeneous and isotropic turbulence theories have been assumed. However, especially in the SBL this assumption is often violated. For instance during night time cooling, the onset of convection generates non-local turbulence. In this case the description of turbulent transport as diffusive process is in principle wrong, because the transport may occur either with or against the mean gradient. This requires a new kind of parameterisation (Stull 1993).

3.4. Turbulence and Marine Ecosystems

One of the main gaps in the understanding how marine and fresh-water ecosystems function, for instance the formation of noxious plankton blooms, is how physical processes affect pelagic primary production and organisms' interactions.

In the *meso-scale*, wind-induced mixing is the most important mechanism for providing 'new' nutrients to the primary producers during the nutrient-limited growth period. In fact, due to meso-scale differences in density stratification, wind generated turbulence has different affects on the vertical transport of nutrients in water masses with different density, creating horizontal patchiness of nutrients and therefore of plankton (e.g. Nelson et al. 1989, Kononen et al. 1996).

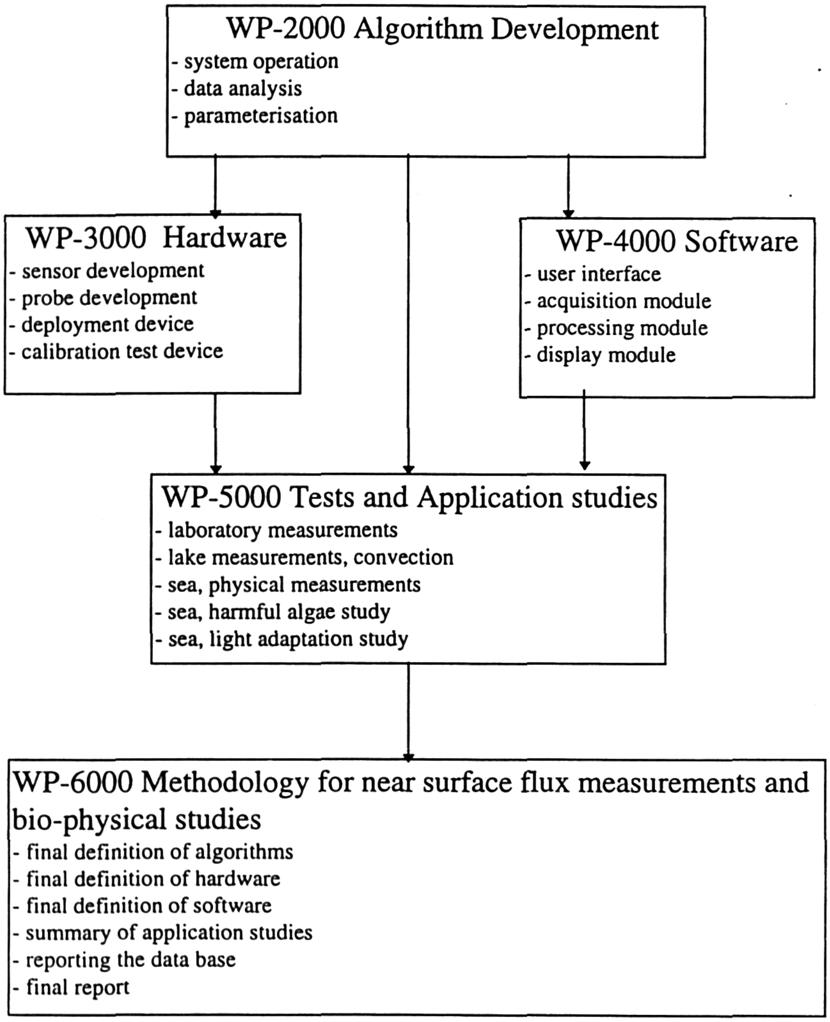
In the *small-scale,* turbulence is critical for plankton because it impacts cells on a scale relevant to biotic and abiotic environmental interactions. Consequently, these small scale processes have important ecological consequences at the larger-scale. Experimental and theoretical considerations published so far indicate that mechanisms of physical-biological coupling are species-specific, and that organisms size, shape and functionality are the key parameters. Turbulence affects feeding of herring larvae (Muelbert et al. 1994), the zooplankton metabolism (Saiz and Alcaraz 1992, Alcaraz et al. 1994) and grazing efficiency (Rotschild and Osborn 1988). Agitation has long been recognised as a growth-stimulating factor for some phytoplankton species, whereas growth of dinoflagellates is inhibited (White 1976, Thomas and Gibson 1990). Fogg and Than-Tun (1960) observed a much increased growth rate of a cyanobacteria when subjected to shaking during growth. When for physiological reasons cells become "too sticky", turbulence increases the probability of collisions of particles, and thus, modifies the aggregation process (Jackson 1990, Kiørboe 1993). As the occurrence of localised micro-gradients of O_2 in cyanobacterial aggregates has been shown, small-scale turbulence can control processes requiring oxic-anoxic transitions (Howarth et al. 1993, Paerl et al. 1995).

4. Work Programm

The objectives form the basic contents of the work packages (WP), comprising:

- WP-1000 (Project Management)
- WP-2000 (Algorithms),
- WP-3000 (Hardware),
- WP-4000 (Software),
- WP-5000 (Measurements and Application Studies), and
- WP-6000 (Methodology).

The flow structure of the intended work programme is shown:



All work packages include by definition an initial specification phase during which the partners participating at a specific task agree on the detailed work plan.

5. The Partnership

The team brought together to fulfil the objectives defined in chapter 2 must be highly skilled not only in microscale technology but over the whole range of applications. It is clearly prudent to plan the actual manufacture of the hardware at a single site. ME Meerestechnik-Elektronik GmbH which is one of the most experienced European manufacturers in this field has been selected. Similar the complete software design and development will be performed by DJL Software Consultancy Ltd, a company with a history of several successful software developments related to marine research.

As project co-ordinator SAI will be closely involved with all stages of the development. Initially SAI will work closely with ME and DJL to draw up specifications for the hardware and software design and development. During the early development SAI will maintain close links with the other partners and be guided by their advice as the prototype system develops.

The methodology for carrying out the initial measurements, data processing and evaluating the overall performance will be developed by a team led by the Swiss Federal Institute for Environmental Science and Technology (EAWAG). Laboratory measurements will be made by personnel of the University Le Havre (ULH). Complementary theoretical and field work on microstructure processes will be carried out by the Institute for Baltic Research (IOW). The Finnish Institute for Marine Research (FIMR) and the National Environmental Research Institute (NERI) will use the system in the final stage of the programme to measure vertical and temporal patchiness in cyanobacterial bloom intensity, and in phytoplankton productivity in relation to the physical factors generating it.

6. Exploitation Plans and Outlook

MITEC is expected to provide a unique and fundamental contribution to environment and climate change research, in the form of an improved understanding of near-surface physical and biological processes. This is relevant to such problems as gas exchange at the air-sea interface and the downmixing of greenhouse gases by turbulent mixing.
The development of the MITEC system will create new capabilities for observing and monitoring the marine environment and will make the European industry more competitive in the world wide market.
Furthermore MITEC will allow the testing and verification of vertical turbulence models with respect to the conditions near to the sea surface. It will also allow comprehensive studies of physical-biological interactions concerning the appearance of noxious plankton blooms, and phytoplankton light adaptation to be made in the near future. In recent years a world-wide increase in the number of noxious plankton blooms has been observed, which had a particular impact on recreation, shell fisheries and fish farming. MITEC will contribute to the future investigation of physical-biological processes that affect such bloom formations. The scientific publications to be produced will introduce a new strategy to study physical-biological couplings and thus create markets for the MITEC profiler.

Furthermore it is planned to organise a workshop on microstructure data analysis and to review and qualify the existing algorithms in order to move towards standardisation. Finally it is intended to organise one general workshop with the intention (a) to publicise the results to the scientific and commercial community in Europe and thus enhance the possibilities of developing the results into a commercial European product and (b) to stimulate other fields of aquatic studies to use the results of the MITEC project.
A WWW page for this project will be maintained which will contain all general information on the project, a software database for searching the data inventories and a database of project related publications. A multimedia data product will be delivered at the end of the project which will highlight the achievements of the project team and also present the project data.

The links between industry (SME's) and academic research will be strengthened in order to improve technology transfer and ensure effective and continuing industrial exploitation of the results of MAST funded research. The development of such technologies will promote ecosystem research. It will also contribute to the understanding of bio-geochemical fluxes, the influence of small-scale physical processes on the dynamics of marine ecosystems, the impact of climatic change on atmosphere-sea exchange processes and the study of the dynamic properties of coastal systems.

After the successful completion of the project there will be a precompetitive prototype available, which will form the base for a forthcoming project, to qualify the system for industrial production (possibly within EUREKA/EUROMAR).
MITEC will provide a new and advanced tool for observations and flux studies in the near surface layer. This tool will help to improve the understanding of the processes in the near surface layer. This will benefit both marine science itself as well as the application of Remote Sensing in the marine environment, by improving the quality of the Remote Sensing derived data products of the marine environment. The European value-added industry will make use of the results to enhance its products and services to governments, marine research institutions and the marine-related industry.
MITEC is expected to support and stimulate research activities in aquatic systems including international marine programmes such as WOCE, JGOFS, GOOS, ELOISE and international lacustrine programmes such as EROS (Black Sea), ALPE (European Program on Alpine lakes), IDEAL (East African Lakes) and BICER (Baikal International Center for Ecological Research).

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References:
Alcaraz, M., Saiz, E. and Calbet,A. 1994. Small-scale turbulence and zooplankton metabolism: Effects of turbulence on heartbeat rates of planktonic crustaceans. Limno. Oceanogr, 39: 1465-1470.

Anis, A. and Moum, J. N. 1992, The superadiabatic surface layer of the ocean during convection. J. Phys. Oceanogr. 22, 1221-1227.

Anis, A. and Moum, J. N. 1995, Surface wave-turbulence interactions: Scaling $\varepsilon(z)$ near the sea surface. J. Phys. Oceanogr. **25**, 2025-2045.

Baker, M. A. and Gibson, C. H. 1987, Sampling turbulence in the stratified ocean: statistical consequences of strong intermittence. J. Phys. Oceanogr. **17**, 1817-1836.

Brainerd, K.E. and Gregg, M. C. 1993, Diurnal restratification and turbulence in the oceanic mixed layer, 1. Observations. J. Geophys. Res. 98, 22645-22656.

Caldwell, D. R. and J. N. Moum (1995). Turbulence and mixing in the ocean, Reviews of Geophysics, Supplement, Pages 1385-1394, July 1995, U.S. national Report to International Union of Geodesy and Geophysics 1991-1994.

Carter, G.D. and Imberger, J., 1986. Vertically rising microstructure profiler. J. Atmos. Oceanic Technol., 3: 462-471.

Craig, P. D. (1996) Velocity profiles and surface roughness under breaking waves. J. Geophys. Res., 101, 1265-1277.

Denman, K.L. and A.E. Gargett (1995). Biological-physical interactions in the upper ocean: the role of vertical and small scale transport processes, Annu. Rev. Fluid Mech., 27, 225- 255.

Dillon, T. M. and Caldwell, D. R., 1980, The Batchelor spectrum and dissipation in the upper ocean. J. Geophys. Res. 85, 1910-1916.

Fogg G.E. & Than-Tun, 1960. Interrelations of photosynthesis and assimilation of elementary nitrogen in a blue-green alga. Proceedings of the Royal Society (B), 153: 117-127.

Gibson, C. H., 1991, Turbulence, mixing and heat flux in the ocean main thermocline, J. Geophys. Res. 96, 20403-20420.

Gibson, C. H. 1991, Laboratory, numerical, and oceanic fossil turbulence in rotating and stratified flows. J. Geophys. Res. 96, 12549-12566

Gibson, C.H. and Schwartz, W.H., 1963. The universal equilibrium spectra of turbulent velocity and scalar fields. J. Fluid Mech., 16: 365-384.

Gregg, M. C., 1989, Scaling turbulent dissipation in the thermocline, J. Geophys. Res. 94, 9686-9698.

Gregg, M.C., 1991. The Study of Mixing in the Ocean: A Brief History. *Oceanography.* **4**, 39-45.

Gregg, M. Turbulence in a Stratified Flow, IUTAM-Symposium, 10-14.9.95, Broome, Australia, 385-398.

Hopfinger, E. J. 1987, Turbulence in stratified fluids: A review, J. Geophys. Res. 92, 5287-5303.

Howarth, R.W., Marino, R., Lane, J. and Cole, J.J. 1988 b. Nitrogen fixation in freshwater, estuarine and marine ecosystems. 1. Rates and importance. Limnol. Oceanogr. 33: 669-687.

Howarth, R., Butler, T., Lunde, K., Swaney, D. and Chu, C.R. 1993. Turbulence and planktonic nitrogen fixation: A mesocosm experiment. Limnol. Oceanogr. 38: 1696-1711.

Imberger, J. and Boashash, B., 1986, Application of the Wigner-Ville distribution to temperature gradient microstructure: A new technique to study smal-scale gradients. J. Phys. Oceangr. 16, 1997-2012

Imberger, J. and Ivey, G.N., 1991. On the nature of turbulence in a stratified fluid, part II: Application to lakes. J. Phys. Oceanogr., 21: 659-679.

Jackson G.A., 1990 A model of the formation of marine algal flocs by physical coagulation processes Deep-Sea Res. 37: 1197-1211

Kiorboe, T. 1993. Turbulence, phytoplankton cell size and the structure of pelagic food webs. Adv. Mar. Biol. 29: 1-72.

Kitaigorodski, S. A. 1993, A note on the influence of breaking wind waves on aerodynamic roughness of the sea surface as seen from below, Tellus, 46A, 681-685.

Kononen, K. 1992. Dynamics of the toxic cyanobacterial blooms in the Baltic Sea. Finnish Mar. Res. 261: 3-36. Department of Hydrobiology, University of Helsinki.

Kononen, K., Kuparinen, J., Mäkelä, K., Laanemets, J. Pavelson, J. and, Nõmmann, S. 1996. Initiation of cyanobacterial blooms in a frontal region at the entrance to the Gulf of Finland, Baltic Sea. Limnol. Oceanogr. 41: 98-112

Lombardo, C.P. and M.C. Gregg, 1989. Similarity scaling of ε and χ in a convecting surface boundary layer. *J. Geophys. Res.* **94,** 6273-6284.

Muelbert, J. H., Lewis, M.R. and Kelley, D.E. 1994. The importance of small-scale turbulence in the feeding of herring larvae. J. Plankt. Res. 16: 927-944.

Nômmann, S. 1990. Physical control of phytoplankton growth in the Baltic Sea. A multitude of spatio-temporal scales. Ph.D.Thesis. Dept. Systems Ecol. Stockholm University. 53 pp.

Oakey, N.S., 1982, Determination of the rate of dissipation of turbulent energy from simultaneous temperature and velocity shear microstructure measurements. J. Phys. Oceangr. **12**, 256-272.

Oakey, N.S. and Elliott, J.A. 1982, Dissipation within the surface mixed layer. J. Phys. Oceangr. **12**, 171-185.

Paerl, H., Pinckney, J. L. and Kucera, S.A. 1995. Clarification of the structural and functional roples of heterocysts and anoxic microzones in the control of pelagic nitrogen fixation. Limnol. Oceanogr. 40: 634-638.

Prandke, H. and Stips, A., 1985, Free sinking probe for horizontal coherence investigation of microstructure. Beitr. Meereskunde, 53, 69-70.

Prandke,H. and Stips,A., 1992: A model of Baltic thermocline turbulence patches, deduced from experimental investigations. Continental Shelf Research 12, No.4/5 643-659.

Shay, T.J. and M.C. Gregg, 1986. Convectively driven turbulent mixing in the upper ocean. *J. Phys. Oceanogr.* **16,** 1777-1798.

Soloviev, A. V. and Vershinsky, N. V. 1982, The vertical structure of the thin surface of the ocean under conditions of low wind speed. Deep Sea Res. 29, 1437-1449.

Soloviev, A. V., Vershinsky, N. V. and Bezerverchnii, V. A., 1988. Small -sclae measurements in the thin surface layer of the ocean, Deep Sea Research, 35, 1859-1874.

Stips, A., 1995: First investigations of the near surface turbulence structure and energy dissipation caused by wind mixing in the Baltic Sea. GOBEX Newsletter, 6/7, 1-10.

Thomas, W.H. and Gibson, C.H. 1990. Effects of small-scale turbulence on microalgae. J. Appl. Phycol 2: 71-77.

Thomas, W.H. and Gibson, C.H. 1990. Quantified small-scale turbulence inhibits a red tide dinoflagellate, Gonyaulax polyedra Stein, Deep-Sea Res. 37: 1583-1593.

Thorpe, S.A., 1978. Turbulence and mixing in a Scottish Loch. Phil. Trans. Roy. Soc. A. 286, 125-181.

Rotschild, B.J. and Osborn, T.R. 1988. Small-scale turbulence and plankton contact rates. J. Plankt. Res. 10: 465-474.

Saiz, E. and Alcaraz, M. 1992. Enhanced excretion rates induced by small-scale turbulence in Acartia (Copepoda: Calanoida). J. Plankt. Res. 14: 681-689.

Stull, R. B., 1993, Review of non-local mixing in turbulent atmospheres: Transilient turbulence theory. Boundary-Layer Meteorology, 62, 21-96.

Yamazaki, H. and Lueck, R., 1990, Why oceanic dissipation rates are not lognormal, J. Phys. Oceangr. 20, 1907-1918.

White, A. W. 1976. Growth inhibition caused by turbulence in the toxic marine dinoflagellate Gonyaulax excavata. J. Fish. Res. Board Can. 33: 2598-2602.

Wüest, A., D.C. van Senden, J. Imberger, G. Piepke, and M. Gloor, Comparison of diapycnal diffusivity measured by tracer and microstructure techniques, *Dyn. Atmos. Oceans* **24**:27-39, 1996a

TITLE: Applications of 3-dimensional electromagnetic induction by sources in the ocean
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AUTOMATIC DIATOM IDENTIFICATION AND CLASSIFICATION
ADIAC

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1. INTRODUCTION

The ADIAC project is a pilot study concerning the application of image processing and pattern recognition tools to the automisation of diatom identification by computer processing. Instead of taking only the shape of the valve outline into account, several methods will be applied in order to employ also the valve ornamentation in the identification process. Important aspects are the development of methods for automatic slide scanning, utofocusing, the realization of big image databases, and the integration into taxonomic/ecological database systems. The project started the 1st of April 1998 and its duration will be three years.

Diatoms have become important in several scientific disciplines not intuitively connected to phycological research. Because of their inert and intricately sculptured shells, and because some have rather narrow ecological preferences, they can be used as ecological indicators. For example, in the study of lake- and sea-bed sediments they can be used to estimate the pH and nutrient status of the water body back in time when the sediments were laid down. They can also be used by archaeologists and forensic scientists to trace ceramic and clothing fragments, and in some cases to establish cause of death. Diatoms are very good indicators of water quality, and authorities responsible for drinking water use them to monitor the quality.

Unfortunately, in many cases the diatom identification is carried out by non-specialists. Furthermore, progress in the use of diatoms in Europe as ecological and palaeoecological indicators, marker fossils, etc., is hampered by the chronic lack of identification tools, inefficient communication, and the slow speed of dealing with samples possibly containing many hundreds of different species. The automisation by computer processing and electronic data exchange will become extremely important if Europe is to have the capacity to monitor and respond effectively to change in aquatic systems, and to remain at the forefront of such research.

Towards computer processing

Diatom research always involves an identification based on morphological properties. This is a tedious work, because microscope slides must be scanned, eventually photographed, and the diatom specimens must be linked to described taxa. The latter is a very complicated task, even for experts, because it requires a profound knowledge of hundreds of genera with often very subtle variations and/or a frequent lookup in atlasses like those of Schmidt, Tempere, Peragallo, and Krammer & Lange-Bertalot. For these reasons the automisation by computer processing is of paramount importance, and we are convinced that computer processing will drastically change diatom research in the near future!

Earlier work on computer identification has always been based on shape descriptors, i.e. parameters which describe the shape of the outline. However, many diatoms cannot be distinguished on shape alone because different species share the same shape, although they may have quite different ornamentations (striae etc) of the valve surface. This is the main emphasis of this ADIAC project: we will develop pattern recognition methods which allow to identify on the basis of both valve outline and ornamentation. Because such methods are only useful in the context of big databases, the construction of such databases together with the classification (group-forming) rules will be studied by experts for a few representative diatom-analysis applications.

This multi-disciplinary project, with a duration of three years, brings together researchers from pattern recognition, diatom applications, and taxonomic databases. It must be seen as a bundled European impulse to push diatom research and to make diatom identification an important and challenging subject in the pattern recognition society.

2. OBJECTIVES

The overall aim of ADAIC is to develop appropriate image databases and analytical methods for the automated identification of diatoms for use in environmental monitoring, micropalaeontology and forensic research etc. The particular objectives are:

1 - To develop diatom image databases with different levels of discrimination complexity. These must be suitable for testing the methods under study in this project as well as for future research. These databases will be made accessible to all interested scientists.

2 - To develop methods for scanning slides automatically, i.e. to detect the positions of diatoms and to ignore debris with some complex structure, to store the calibrated positions, and to take high-magnification images at those positions. Autofocusing will be an integral part of slide scanning, especially at high magnifications.

3 - To develop methods for a complete, graphical, diatom image description, i.e. of both valve shape and ornamentation, yet with a limited number of parameters which allows for a reliable identification.

4 - To develop a diatom identification system based on graph matching that takes all available morphological information into account and that produces a sorted list with best matches.

5 - To test the methods developed, using the databases, in order to access the overall performance of the algorithms and to optimise them by including expert knowledge.

6 - To integrate the identification methods into taxonomic and ecological database systems which include high-quality diatom images but also graphical representations of these consisting of the skeletons and characteristic points as derived from the model-based analysis methods.

As a result, this project will generate software systems for slide scanning, autofocusing, image analysis, and pattern recognition, all optimised for diatom valve images. In parallel, huge image databases will be created, together with taxonomic and ecological descriptions. All software and the image databases will be integrated into taxonomic and ecological database systems, which enable to identify diatoms by means of computer image processing.

3. WORKPLAN

The foregoing objectives can be directly mapped onto a number of tasks and subtasks. We present only a schematic organisation of the tasks in order to give a coarse idea about how ADIAC will function:

TASK 1: Image preprocessing and databases
Subtask 1a: Test image databases
Subtask 1b: Image preprocessing and compression
Subtask 1c: General image databases

Image preprocessing serves to normalise the contrast etc in order to facilitate feature extraction. Test image databases contain selected diatom images with increasing discrimination complexity. These serve for testing the image analysis tools. General image databases will contain samples of all relevant diatom species to be included in the taxonomic and ecological systems. These images will also be made available, together with analysis software, to all interested scientists.

TASK 2: Slide scanning, diatom localisation and autofocusing
Subtask 2a: Automatic slide scanning
Subtask 2b: Autofocusing

In this task the automisation of slide scanning for diatoms will be studied by linking a computer to a microscope and CCD camera. Because of the fact that diatom valves are three-dimensional (3D) objects, the exact criteria for autofocusing will be studied in order to obtain the best images suited for a 2D analysis.

TASK 3: Diatom analysis and identification
Subtask 3a: Classical analysis
Subtask 3b: Mathematical morphology
Subtask 3c: Gabor wavelets
Subtask 3d: Graph matching

In task 3 several image processing tools will be implemented and their performace will be evaluated in order to obtain the best algorithms. On the basis of a hierarchical graphical representation, graph matching will be applied to link an unknown diatom to possible matches in the databases.

TASK 4: Identification tests

This task is the formal testing and evaluation work related to task 3. It will provide the feedback necessary for the algorithm optimisation.

TASK 5: Integrated database systems

By integrating the best image analysis algorithms, graph matching and the image databases, a complete system will be created. In order to include also non-image information in the identification process, taxonomic and ecological factors will be employed by linking the identification software with existing taxonomic and ecological database systems.

TASK 6: Data management

Data management will not only concern the exchange of images etc between the partners, but also to provide them to other interested researchers, both from taxonomy and from pattern recognition. A couple of months after the project start, there will be created an ADIAC homepage with image databases and software.

4. A FINAL WORD ABOUT COLLABORATIONS AND THE FUTURE

The ADIAC project is a pilot study concerning the automisation of diatom identification by means of computers. During the three years it is expected to realise state-of-the-art algorithms and huge image databases. However, it is the first project in which the diatom valve ornamentation will be explored. Hence, it is expected that the project will need a continuation to further improve the analysis tools, but mainly to establish image databases that contain all diatom species relevant for certain applications/sites.
The ultimate outcome of ADIAC could be that one or two supercomputer centres have the best analysis programs and all image databases, such that all interested researchers could send images in specific formats and get back a sorted list with the best matching species. In order to explore such a futuristic idea it is extremely important that ADIAC creates also a database with potential users. ADIAC will try to contact many diatomists who could profit from the project. Because this is a very time-consuming task, we invite individual researchers, institutions and companies to contact us and to let us know what their applications are and what they would expect. ADIAC will organise several workshops for which interested researchers will be invited, and we hope to establish active collaborations beyond the ADIAC project partnership.

III.1.2. Underwater communication and orientation

TITLE: Propagation Channel Simulator (project **PROSIM**)

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PROPAGATION CHANNEL SIMULATOR PROSIM

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SUMMARY

This paper presents the results obtained in the PROSIM project after two years of work. They concern modelling and simulation of sound speed random fluctuations due to internal waves in a shallow water waveguide, characterization of sea surface waves and entrained bubbles and simulation of propagated broadband acoustic waves in a channel subject to such random fluctuations.

Two experiments at sea have been performed, showing that the shallow water channel can be highly variable if the full PROSIM bandwidth (100 Hz - 10 kHz) is considered, even on short terms, which makes any attempt to model the fine structure of the acoustic field hardly possible and emphasizes the need for stochastic modelling.

1. INTRODUCTION

The objective of the PROSIM project is to develop a software package able to simulate the effect of a shallow water propagation channel to an input acoustic signal in the band 100 Hz-10 kHz.

For this purpose, the simulator is intended to provide realizations, for a set of given input functions, of the output pressures on an array of sensors at realistic long range (maximum 100 km), two dimensions, shallow water (50 to 300 meters) acoustic channel with random fluctuations due to major medium effects : internal waves and sea surface agitation.

Provided the simulator is sufficiently realistic, this is why stochastic propagation effects are introduced, such a generic tool would be extremely useful to test and evaluate by simulation signal processing algorithms of all kinds related to underwater acoustic systems for a number of applications : communications, monitoring ocean processes by tomography, localization of underwater vehicles by exploiting the multipath structure of propagation, classification and localisation of transients noises produced by all kind of sources, including marine animals.

For such applications either active or passive, a detailed knowledge of received waveforms is required, the channel simulator is a tool which would allow the user to see what deformation to the input signal the medium will produce.

The channel simulator will improve the efficiency in the design of new marine acoustic systems by reducing to a minimum expensive experiments at sea.

The main innovations are in the stochastic aspects which are introduced, related to oceanic fluctuations, and to the wide band aspect which makes unavoidable the development of efficient and fast algorithms for modes calculation and interpolation techniques.

In order to support the theoretical work done in oceanographic and in acoustic modelling, two experiments at sea have been performed : one in the Mediterranean Sea near the Elba island and the second one in the Clyde Sea. Both of these experiments proved to be very successfull, they allowed a lot of environment and acoustic data to be collected.

A preliminary version of the PROSIM software already exists including the deterministic propagation module, the bistatic reverberation module and the ambient noise module. The software works on a standard workstation under UNIX and uses MATLAB for input - output operations.

2. STRUCTURE OF THE PROJECT

The project is organized around the following four main tasks:

1. **Oceanographic modelling**: This task is performed mainly by the University of North Wales in Bangor. The objective is to provide environmental parameters as input to acoustic models. The parameterisations will include characteristics of the fluctuation of the sound velocity profile, sea surface spectra and bubble density due to breaking waves.
2. **Acoustic modelling**: This task is done by SACLANTCEN and TMS.SAS. The objective is to develop numerical models for broadband deterministic propagation and time-space moments of the acoustic field which are necessary to obtain realizations of the two points time varying impulse response of the medium.
3. **Experiments at sea:** Two experiments at sea were planned and have been performed: one in the Mediterranean sea performed by SACLANTCEN and the other one in the Clyde sea with participation of Heriot-Watt University, University of North Wales and TNO-FEL. The objective is to acquire experimental data to support the oceanographic and acoustic modelling, and to provide indications on in which circumstances the simulator gives usefull results.
4. **Software finalization**: This task is done by TNO-FEL, the objective is to implement the algorithms in an appropriate software package of professional standard able to be used by other users (including non-specialists) and to be maintained, updated or extended efficiently.

3. EXPERIMENT IN THE MEDITERRANEAN SEA

SACLANTCEN performed the first PROSIM experiment in the area South of Elba, Mediterranean Sea, in the time period 15-23 May, 1997. During the experiment it was emphasised to acquire environmental data at the same time as the acoustic data; therefore, different environmental equipment was moored in the area where the acoustic measurements were performed.

The acoustic experiment was divided into three main parts as follows:

1) Measurement of sound propagation along a nearly range-independent bathymetry track by towing an electrical source and dropping explosive charges.

2) Measurement of sound propagation along a range-dependent bathymetry track by dropping explosive charges.

3) Measurement of time-dependent sound propagation along a fixed path for 42 hours (36 hours linear frequency modulated signal transmission and 6 hours continuous waves transmission) by using a moored electrical source.

All the acoustic measurements covered a broad frequency band ranging from about 50 Hz up to 7.5 kHz depending on the actual source. In the case of the electrical sources both linear frequency (LFM) sweeps and continuous waves (CW) signals were applied with different time durations of the pulses. The received time series were recorded by using two portable vertical arrays (PA1 and PA2) and the SACLANTCEN vertical receiving array (SVA) positioned at different locations.

The main tracks shown in Fig. 3.1 are noted as Track A for the nearly range-independent bathymetry track along the Formiche-Elba transect, and Track B for the range-dependent bathymetry track. Track B has an almost constant sloping bottom with increasing depth towards the Montecristo-Giglio Islands.

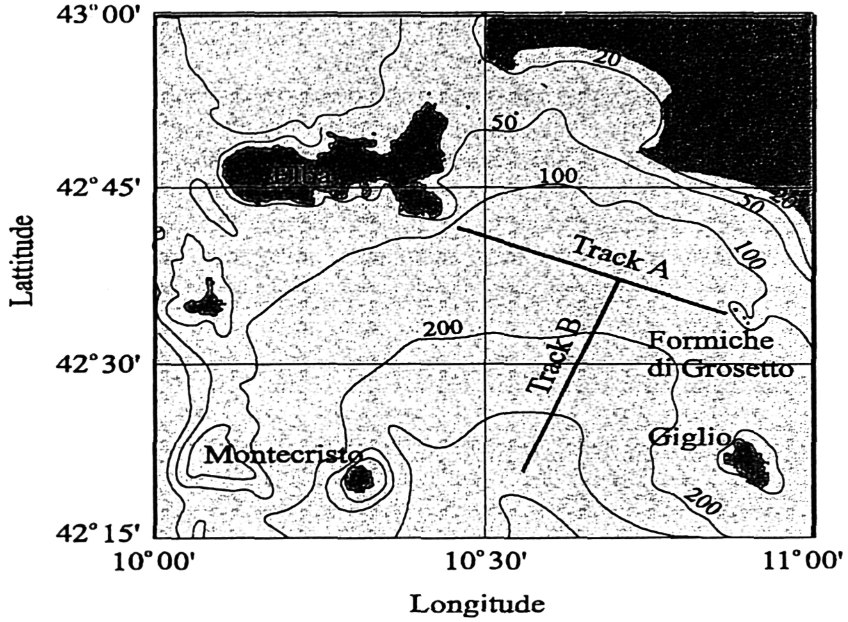


Figure 3.1 *Experimental area including acoustic tracks.*

Two vessels were involved in the experiment: R/V Alliance and R/V Manning from SACLANTCEN. The area of interest shown in Fig. 3.1 is well-known from previous sea trials. Previous measurements showed that the upper about 10 m of the bottom consists of a soft clay layer with a sound speed at the water-bottom interface lower than in water. This sediment layer is overlying a sub-bottom with a higher sound speed

3.1. Oceanographic data

Emphasis was given to measurements of variability of the ocenographic parameters which have a strong influence on acoustic, namely temperature, velocity profile and surface agitation. Some of the more significant data collected are presented below:

Temperature:

The temperature variations are present both as a function of time at a fixed position and as a function of position at a fixed time. The thermistor chains and current meters moored along Track A, see Fig. 3.1, measured the temperature continuously versus time at discrete depths. The measured temperature over 7 days for three selected depths is shown in Fig. 3.2.

As seen in Fig. 3.2 there is a small variation of the temperature between the four locations along the track. The maximum temperature appears close to the sea surface and is decreasing as a function of depth. However, the temperature measured at T2 seems to be slightly higher at the two deepest sensors compared to the temperature at the other locations. This indicates a weakly range-dependent sound speed profile which is almost constant in time.

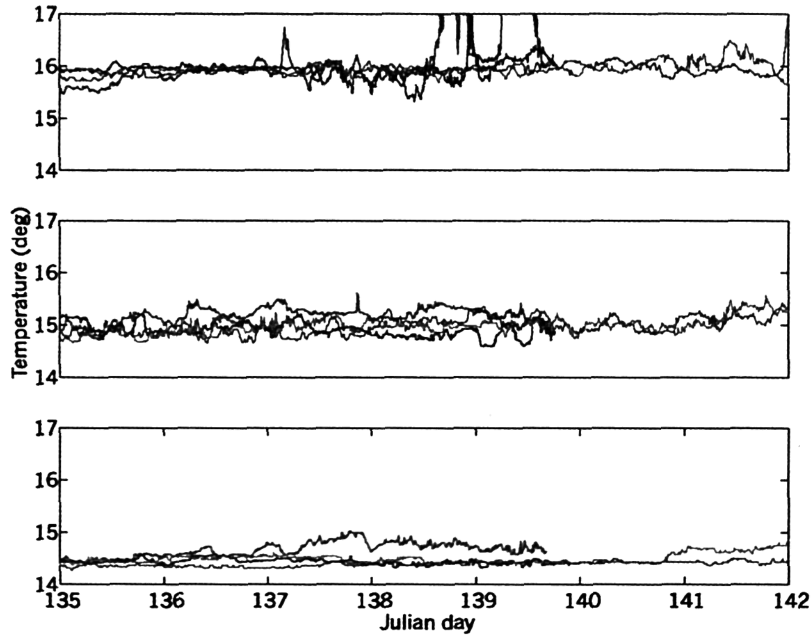


Figure 3.2 *Temperature as a function of time for three selected depths measured by using the thermistor chains (T1 and T2) and temperature sensors on the current meters (CM1 and CM2). Red curve: T1 for depths 17.5, 35.5 and 60.5 m. Green curve: T2 for depths 19.5, 35.5 and 57.5 m. Blue curve: CM1 for depths 15.0, 37.0 and 59.0 m. Cyan curve: CM2 for depths 17.5, 37.0 and 60.0 m. The depth increases from the top figure and down.*

Sound speed:

Extensive measurements of the sound speed in the experimental area have been performed. A clear variation of the sound speed as a function of range has been observed. Especially in the upper 30 m some variability can be seen, while for depth greater than about 40 m the sound speed is almost constant as a function of range. There is a remarkable change in the sound speed structure close to Formiche after about 22 km. This characteristic is seen for all the towed CTD-chain data no matter if the acquisition started at Formiche towing the chain towards Elba or vice versa. This indicates a kind of front close to Formiche which seems stable in time.

3.2. Acoustic data

Received time series:

The equalised received time series in ~ 31 hours transmission and approximately 1 hour sampling over a propagation path of 15 km for the frequency band 300-850 Hz is shown in Fig. 3.3. The pulse duration is 203 ms and the receiver depth is 66 m (~ mid-water column). In Fig. 3.3 for the low-frequency sweep it is clearly seen that the received signal changes as a function of time, but it seems only to be an amplitude modulation. The arrival time is constant over the 32 hours, and it is possible to identify certain time periods, where only a small variability appears. This is especially clear in the first 5 hours, from hour 10 to 13 and the last two hours, where some stable features can be identified. In these time periods it may be possible to simulate the received time series by applying a deterministic broad-band propagation model.

Propagation losses:

Another measure for the time variability is the total energy contents in the received signals as a function of time. The received energy at a depth of 66 m for 31 hours transmission for the two frequency bands 300-850 Hz and 850-7500 Hz is found to vary ~ 4 dB over 31 hours at a depth of 66 m, and this variation is almost the same for the two frequency bands except for one transmission after 22 hours (noisy signal) at the high-frequency band. The same conclusions can be said for a shallower receiver, but slightly more variability is seen as the receiver is close to the thermocline. It should be noted that the energy levels for each receiver may have a 24 hours periodicity, which can be found both for the low and high frequency signals. This is close to the periodicity of the current.

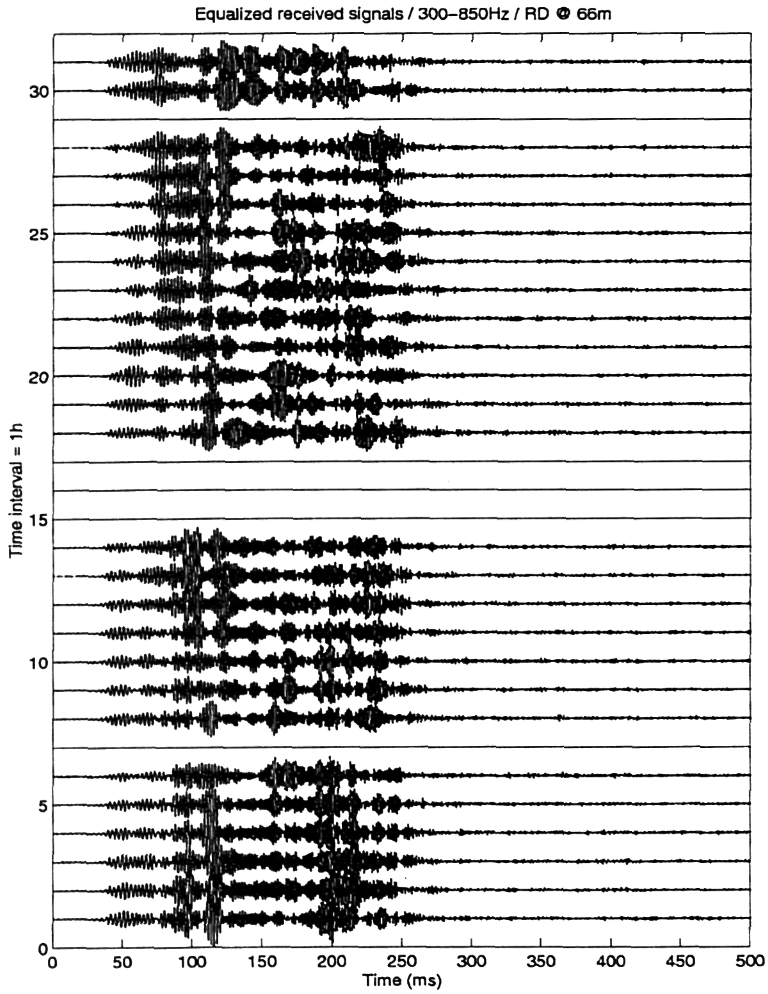


Figure 3.3 *Equalised received time series covering the frequency band from 300\ to 850 Hz at a range of 15 km and a depth of 66 m. The time period covered is ~ 31 hours with about one hour sampling.*

4. EXPERIMENT IN THE CLYDE SEA

The Scottish Water trials took place in the Clyde Sea during the period of July 24th to August 7th 1997. Operating from Troon Harbour, the R.V. Calanus was used as the acoustics receive ship and the R.V. Prince Madog was the acoustic transmit ship.
The R.V. Prince Madog maintained a relatively fixed Tx position, located in an average water column depth of 55 metres, just on the edge of a sand bank. The range variation between vessels was achieved through moving the position of the R.V. Calanus using either of two tracks, a 188° bearing, or a 234° bearing.

4.1. Oceanographic data

In order to sample both the spatial and temporal variability of the water column a combination of CTD sections and time series measurements was made during the experiment. As an example, a yo-yo experiment made from the Calanus was performed on 6/8/97 in which 16 casts were made over the 2-20 meter depth range in 30 minutes. The data obtained is plotted in Figure 4.1; internal waves with a period of ~10 minutes are clearly visible.

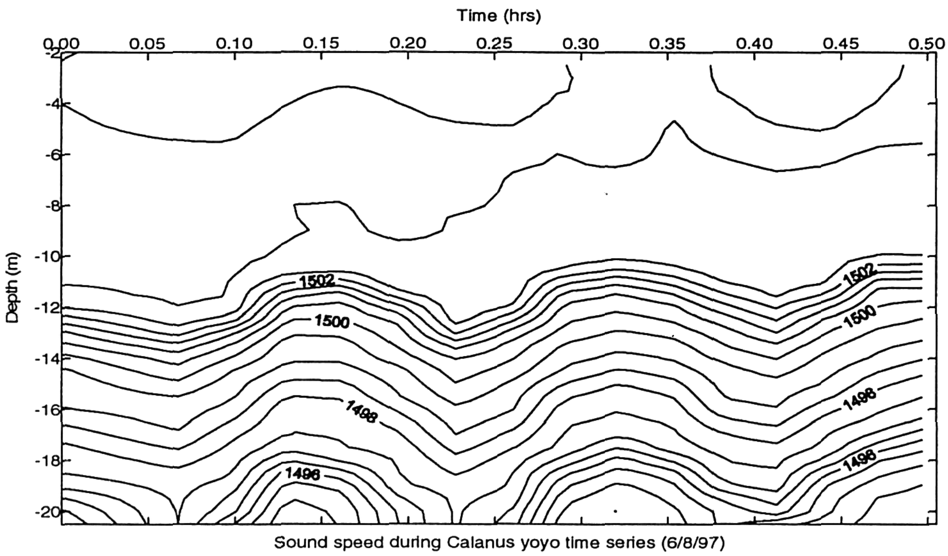
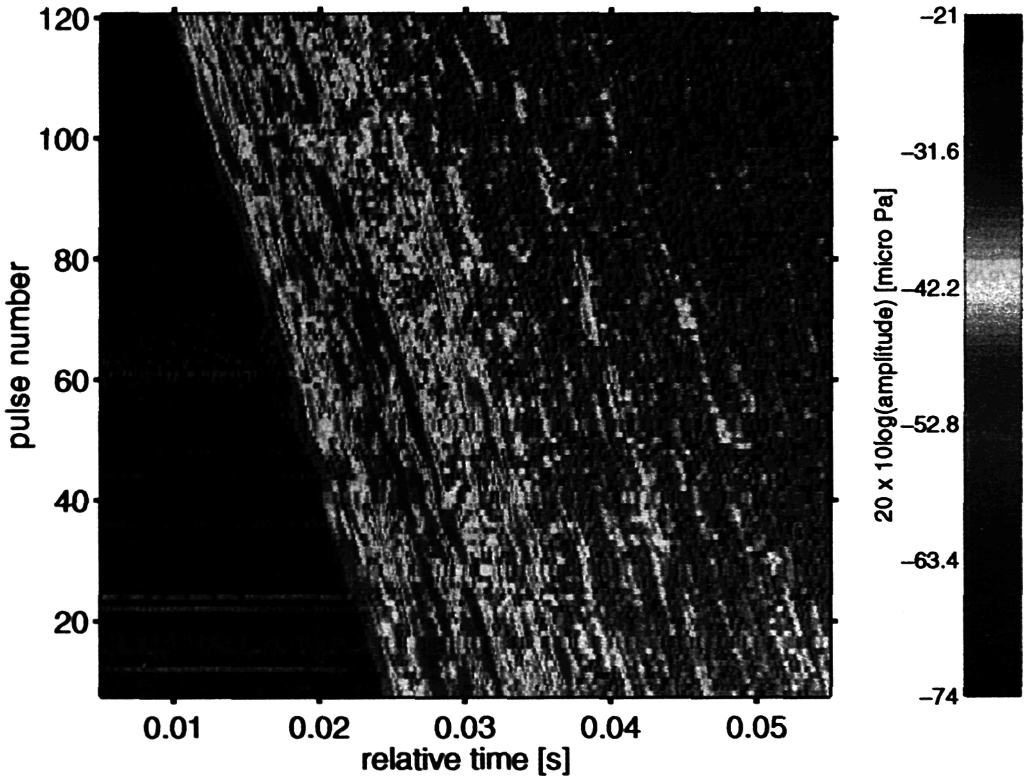


Figure 4.1. Variation in sound speed during the half-hour CTD yo-yo experiment performed on 6/8/97 (0930 - 1000). Contours are at 0.5 ms^{-1} intervals. Internal waves with a period of ~10 minutes are clearly visible.

4.2. Acoustic data

The investigation of pulse to pulse variability is done by looking at the arrival structure estimated by cross correlating the received signal with a replica of the transmitted waveform (FM sweep in the full bandwidth 1-8 kHz). As an example, the results obtained at 5 km range are displayed in Fig 4.2. The figure indicates that the relative arrival times of the pulses decrease a little with increasing pulse number. This is due to a very small clock error (a difference of about 16 ms over 120 pulses, (70 minutes)).

One can see that the arrival structures change significantly from pulse to pulse. For instance, the last strong arrival is clearly visible for most of the pulses up to about 85, but has disappeared for the pulses corresponding to higher numbers.



5. OCEANOGRAPHIC MODELLING

5.1. *Internal waves in shallow water*

A simulated Internal Wave Field is assumed to be a linear combination of plane waves of the form

$$w = \sum_{j=1}^{j_{max}} \sum_{\omega=f}^{N} A(\omega, j) \cdot \sin(kx - \omega + \phi) \cdot W_z(\omega, j) \cdot \sqrt{\delta\omega}$$

where w is the total vertical velocity due to all internal wave components. W(ω,j) is the vertical mode structure which is calculated from the buoyancy frequency profile. The only

unknown in the formula is the amplitude A(ω,j) of the wave. This can be specified from the GM equations following the method of Saunders and King [1].
To produce a simulated internal wave field a summation is made over a number of modes and frequencies. Each mode is assumed to be uncorrelated, hence the phase φ of each component is chosen at random between 0 and 2π. The inclusion of a factor of $(\delta\omega)^{1/2}$, where δω is the bandwidth represented by a particular frequency, ensures that the integral of B(ω) over the interval (N,f) is equal to 1, as required by the GM equations.
The effect of IWs on the sound speed is then found by using a simple advection model to move temperature and salinity surfaces in response to the wave field. Sound velocity (a function of temperature, salinity and depth) is calculated from the perturbed temperature and salinity fields using the standard formula of Chen and Millero [2], and sound speed fluctuations are obtained by subtracting off the initial sound speed profile. The IW simulation method presented above is based largely upon that developed during the LORACOM project [3].
A preliminary calculation of the expected sound speed fluctuations in the Clyde Sea has been made. Results are available in [4]. It is found that larger fluctuations occur in the upper part of the water column due to 1) the increased sound speed gradient, and 2) the increased buoyancy frequency, which causes high frequency IW modes to have larger amplitudes.

5.2. *Surface wave spectrum*

The Pierson-Moskowitz (P-M) spectrum has been used extensively for scientific and engineering studies since it represents a limiting condition for wave development under stationary conditions. Since it was derived from mainly open ocean observations of waves the P-M spectrum is not thought to be valid in shallow water regions.
Barnett and Wilkerson [5] collected wave data using a radar altimeter in an aircraft and observed that at a particular frequency the waves did not grow monotonically but exhibited an overshoot before reaching the final saturation value. This behaviour suggested that a non-linear process was responsible for the overshoot. Consequently, a major international experiment (the Joint North Sea Experiment or JONSWAP) took place in the southern North Sea off the coast of Denmark (Hasselmann *et al.,* [6]). Measurements were made at 13 stations up to 160km from the coast under a variety of wave conditions and the wave spectra computed. The results showed an enhancement of the spectral peak and the transfer of energy from high to low frequency components by non-linear interaction processes.
The peak enhancement factor, γ, describes the overshoot phenomenon and leads to a general expression for the JONSWAP spectrum in the form

$$E(f) = \alpha\, g^2\, (2\pi)^{-4}\, f^{-5}\, \exp(-5/4\, (f_m / f)^4)\ \gamma^q$$

where $q = \exp\left[\dfrac{-(f-f_m)^2}{2\sigma^2 f_m{}^2}\right]$, and $\sigma = \sigma_a$ for $f < f_m$ or $\sigma = \sigma_b$ for $f \geq f_m$

This expression contains five free parameters: f_m, α, γ, σ_a and σ_b. The peak frequency of the spectrum is represented by fm and α is a parameter that corresponds to the 'constant' of the saturation range. The parameters γ, σ_a and σ_b describe the shape of the spectrum with γ defined as the ratio of the peak in the JONSWAP spectrum to that of the peak in the P-M spectrum with the same values of α and f_m. Since it is derived from shallow water wave data the JONSWAP spectrum is adopted for PROSIM applications.

5.3. *Whitecap and bubble plume model*

The goal of the whitecap and bubble plume modelling is to produce realistic simulations of the number, size and distribution of gas bubbles produced by breaking surface waves in order to be able to evaluate the impact of the bubble distribution on sound speed. As we are only interested in the effects of bubble clouds on acoustic signals, it is sufficient to consider just the distribution of bubbles along a vertical (x, z) slice of ocean, corresponding to an acoustic transmission path. Thus the output required from the model is a range/depth array consisting of bubble populations for each of a discrete set of bubble size intervals.

The formulation of the whitecap and bubble plume model uses surface whitecap coverage equations to calculate directly the intersection of an acoustic path with a volumetric distribution of bubble clouds. The change in the distribution of bubble sizes with depth as a bubble plume ages is obtained from published literature on the behaviour of gas bubbles in seawater.

Equations for bubble concentration and vertical distribution as a function of wind speed are given in [4]. The requirement for the bubble plume model is to define the typical number and size distribution of bubbles in a plume as a function of age.

An individual whitecapping event injects a broad size range of bubbles into the water column. These bubbles interact with seawater in several ways; smaller bubbles rapidly lose gas to the surrounding water, large bubbles rapidly return to the surface due to their buoyancy, and turbulence within the water column redistributes bubbles both vertically and horizontally.

The FORTRAN implementation of the bubble plume model is currently under development.

6. ACOUSTIC MODELLING

6.1. *Deterministic wideband propagation model*

The development of a numerical broad-band (10 Hz - 10 kHz) channel simulator requires special attention on versatility, robustness and efficiency of the deterministic acoustic modelling module. These requirements can at present only be fulfilled by using models based on wave theory. The propagation of broad-band signals may in general be determined by applying the brute-force technique. In this case the transfer function of the wave-guide is established during a frequency-by-frequency analysis using a single frequency propagation model. The received time signal is then calculated by using Fourier synthesis. However, the Fourier synthesis is not applicable for predicting very broad-band sound propagation as in this case because of the computation time required to perform the frequency-by-frequency analysis.

An extensive search has been performed by SACLANTCEN to investigate the state-of-the-art development within broad-band propagation models. A numerical propagation model called ORCA recently developed by Westwood *at al*, [7], has shown the necessary performances for application in the PROSIM channel simulator. ORCA is based on a layered normal-mode approach assuming that the inverse of the sound speed squared varies linearly with depth in each layer. The acoustic field is then given analytically as a sum of two independent Airy functions within each layer. A characteristic equation is defined by using propagator matrices which is solved for the eigenvalues. The derivative of the characteristic equation with respect to wavenumbers is calculated and used in an interpolation scheme for finding the eigenvalues efficiently. Furthermore, the derivative of the wavenumbers with respect to frequency is given analytically, which is utilized to interpolate the mode functions in frequency accurately and efficiently.

Fast mode solver:

Shear properties of the ocean bottom were neglected at the starting point of the model development. Furthermore, it was decided to consider only real wavenumbers in the PROSIM deterministic module for mode determination to optimize the efficiency of the model. Therefore attenuation effects are introduced by using perturbation theory resulting in an additional imaginary part on the mode wavenumbers. The eigenvalue solver is the same regardless of whether single frequency or broad-band calculations are required. In the broad-band calculations the eigenvalue solver is called repeatedly until a necessary number of frequency components have been analyzed. The eigenvalue solver is in general based on altering between bisection and cubic spline of the characteristic equation. As an eigenvalue is bracketed by the bisection method, the characteristic equation is approximated by a cubic spline, and an estimate of the eigenvalue is given by the root of this spline. There is a complex procedure to check if the estimated eigenvalue is found with sufficient accuracy, and if any modes are missing in the search interval.

Range-dependent environment:

The range-independent PROSIM simulator has been extended to handle *range-dependent* environments. A range-dependent environment is simulated by dividing the full range into a number of segments, where each segment has different range-independent properties. The wavenumbers and the corresponding mode set in each segment are then computed. Hence there is no significant difference between computing modal properties for range-independent and range-dependent environments. The PROSIM range dependent propagation model is based on adiabatic mode theory. It is assumed that in going from one range to the next the modes will couple adiabatically, i.e., without any transfer of energy to higher or lower-order modes. This approximation leads to the following well-known expression for the complex pressure (see Ref. [8] for details on the adiabatic approximation)

$$p(r,z) \cong \frac{i}{\rho(z_s)\sqrt{8\pi}} e^{-i\frac{\pi}{4}} \sum_{m=1}^{M} \psi_m(0,z_s)\, \psi_m(r,z) \frac{e^{i\int_0^r k_m(r')dr'}}{\sqrt{\int_0^r k_{rm}(r')dr'}} \qquad (1)$$

where M denotes the lowest number of modes existing in any two adjacent segments, and k_{rm} is the m'th eigenvalue at range r. The eigenvalues and eigenfunctions are normally evaluated at a discrete set of ranges, whilst the values at intermediate ranges are calculated by linear interpolation.

As seen in Eq. (1) the integral of the wavenumbers is used in the adiabatic formulation of the range-dependent environment. This integration is always performed analytically assuming that k_n varies linearly between two adjacent segments.

The environment is approximated by a series of range-independent segments with a sloping bottom described by a staircase approximation.

Numerical Verification:

A simple acoustic environment has been defined in order to verify the implementation and validity of the adiabatic approach in the PROSIM model. At present it has been chosen only to consider the bathymetry as range-dependent, but PROSIM can handle any range-dependent environment, e.g. sound speed profile, density, attenuation, sediment thickness etc., within the limitations of the adiabatic approximation. The SACLANTCEN coupled normal mode model C-SNAP, see Ref. [9], is used to generate the reference solution in terms of the received time series.

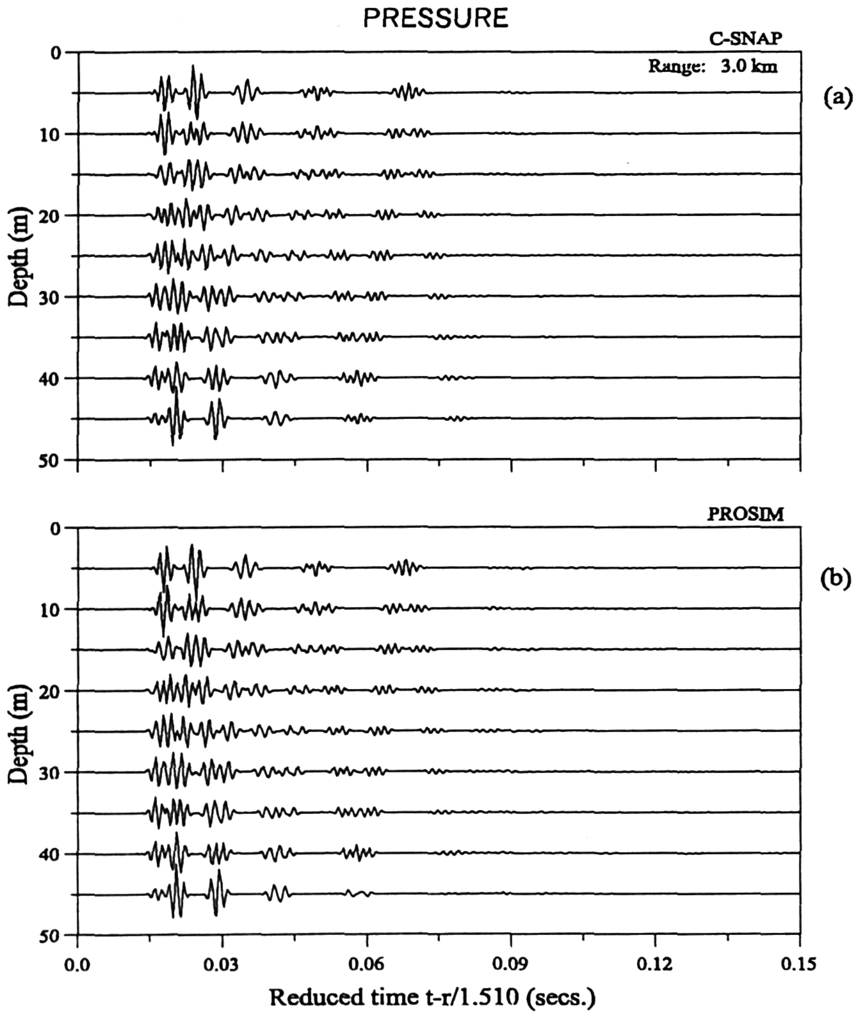


Figure 6.1: *Depth stacked received time signal at a range of 3 km for the range-dependent environment obtained by (a) C-SNAP and (b) PROSIM.*

C-SNAP treats the attenuation in the bottom by a perturbation theory as the PROSIM model. However, the C-SNAP model allows coupling of energy between modes, and hence a notable difference between the solutions obtained by PROSIM and C-SNAP is expected for strongly range-dependent environments.

The source pulse used is a signal constructed of a Hanning weighted sine wave at 600 Hz with a duration of about 5 ms corresponding to the desired bandwidth.

The received time signals for the 1° up-slope bathymetry obtained by using the two numerical models are shown in Fig. 6.1.

The received time signals in Fig. 6.1 show excellent agreement between C-SNAP and PROSIM. This is important since the range-dependent approach used in the two models is different, i.e. adiabatic and coupled mode solutions. Therefore, it may be concluded that mode coupling is not important in this specific environment.

The PROSIM model is found to be about 35 times faster than C-SNAP by using an efficient eigenvalue finder, mode solver and frequency interpolation techniques.

6.2. Stochastic propagation model- Effect of surface waves

The stochastic part of the PROSIM channel simulator is intended to provide realisations of received pulses after propagation in a random fluctuating environment. The major effects considered are random perturbations produced by internal waves and interactions of the sound with the rough moving sea surface due to wind action. The first effect is modelled by a technique developed in a previous MAST project LORACOM and is described in [4]. In the following section, the effect of surface waves is presented with more details.

The surface elevation $\zeta(x,y,t)$ is a random process assumed to be normal, uniform in the horizontal plane and stationnary. The impulse-response $h(t;t_0)$ is then a realization of a time-dependant random process, characterized by moments and statistical properties. Only the following two moments are considered:

- the **mean impulse-response** $<h(t,t_0;\, \underline{r}_R,\, \underline{r}_S)>$ (first order moment) ;

- the **cross-correlation**, or **covariance**, function $< h(t_1,t_1^0;\underline{r}_1^R,\underline{r}_1^S)\; h^*(t_2,t_2^0;\, \underline{r}_2^R,\underline{r}_2^S)>$ (second order moment).

These moments are estimated through the computation of the mean luminance (mean space-and-time Wigner transform of the impulse response) which relies on two properties :

- Away from the boundaries, the conservation (or transport) of luminance along some paths which can be computed (and which are actually the ray-paths of geometrical acoustics (see Ishimaru, ref.[10] and Wilson & Tappert, ref.[11])
- The application at each encounter with a boundary of a **scattering kernel** W.

Intrinsically, the problem reduces to the numerical evaluation of an integral; an exotic but classical technique for such calculations is the **Monte-Carlo method**, which consists in generating pseudo-random numbers, in a way concieved *ad hoc* so that their average is equal to the desired integral (see Sabelfeld, ref.[12]). Applied to the considered problem, quite simple a technique, roughly illustrated by the Figure 6.2, the main principles of which we originally borrowed from Wilson & Tappert (ref.[11]), is:

1 / a ray is launched along some initial values (transmission angle, frequency...) ; it is followed up to its first encounter with a boundary ;

2 / the attenuation coefficient of the specular part being evaluated, (as a function of frequency and of the incident parameters of the boundary encounter), a first pseudo-random number X is drawn, with a uniform probability density over the interval [0,1] ; if X is less than R, the ray is specularly reflected ; if X is higher than R, the ray is reflected, with a random deviation in reflection direction, and a frequential shift, the probability density of which is the kernel W, renormalized so that its integral is 1, necessary condition for an interpretation as a probability density.

3 / the ray followed again up to the next boundary encounter, where, if necessary, the procedure 2/ is performed again.

This recipe is applied up to the satisfaction of some stop criterion. The result of this procedure is a randomly perturbed channel, which random jumps at each boundary encounter ; the luminance is conserved along this path ; the mean energy is then summed up over bins in the fictive space {locations $\underline{r}$, directions $\underline{d}$,times t, frequency f} by evaluation the length of the intersection between the box and the path (ref.[11]). Then the ray is launched again, the procedure is followed again, and a new random path is generated ; the energy in the bin is. At the end, the mean energy in this bin asymptotically tends,when the number of random perturbed paths is high enough, toward the sum of the mean luminance over the considered bin. A numerical method for evaluating the second-order moments is so developed, which does not require important storage space involving only sums of positive terms; it is a technique which converges uniformly toward the solution (increasing the number of realizations of random paths necessarily improves the quality of the final result).

This Monte-Carlo method, summarized on Figure 6.2, finally provides numerical estimations of the second-order moments of the random fluctuations of acoustic impulse response, summed up over bins, and, among them, the elements required for the mean time Wigner transform of the impulse response on a receiver located at a fixed location; the final step, from this previously computed and stored moment, is to call a routine for drawing realizations of non-uniform or non-stationary random processes depending on two variables, draws realizations of the impulse response, assumed a normal process (function of two times t and t_0).

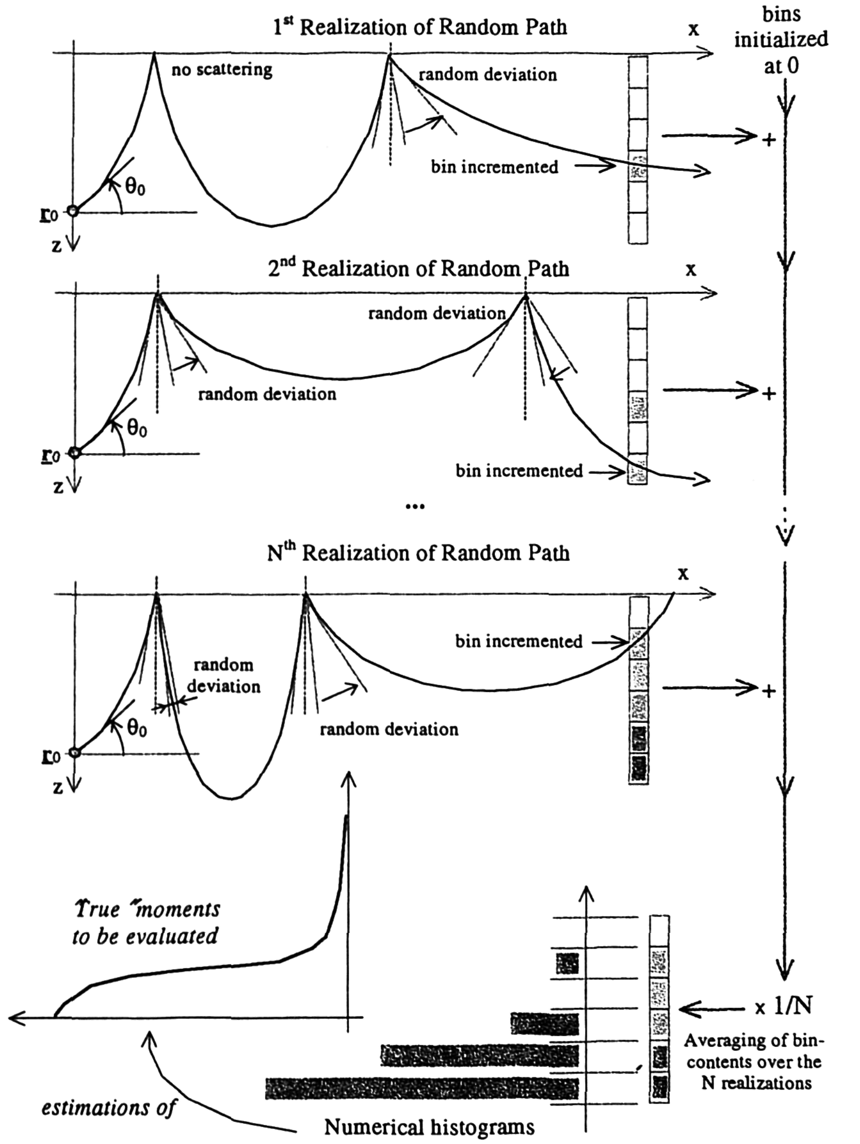


Figure 6.2 : principles of the numerical Monte-Carlo technique used for evaluating second-order moments of acoustic fields

6.3. Bistatic reverberation and ambient noise

Random time series which simulate bistatic reverberation and ambient noise are also introduced in the PROSIM simulator, based on a modelling done by TNO-FEL. This modelling includes spatial coherence of both of these background signal components and is described in [4].

7. SOFTWARE DEVELOPMENT

A first version of the PROSIM simulator already exists which incorporate the deterministic model developed by SACLANTCEN, and the ambient noise and reverberation models developed by TNO-FEL. The structure of the simulator is such that it is relatively easy to add the other models, without extensive modifications to the user interface.

7.1. PROSIM software design.

The PROSIM simulator is composed of three layers, an input layer, a computation layer and an output layer. Each layer communicates with the other layers via an internal interface, hidden from the user. Each of the three layers has to adhere to certain requirements.

- Input layer: The input layer communicates directly with the user. The user should be able to specify the problem, i.e. environment and pulse definition, in a natural way. Moreover, it should be possible to process the input-data so that the user can immediately enter and manipulate raw, measured data. This is particularly the case for measured sound speed profiles and bathymetry information. Further, checks must be performed to determine whether the specification of the problem is legal, consistent and complete. If this is the case, the processed data can be communicated to the different models in the second layer. In practice this is done via files.
- Computation layer: The different models read the output data of the input-layer from file. The aim is not to change the original interfaces of the models. In some rare cases, however, this will be necessary e.g. to keep the pulse definitions and sample frequencies in the different models consistent.
- Output layer: The output layer reads the output of the model calculations from file. Most models will output the signals received by the hydrophones. An exception is the deterministic model, which yields a transfer function. Hence, a fourier synthesis has to be made to compute the received signal. The different signals must be represented in a clarifying way: for example the noise and direct propagation signals must be plotted in the same picture to get an impression of the signal-to-noise ratio. If one wants to investigate multipath arrival structures, an FM-pulse should be transmitted and the received signal should be matched filtered to better distinguish the different arrivals.

The ideas sketched above have been implemented in MATLAB, a trademark of the MATH WORKS Inc. Although this package is commercial, it is so widely used that this choice assures a high level of portability.

7.2. The graphical user interface.

The graphical user interface is structured around three main menu's, the 'Environmental input parameters' menu, the 'Pulse definition' menu, and the 'Options' menu.
The menu entitled 'Environmental input parameters' is used for entering all environmental parameters: sound speed profile (SSP) in the water column, sediment and subbottom, bathymetry information, noise parameters, and attenuation and density in the sediment and subbottom.

The second section is called 'signal processing' and specifies the minimum and maximum frequency of interest and the observation time. The third section, called 'source receiver configuration' specifies the location of the projector and the hydrophones.

7.3. Example.

The features of the PROSIM simulator is illustrated for the situation as measured during one of the Firth of Clyde experiments. As an example we will take an FM sweep of one second from 700-1300 Hz, transmitted on August 2, between 20:30 and 20:50. The range between Transmitter and Receiver was about 10 km and the ships were moored along the primary track. The transducer was positioned at a depth of approximately 40 meters.
Figure 7.1 displays the simulator output waveforms obtained in this case.

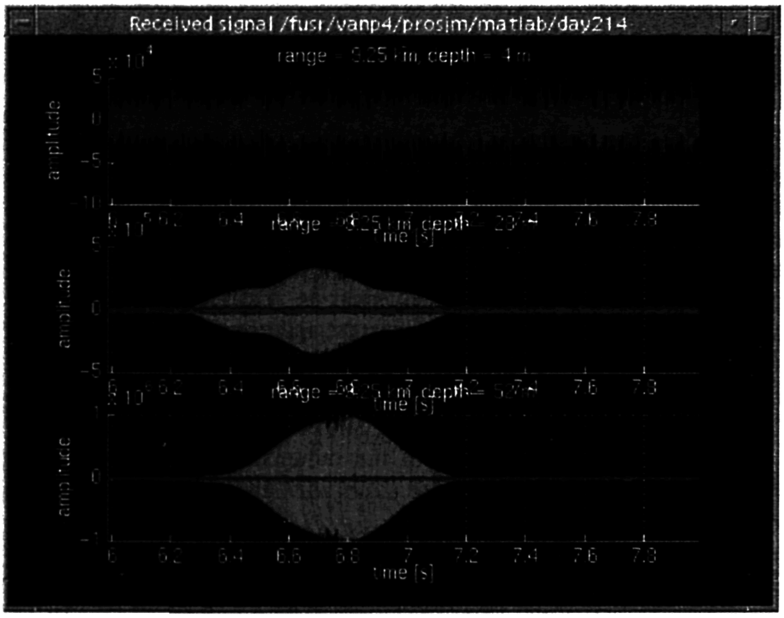


Figure 7-1. Noise (blue) and direct propagation (red) signals on the three hydrophones.

The first hydrophone shows a poor signal to noise ratio, but the other two are clearly better. This is in line with the actual measurements in the Firth of Clyde.

It is also possible to process these simulated waveforms by match filtering to better identify the various arivals.

8. CONCLUSION

During the second year of the PROSIM project, two milestones have been passed successfully: the two experiments at sea. A lot of data was collected during these experiments, both environment data and acoustic data. According to the first analysis, the Clyde sea data exhibit more variability in terms of environment parameters, mainly temperature profiles than the Meditreeanean Sea data; not surprisingly, the estimated channel impulse response is found to be also more fluctuating in the case of the Clyde Sea.
The deterministic propagation model, together with the reverberation and noise model are already implemented in a first version of the simulator which works on a standard workstation in a reasonable computing time. The stochastic simulator is not ready yet in an easily usable version, therefore no comparison with experimental data was attempted to explain the observed variability and try to see if the statistics of the experimental impulse

responses correspond to the predicted ones. This work is planned for the third year of the project, together with the full exploitation of the experimental results.

9. ACKNOWLEDGEMENTS

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The present paper includes contributions of all the partners of the project.

10. REFERENCES

[1] Saunders, K. D. and D. B. King (1991). Simulating temperature, salinity and currents in the ocean. In *Ocean Variability and Acoustic Propagation* (ed. J. Potter and A. Warn-Varnas), Kluwer Academic, pp 561-577.
[2] Chen, C.-T. and F. J. Millero (1977). Speed of sound in sea-water at high pressures. *Journal of the Acoustical Society of America*, 62, 1129-1135.
[3] Long range acoustic communications (LORACOM) project, Second year technical report ref TMS SAS 96/S/EGS/NC/028 AP, 150 pp. EC contract number MAS2-CT94-0080.
[4] PROSIM First year technical report. Ref TSM SAS 97/EGS/NC/084 AP
[5] Barnett, T. P. and Wilkerson, J. C. (1967). On the generation of ocean wind waves as inferred from airborne radar measurements of fetch-limited spectra. *Journal of Marine Research*, 25, 292-328.
[6] Hasselmann, K. *et al.* (1973). Measurements of wind-wave growth and swell decay during the Joint North Sea Wave Project (JONSWAP). *Dt. Hydrogr. Z.*, 12 (8).
[7] E. K. Westwood, C. T. Tindle, and N., R. Chapman, "A normal mode model for acoustic-elastic ocean environments", Journal of Acoustic Society of America, **100** (6), December 1996 pp. 3631-3645.
[8] F. B. Jensen, W. A. Kuperman, M. B. Porter and H. Schmidt, "Computational Ocean Acoustics" AIP Series in Modern Acoustics and Signal Processing, American Institute of Physics, New York, 1994
[9] C. M. Ferla, M. B. Porter and F. B. Jensen, "C-SNAP: Coupled SACLANTCEN normal mode propagation loss model", SACLANTCEN Memorandum serial No.: SM-274, December 1993.
[10] A. Ishimaru *WAVE PROPAGATION AND SCATTERING IN RANDOM MEDIA* (2 vol.) Academic Press, Orlando, Florida (1978)
[11] H. L. Wilson & F. D. Tappert *"Acoustic propagation in random oceans using the radiation transport equation"* in J.Acoust.Soc.Am.95(4), pp.256-274, 1979
[12] K. K. Sabelfeld *MONTE CARLO METHODS IN BOUNDARY VALUE PROBLEMS* Springer-Verlag, Berlin-Heidelberg, 1991

TITLE: The Development of a Hydro-mechanical, Multi-sensor Towed Undulating Oceanographic Vehicle for deployment from Ships-of-Opportunity (project **HYTOV**)

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THE DEVELOPMENT OF A HYDRO-MECHANICAL, MULTI-SENSOR TOWED UNDULATING OCEANOGRAPHIC VEHICLE FOR DEPLOYMENT FROM SHIPS-OF-OPPORTUNITY HYTOV

SUMMARY

The object of the project is to develop an inexpensive towed undulating oceanographic vehicle for deployments from Ships of Opportunity (SOOP). HYTOV is mechanical requiring no power or control from the towing vessel and should be easy to handle and deploy. It is to be capable of attaining an undulation aperture of 35 metres at a wide range of tow speeds. The payload will be highly flexible with the capability of accommodating a range of oceanographic sensors. The project commenced in July 1998 and runs for 30 months. The main aims of HYTOV are described and the work plan is outlined. The user requirement has been produced and is incorporated at Enclosure 1 whilst some of the initial hydrodynamic analysis is at Enclosure 2.

1. INTRODUCTION

The aim of the project is to design and develop a very low cost towed undulating oceanographic vehicle, deployed from a diverse range of ships-of-opportunity (SOOP), for the acquisition of large, high quality, long-term profiled data sets of upper ocean environmental parameters.

Current technology has produced towed oceanographic vehicles which either (i) have the ability to undulate through the water column using sophisticated control systems or (ii) are simple fixed depth systems.

The Continuous Plankton Recorder (CPR) is a robust fixed-depth towed oceanographic vehicle that has been used to provide information about plankton abundance and distribution in the Northeast Atlantic Ocean since 1931. It is the most widely used vehicle for SOOP deployment, the fleet numbering 120 or so world-wide. In recent years, several of the CPR fleet have been successfully instrumented with self-powered, self-logging CTD-fluorimeters. These deployments have furnished useful fixed depth data sets and valuable operational experience. The instrumented CPR approach to data gathering, whilst providing a valuable contribution to the collection of long-term data sets with wide oceanographic coverage, still leaves the scientific community with little or no information about the vertical distribution.

Some of the commercially available undulating towed vehicles are capable of deployment from SOOP. However they invariably do not address the requirement for wider, more frequent oceanographic coverage for a number of reasons :

- system costs vary from 25 000 ECU to 50 000 ECU depending on the sensor payload
- the vehicles are presently operated only from a limited number of SOOP
- whilst reasonably robust, great care is required during deployment and recovery
- the towing loads are higher than for the CPR's
- operation of these vehicles has a greater impact upon the daily operation of the host ship than CPR systems.

HYTOV is an RTD programme leading to the design and development of a very low cost purely mechanical CPR-type vehicle with optimised cargo space for the deployment of multiple sensors, capable of undulating through a 35 metre aperture and of being deployed from a diverse range of SOOP with particular reference to cargo ships and ferries drawing on the experience of the SOOP used in other programmes. The programme requires an advancement in towed body hydrodynamics, vehicle deployment, mechanical control systems and, particularly, data portability and dissemination.

The specific goals include:

- to design a small towed vehicle that has the hydrodynamic capacity to give a 30/35 metre undulation aperture when towed on an unfaired cable. Typical passage speeds will be 15 knots, however maximum speeds of 20 knots will be considered, allowing deployment from vessels such as high speed ferries
- to produce a robust design for use on SOOP
- to design and develop a mechanical, impeller driven elevator/wing actuator and control system
- to design for simplicity of manufacture
- to optimise cargo space for easy payload integration and good water flow over/around sensors

The vehicle will be designed to accommodate a limited oceanographic payload which will be demonstrated and assessed during the sea trials. For trials purposes the payload will be based upon:

- CTD sensors
- miniature Chlorophyll fluorimeter
- Integral data logger, developed for the specific requirements of easy data portability and possibly a continuous plankton recorder or nutrient sensor.

2. WORKPLAN

The programme will involve the formulation of detailed specifications covering the operational and logistical requirements of the SOOP provider and the scientific requirements of the oceanographic community. From this basis, vehicle design parameter research will be undertaken to optimise the load carrying capacity, hydrodynamic loading, stability and deployment logistics of the vehicle. The feasibility of impeller driven, low complexity, high reliability mechanical depth control systems will be investigated and detailed design of the control system undertaken with particular regard to the safe vehicle operating envelope.

Detailed vehicle hydrodynamic design and control system design will lead to the construction of a prototype vehicle, whose performance characteristics will be assessed using the consortium's experimental facilities, in conjunction with an advisory peer group drawn from associated oceanographic laboratories; research organisations and institutions; and centres of excellence. Full field trials of the system using SOOP will be undertaken.

Work Package 1 Formulation of requirements specification

This was a predominantly consultative phase to determine the detailed specifications covering the operational and logistical requirements of the SOOP provider and the scientific requirements of the oceanographic community. The requirements specification is at Enclosure 1.

Work Package 2 Research the vehicle design parameters

The body design of the proposed vehicle will be dependant upon the required payload volume and weight; the desired undulation aperture; the operational speed range of host vessels; the towing cable characteristics; limitations imposed by deployment and recovery logistics; and the power requirements of the control system. The specification formulated in Work Package 1 will address both the operational and logistical requirements of the SOOP operator, and the payload scientific recommendations of the user community.

Building on the hydrodynamic designs of both the CPR and commercial undulating towed vehicles, Work Package 2 will research the hydrodynamic design parameters to optimise the load carrying capacity, hydrodynamic loading, stability and deployment logistics of the vehicle to meet these requirements specification.

Initial theoretical analysis by HSVA suggests that for high towing speeds, a method of active roll stabilisation will be required. Provisional CFD (Computational Fluid Dynamics) and Lift/Drag plots have been generated for the proposed vehicle design, Enclosure 2. Further computational analysis will be used to determine the forces required to operate the control surfaces and to give an indication of the response time required. This computational data will be used as the primary basis for the vehicle design.

Work Package 3 Research the feasibility of an impeller-driven mechanical control system

For a given payload, tow cable configuration and host vessel speed, the vehicle depth is determined by the elevator position. The vehicle will undulate through the water column when the elevator is cycled between limiting positions, setting the upper and lower depth limits. This Work Package will research the feasibility of impeller driven mechanical control systems to cycle the elevator position.

To prevent damage both to the vehicle and to the sensor suite, it will be of critical importance that the vehicle should at no time break surface during a towed deployment. A safety override system will be designed and tested to constrain the elevator to a dive position at shallow depths.

Cost has been identified as a key factor in the commercial success of the proposed undulating towed vehicle. The design activities will focus on reliability, robustness, ease of manufacture and cost considerations.

Work Package 4 Detailed vehicle and control system design, prototype construction and test

The outcome of Work Packages 2 and 3 will form the outline design specifications for the vehicle body and the vehicle mechanical control system. Work Package 4 involves the detailed system design, prototype vehicle construction, test plan formulation and prototype vehicle test using the experimental facilities of HSVA.

Work Package 5 Field trials of an instrumented prototype system

A prototype vehicle complete with CTD-fluorimeter, attitude sensors and data logger is trialled to 'dry run' the data dissemination aspects of the programme. The vehicle will be subjected to full ocean-going field trials utilising SOOP to evaluate the success of the full system. Any deficiencies and enhancements required to enable the operational system to undertake full scientific missions will be identified.

ENCLOSURE 1

1 Work Package Title

Requirements Specification

2 Introduction

The purpose of the Requirements Specification is to provide a comprehensive 'design brief' that will enable work to start on work packages 2 and 3. This document is not a vehicle design specification, but does identify the primary oceanographic / scientific user requirements and target performance parameters for a robust 'stand-alone' towed underwater vehicle for Ship of Opportunity (SOOP) deployment.

3 Sensor Payload

The vehicle design should allow for the internal mounting of the following instrument options:

Core Sensors	Optional Additional Instrumentation
Conductivity	Continuous Plankton Recorder (CPR)
Temperature	Phosphate / Nitrate Sensor
Depth	PAR
Fluorimeter (Chlorophyll *a* and Turbidity)	

The sensor requirements are derived from extensive consultation with the European science base.

Due to the harsh deployment and recovery conditions typically encountered during SOOP operation, it is anticipated that the entire sensor payload - including sensor heads - will be contained within the overall vehicle envelope. Ducted water flow will be distributed to the instrumentation payload.

4 SOOP Installation and Operational Logistics

4.1 Transhipment

The vehicle will not routinely be transported by air - if, for example, a diaphragm or differential pressure system is used for vehicle control surface actuation, special criteria for air transport will be considered.

The packing crate will have high visibility, be robust enough for extended re-use, and include both slinging points and standard fork-lift slots. Consideration in the design of the packaging will be given to the corrosion implications of a salt-spray environment.

The vehicle tow cable will be packed with the vehicle - see 4.3. The core sensor pack and any additional sensors will be packed separately form the vehicle.

4.2 Sensor System Installation

It is expected that the core sensors, as defined in 3, will be mounted in a ruggedised cassette capable of quick and easy installation by the SOOP deck crew if necessary.

All core sensor system power requirements will be derived internally, or from a separate battery pack from within the 'cassette' system, so that no instrument underwater harness interconnections will have to be made by the SOOP operators.

It is envisaged that additional instrumentation will be mounted in the vehicle using quick-release clamps on a 'plug-and-play' basis.

It is a requirement that all underwater harnesses are routed within the vehicle, maintaining a clean external profile.

4.3 SOOP Vehicle Installation

In addition to the packing crate slinging points, it would be considered an advantage for the vehicle itself to have integral slinging points, for deck handling / securing, and as 'grab' points during deployment and recovery.

The vehicle must be easily deck-handled, and should not require a special deck 'skid'.

In the majority of towed underwater systems the tow cable is one of the most dominant factors: diameter / drag, dynamic bend radius, torque, weight in seawater - all significantly affecting hydrodynamic performance of the vehicle. In order to assist in the modelling of the vehicle, a suitable tow cable will be identified and supplied as part of the vehicle system. This also removes the safe-working-load safety concerns and difficulties in terminating existing SOOP winch / cable combinations.

In order to assist in transferring the tow cable to the towing vessel winch or mooring capstan, it will be supplied on a basic hand-driven reel and stand - a simple traversing arm to ensure a satisfactory 'cable-lay' on the drum would be considered an advantage. The tow cable handling sub-system could be an integral part of the packing crate.

4.4 System Deployment and Recovery

Safety regulations will require a minimum of two crew for deck handling operations. The vehicle design will therefore aim for a two-man deployment / recovery procedure.

Target Deployment / Recovery conditions:

Sea State	5 - 6
Min. Deployment Speed	8 kts
Max. Deployment Speed	20 kts*
Max. Recovery Speed	20 kts (using sea anchor)

*The vehicle should be capable of rapidly 'pulling' itself under the sea surface at this deployment speed, minimising cable-snatch and high shock loads on the vehicle and sensor payload.

Consideration in the vehicle design must be given to isolating the sensor payload from shock-loading during the deployment / recovery phase.

It is a requirement that the vehicle can be deployed using the existing davit, shreave and snatch-block arrangement used by SAHFOS SOOP's which are rated to 2000Kg SWL. An 'A'-frame should not be required .

Ideally, snatch loads during deployment at 20kts should not exceed 1500Kg.

SMHI experience suggests that they might do several short deployments of 2-3 hours in a 24 hour period - to assist in easy frequent deployment the fully instrumented vehicle should not exceed 80kg weight in air.

4.5 Undulation Profile 'Programming'

The adjustments to change the undulation profile of the vehicle will not be undertaken by the SOOP personnel, but pre-set prior to transhipment.

4.6 Vehicle Hydrodynamic Performance

The requirement in terms of pitch, roll and yaw, is to provide a 'stable' instrumentation platform, with a repeatable undulation profile within the following *target* parameters:

Min. Operating Depth	5 metres
Max. Undulation Aperture	35 metres
Towing Accuracy	±1.5 metres
Max. Rate of Change of Depth	35m in 1km @ 15 kts (≈ 0.25m/s)

5 SOOP Data Handling Requirements

5.1 Data Logger

The data logger should be capable of storing a minimum of 24 hrs data at the appropriate data logging rate to give meaningful spatial resolution with respect to the ship's track and speed, and vehicle undulation rate and profile.

5.2 Data Correlation with Ship Position

It is envisaged that for those deployment vessels without GPS, a GPS system and logger would be provided as part of the overall system.

'Physical' deployment protocols should be considered to ensure that GPS logging is started at the time the vehicle is deployed. This might involve removal of the towing connection from the GPS system initiating logging, or removal of a flag from the vehicle.

The Real Time Clock (RTC) in the both the core sensor data logger and GPS logger will be factory pre-set to GMT.

5.3 Data Extraction from the Vehicle

Primary emphasis will be placed on an easily removable 'sensor cassette', which, together with the GPS logger, can be returned to the laboratory for up-loading data, instrument re-calibration and replacement of the battery pack.

For 'near-real-time-users', time-stamped stored sensor data will be up-loaded to a host computer via a serial link . GPS will be incorporated with the sensor data in the host computer prior to subsequent onward transmission via satellite, radio telemetry or other appropriate transport mechanism. The data logger, or core sensor cassette will be removed from the vehicle and a serial link to the host computer established via a rugged underwater harness, so that the logger pressure seal is not compromised.

6 SOOP System Maintenance

6.1 Deployment Cycle

SAHFOS CPR SOOP deployments suggest that vehicles are usually returned to the laboratory within a 10 day period; but this period can be extended up to a maximum of 6 months.

Materials selection and mechanism / control surface design should allow for stowage in a salt-spray environment for up to 6 months.

6.2 Maintenance Requirements / Schedule

The vehicle must be designed for 'minimum-at-sea-maintenance' - after deployment the SOOP operators will only be expected to rinse the vehicle with fresh water and remove any obvious trapped debris.

This rudimentary level of maintenance, for a period of up to 6 months, must be taken into consideration during the design phase of the undulator - potential salt water and debris traps should be avoided, mechanisms, bearings and other moving parts should be sealed and not require regular lubrication or servicing.

Wherever possible moving parts and body components should be designed for quick and easy on site replacement. A modular approach which involves replacement of sub-assemblies - not individual components - would be considered an advantage.

7 Safety

Consideration must be given to safety during all activities and operations associated with the design, manufacture, installation and use of the equipment. The equipment must be designed so that hardware does not pose any known threat to the safety of personnel engaged in the design, manufacture, installation or operation of the system.

The third party liability implications of installing and operating *HYTOV* on ships of opportunity will be investigated.

8 Standards

All work must be carried out to the standards laid down in ISO 9001.

ENCLOSURE 2

Initial design considerations lead to a vehicle configuration which is based on existing designs, e.g. Chelsea Instruments Nu-shuttle / Aquashuttle, but aiming at an enhanced hydrodynamic performance in terms of lift, drag and especially course stability at higher speeds. These ideas lead to an initial body design which is shown below:

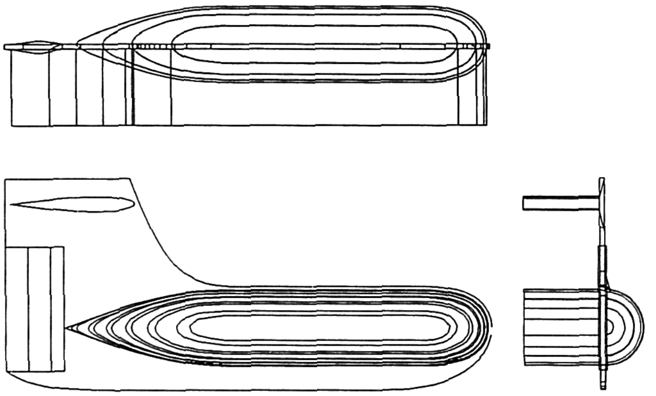

Fig. 1.0 'HYTOV - initial design configuration'

Dimensions and proportions of the vehicle body were derived from estimation formulae for the lift and drag of the main body. The aspect ratio of the body was chosen so that basic requirements regarding desired lift and payload capacity can be fulfilled.

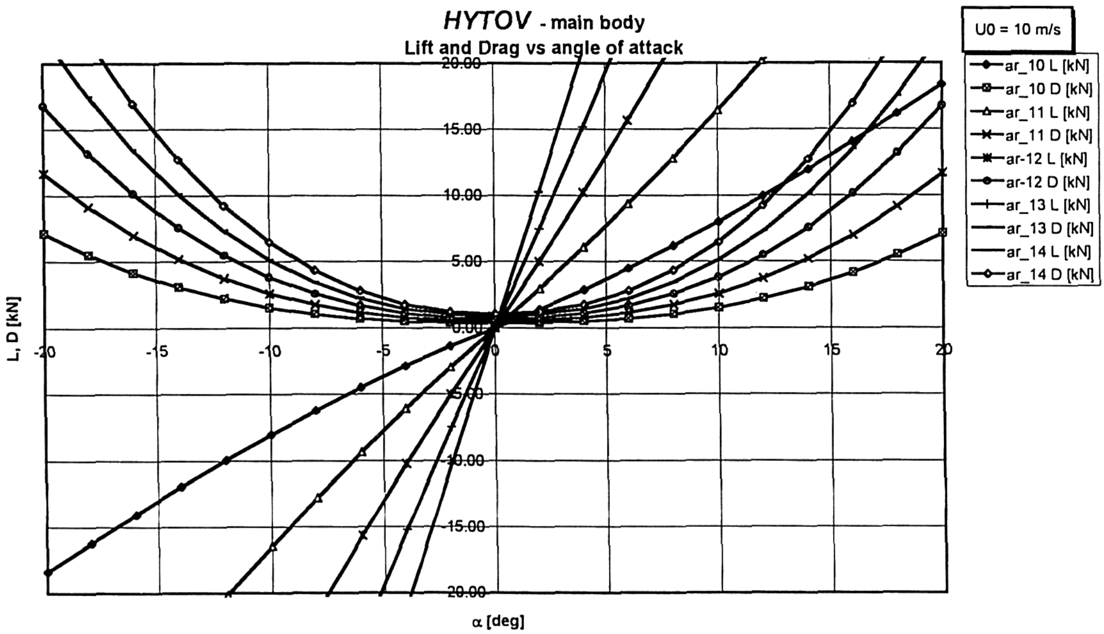

Fig. 2.0 'Lift and drag of the HYTOV main body vs. angle of attack'

Parameter for all curves is the aspect ratio B/L, i.e. body width vs body length. This varies from 0.33 (ar_10) to 1.00 (ar_14). Lift and drag are given in [kN] for a free stream velocity (ship speed) of 10m/s. Forces scale approximately to the square of the velocity, therefore smaller velocities will yield substantially lower drag and lift forces.

A CFD (Computational Fluid Dynamics) analysis of the initial configuration is being performed using a viscous flow code solving the Reynolds Averaged Navier-Stokes Equations (RANSE). The following figures show pressure contours & velocity distributions for different angles of attack. The pressure distribution at the centre plane of the HYTOV vehicle is shown in Figs. 3.0 and 3.1. The plots indicate contour levels of the pressure coefficient

$$c_P = \frac{P - P_0}{\frac{\rho}{2} U_0^2}$$

Only the dynamic part of the pressure is plotted, the hydrostatic contribution

$$P_{stat} = \rho \bullet g \bullet h$$

needs to be added according to the actual depth of the vehicle:

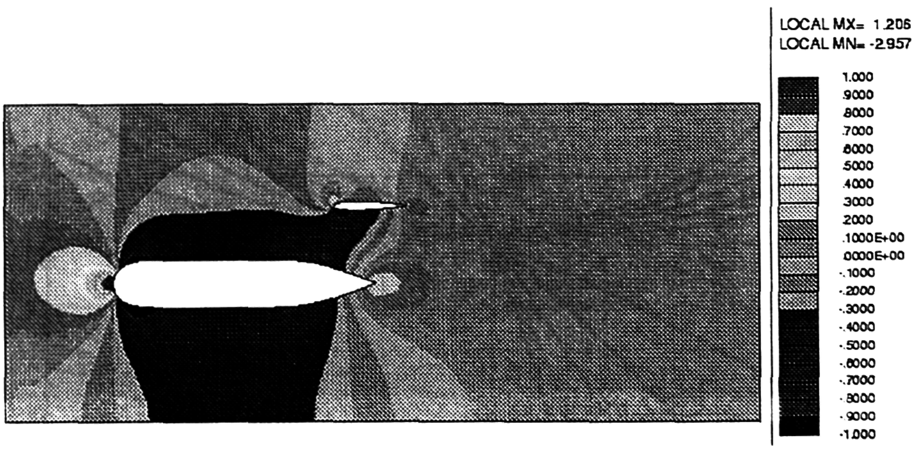

Fig. 3.0 'Pressure distribution at HYTOV centre plane'

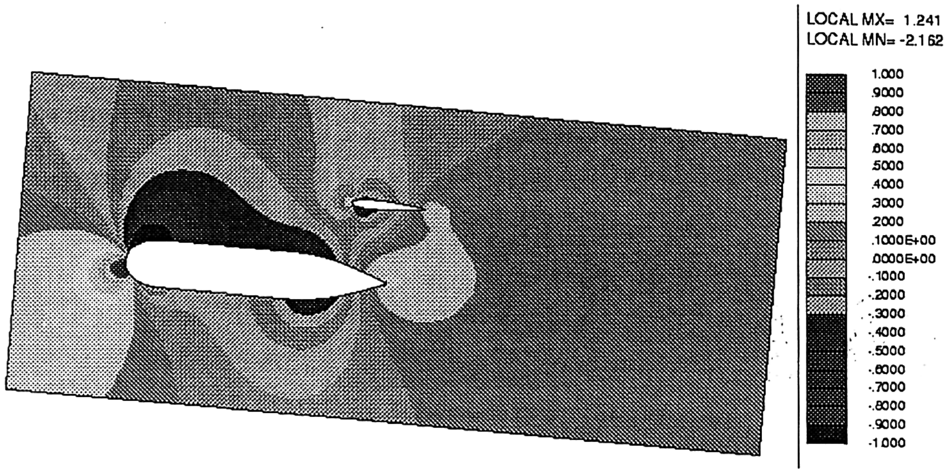

Fig. 3.1 'Pressure distribution at HYTOV centre plane'

Figs. 4.0 and 4.1 indicate the velocity field around the vehicle. These plots show contour levels of the velocity magnitude V/V_0

$$\frac{V}{V_0} = \frac{\sqrt{V_x^2 + V_y^2 + V_z^2}}{V_0}$$

again in the centre plane of the vessel.

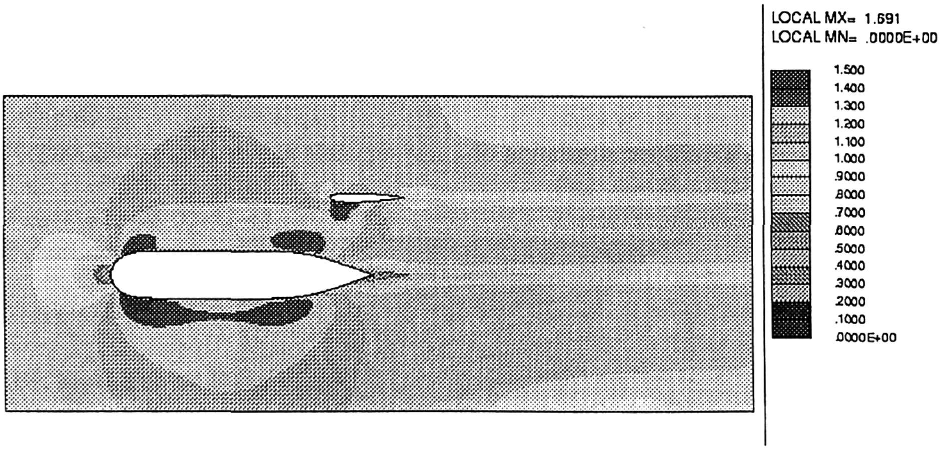

Fig 4.0 'Velocity distribution at HYTOV centre plane'

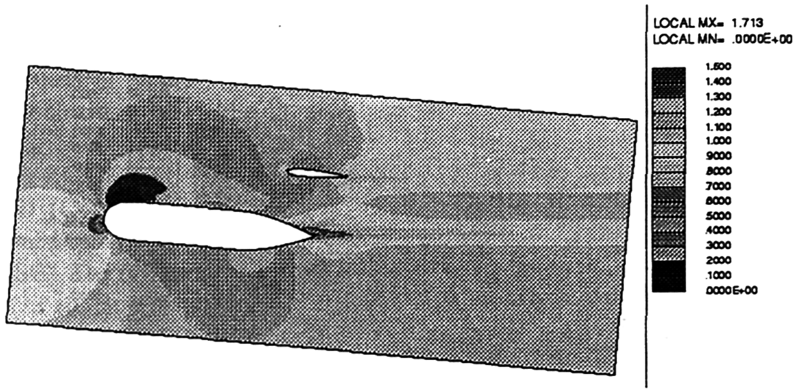

Fig 4.1 'Velocity distribution at HYTOV centre plane'

The velocity plots indicate that the boundary layer on the body is rather thin and no flow separation is to be expected in the cases shown.

Another main area of work in the hydrodynamic design part of the vehicle is the **simulation of the transient behaviour of the entire towed system**, i.e. the towing cable and the vehicle, behind a ship. An existing simulation method has been further amended for this purpose. The method includes a novel approach to the simulation of the towing cable. In order to simulate the transient motion of the towing cable, the cable is divided into elements. The elements are idealized as cylinders with joints at their ends. The equations of motion of the elements, which are assumed to be rigid, are obtained from the equations of linear and angular momentum. The forces considered are the weight, the hydrostatic buoyancy, the drag, the lift and the coupling forces at the joints to the neighbouring elements. A linear equation system is completed by kinematic conditions at the joints between the neighobouring pieces. The tip of the first element of the cable is assumed to have the ship velocity in an earth fixed coordinate system, an additional mass - which is a sphere in the present case - is positioned at the end of the cable.

The following plot shows a transient simulation of a towing cable at v = 10m/s using only 10 elements.

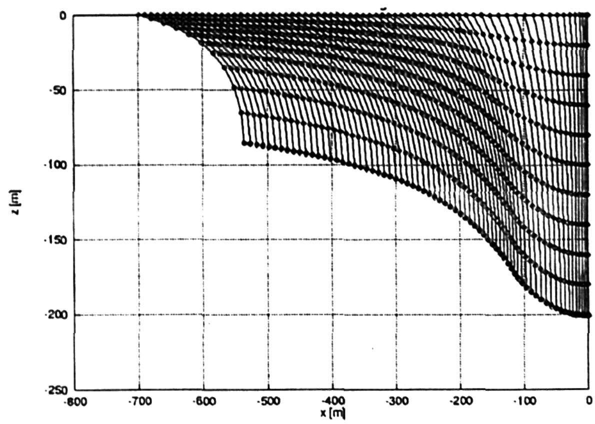

Fig. 5.0 'Transient simulation of a towed cable with additional mass at the end'

TITLE: Long Range Telemetry in Ultra-Shallow Channels **(LOTUS)**

CONTRACT NO: MAS3-CT97-0099

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LONG RANGE TELEMETRY IN ULTRA-SHALLOW CHANNELS

LOTUS

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SUMMARY

The main objective of the LOTUS project is to develop the technology for a long to medium range acoustic communication network capable of operating over distances far larger than the water depth. The network would provide greatly increased capability for monitoring, and acquiring data from, a set of unsupervised sub-sea instruments and long range AUVs, via a ship or a surface buoy providing a radio/satellite link to land. Acoustic communication is the only realistic option for such a network since optics and radio waves are too highly attenuated in sea water. The proposed experimental network would achieve a point-to-point link of up to 10 km using an 8 kHz carrier, with target data rates of up to 4 kbits/s on each link using coherent modulation. Scaling for longer ranges will be considered by modelling.

The main technical difficulties arise from the strong, time varying reverberation present in the very shallow channel (causing both fading and intersymbol interference), and from co-channel interference from units transmitting simultaneously. Promising results have been achieved with receivers based on recent signal processing methods that exploit the uncorrelated fading characteristics on all the various signal paths to provide separation at the receiver. These innovative multi-access methods, in combination with traditional techniques, will be investigated by using a 3-D array of transducers at the receiver to achieve spatial diversity, with an adaptive combiner optimised for each signal source. The design of the adaptive combiners will be an important part of the investigation in which modern cyclostationary and higher order signal statistics will be exploited.

The concept of a network has several other advantages: (a) by using a number of short hops, much longer overall range can be achieved; (b) if data from an instrument can be obtained by more than one route, this higher level of diversity can be used to improved reliability; and (c) the multiway flow of data can be used to control how the network adapts to changing conditions and requirements.

The project is divided into 9 tasks, which include the development of new transducers and the construction of an experimental system to be used in two sea trials to test the principles. The consortium, of 5 partners and a sub-contractor, includes 2 universities with many years experience of underwater acoustic communications and modelling, 3 SMEs currently producing acoustic communication equipment, and a large oil company representing the end user.

A communication system of the type described would not only be of benefit to ocean science, but could prove to be of enormous commercial and environmental value, improving

the safety and efficiency of underwater oil production, enabling new approaches to managing fisheries, enhancing pollution monitoring, and contributing to preservation of the marine environment.

1. INTRODUCTION

Existing commercial, sub-sea acoustic communication systems fall very far short of theoretical performance - by around two orders of magnitude - and are simple point-to-point links with no network capability. The network in LOTUS will be achieved thanks to three recent developments: (a) the use of recently developed wideband coherent acoustic communication methods, which use receiving arrays and adaptive spatial diversity combining to discriminate between wanted and unwanted signals; (b) improved transducer technology; and (c) multi-access techniques developed for land based mobile telephone networks.
The network will use acoustic communications, and packet store-and-forward networking methods. This will provide much greater ranges, by hopping from unit to unit, and also much improved reliability due to the diversity of different routes that information can take. The design methodology will concentrate on "small" coastal regions of some 50km square, where the depth may be only 50m or less, but will also consider "large" ocean basins of several hundred km square, with depths up to 1000m or more. The longest point-to-point links, and those to shore, will be implemented by surface buoys with radio/satellite links. Sea experiments in a coastal region will be used for data gathering and verification of the network design, whilst modelling will be used for investigating networks suitable for large ocean basins.
The capability to communicate with instruments without cables or locally stationed ships will enable the use of much greater numbers of sub-sea instruments at much lower cost than is currently possible. Continuous data monitoring could be achieved, with data being relayed back to shore on-line. The position of AUVs or drifting instruments could also be estimated with this type of network. Such technology is vital for future sub-sea exploration.

2. TECHNICAL CONSIDERATIONS

Several technical problems present themselves to those attempting to produce reliable sub-sea commmunications systems the most notable of these are multipath, time varying channel response and acoustic noise.

- **Multipath:** It is well known that a long-to-medium range, shallow, underwater, acoustic channel has a complex multipath characteristic [1]. This can be represented and measured by its impulse response, which will show substantial "time-spread". For ranges of 10 km or so the response is generally spread over a few milliseconds, with several closely separated significant arrivals accompanied by long reverberation of lower energy. These multiple signal paths are due not only to reflection or scatter from the sea surface and bottom, but also to the many "micro-multipaths" that are supported in the body of the water column. These "micro-multipaths are due both to the range dependant velocity profile and, in coastal region conditions, also to surface layer effects caused by waves and bubbles. Furthermore, it is quite possible for two distant transducers to be in "shadow zones" where refraction results in there being no transfer of energy between them.

- **Time varying characteristics:** Of very particular importance in these channels, is the fact that the response is significantly time-varying. This is due to several factors:
  - the moving sea surface and varying bubble concentration;
  - short term (seconds or minutes) and small, but significant, fluctuations in the velocity profile - over ranges of a few kilometres these temporal changes are spatially uncorrelated;
  - long term (month) fluctuation in the velocity profile from season to season; and
  - the movement of both transmitter and receiver, due either to substantial movement of a boat or AUV, to the small movements of suspended or floating transducers.

- **Acoustic noise:** At the frequencies of interest there is significant acoustic noise present in the sea. Some of this is caused by natural events such as breaking waves or rain, whilst some is man-made from ships, sonar and various off-shore or ocean activities.

The combination of time-spread and time-variability and acoustic noise causes several effects which are major problems for a communication link. These are: signal fading at a receiver transducer - both short and long term; Doppler phase or frequency shift; severe, time varying inter-symbol interference (ISI) - the effect of reverberation interfering with the main signal arrival; and poor signal-to-noise ratio (SNR) at the receiver. Similar problems exist with radio systems. However, the very low bandwidths available in sub-sea communications means that the techniques employed in radio systems appear very inefficient when transferred to the underwater environment.

It is intended to overcome the problems outlined above by developing a sophisticated receiver structure. The receiver will consider the following techniques:

- **Spatial diversity combining:** The problem of fading on a time-varying multipath channel can be countered in the receiver by using diversity (i.e. more than one transmission of the same data). One way of achieving this is to divide the available frequency band into multiple narrow bands, and transmit on each simultaneously. However, not only is this bandwidth inefficient, but since symbol rates are low in each narrow band, non-stationarities in the channel often result in loss of phase coherence from one symbol to the next. Spatial diversity with a single wide frequency band is an alternative approach in which signals are transmitted from a single transducer, but received on multiple transducers. If positioned suitably, the outputs have uncorrelated fading characteristics and can be combined to form a fade-free resultant for the "direct path" signal arrival. Not only this, but since fading of the multipath signals is uncorrelated to that of the direct path, the combining can also be arranged to cancel multipath and hence reduce ISI. Adaptive FIR filters placed behind each receive element may be used as adaptive combiners and, if operating correctly, automatically compensate for, and track, the fading characteristic of each path. The optimum number of receive elements appears to be 4 to 5 [2], but this and the best geometric configuration for the non-rigid array is not well understood at present. If the elements are close enough together, spatial beamforming and multipath nulling may take place. However, if they are too close together, there may be insufficient diversity. Spatial diversity has the advantage of not compromising data rate.

- **Equalisation, phase tracking and sequence detection:** The output of the combiner is passed to a combined forward and decision-feedback adaptive equaliser. The decision-feedback section operates as an echo canceller by subtracting delayed versions of the sequence detector output from its input, thus further reducing ISI. The forward section operates as a linear equaliser, and also achieves phase tracking and symbol synchronisation. However, if time variations are too severe. separate phase trackers may be required for each transducer receive element. High order phase-locked-loops can be used [2], or differential demodulation methods [3] which are particularly robust in cases of high Doppler phase shift.

- **Adaptive filter techniques:** Since time-spread in the underwater channel is long and rapidly time-varying, modern fast adaptive filter methods for the above structures are required based on the LMS, RLS and other algorithms. The adaptive filter coefficients in this structure are usually updated continuously by using a decision-directed error derived from the sequence detector but an occasional transmitted training sequence may also be used (and required) to determine initial values. Use of "blind" adaptive techniques (i.e. requiring no training sequence) is also of interest in our application.

- **Wide band modulation:** A further important method of increasing adaptive filter tracking rates is the use of wide-band modulation methods, rather than multiple narrow bands. This increases the data rate and hence increases the tracking rate of the adaptive filters and phase estimators.

- **Non-coherent modulation:** For the most severe channels it may never be possible to achieve coherent modulation successfully and more conventional non-coherent methods, such as frequency-shift-keying (FSK) or chirp modulation [4], will then be used. For FSK, adaptive diversity would still be a valuable technique for overcoming fading.

Further problems arise from a consideration of the network aspects of the proposed system. In a network with multiple transmitters there will inevitably be significant co-channel interference - i.e. signals received simultaneously from more than one network node. In these circumstances, multi-access techniques are necessary to separate the simultaneous transmissions. Two main approaches will be investigated; original research on reduction of co-channel interference using adaptive spatial combining and/or beamforming from multiple receive elements, a method with proven success in reducing multipath interference, and the use of traditional multi-access techniques using CDMA, TDMA, FDMA and cellular methods. Some possible approaches to this problem are:

- **Space-time processing principles:** The receiver structure will be based on that proposed by Stojanovic [5]. It has several adaptive spatial combiners, one for each signal source of interest. If the elements of the receive array are suitably positioned, each adaptive combiner provides enhancement of one of the source signals whilst cancellation of the others - thus delivering both linear equalisation and source separation. The sequence detector becomes a multiple-input-multiple-output decision feedback equaliser, which allows reverberation of each source signal to be subtracted from the others.

- **Receive array geometry:** In order for these space-time processing techniques to be effective both for multipath, which is predominantly in the vertical plane, and for source separation, which is predominantly in the horizontal plane, the receive array must have elements spaced in a 3-D pattern. Furthermore, if some of the elements are close together, the array will behave as a beamformer in much the same way as a terrestrial antenna and spatial nulling of interferers such as ships or other "point sources" can then occur. The optimal array geometry is unknown at present but it is important to note that the adaptive combining/beamforming techniques proposed allow the array to be non-rigid and of imprecise dimensions.

- **Signal processing innovation:** Since the channels are time-varying and unknown at the receiver, one of the important signal processing issues to investigate is the initial acquisition phase, and subsequent tracking phase, of all the adaptive filters. Acquisition may be accomplished by use of a training signal, by the use of signature waveforms (spread spectrum), or by the use of modern so called "blind" methods which exploit the cyclo-stationary or higher order statistics of the received signal [6, 7, 8, 9, 10, 11, 12].

- **Traditional multi-access techniques**: CDMA (Code Division Multiple Access), TDMA (Time Division Multiple Access) and FDMA (Frequency Division Multiple Access), all well used in land based communication networks. In addition, land based mobile cellular systems make use of the limited propagation distance of UHF signals. These methods will be investigated in the context of the underwater acoustic communications channel, which has different characteristics from radio channels.

The network structure will also demand consideration of network protocols. For reasons of bandwidth efficiency and reliability it is important to reduce network protocols to a minimum. However, there are several aspects which have an important effect on the performance of the system as a whole, these are:

- **Maximum range:** Because a network can relay data in a sequence of short hops, they can operate over a much longer overall range - in theory around the world with enough network nodes! By making one or more nodes a surface buoy with a radio link, data can be relayed to shore-based scientific workstations thousands of miles away.

- **Network redundancy:** In a network with more than two nodes, there is redundancy in the route by which data may reach its destination node. Effective exploitation would significantly increase the reliability of communications as additional nodes are added.

- **Adaptivity:** Within a communication system such as that proposed, there are many parameters whose optimum value will depend on time-varying operating conditions. Apart from the adaptive filter coefficients, these include; type of modulation, symbol rate and transmitted power level (to achieve the best overall bit-error rates including the effects of co-channel interference). These require that the transmitter has knowledge of the various channel properties which it can only acquire by exchanging information with each of the other nodes in the network. This requires the development of suitable algorithms to determine the optimum communication parameters, and of a suitable protocol for information exchange.

3. PROJECT STRUCTURE

As well as Task 0 – Project Management, the LOTUS project is divided into eight tasks. These interlink as shown in Figure1.

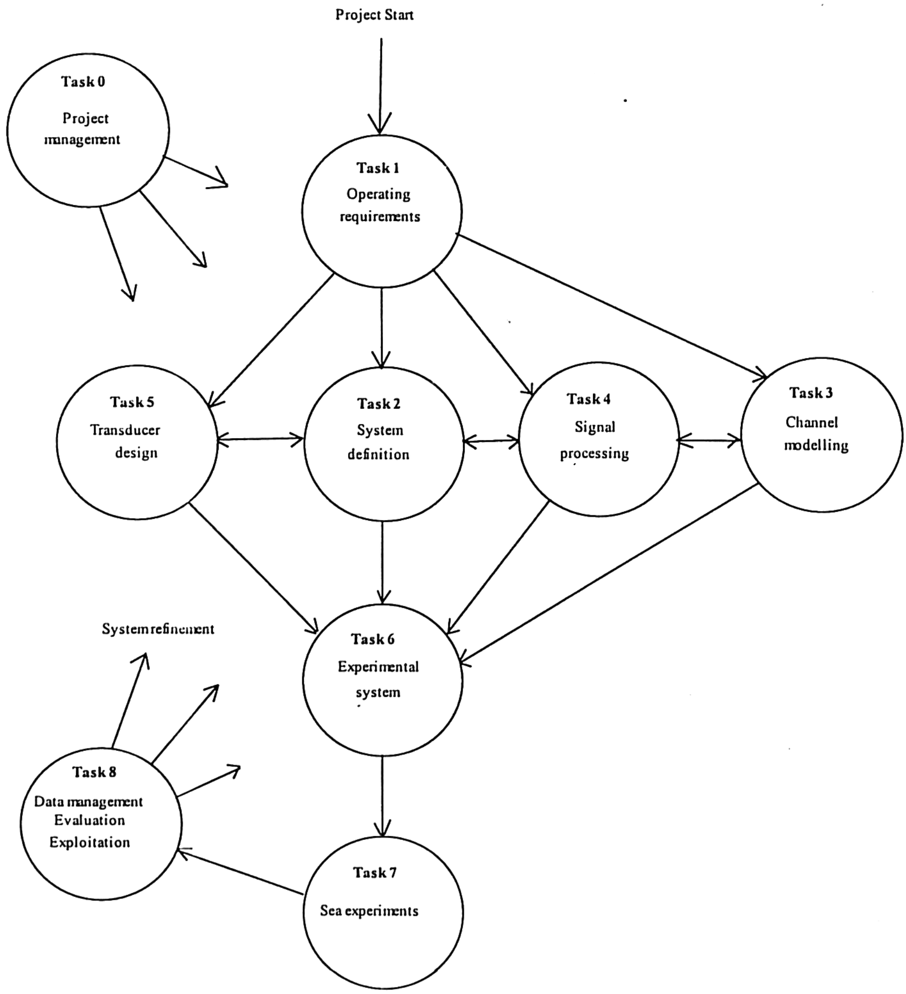

Figure 1

3.1 TASK 1 - OPERATING REQUIREMENTS AND CONDITIONS

This task will set up an Advisory Panel of end users representing the offshore industry, hydrographers and monitoring agencies. This Advisory Panel will specify the requirements of the acoustic communication system from the point of view of end users. This task will also provide environmental information on the typical acoustic channel for the applications identified. A large number of applications exists for the type of communication system described in this proposal, resulting in different requirements for system parameters such as operating range and depth, data rate and power limitations. Scenarios for communication between the sea-bed unit and a ship or a fixed platform will be defined. The study being carried out in this task is seen very much as driven from user requirements rather than system capability. The characteristics of the acoustic channel itself vary widely, with important parameters being acoustic noise levels, proximity of sea-bed structures, velocity profiles, temperature, salinity, wave conditions, stability of transducer platforms, etc. These will be determined using existing available databases, and making direct measurements.

3.2 TASK 2 - SYSTEM DEFINITION

This task will provide a detailed specification of the systems to be used for the two sea trials identified later. Specifications and predicted performance will be prepared for each of the systems to be used for the sea trials. These specifications will provide, from the outset, a clear framework for harmonisation between tasks. The structure and aims of the first sea trials will be defined in detail. Based on the sonar equation, prediction from available publications and preliminary assessment of normal operating conditions, a realistic and detailed definition of the target system parameters and performance will be prepared. This will define the range of values required on parameters such as; operating frequency, transmitter source level, transducer dimensions, receive transducer sensitivity, signal bandwidths, symbol rates, modulation types, etc. Interface between other tasks will also be defined to ensure compatibility.A similar procedure will be followed for the second system, taking into account the results of the first sea trials, analysis of acquired data, and performance predictions from Tasks 3 (Channel Modelling) and 4 (Signal Processing).

3.3 TASK 3 - CHANNEL MODELLING

The aim of the channel modelling task is to develop an accurate model of the propagation of acoustic signals at medium-range frequencies (5-20kHz) in the ultra-shallow water communication channel (USWCC). The channel model will be used to determine the influence of the physical conditions on the design and performance of the communication system. A three-dimensional acoustic propagation model will be chosen as a basic tool. This model will include well-known attenuation factors, such as geometrical spreading, volume absorption, bottom reflection loss and rough boundary scattering losses. The model will also include acoustic background noise, both from natural and man-made sources, and will support investigation of communication network performance for various velocity profiles, network architectures, and transducer geometries. The model will be modified to include temporal variations of signal propagation. On-going research predict time-domain fluctuations caused by reflections from the moving sea surface and from fluctuations of the sound speed profile caused by ocean currents and bubble layers. These results will be included in the model. The modelling of the USWCC will be a difficult challenge owing to the extreme geometrical shape. The reflection and scattering from the rough boundaries occurs at very low grazing angles in the geometry of the USWCC. Presently, high frequency scattering from a rough surface, particularly the time-varying ocean surface, can only be predicted at grazing angles above 20 deg. A solution to this problem is the most ambitious goal of Task 3. Each stage of the model will be compared to the real data from the sea trials in the project.

3.4 TASK 4 - COMMUNICATIONS SIGNAL PROCESSING

The objective of this task will be to investigate and develop transmit/receive algorithms for use in the communication system. These algorithms will be implmented in software in a form suitable for porting to the experimental system of Task 6. The design of a surface buoy to link the subsea network with radio and satellite systems will be investigated. One of the important innovations in the project is the development of new concepts in adaptive spatial combining to separate multiple signal sources, and the way these new methods can be combined with traditional multi-access methods. The research in this task will involve development of algorithms and software, and testing using data obtained from the following sources; (a) synthetic, by linear addition of modulated signals, multipath and noise, non-stationarities with realistic statistics will be introduced as this information becomes available both from Task 3 (Modelling), and from Task 7 (Sea Experiments), (b) signals available from previous EU projects, recorded from a receive array and a single transmitting source, (c) real signals obtained during the sea experiments in this project.

3.5 TASK 5 - TRANSMIT TRANSDUCER DESIGN

A new transmit transducer will be designed specifically for the underwater acoustic communications system. The transducer required for transmission in a test system to be mounted on a sea-bed node will be developed, as it is not available commercially. The requirements include a vertical beam of 10 to 30 deg, horizontal opening angle of 360 deg, vertical sensitivity approximately -10dB ref horizontal maximum (to allow communication to the surface at close range), bandwidth 30% of centre frequency, low weight and drag, high efficiency (30%) and depth rating (500m). It is primarily the beam pattern and the shape/weight constraints that dictate new transducer design. The impact on the beam pattern on transducer mounting design will be studied. A standard transducer design would use a ceramic ring but a new design will be investigated based on a metal structure driven in its radial mode by a number of special tonpiltz elements.

3.6 TASK 6 - DESIGN AND IMPLEMENTATION OF EXPERIMENTAL SYSTEM

This task aims to design and implement a test system, consisting of a transmit node, an intermediate single network transmit/receive node and a receive node to be deployed from a boat, with the capability to test the signal processing algorithms developed in Task 4. Both transmitters and receivers will be packaged in watertight containers to facilitate the experiments. For the 1st sea experiment, the system will consist of a receive node deployed from a small boat (i.e. trawler sized) or tethered buoy and one or two sea-bottom mounted transmit nodes. The receive node will have multiple receive transducer elements and multi-channel data recording capability. The bottom mounted transmitter unit will be self-contained and controlled by an umbilical to the surface. The purpose of this is to acquire sufficient real data for off-line development and testing of the spatial diversity techniques to be developed. For the 2nd sea experiments, the ship and seabed nodes will be upgraded to have transmit/receive capability, thus an intermediate relay node can be included.

3.7 TASK 7 - SEA EXPERIMENTS

The experimental system and communications algorithms will be tested during two sea trials. These trials will also acquire data for off-line processing. Experimentation in realistic sea conditions is essential to evaluate the communication system proposed. In these experiments, up to three transmitting nodes will be deployed. Either two transmitters to a single receiver or one source transmitter a main receiver and an intermediate relay. These experiments will be carried out in the second and third years of the project and are likely to take place in the North Sea.

3.8 TASK 8 - EVALUATION, EXPLOITATION, AND DATA MANAGEMENT

This task will evaluate the system as a whole, to ensure exploitation of the results, and to undertake sound data management. This task is primarily concerned with exploitation of the results of the project and consists of three main elements; evaluation and market survey, data management and banking, and future exploitation plans. A Consultative Group of End-Users will be established during the project lifetime, whose role will be to provide valuable input on the suitablility of the product in various application domains. The issue of how exploitation in the form of possible products can be facilitated both before and after formal termination of the project will be specifically addressed.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

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1 Catipovic J.A., "Performance Limitations in Underwater Acoustic Telemetry", *IEEE Journ. of Ocean Eng.*, Vol. 15, No. 3, July 1990, pp. 205-216.

2 Stojanovic M., Zvonar Z., Catipovic J.A., Proakis J.G., "Adaptive multichannel combining and equalisation for underwater acoustic communications", *J. Acoust. Soc. Amer.*, vol. 94(3), pt.1, Sept. 1993, pp.1621-1631.

3 Hinton O.R., Howe G.S., Adams A.E., Holt A.G.J., "An underwater acoustic telemetry link operating at 10 Kbaud using PSK modulation", *UDT '92*, London, June 1992

4 Ayela G., Nicot M., and Lurton X., "New innovative multimodulation acoustic communication system", *OCEANS 94*, Brest, Sept 1994

5 Stojanovic M, Zvonar Z, "Adaptive spatial/temporal multi-user receivers for time-varying channels with severe ISI", in Proc. *28th Annual Conference on Information Sciences and Systems*, Princeton, NJ, March 1994.

6 Schell S.V. et al, "Blind channel identification using 2nd-order cyclostationary statistics", *EUSIPCO-94*, Edinburgh, Sept. 1994, pp.716-719.

7 Nikias C.L. and Mendel J.M., "Signal Processing with higher-order spectra", *IEEE Signal Processing Magazine* July 1993, pp.10-37.

8 Hatzinakos D. and Nikias C.L., "Estimation of multipath channel response in frequency selective channels", *IEEE Journal Selected Areas in Communications*, 7(1), January 1991, pp.12-19.

9 Scarano G. and Jacovitti G., "DOA estimation based on generalised eigen-decomposition of generalised cumulants", *EUSIPCO-94*, Edinburgh, Sept. 1994, pp.179-182.

10 Moreau E. & Macchi O., "Complex self-adaptive algorithm for source separation based on high-order contrast", *EUSIPCO-94*, Edinburgh, Sept. 1994, pp.1157-60.

11 Liu H., Xu G., Tong L., Kailath T., "Recent developments in blind channel equalisation: from cyclostationarity to subspace", *Signal Processing*, vol.50, Elsevier, p.83-99, 1996.

12 Moulines E., Dohamel P., Cardoso J., Mayarargue S., "Subspace methods for the blind identification of multi-channel FIR filters", *IEEE Trans. Sig. Proc.*, vol. 43(2), pp.516-525, Feb 1995.

TITLE: Shallow Water Acoustic Network (SWAN)

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SHALLOW WATER ACOUSTIC NETWORK SWAN

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SUMMARY

The SWAN (Shallow Water Acoustic Network) project aims at synthesising signal processing techniques for achieving robust and unsurveilled coherent communication in shallow-water. In particular, the project entails the investigation of innovative solutions at the physical communication layer for the basic building block of a communication network node, that is a Multi-Element Multi-User (MEMU) array receiver.

The project, started in January 1998, has a 36-month duration. Its activities are:

♦ Outlining a communication network.

♦ Modelling of the shallow water propagation channel and generation of time signals at the receiver input.

♦ Network receiving node processing, entailing:
  * Upgrading of the decision-directed array processing algorithms of a receiving network node, combining jointly synchronization and fractionally-spaced decision feedback equalization.
  * Introduction of channel identification to update the parameters of a MEMU equalizer.
  * Analysis of cyclostationary blind array techniques for self-start and self-recovery capability.
  * Combination of blind and decision-directed techniques in dual-mode processors.

♦ Performance evaluation obtained first on synthetic data then on data gathered at sea.

The expected achievements of the project are:

♦ to characterise the acoustic propagation in shallow water;
♦ to synthesize innovative array processing algorithms not adopting training sequences;
♦ to quantify the capability of fast self-start and self-recovery;
♦ to implement a channel and node simulator, for performance evaluation on synthetic data;
♦ to acquire sea trials data in two campaigns for off-line testing and performance evaluation of the processing and the modelling.

1. INTRODUCTION

The increasing need of fixed and mobile measuring platforms in the continental shelf sea-floor requires new reliable solutions for bandwidth-efficient data communication, increased data rate, full utilisation of the underwater acoustic channel with simultaneous transmissions in the same band and capability of fast self-start, and self-recovery in the event of unexpected communication breakdown. These solutions must cope with the harsh propagation conditions

which may occur in shallow water (extensive multipath, strong influence of sea-state conditions and of sea-bottom topography, ship noise interference, etc.).
Recent advances in signal processing algorithms indicate that great reliability can be achieved, especially in the presence of rapidly varying time-dispersive channels and fading, by blind array processing in the receiver. The blind processing is particularly attractive in a network for: - the capability of fast self-start when the communication link is established, - the capability of fast bootstrapping the system back to normal operation in the event of unexpected communication breakdown, - the effective capability to track time-varying channels, and - the bandwidth savings resulting from the elimination of training sequences.
The project broad objective is to advance the shallow-water coherent communication signal processing techniques at the physical layer level of the communication hierarchy, especially those leading to robust and unsurveilled operation.
One of the goals is to adopt the same coherent signalling waveforms in each network node, by exploiting in the processing the distinctive features of the distorted replicas of distinct signals at the receivers that are the respective channel responses, to achieve the received message separation in the presence of the multiple access interference and a high throughput.
The project specific objective is the investigation of innovative solutions for the basic building block of the communication network, that is the Multi-Element Multi-User (MEMU) array receiver.
The project partial objectives and the methods to achieve them are:

- characterising and modelling the propagation channel in shallow-water; implementation of channel and node simulators for performance evaluation on synthetic data;
- synthesising and intercomparing a set of innovative array processing algorithms in a MEMU receiver node: - decision-directed algorithms, combining jointly synchronization and fractionally-spaced decision feedback equalisation; - introducing channel identification to update the parameters of a MEMU equalizer, to improve performance especially at low signal-to-noise ratio; - analysis of blind cyclostationary algorithms: candidate techniques to be investigated entail either channel identification and/or equalisation, or symbol sequence estimation with no need of channel estimation/equalisation; - the blind and decision-directed techniques combination in dual-mode processors;
- acquiring data in sea trials in the North Sea for off-line evaluation of the processing and the modelling; a data management structure is implemented according to the code on Data Management in MAST-III projects to gather, validate, publish and distribute data;
- evaluating the performance, first on synthetic data generated by a channel and node simulator, then on data gathered at sea;
- disseminating the collected data for beneficial of the Community RTD, and exploiting the project results after the project completion.

Configurations are analysed with water depth from 20 to 50 meters and distance-to-depth ratio greater than 40. The maximum distance between adjacent network nodes are a few km, in agreement with potential users' requirements, and the centre frequency around 10 kHz.

2. STRUCTURE OF THE PROJECT

The tasks and their mutual relations are illustrated in the project flow chart in Fig. 2.1, where circles indicate tasks, labelled with the description of activity, their number, and responsible partner; rectangles indicate the task outputs; links between tasks are also indicated.
User needs are employed to outline the features of a coherent communication network in shallow water (task 1) and to point out the characteristics of the basic block of the network

node, which is the Multi-Element Multi-User (MEMU) array receiver operating in the presence of simultaneous users in the same band.
In task 2, after review of coastal water fluctuation processes, the deterministic and the stochastic propagation model are analysed, channel response is modelled and time signals at the channel output are simulated. The model architecture and software is then reviewed and finally tuned.
The processing in a network receiving node (task 3) analyses three distinct items: adaptive processing techniques employing decision-directed equalisation, blind cyclostationary techniques, and dual-mode techniques collating the two techniques generated above. These three kinds of processing undergo a first review on real and synthetic data, and are eventually tuned and finalised.
Task 4 concerns sea trials and the first-level Quality Control (QC) of experimental data. After the specification and planning of the sea trials and of the instrumentation, two subsystems, the first for collecting sea trials data and the second for the data QC, first level are developed and tested.
Validation of results and data entails validating the channel modelling and the network node processing schemes and software on the basis of the data gathered. A data QC (2nd level), management and databanking is planned in order that data from the first and second sea-trials are quality-controlled (2nd level), internally distributed and finally banked for external distribution.

2.1 Co-operation with other MAST III projects

A co-operation between SWAN and ROBLINKS, an other MAST III project on shallow water communication, is envisaged, to a limited extent in signal processing, and to a greater extent in sea trials.
The first SWAN sea trial and the unique ROBLINKS trial will be carried out during the same time frame, and both parties will install their own equipment on the same North Sea platform. ROBLINKS equipment can be regarded as a back-up for SWAN trials (and vice-versa), and used to acquire data if necessary.
The gathered data of the two projects will be circulated between the two project teams.

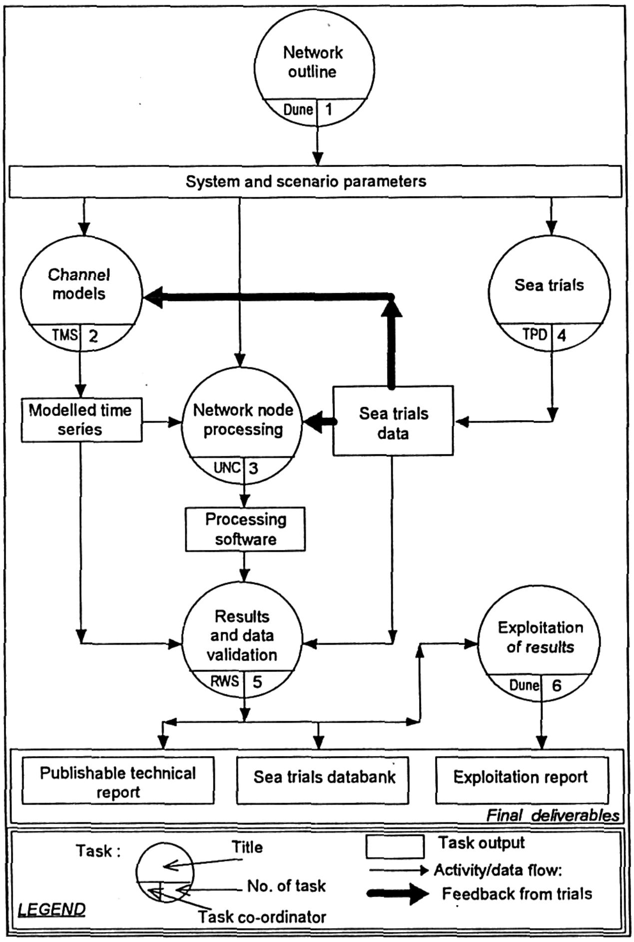

Fig. 2.1. Task structure of the project.

The code for responsible and participant partners in Fig. 2.1 is indicated as follows:

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3. PROJECT ACTIVITIES

The planned project technical activities, summarised in sect. 3, are now described in more detail.

3.1. NETWORK OUTLINE [1-2]

The range of applications for a shallow-water acoustic network will be analysed to match the requirements with a user survey. Network structures and access techniques will be reviewed,

taking especially into account of the significant latency and multipath affecting acoustic communications in shallow waters.
The network parameters will be analysed: number and spacing of nodes, frequency and bandwidth of operation, source level of transmitters, ambient noise levels, influence of shadow zones or ducting regions, etc. The scenario will also take into account noise pollution arising in the proximity of harbours.

3.2 CHANNEL MODELS [3-5]

In order to generate synthetic signals featuring the random distortions due to propagation in the acoustic channel, a simulator will be developed, based on previous work. Starting from the input signal, this simulator will be used to generate realizations of the signal output at the receiver, reproducing the common and most important effects representative of shallow water propagation. In this view, the channel simulator will not try to match a particular physical situation corresponding to a precise geographical location and time in the year, but will consider a simplified medium supporting only characteristic effects of guided propagation: high number of multipaths, surface agitation, volume fluctuations of sound velocity responsible of time fluctuations and fading. Since the frequency range of interest is around 10 kHz, ray theory is applicable. Furthermore there is no need to consider wave propagation into the bottom, so the bottom interaction reduces to a reflection loss effect.
During the course of the project, the channel simulator will be developed in subsequent phases, starting from the most simple case, for the purpose of delivering results to the other partners already a few months from the start.

- A deterministic simulator (no random fluctuations of sound speed, no roughness) corresponding to a layered medium (flat bottom and range independent velocity profile).
- A first version of a numerical simulator, based on previous work, of randomly fluctuating acoustic impulse response of shallow water channels adapted to the high frequencies and moderate propagation ranges (a few kilometres) involved in the project.
  The phenomena responsible for channel fluctuations, the effects of which are simulated, are:
  * Fluctuations in the volume of the water layer (such as sound-speed fluctuations associated with internal waves, tidal currents, etc.), in terms of time fluctuations of the space distribution of sound-speed, or of some effective sound-speed; acoustic effects of layers or clouds of discrete scatterers, and main contributions from sub-surface layers of bubbles will also be introduced through an equivalent sound-speed.
  * Effects of scattering by surface and bottom roughness.
  * Movements of source and receivers.
- Statistical moments will be numerically evaluated in the random channel assumed statistically stratified and sequences of channel impulse responses will be generated from the previously computed moments.
  Effects due to roughness of the sea surface will be modelled under Kirchhoff approximation. A first unified version of the simulator, integrating under a common geometrical approach effects of surface roughness and sound speed fluctuations will be developed.

3.3 NETWORK NODE PROCESSING

Schemes concerning non-blind and blind (scalar and) array receiver processing in the absence of periodic training sequences will be investigated, upgraded and integrated in a Multi-Element Multi-User (MEMU) array receiver. The task entails into three distinct activities, the first two of which, processing adopting decision-directed equalization and blind processing, can initially be worked on independently. The results of these two activities will be collated in a dual mode processor in the third activity.

Decision-directed equalization [6-13]

Two adaptive implementations of the Multi-Element Multi-User (MEMU) array receiver in the network node will be investigated:

- *Decision Feedback Equaliser* (DFE): evaluation of the performance of
  - the adaptive implementation, also when tracking a time-varying channel,
  - Least-Mean-Square (LMS) or/and Recursive-Least-Square (RLS) algorithms,
  - the combiner vs beamformer modality of operation.
- Channel identifier: performances are compared with the processor not adopting channel identification.

In the *unrealistic* hypothesis that the channel response were known, the optimal Multi-Element Multi-User (MEMU) array receiver would consist of a combiner/beamformer for each transmitter, followed by a sequence detector which is a Maximum Likelihood Sequence Estimator (MLSE). To reduce the computational complexity in the sequence detector implementation, a suboptimal processor, that is a MEMU *equaliser*, is adopted instead of the Maximum Likelihood Sequence Estimator.

In the *realistic* hypothesis that the channel response is not known, the combiner and detector/equaliser parameters are unknown and have to be estimated adaptively. The implementation of the adaptive channel identification will be performed in two ways:

- implicitly: the detector is a MEMU *Decision Feedback Equaliser* (DFE) in which decision from the output symbol error of one user are fed-back to all the other users, for updating the processor parameters;
- explicitly: the detector includes a channel identification block, that feeds the channels parameters to the equalizer.

A MEMU receiver of this type operates either as a combiner when the combiner filters seeks to match the channel, or as a beamformer when the beam pattern seeks to null the multipath and interferers.

Blind processing [14-31]

The following receiver array processing techniques (a., b., below) based on the cyclostationary structure of the transmitted signals will be investigated.

a) *Channel estimation/equalisation, followed by symbol sequence estimation.*

a1) *Statistical techniques.*

The goal is to estimate the channel with a short sequence of received data, by exploiting the statistics of cyclostationary signals, and then to apply the identified channel to perform equalisation on the receiver outputs for subsequent symbol estimation.

a2) *Subspace-based identification techniques.*

The input signal is treated as an unknown deterministic signal, and identification, accomplished without the use of any statistics, provides exact channel estimation using only a finite number of observations.

b) *Symbol sequence estimation, with no need of channel estimation/equalisation.*

b1) *Statistical technique.*

The source correlation is estimated from the receiver output observation via orthogonalization of the channel (without knowing the channel) and the Viterbi algorithm is applied to recover the input signals.

b2) *Deterministic technique.*

The estimation of the unknown deterministic input signal sequence is accomplished without the use of any statistics and using only a finite number of observations.

Dual mode processing
Dual-mode processors, that combine blind techniques based on cyclostationarity with decision-directed techniques, are synthesised from the decision-directed and blind schemes developed previously. It will be investigated which advantages, such as decrease in computation and finer performance, are gained by the switch to decision-directed rules, viable for slowly-varying channels.

3.4 THE SEA TRIALS

Two sea experiments will be carried out to validate the models and algorithms developed and implemented under the previous tasks.
The North Sea has been designated as major test location, as it is a representative shallow-water coastal area, with variable marine climate conditions, high range-to-depth ratio, 'difficult environment' like ship traffic, particular salinity profile, etc. The permanent measuring platform "Meetpost Noordwijk" in the North-Sea will be made available for the SWAN sea trials. The water depth at the platform is 18 m.
The sea trial preparation entails a specification and planning phase, to define in detail the following aspects: * experimental set-up and requirements, * wet-end instrumentation, * dry-end instrumentation, * campaign and cruise planning.
The specification shall comply with the technical needs of the project in one hand and with the technical and environmental feasibility on the other hand. Items like precise choice of the experiment location and lay-out, period of the year, instrumentation to be deployed, etc., represent issues to be specified at this stage.
The layout of the experiments will be based on a simple configuration with two 'users' (i.e., two transmitters), placed onboard a ship and/or on an autonomous battery-packed station, transmitting toward a single receiver station, equipped with an array, set at a fixed location, either on a research platform or on a vessel: for the proposed network concept the communication problems at the physical layer level are all present in the envisioned set-up.
The instrumentation consists of the hardware for transmission (for two users) and reception (one receiving array) and of the hardware and software for data acquisition, quality control (1st level) and mass storage. The required equipment selected on the basis of the specifications and the availability of equipment at the project partners, will be gathered, integrated in the sea trial system and tested: before installing the equipment at the measuring location, a functional test will be carried out in an indoor water basin. Alternative solutions for key instruments will be defined in order to reduce the risk of delays due to equipment failure.
The heart of the reception system consists of a multi-channel data acquisition system integrated in an acoustic/sonar workstation which was designed as a research tool for the generation, acquisition, quality control and storage of acoustic signals for a variety of applications (both on land and underwater). Data acquisition, i.e., for A/D conversion and data storage, and for initial data processing, visualisation and real-time DQC1 (data quality control 1st level) will be carried out on this workstation on board the off-shore platform, to detect and possibly assess errors of various kinds in the acquired data at an early stage. All acquired data will then be properly backed-up.

3.5 RESULTS AND DATA VALIDATION

The task is composed by: results validation; data quality control (2nd level), management and banking.
The activities for modelling and processing evaluation and tuning, performed in tasks 2 and 3 on the data from each sea trial at different stages in the project, are co-ordinated in this task. The preliminary results (channel modelling process compared with data, performance comparison of network node processors, data quality assessment), are summarised and integrated in the context of the complete system performance evaluation. On the basis of the results achieved from the first sea trial, recommendations for the second sea trial will be defined.
The 2nd level quality control steps are defined, planned and carried out. A detailed data acquisition and management plan will be made at an early stage. Consequently, a proper data bank will be designed and implemented to make project acquired data publicly available as a foreground result, according to EC-MAST III Code data management.

3.6 EXPLOITATION AND DISSEMINATION OF RESULTS

A detailed plan for result exploitation is set-up, and consequent actions are started to:

- outlining a common exploitation strategy among partners,
- planning contacts with target groups potentially interested to the project results,
- promote the presentation of papers and articles on the scientific achievements of the project,
- support the distribution of the banked data at a European level,
- provide updated information on the project results to the scientific community at large.

A project Web site has been designed and set-up (URL: http://www.mclink.it/com/swan, [Web]) and will be continuously updated to give public access to:

- inventories of planned, submitted and published papers,
- the banked data, after the project time span, with eventual adaptation to specific standards, and integration of the databank into larger databanks,
- on-line disclosure of non commercially-sensitive achievements of the project.

The results will be beneficial for the underwater acoustic communication and propagation research communities. The acquired data will be beneficial for the oceanographic community, and for researchers in the dynamics of the coastal waters and in the acoustic propagation and modelling.

4. ACKNOWLEDGEMENTS

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5. REFERENCES

[1] J.Jubin, J.D. Tornow, "The DARPA Packet Radio Network Protocols", *IEEE Proc.*, vol. 75(1), Jan. 1987, p. 21-32.

[2] J.L.Tavalage, T.E.Thiel, D.Brady, "An Efficient Store-and-Forward Protocol for A Shallow Water Acoustic Local Area Network", *Proc. Oceans'94 Conf.*, Brest, F, Sept. 1994, vol. I, p. 883-888.

[3] S.McDaniel, "A small-slope theory of rough surface scattering", *J.Acoust.Soc.Am.*, vol. 95(4), pp.1858-1864, 1994.

[4] M.Spivack, "A numerical approach to rough surface scattering by the parabolic equation method", *J.Acoust.Soc.Am.*, vol. 87(5), pp.1999-2004, 1990.

[5] M.Spivack, "Coherent field and specular reflection at grazing incidence on a rough surface", *J.Acoust.Soc.Am.*, vol. 95(2), pp.964-970, 1994.

[6] M. Stojanovic, "Recent Advances in High-Speed Underwater Acoustic Communications", *J. Ocean. Eng.*, spec. Issue on *"Underwater Acoustic Communications"* vol. OE-21(2), Apr. 1996, pp. 125-136.

[7] M. Stojanovic, Z.Zvonar, "Multi-Channel Processing of Broad-Band Multi-User Communication Signals in Shallow Water Acoustic Channels", *J. Ocean. Eng.*, spec. issue *"Underwater Acoustic Communications"*, vol. OE-21(2), Apr. 1996, pp. 156-166.

[8] M. Stojanovic, J.A. Catipovic, J.G. Proakis, "Reduced Complexity Spatial and Temporal Processing of Underwater Communication Signals", *J. Acoust. Soc. Amer.*, vol. 98(2), pt. 1, Aug. 1995, pp. 961-972.

[9] M. Stojanovic, Z.Zvonar, J.A. Catipovic, J.G. Proakis, "Spatial Processing of Broadband Underwater Acoustic Communication Signals in the Presence of Co-channel Interference", *Proc. Oceans'94 Conf.*, Brest, F, Sept. 1994, vol. I, pp. 286-291.

[10] M. Stojanovic, J.A. Catipovic, J.G. Proakis, "Fast Coherent Digital Communication for Underwater Acoustic Channels", *J. Ocean. Eng.*, vol. OE-19(1), Jan. 1994, p. 100-111.

[11] M. Stojanovic, Z. Zvonar, J.A. Catipovic, J.G. Proakis, "Adaptive Multichannel Combining and Equalization for Underwater Acoustic Communications", *J. Acoust. Soc. Amer.*, vol. 94(3), pt. 1, Sept. 1993, pp. 1621-1631.

[12] O.R. Hinton, *et al*, "Performance of a Stochastic Gradient Adaptive Beamformer for Sub-Sea Acoustic Communications", *EUSIPCO-94*, Edinburgh, Sept. 1994, p.1540-43.

[13] T.Shimamura, S. Semenani, C.F.N. Cowan, "Equalization of Time-Variant Communication Channels: Channel Estimation Based Approach", *IEEE Conf. on Acoust. Speech and Signal Processing (ICASSP'96)*, May 1996, Atlanta, p. 1703-06.

[14] L.Tong, G.Xu, T.Kailath, "Blind Identification and Equalization Based on the Second-Order Statistics: A Time-Domain Approach", *IEEE Trans. on Inform. Th.*, vol. IT-40(2), Mar. 1994. p. 340-349.

[15] H.H.Zeng, S.Zeng, L.Tong, "On the Performance of Blind Equalization Using the Second-Order Statistics", *IEEE Conf. on Acoust. Speech and Signal Processing (ICASSP'96)*, May 1996, Atlanta, GA, p. 2427-30.

[16] G.Xu, H.Liu, L.Tong, T.Kailath, "A Least-Squares Approach to Blind Channel Identification", *IEEE Trans. on Sign. Proc.*, vol. 43(12), p. 2982-93, Dec. 1995.

[17] E.Moulines, P.Dohamel, J.Cardoso, S.Mayarargue, "Subspace Methods for the Blind Identification of MultiChannel FIR Filters", *IEEE Trans. on Sign. Proc.*, vol. 43(2), p. 516-525, Feb. 1995.

[18] L.A.Baccala, S.Roy, "A New Blind Time-Domain Channel Identification Method Based on Cyclostationarity", *IEEE Sign. Proc. Letters,* vol. 1(6), p. 89-91, Jun. 1994.

[19] H.Liu, G.Xu, L.Tong, T.Kailath, "Recent Developments in Blind Channel Equalization: From Cyclostationarity to Subspace", *Signal Processing,* vol. 50, Elsevier, p. 83-99, 1996.

[20] A.Chevreuil, P.Loubaton, "On the Use of Conjugate Cyclostationarity: A Blind Secon Order Multi-User Equalization Method", *IEEE Conf. on Acoust. Speech and Signal Processing (ICASSP'96)*, May 1996, Atlanta, GA, p. 2439-43.

[21] L.Tong, "Blind Sequence Estimation", *IEEE Trans. On Commun.*, vol. COMM-43(12), p. 2986-94, Dec. 1995.

[22] L.Tong, "Joint Blind Detection and Carrier Recovery over Fading Channels", *IEEE Conf. on Acoust. Speech and Signal Processing (ICASSP'95)*, May 1995, Detroit, MI, p. 1205-08.

[23] H.Liu, G.Xu, "A Deterministic Approach to Blind Symbol Estimation", *IEEE Sign. Proc. Letters*, vol. 1(12), p. 205-207, Dec. 1994.

[24] H.Liu, G.Xu, "Closed-Form Blind Symbol Estimation in Digital Communications", *IEEE Trans. on Sign. Proc.*, vol. 43(11), p. 2714-23, Nov. 1995.

[25] D.J.Reader, W.G.Cowley, "Blind Maximum Likelihood Sequence Detection", *IEEE Conf. on Acoust. Speech and Signal Processing (ICASSP'95)*, May 1995, Detroit, MI, p. 2694-97.

[26] V.Weerackody, S.A.Kassam, "Dual Mode Type Algorithms for Blind Equalization", *IEEE Trans. on Comm..*, vol. COMM-42(1), Jan.1994, pp. 22-28.

[27] A.G. Bessios, "Compound Compensation Strategies for Wireless Data Communications over the Multi-Modal Acoustic Ocean Waveguide", *J. Ocean. Eng.*, spec. Issue on "*Underwater Acoustic Communications*" vol. OE-21(2), Apr. 1996, pp. 167-180.

[28] H.H.Zeng, L.Tong, "Blind Channel Estimation Using the Second-Order Statistics: Algorithms",*IEEE Trans. on Signal Processing*, Aug. 1997, vol. SP-45(8), p. 1919-30.

[29] H.H.Zeng, L.Tong, "Blind Channel Estimation Using the Second-Order Statistics: Asynthotic Performance Limitation", *IEEE Trans. on Signal Processing*, Aug. 1997, vol. SP-45(8), p. 2060-71.

[30] H.Liu, G.Xu, "Smart Antennas in Wireless Systems: Uplink Multiuser Blind Channel and Sequence Detection", *IEEE Trans. on Signal Processing*, Feb. 1997, vol. SP-45(2), p. 187-198.

[31] K.Abed-Meraim, W Qiu, Y. Hua, "Blind System Identification", *Proc. IEEE*, vol. 85(8), Aug. 1997.

[Web] More updated information on the project can be found on-line at http://www.mclink.it/com/swan.

TITLE: Long range Shallow Water Robust Acoustic Communication Links (**ROBLINKS**)

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LONG RANGE SHALLOW WATER ROBUST ACOUSTIC COMMUNICATION LINKS ROBLINKS

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1. INTRODUCTION

Underwater Acoustic (UWA) communication has become increasingly important in recent years. The reason is that it is a topic of significant generic importance for the underwater activity in general, whether it concerns remote control or data transfer applications for undersea instruments, ships, underwater vehicles, robots or even divers. Among the difficulties encountered in UWA communication, the major problem generated by the long ranges and the shallow water channel is the large value of the time spreading, which occurs in the form of strong multipath propagation in addition to high temporal (phase) and spatial variability.
The scientific innovation of the ROBLINKS project is to focus on new techniques of UWA communications, based on continuous parallel identification of the channel response, with the aim to provide self-adaptive algorithms insensitive to the channel characteristics. This feature should considerably improve the robustness of the communication links: the channel identification provides automatic assistance to update permanently the parameters of the symbol restitution process. As such it fundamentally differs from the standard equalization approach which needs fine receiver tuning in order to adjust its parameters to the channel conditions and often requires operator assistance.

2. OBJECTIVES

The overall aim of ROBLINKS is to develop and test robust coherent acoustic communications at long ranges in shallow water at data rates in excess of 1 kbit/s. Here, robust means insensitive to the acoustic channel conditions. Present communication systems have good performances but either they have poor bandwidth efficiency (non coherent methods) or they are limited by time spreading which is less than the signal duration (differentially coherent) or they require operator assistance to adjust the receiver's parameters to the channel characteristics (coherent methods with standard equalization approach). A robust coherent communication system should fulfil a very large range of possible missions which involve remote control of underwater instrumentation and vehicles or collection of data from undersea equipment: surveying and monitoring of the marine environment in shallow water (coastal zone) but also in the deep sea, sea resources exploitation, etc.

The specific objectives of ROBLINKS are:

1. To develop new signal concepts and algorithms for optimal coherent signal processing in the time domain to achieve reliable Long Range (tens of km) UWA Communication in shallow waters with high range/depth ratio (in excess of 100). The aim is to achieve this at reasonable data rates (aim > 1 kbits/sec) , using frequencies in the range 1-10 kHz. The proposed algorithms will be designed to be insensitive to imprecise a priori environmental information. The word "robust" in the project title is used in this particular sense.
2. To evaluate experimentally the performance of these waveforms and processing algorithms under real shallow water conditions (North Sea). Selected waveforms and processing algorithms will be implemented on a real-time system and the real-time performance will be evaluated from data, recorded during the sea trial.

3. METHODOLOGY IN RELATIONSHIP WITH THE WORKPLAN

The project is focused both on the basic concepts of Communication Techniques and System and Experimental demonstration at sea.
The scientific content of ROBLINKS is divided into 8 main Tasks:

Tasks 1 and 2: Design of robust coherent communication schemes based upon the permanent identification of the channel response.

Two competing strategies will be investigated: identification with parallel monitoring and blind identification.

The first strategy (Task 1) consists of using a permanent monitoring signal, which avoids the traditional learning sequences periodically injected into the communication channel. The general idea is that this permanent signal should firstly allow to track fast variations of the acoustic channel impulse response and secondly exclude symbol errors from the channel estimation process. Additionally, the synchronisation can be aided significantly by carefully chosen monitoring signals. The loss of signalling space devoted to communication will hopefully be recovered by a better channel identification ability and a better reliability of the communication, which in turn allows to use efficient coding schemes.

The second strategy (Task 2) is (permanent) blind identification. This strategy does not use any known monitoring signal, but makes use of the statistical features of the communication signals themselves. The a priori knowledge about the distributional properties is exploited.

The algorithms developed will be implemented on scientific computers. Fine tuning and testing of the algorithms will make use of existing software channel simulators.

Task 3: Real-time UWA communication system.

For real-time demonstration a communication system will be realised, based on a PC computer for low power signal processing at transmission and reception. An acoustic source in the frequency range 1 kHz- 15 kHz will be used at transmission and a vertical line array at reception. The transmission options, which will be applied during the sea trial (in Task 4), will include both strategies mentioned previously. The first data analysis (in Task 5) of the sea trial will lead to the selection of one complete communication scheme (transmission and reception), using identification with parallel monitoring, which will be implemented on the real-time system for testing in laboratory (during Task 6).

Tasks 4, 5 and 6: Experimental analysis

To assess the performance of the new processing schemes under real conditions, a sea trial (Task 4) will be performed around the mid term of the project duration for data collection and real-time demonstration in shallow water and long range. The North Sea is designated as test location, because it will provide required environmental conditions (shallow water coastal areas, difficult conditions like ship traffic) and facilities (environmental measurements from stations in network configurations). Analysis of this trial will provide the required insight into the performances under real conditions.

Objectives of trial data analysis (in two steps: data analysis 1 = Task 5 , data analysis 2 = Task 6) are:

- to provide experimental data to test and compare off-line the performance of the signalling schemes and algorithms developed under Tasks 1 and 2 under real conditions (in data analysis 1 after the trial execution);
- to select the overall communication and identification scheme with parallel monitoring which will be implemented in the real-time communication system (in data analysis 1);
- to improve the algorithms of both parallel monitoring and blind identification strategies for further off-line processing and estimation of performances (in data analysis 2, at the end of the project);
- to demonstrate the performances of the real-time UWA communication system that can be obtained in practice (in data analysis 2);

Task 7 : Synthesis on long range coherent communications and project achievements.

This Task aims at finalizing all the conclusions and findings out of the ROBLINKS project. This includes:

- the channel responses analysis (long range, shallow water);
- the evaluation of the two principles of coherent communications, both based on permanent channel identification, which have been studied during the project;
- the communication performances which are achievable or should be achievable;
- the signalling schemes which are recommended;
- the receiver design rules with recommendation on identification, word and carrier synchronization, channel inversion;
- the evaluation of the real-time communication system.

Task 8: Data management.

Prior to the sea trial a detailed management plan and measuring plan will be made. The data will be collected according to the procedure, described in these plans. Selected quality checked data will be made available for future use by other EU parties. All data will be well documented.

III.1.3. Underwater viewing

TITLE: Detection of Embedded Objects (project **DEO**)

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DETECTION OF EMBEDDED OBJECTS
DEO

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SUMMARY

The DEO project is a study of acoustic scattering from man-made objects lying either partially or completely embedded in seabed sediments. Initial experimental and theoretical results reveal that such echoes have strong characteristic features, indicating that their detection and classification could eventually be achieved by a remote sensing method.

1. INTRODUCTION

GENERAL CONTEXT

Many man-made objects lie on or under the seabed, either because they have been deliberately placed there, such as pipes or containers, or by accident, such as shipwrecks. To locate, identify and characterise these objects, perhaps prior to retrieval, some kind of remote sensing technique is necessary. When the object lies on the bottom, the use of imaging techniques is feasible. But when an object is partially or completely embedded, there is no satisfactory method available to find it. The aim of the DEO project is to demonstrate the feasibility of a method to detect and classify objects buried in marine sediments. *A fortiori* this method will also apply to objects that are not buried.

It has long been known that acoustic scattering from underwater objects yields information about the object that can be used for detection as well as identification. However, when these objects are buried in sediments, the problem is more difficult because effects of the local acoustic environment mask the target. These effects include reverberation of the water-sediment interface, multiple reflections occurring between the object and the interface, and modification of the acoustic response of the object due to intrinsic properties of the sediment (attenuation, shear rigidity, porosity).

METHODOLOGY

The research programme involves:
a) the application of recent theories of diffraction to understand and explain the main physical

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processes of diffraction by buried objects;
b) the analysis of bottom scattering reverberation;
c) the use parametric sources to make experimental measurements over a large relative frequency bandwidth and a linear receiver array to improve the data signal to noise ratio;
d) the application of signal processing techniques to improve the signal-to-noise ratio and neural networks to identify the objects.

Before studying the case of embedded objects, the acoustic properties of non-embedded objects must be well known and need to be carefully studied. Consequently the project is divided in two main phases:

First phase: Study of echoes from objects in the free field.

Second phase: Study of objects buried in sediments.

The first phase precedes the second stage in order to provide a reference with which buried targets data can be compared.

EXPECTED ACHIEVEMENTS

It is expected that remote sensing will be used tto detect and classify all kinds of objects, whether they are on the sea bed or buried in sediments, with the following limitations:

- minimum object dimension: a few cm.
- maximum buried depth: from several millimetres for the smallest objects, to several metres for objects of more than 1 metres dimension, even 10 to 20 metres for very large objects.

In order to make a coherent report, and because of the importance of the first phase, the present paper is limited to the analysis of targets in the free-field. Few experimental results for buried objects are presented, since these have not been fully analysed; also, no modelling of bottom scattering is presented, because this comes in the second phase.

2. EXPERIMENTS

Realistic sea experiments are fundamental to the success of this project. To enhance the chances of success, sea trials are carried out by two teams in two different sites. SACLANT Centre operates in the North Tyrrenian Sea, Italy, for full-scale sea experiments, but with a high degree of control over the geometry. Loughborough University (LU), jointly with TNO Physics and Electronic Laboratory (TNO), operates on Loch Duich, Scotland, in an environment that is more difficult to control but closer to operational conditions.

Both teams use a different parametric array. The LU system, working at a central primary frequency of 75 kHz, was tested during the MAST-II **REBECCA** Project [Woodward 94]. The TOPAS Sonar system used by SACLANT Centre [Deo 97], has a corresponding frequency of 40 kHz. Both emit a variety of Ricker, chirp and sine wave pulses at secondary frequencies in the band 1 to 15 kHz. This large bandwidth was selected to allow the extraction of target classification clues. Targets are steel cylindrical shells with flat end-caps, and with a 6-mm wall thickness. The LU targets are 1 m long, 25 cm diameter. The SACLANT Centre targets are 2 m long, 50 cm diameter. They are filled with air, sea water, or a mixture of sand and concrete. The covered "*ka*" domain is 1 to 15.

2.1. LU AND TNO TRIALS. FIRST PHASE: FREE-FIELD TARGET

For the first sea trial of the project carried out by LU in May 1997, the parametric sonar array

and the target were deployed as shown in figure 1. The target was rotated under computer control in a horizontal plane through 360° in the free field of the source. Scattered and reflected signals were monitored using a 20-hydrophone linear array deployed either horizontally or vertically by TNO-FEL and a hydrophone deployed by LU.
Recordings of secondary frequency signals of both direct and scattered signals were made following detection with the 20-element array. Signals were transmitted ashore for processing via a sea-bed cable to the TNO base. An additional hydrophone was placed between the parametric source and the target and received signals were captured on board the source raft by the LU data capture system.

Transmitter and receiver logging systems were synchronised using a GPS receiver for post-correlation of transmission and received data. Additional transmission signal data and target angle information were sent ashore via a fibre optic link for display at the TNO base.

Initial measurements were made for system calibration and to determine ambient noise levels. The target was then rotated, with its axis horizontal, through a range of aspect angles, and recordings of both primary and secondary frequency signals for both incident and scattered data were made.
Target-free bottom scattering measurements were then carried at grazing angles between 24° and 36°. Additional measurements, including sound velocity and depth profiles, as well as raft stability measurements, were also made.

On-line displays in both the time and frequency domains were monitored by TNO and LU using separate systems. Post-data organisation of trial data, including acoustic signals captured by the TNO system and the LU data capture system, also took place. Sampled acoustic recordings were made by LU using a single-element hydrophone. Recordings were made directly to a PC hard disc and to a digital oscilloscope capture system. The displayed results showed the secondary frequency components of both the direct and scattered signal received at a single hydrophone for each type of transmitted pulse.

2.2. SACLANT CENTRE TRIALS, FIRST PHASE: FREE-FIELD TARGET

Considerable effort was made to control the trial geometry, especially the incident grazing angle and target aspect angle. In order to achieve a good reproducibility, the selected trial location was a dry-dock in the Military Arsenal of La Spezia, Italy (see set-up scheme in figure 2) [Deo 97].

The parametric array was mounted at the top of a fixed tower and could be remotely controlled by a pan and tilt assembly whose rotations were measured by a Seatex Motion Reference Unit (MRU). The targets were suspended in the water column from a floating frame equipped with a rotation mechanism. The raft was secured to the sides of the basin with lines. The distance between the transmitter and the targets was such that the targets were in the far-field of the sonar according to the TOPAS calibration [Deo 97].

As a receiver a single omnidirectional hydrophone was placed in the near-field of the targets for most of the examined frequency range (i.e., for f>4 kHz). This was necessary because as the receiving hydrophone moves closer to the transmitter in space, the interfering reflections from the surface and bottom move closer to the specular response in time, thereby obscuring the signal resonance component.

The free-field experiments were successful both in terms of reproducibility, as most of the geometrical parameters were controllable and measurable, and in terms of data quality, as a large set of good data with high SNR values was provided for each target used.

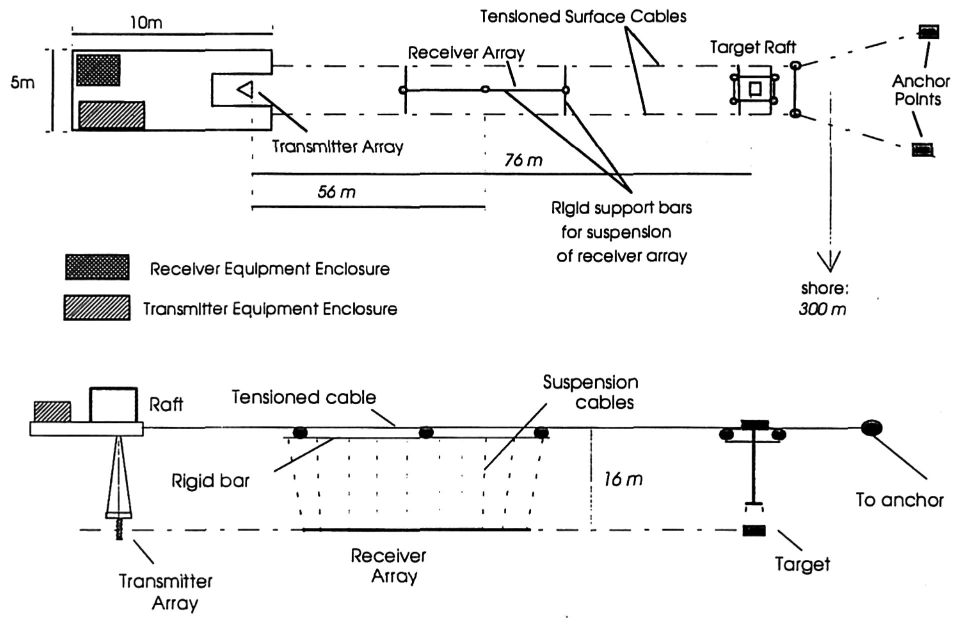

Figure 1: The free-field LU / TNO experiment configuration

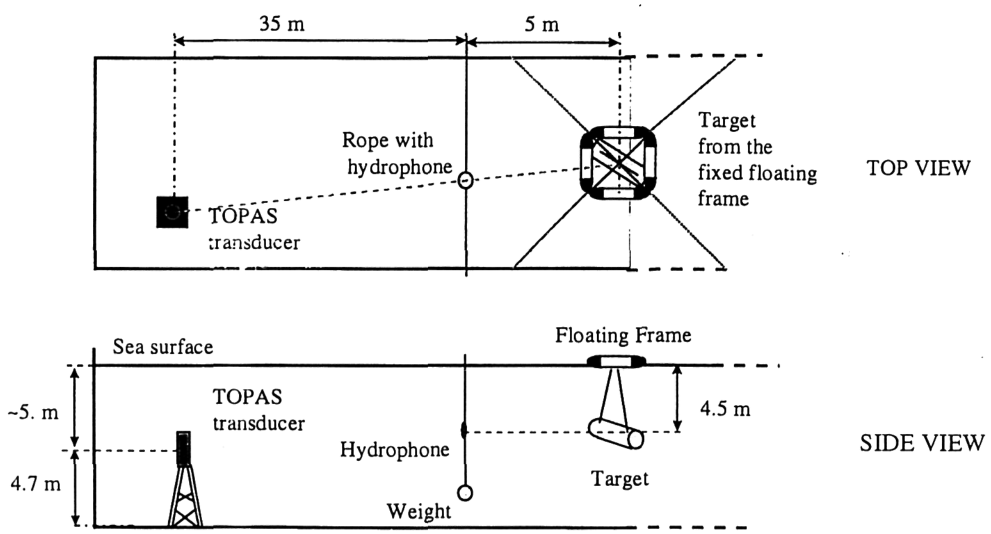

Figure 2. The free-field SACLANT Centre experiment configuration.

2.3. SACLANT CENTRE TRIALS. SECOND PHASE: BURIED TARGETS

The experiments took place in December 1997 close to the northern coast of Elba Island, in an area chosen for its water depth (~15 m) and bottom (low-relief, sandy) conditions.
The TOPAS parametric array was also used as the transmitter in this second trial. The receiver was a 16-element linear array oriented vertically [Deo 98].
As a target, the same water-filled cylinder that was used in the free field trial was employed almost flush buried (about 10 cm below the bottom surface).
For acquisition under variable conditions of trial geometry (figure 3), the transmitter was mounted on a variable-height chassis which in turn was mounted on a 24 m linear rail deployed on the bottom, along which it could be moved in a precise and controlled manner [Deo 98]. The rail was equipped with a motor which allowed accurate and reproducible displacement of the chassis along the rail. In order to have only one parameter (i.e., the grazing angle) changing together with range (while the sonar tower moved along the rail), the target was buried with its cross-section aligned with the rail 15 m away from one end of the rail itself, as shown in figure 3. Data back-scattered by the target at broadside were acquired at various low grazing angles slightly above the nominal sand critical angle (~26-28°). Low grazing angles were selected for limiting the backscattered bottom reverberation level [Deo 98] [Maguer 97].

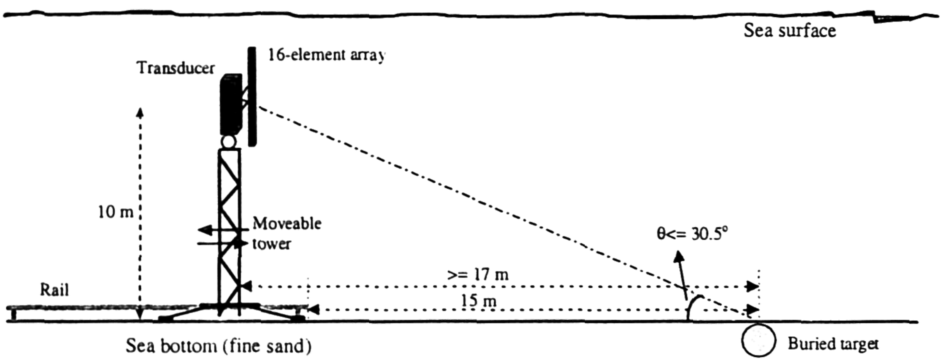

Figure 3. The second phase trial geometry (section).

3. RESULTS

An example of a received signal from LU/TNO experiments is shown in figure 4.
An example of data from the first SACLANT Centre trials is provided in figure 5. It refers to the time and frequency response of the water-filled cylinder insonified by a Ricker-8 pulse (i.e., with central frequency = 8 kHz) as a function of azimuth aspect angle. In these trials the SNR mean value is estimated more than 30 dB at broadside in a large frequency bandwidth.

For the data scattered by the buried shell insonified at broadside, an example is shown in figure 6. The mean Signal to Reverberation Ratio is estimated around 10-15 dB.

3.1 COMPARISON BETWEEN EXPERIMENT AND THEORY.

It has long been known that acoustic diffraction from underwater objects yields information

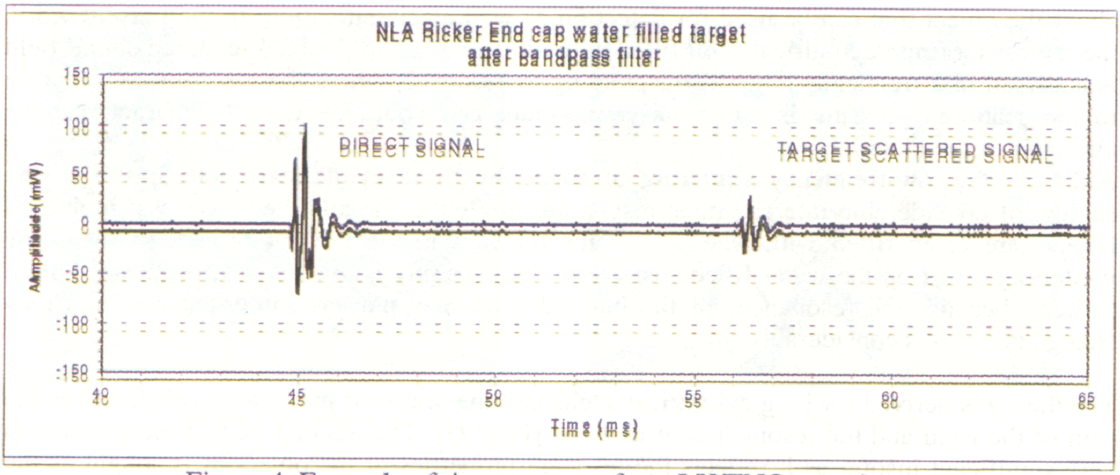

Figure 4. Example of time response from LU/TNO experiment

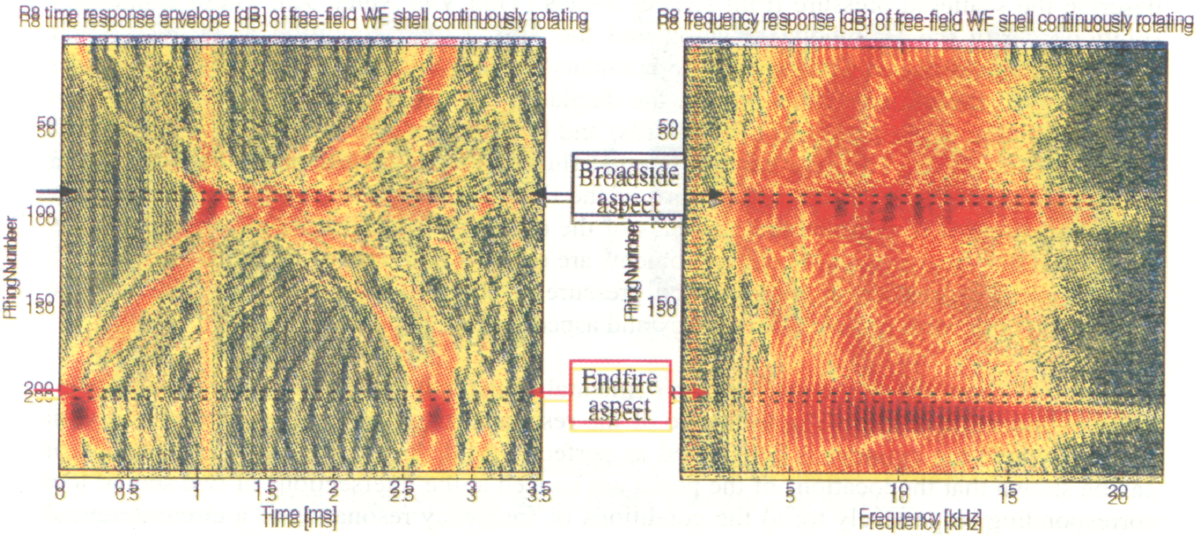

Figure 5. Time and frequency response of the rotating water-filled cylindrical shell.
Color scale: [-80(dark blue), 0(brown)] [dB] for both plots.

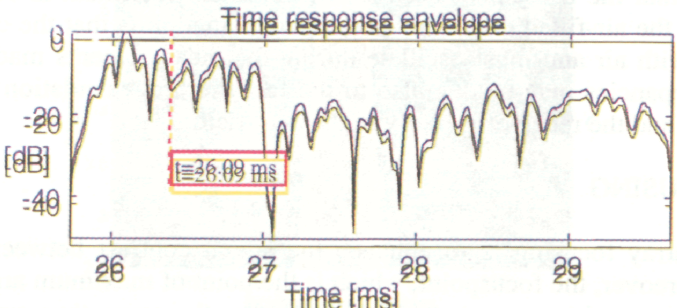

Figure 6. Beamformed response to a Ricker pulse 8 kHz by the buried shell at broadside (selected grazing angle = 31°).

about the object that can be used for detection as well as classification. In the present work, the target is a finite cylindrical shell filled with air or water and the backscattered sound field is analysed in a frequency range corresponding to $1<ka<12$, where the resonant contribution to the scattered pressure is particularly important and could be used to characterise the elasticity of the material.
Although the low-frequency scattering of sound by finite cylindrical shells has been the subject of considerable interest, most results are limited to the air-filled case. As the buried objects are often filled with water, it seems relevant in the present project to focus the modelling effort on the water-filled case. Note that it is physically and mathematically more difficult because the resonances of the internal fluid may play an important role and may change the results considerably.

The theory is derived for long cylindrical shells and the scattered pressure is calculated as the sum of the rigid and the resonant contributions [Deo 97]. This second part of the pressure is very significant insofar as it reflects the resonant properties of the object; it is sought as the sum of a continuous superposition of sectorial spherical harmonics which have been used to describe the scattered pressure from slender bodies [Tran Van Nhieu 92]. These elementary solutions verify the Helmholtz equation and the Sommerfeld condition at infinity. Their strengths are determined by applying the boundary conditions on the surface of the cylinder (continuity of the normal component of the displacement and vibration of the shell surface under the action of the pressures of the fluids) and by using the thin bending cylindrical shell theory [Kennard 53]. The external and internal fluids and the displacements of the cylinder surface are expanded in the eigenfunctions of the finite cylindrical shell. In order to simplify the calculations, it is assumed that the ends of the cylindrical shell are simply supported, i.e. the normal displacement and bending moments are equal to zero at both ends.
An analytical expression of the scattered pressure is obtained and is used to calculate the transfer function with respect to frequency and aspect angles.

Figures 7-8 show the numerical and experimental transfer functions for the air and water-filled cylinders, 2 m long. The influence of the resonances can be seen by comparison with figure 9 where the cylinder is considered as perfectly rigid. In the frequency-angle space, it can be shown that the locations of the peaks are located at the intersections of two sets of loci corresponding respectively to: a) the conditions of frequency resonance of a circumferential mode; b) the condition of spatial coincidence with one of the axial mode of vibration of the shell.

It is readily seen that the agreement between theory and experiment is better in the water-filled case than in the air-filled case. One possible explanation is that the cylinder is instable when it is filled with air and must oscillate during the measurements made during rotation. Parts of the discrepancies may be due also to the fact that the calculation is done in the far field of the target, and the receiver is partly in the near field.

3.2 ARRAY FOCUSING

The purpose of array focusing is to enhance the signal contrast between target echo and reverberation. Moreover, the focuspoint, which is the point of maximum array sensitivity, can be steered in bearing and in range to scan the area near the array, thus creating an image of highlights in this area.
Array focusing is applied in the near field of the array, where significant wave front curvature

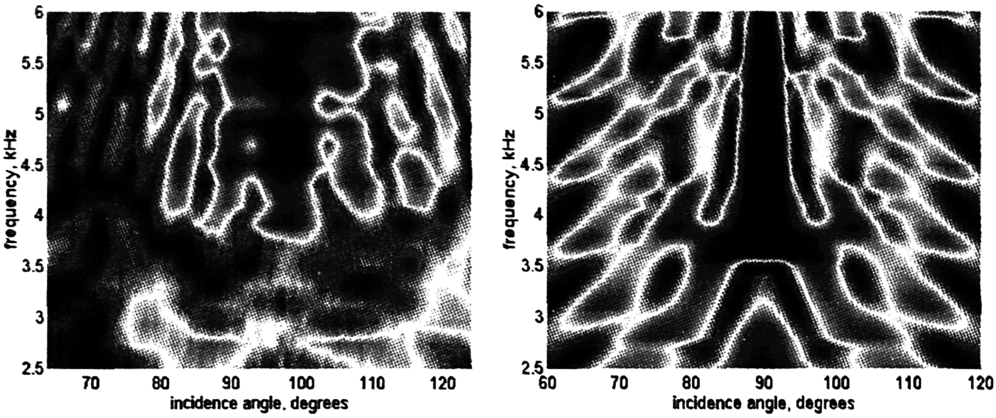

Figure 7. Air-filled cylinder. Left: experiment. Right: theory.
Color scale: target strength - 20 to 0 dB

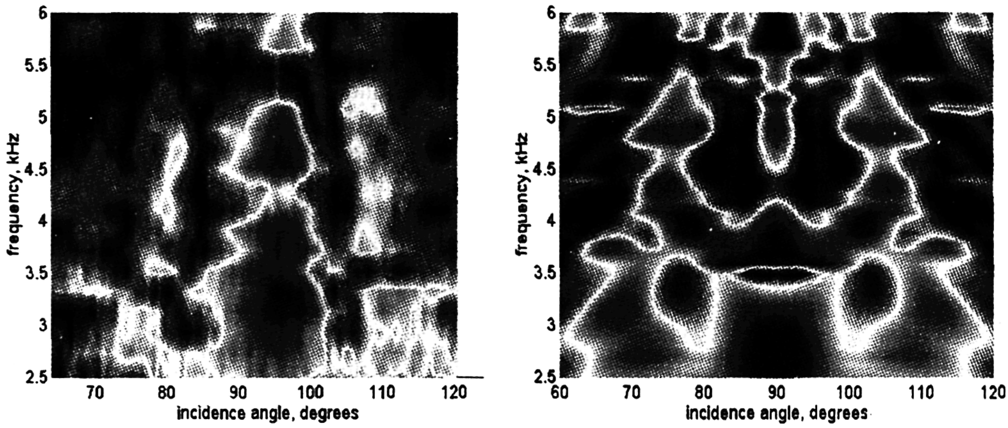

Figure 8. Water filled cylinder. Left: experiment; color scale: target strength - 25 to 0 dB.
Right: theory; color scale: target strength - 20 to 0 dB.

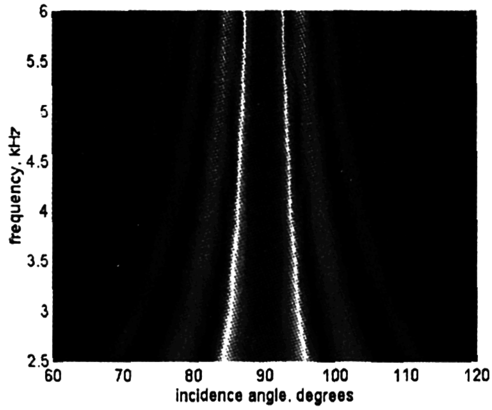

Figure 9. Rigid echo. Color scale: target strength - 20 to 0 dB.

occurs (i.e. where $R < L^2/\lambda$, with R: range, L: array length, λ: wavelength). The difference with far-field beam-steering is the addition of the range dimension. The array output signal is obtained by delay-and-sum steering, where the time delays are determined from the difference in path length relative to the central hydrophone. Time shifts are applied in the frequency domain to allow sub-sample accuracy in the delays. The shifted signals can simply be summed if the echo is assumed to be coherent along the length of the array. If this assumption is not valid, the signals should be pre-processed before summation, for instance by matched filtering. A Matlab program has been written to perform these functions interactively, using the data obtained from the 1997 Loch Duich trial.

If a target in the bottom is to be detected, refraction of the sound rays at the boundary between the two media should be taken into account, as a consequence of the different sound speeds in water and in the sediment.

Figures 10 and 11 show an example of a broadside view of the air-filled cylinder with the array in vertical position, where the signal of each hydrophone is matched filtered prior to focusing. By aiming the focuspoint either at the target or at its surface reflection, a clear distinction in strength can be made between the two signals.

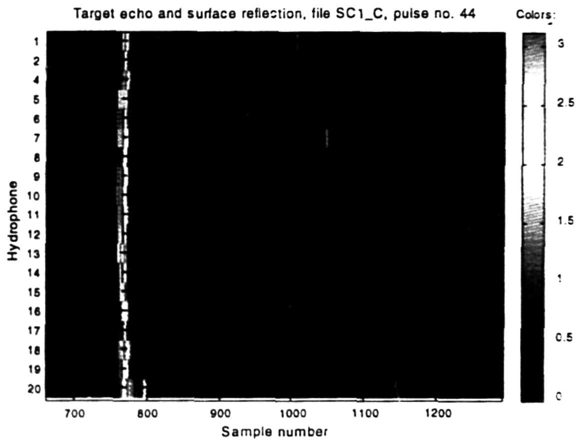

Figure 10 Matched filter output of separate hydrophones.

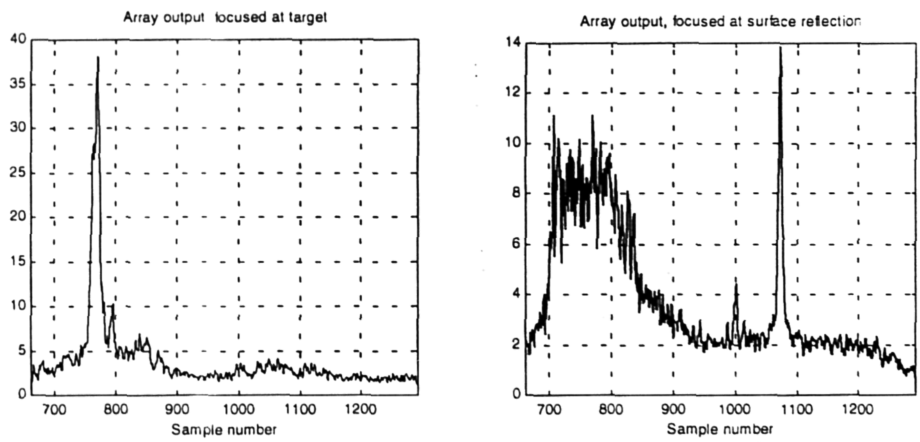

Figure 11. Array output when focusing at target (left) or at surface reflection (right).

3.3. DETECTION

In this section, the problem of detecting a target response embedded in strong reverberation is addressed in relation to active sonar systems. The first consideration is that when the noise is coloured and non-stationary (as reverberation typically is) the conventional matched filtering yields performances lower than an optimal detector. The latter is composed of a pre-whitener (deduced from the knowledge of the noise covariance function) followed by a matched filter adequately modified. In other words, we will consider a classical approach to the detection of transient signals in presence of coloured noise: to turn the problem, owing to the action of the pre-whitening filter, toward a standard detection of a signal embedded in additive white Gaussian noise. The pre-whitener filter that we adopted is based on an auto-regressive (AR) model: this accurate model allows us to easy estimate the noise covariance function on very short record of data also. To perform this estimation, despite the non-stationarity of the reverberation, we can assume that reverberation is a phenomenon slowly varying compared to the duration of the transient signal and, thus, the shape of its spectral density is locally stationary. This is equivalent to suppose that the AR parameters (estimated inside a data block lasting as the transient signal to be detected) vary slowly moving from a data block to the successive one. The success of the whitening operation depends on the validity of the above-discussed assumption of local stationarity [Carmillet 96] [Kay 90].

To exploit the above-mentioned concepts, it is necessary to model the noise as an AR process and to design a whitening MA filter based on the estimated AR coefficients. The noise $n(t)$ (i.e. reverberation) is modelled as a zero-mean complex Gaussian AR process of order p. The assumption of $n(t)$ as a zero-mean complex Gaussian process can be justified by the central limit theorem as reverberation is the sum of the effects produced by many homogeneous scatterers. The order p of the model coefficients can be fixed according to the Akaike information criteria [Makoul 75]. Let us call $n(t)'$ the prediction of $n(t)$ and $e(t) = n(t) - n(t)'$ the prediction error. Like $n(t)$, also $e(t)$ is a random signal. The least squares method is used. The p coefficient values are obtained by minimising the expected value of the square error.
After their calculation, we design the desired whitening filter, by writing it in the domain of the Z-transform.

To practically face the problem, we can assume that: (1) the signal to be analysed is cut into blocks having a time equal to the duration of the echo to be detected. So, we have to search for echo presence in each block; (2) the first block contains reverberation only; (3) in the first K block, the target echo presence has not detected yet.
From the local stationarity assumption, it is reasonable to filter the data of the block K+1 by using the whitening filter that has been deduced on the basis of the reverberation signal in the block K. The detection problem related to the block K+1 can be stated as the choice between the hypothesis H_0 (i.e., target absence) and H_1 (i.e., target presence). Data of the block K+1, which could contain the target echo, are whitened by the whitening filter whose coefficients were estimated at the block K. Under the hypothesis that such data have been perfectly whitened, we attain the classical problem of detecting a transient signal (partially known) embedded in additive white Gaussian noise. The solution of this problem relies in a bank of matched filters in order to keep into account all the potential waveforms that the partially know signal may assume.

The real data used to test the detector have been extracted from the rotating target experiment performed by the SACLANT Centre. In such experiment, about 240 pings of a 4 kHz Ricker pulse were emitted and the back-scattered signals acquired while a cylinder was rotated by

180°. From each back-scattered signal (i.e., the echo at a given target aspect angle) we can extract a portion of it that corresponds to a reverberation phenomenon. By adequately sequencing and windowing the 240 reverberation portions we have generated reverberation signals that are 80 ms long. Then, we mark a reverberation signal by data blocks of 3 ms each. To simulate a buried object echo, we have added a free-field target echo (2.4 ms long to consider both reflection and resonance contributions) taken at 0° aspect angle to a reverberation signal. By tuning the amplification applied to the normalised target echo, it is possible to modify the signal-to-noise ratio (SNR) of the buried object echo that we are building. As we have to detect the echo of a buried target of which we do not know the view angle *a priori*, we have to take into account the target echoes at different aspect angles. Therefore, we decided to design 5 matched filters working in parallel on the signal that is the output of the whitening filter. As impulse responses, we selected the target echoes at aspect angles of 0°, 22.5°, 45°, 67.5°, and 90°.

Figure 12 shows the spectral efficiency of the whitening filter. Panels show the waveform before and after the whitening operation and the related spectra of a block (3 ms long) of a reverberation signal. One can notice that after the filter, a spectrum very close to flat is obtained.

Figure 13 shows the output from a matched filter of the bank. Such a filter is matched to the target echo at 22.5° and signals obtained with and without the whitening filter are shown. The output signals have been obtained by processing data arranged as follows: the 0° echo target was added to the long reverberation signal so that the SNR is about -5 dB. The position of the target echo is around 55.5 ms. After matched filtering, a square-law filter was applied to extract the envelope profile and a normalisation to one was always performed. Observing all the outputs from the filter bank, we can notice that all the output signals after the whitening operation show a steep and very clear peak corresponding to target echo. This is an indication of great robustness with respect to unpredictable differences among expected echoes and the received echo. As foreseen, the signal with the best ratio between amplitude of the wanted peak and amplitude of the highest spurious peak is the one processed by the filter matched to the 0° angle. Also, without whitening, some matched filters provide a steep peak corresponding to the target echo, but unfortunately many other peaks are present in the signal and are taller than the wanted peak. This fact is due to the highly correlated nature of reverberation phenomenon together with low SNR. One can conclude that the presence of the whitening block before the bank of matched filters is highly recommended in facing this kind of problem.

3.4. CLASSIFICATION

A few considerations and results concerning classification are now presented, with emphasis to a Neural Network Classifier (NNC) design.

As shown above, resonances are present in the back-scattered parametric sonar signal. The ringing, which is object specific, is especially visible when the object, cylindrical in shape, is placed at the broadside or at endfire relative to the transmitter. The data set used [Deo 97] comes from the SACLANT Centre experiment. As far as classification is concerned, two classes are defined, identified in the following as the "target class" and the "non-target class". The criteria to partition the available experimental data set are described here. The members for the target class are chosen among the cylinder echoes around the broadside position (the

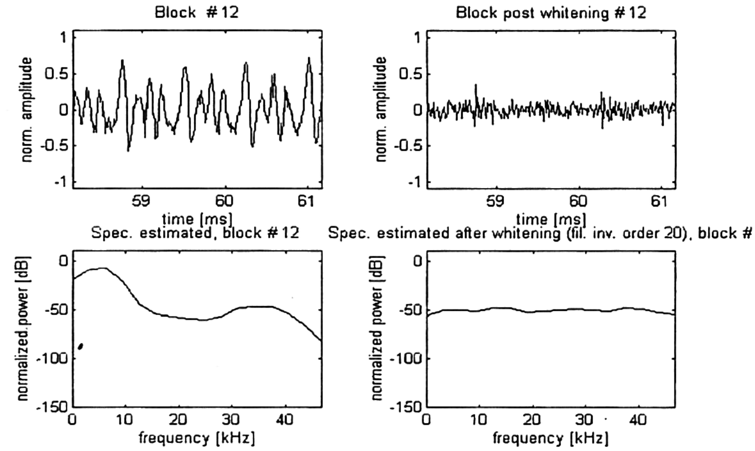

Figure 12. Spectral efficiency of the whitening filter

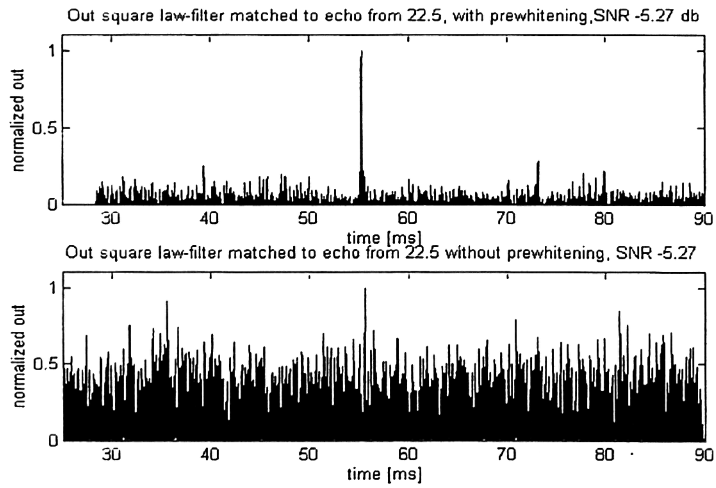

Figure 13. Output signals from a matched filter with and without the whitening process.

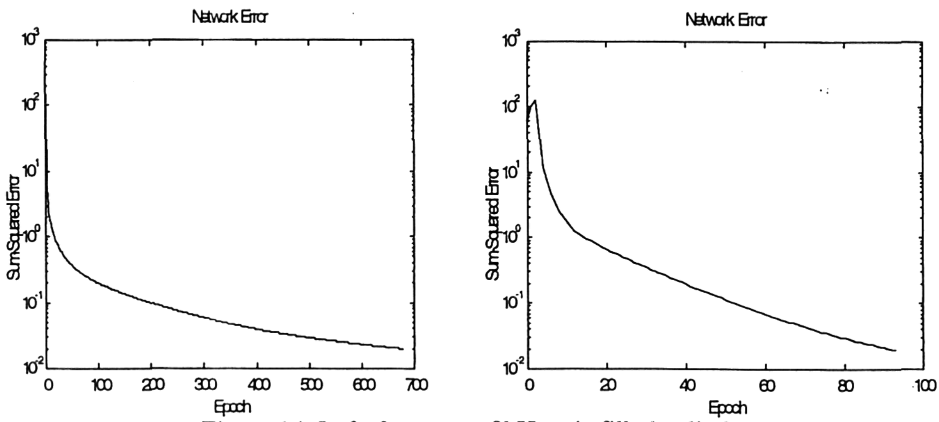

Figure 14. Left: frequency 8kHz; air-filled cylinder.
Right: Frequency 4kHz; water-filled cylinder.

rotating cylinder is well suited for this purpose) while the members for the non target class come either from the object in the off broadside position or from the surface and bottom reverberation returns. Several data set can be built as a function of the pulse characteristics and of the target type. Each data set can be further partitioned into the "training set" and the "data set", with members of the two classes represented in each set; the first one allows computation of the NNC parameters, the second one is used to check the classification capabilities.

The network has two layers of neurons plus a thresholding stage not directly involved with the training procedure. The first layer is built with biased tan-sigmoid neurons [Bose 96]. Each neuron is fed with 220 weighted inputs. The inputs number has been chosen taking into account the target size and the sampling frequency, while about 20 neurons have, with a trial and error tuning, proved to be necessary as a trade-off between performances and computing time. The second layer is built with just one biased linear neuron whose output is related to the previously described "target / non target" classes; the linear output is then thresholded in order to get on/off information about the class of belonging.

The tan-sigmoidal response of the neurons is a differentiable function that makes it suitable for the back-propagation algorithm to be applied for the training phase [Bose 96]. The network topology described can take advantage of the Nguyen-Widrow method [Nguyen 90] to find initial conditions which make the training faster, provided of course that the chosen classes can actually be taken apart. Given the previously stated network size, 4420 weights (220 inputs * 20 first layer neurons + 20 inputs * 1 2nd layer neuron) plus 21 biases have to be computed. The coefficients are found by minimising the errors between the wanted outputs and the computed outputs when the network is fed with the training set. A gradient-based minimum seeking algorithm is recursively applied. If the training is successful, any vector in the training set will of course give the correct answer, moreover unknown vectors from the data set can be presented to the trained NNC to be taken apart into the two target/non target classes previously defined. Figure 14 show the training results for the air filled, 8 kHz pulse, and water filled, 4 kHz pulse, cylinders. The error between the wanted and computed outputs decreases steadily (after the first steps) for each training epoch, until the error goal, conventionally placed at .02, is successfully reached. The computed weights are of course different as a function of the target features and of the transmitted sonar signal.

In order to evaluate the performances the trained NNC is monitored when presented with the data sets. The achieved correct classification result is around 95%. The method seems to be promising though it is not possible to draw final conclusions at this stage because of the relatively small number of independent patterns available. Besides in the free-field experiment the achieved S/N ratio is good and of course this could not be the case for buried objects.

4. CONCLUSIONS

In summary, at the present time, the main project results are as follow:

- Good and almost noiseless experimental data have been obtained with several kinds of cylinders for various aspects angles.
- It is possible to differentiate between an elastic shell and a rigid object acoustically by analyzing the transfer function in the frequency-angle space.

- Resonance back-scattered pressure is more important than specular reflection when the conditions of frequency and spatial resonances are met.
- Simulation tools are validated by comparison with experiments and they could be useful for the selection and extraction of features significant for target classification purposes.
- Array focusing, detection, and classification results represent an encouraging step toward obtaining good detection performance on the echoes of targets buried in the sea bed.

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REFERENCES

[Bose 96] Bose, Liang. *Neural Networks Fundamentals with Graphs, Algorithms and Applications*; Mc Graw-Hill (1996).

[Carmillet 96] V. Carmillet, G. Jourdain. Wideband sonar detection in reverberation using autoregressive models. *Int. Conf. Oceans 96 MTS/IEEE*, Fort Lauderdale, FL (USA), 1435–1440, 1996.

[Deo 97] Contract MAS3-CT95-0009. *First Year Technical Report*, European Community (1997).

[Deo 98] Contract MAS3-CT95-0009. *Second Year Technical Report*, European Community (1998)

[Kay 90] S. Kay, J. Salisbury. Improved active sonar detection using autoregressive prewhiteners. *J. Acoust Soc. Am.* 87 (4), 1603-1611 (1990).

[Kennard 53] E.M. Kennard. The new approach to shell theory: circular cylinder. *J. Appl. Mech.* 20, 33-40 (1953)

[Makoul 75] J. Makoul. Linear prediction: a tutorial review, *Proceedings of IEEE*, vol. 63, no 4, 1975.

[Maguer 97] A. Maguer, S. Fioravanti, A. Lovik. Below critical angle detection of buried objects. *IEEE Proc. of OCEANS'97*, 1, 512-517

[Nguyen 90] Nguyen, Widrow. Improving the Learning Speed of 2-Layer Neural Networks by Choosing Initial Values of the Adaptive Weights. *International Joint Conference of Neural Networks*, vol 3, 21-26, July 1990.

[Tran Van Nhieu 92] M. Tran Van Nhieu. Scattering from a finite cylindrical shell. *.J. Acoust. Soc. Am.* 91 (2), 670-679 (1992)

[Woodward 94] B. Woodward, A.D. Goodson, J.C. Cook, P.A. Lepper. Sea-bed characterisation using non-linear acoustics. *Proc. 2nd European Conf. on Underwater Acoustics*, Copenhagen, Denmark, 4-8 July 1994.

TITLE: Three-Dimensional Sediment Transport Measurements by Acoustics (project **TRIDISMA**)

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THREE-DIMENSIONAL SEDIMENT TRANSPORT MEASUREMENTS BY ACOUSTICS (TRIDISMA)

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SUMMARY

The TRIDISMA Project's primary objective has been the development of a high frequency acoustic instrument capable of resolving profiles of the three components of current velocity together with profiles of suspended sediment concentration and size in wave and current dominated benthic boundary layers. The first twenty months of the project has resulted in the design and construction of the TRIDISMA instrument hardware and software, culminating in trials of a laboratory version of the instrument in a large scale wave and current flume. The results of these trials are discussed in this paper and highlight the capability of high frequency acoustics as a tool for resolving transport processes under waves and currents. The project is now entering it's third year during which a deployment of a field ruggedised version of the instrument will be made and the data analysed.

1. Introduction

Accurate measurements of sediment transport are a baseline requirement for most of Man's attempts to understand or engineer the behavior of our coastal regions and it's quantification is fundamental to numerous practical applications, from assessing the stability of dredge spoil sites and harbour siltation, to the morphodynamics associated with beach erosion and coastal protection.

Sediment transport in the marine environment is, however, notoriously difficult to measure, since the relationship between the net flux of sediment and transport under combined waves and currents is still poorly understood. To some extent this has been exacerbated by our inability to quantify the temporal and spatial scales of the transport process since most existing instrumentation rely on point measurements of flow (in one or more directions) together with co-located measurements of suspended sediment, usually obtained by means of sediment traps, pump-samplers, transmissometers or optical backscatter probes. Profiles are thus obtained by stacking such instruments together in vertical arrays.

These point measurement systems are unable to rapidly parameterize profiles of sediment concentration, size and the flow components right down to the bed, a necessary requirement for accurate measurements of sediment transport, and often have large uncertainties associated with them particularly close to the sea bed where their presence disturbs the flow.

Acoustic instrumentation has distinct advantages in their application to studies of sediment transport as they are both non intrusive and are capable of providing profile measurements with a high temporal and spatial resolution.

Initial developments of acoustic instrumentation to measure the sediment transport processes focused on the use of backscattered sound to determine the concentration profile of suspended sediment. The backscattering of high frequency sound from sediment particles suspended in water is determined by a complex but reasonably well described function of the acoustic wavenumber of the ensonifying pulse and the radius of the scattering particle. This wavenumber dependence has been developed such that the strength of the backscattered signal obtained by ensonifying the same suspension profile with two or more widely-spaced acoustic frequencies can be used to derive profiles of sediment size by taking the either the ratio or the difference in received signal strengths at different frequencies and matching them against theoretical form function ratios or differences. These methods have been validated in a number of laboratory, marine and theoretical studies which confirm that the size and concentration of profile sediment can be obtained from backscattered signals to an accuracy of order 10 %, Thorne et al. (1997).

Concurrent with these technical developments in the processing of backscattered sound advances in digital signal processing are providing methods of using backscattered signals to derive high resolution (centimetric) velocity measurements. Techniques that have shown promise include both coherent Doppler current profiling and acoustic correlation techniques, both of which have been evaluated in the recent MAST II project VERIPARSE, Kaminga et al. (1995).

The TRIDISMA project was established to develop and test a prototype instrument, using the aforementioned acoustic methods, which could be used to evaluate the basic technologies associated with acoustic backscattering and explore the possibilities offered by coherent Doppler and Acoustic Correlation techniques in quantifying and resolving flow structure under the action of both waves and currents.

In order to achieve the desired measurements it is necessary to understand the interaction of sound with the phenomenon under investigation and to develop methods by which information can be extracted from the backscattered signals. This field has been the subject of extensive research over the last 10 years and considerable progress has been made in understanding the interaction of the backscattered signal with sediment scatterers and it's frequency dependence to a first order approximation. This first order approach is thought to break down where particles concentrations are high such as in the sheetflow layer close to the bed in a wave current boundary. In this near bed region, the inversion of acoustic data to obtain concentration and size becomes problematic as a result of errors arising from small uncertainties in the form function and attenuation terms rapidly accumulating. Further problems arise from second order scattering effects such as particle shading and the existence of multi-paths. These errors combine and resulting in the over prediction of the concentration profile. For this reason a programme of experimental and theoretical study aimed at resolving the higher order interactions of the backscattered sound has run concurrent with the design and construction of the TRIDISMA Instrument with the objective of predicting their likely contribution to the backscattered pressure field.

As well as advancing the general understanding of acoustic scatterer interactions, results from trials of the TRIDISMA instrument will ultimately provide a specification that can be used for the future development of a commercial device to fill this important gap in available instrumentation. Data produced from the early trials of the TRIDISMA instrument are already proving to be of great scientific value by elucidating the flow structure and it's relationship to sediment flux.

TRIDISMA has drawn together European partners with wide and extensive experience in the design, construction and use of high-frequency acoustic instrumentation as measurement tools. The Partners come from Industrial, Research and Academic areas, indicating the wide appeal of this project; several partners have already experience of using acoustic instruments to obtain measurements of suspended sediment concentration and velocity in coastal, beach and estuarine environments some of which were conducted under previous EU programs. The industrial partners have an existing presence in the marine acoustic market and view this prototype instrument development as leading to new products helping to maintain the competitiveness of European Industry in marine ultrasonics.

2. Technical Description of the TRIDISMA APRI

The brief for the development of the TRIDSIMA APRI (Acoustic Prototype Research Instrument) was to produce a system capable of measuring sediment transport profiles in the benthic boundary layer to a height of approximately 1 meter above the bed, by simultaneously measuring profiles of the suspended sediment concentration, particle size and the three components of the fluid velocity. This has been achieved by using an acoustic correlation method to resolve the horizontal velocity components and the coherent Doppler principle to resolve vertical velocities whilst measurement of the backscattered pressure at four different frequencies allows inversion of the recorded data to derive sediment size and concentration profiles.

The APRI Hardware has been designed for deployment at a beach or sandy estuarine location and comprises an underwater unit which houses the main transmit and receive electronics together with the 6 acoustic transducers attached via 5 m cables, and a data acquisition system presently connected to the underwater unit by 200 m of multi-core cable. This latter system consists of a junction box housing signal conditioning electronics and a PC based data logging system based around an 8-channel high performance 12 bit analog to digital conversion board. This board uses the 32-bit PCI bus to allow very high speed streaming of A/D samples to host memory or disk. All eight converters are triggered in parallel to allow simultaneous sampling and A/D conversion of the 7 channels of the APRI. Digital output channels provided by the A/D board are used to configure the APRI via a serial communication line and to synchronise the transmitted pulses.

The TRIDISMA APRI uses a monostatic transducer configuration throughout and thus each transducer is used to both project and receive the backscattered signal. This presents a number of ancillary problems in that the transducers ring for several cycles after transmission during which time no acoustic reception can take place.

The choice of acoustic frequencies for use by the APRI has been the subject of extensive research and takes into account previous experimental experience gained with coherent Doppler sonar, Hardcastle (1995), together with an optimisation study for the inversion of backscatter data to derive profiles of particle size for sand sized sediments. This has resulted in the following frequencies being selected:

- 0.5 MHz for coherent Doppler profiling
- 0.5, 1.0, 2.0 and 4.0 MHz for backscattering analysis
- 2.0 MHz for correlation profiling

Transducers used in the APRI have been selected from commercially available units but have been optimised in terms of their transmit/receive sensitivity, beam pattern, maximum power output, acoustic impedance and bandwidth. Each transducer has also been calibrated individually in terms of its beam pattern, transmit and receive gain in order to allow data to be reduced directly to acoustic pressure.

The main specifications of the APRI are summarized in the following table.

Parameter	Doppler	Correlation	Backscatter		Units
Frequency	500	2000	1000	4000	kHz
Number of transducers	1	3	1	1	
Q	2 - 5	10	5 – 10	5 - 10	
Pulse length	64	16 - 64	16 – 64	16 - 64	μs
Beamwidth	5°	2°	3°	2.2°	deg
Data logged	Quad. in phase, out phase Amplitude	Amplitude	Amplitude	Amplitude	
Digitisation rate	18.75	75	75	75	kHz
Digitisation	11 bits + sign	12 bits	12 bits	12 bits	
Gain Control	Log amplifier	Log amplifier	Log amplifier	Log amplifier	
Gain	80	80	80	80	dB
Bandwidth	200	200	200	200	kHz
Principle	Complex demodulation	Envelope Detection	Envelope Detection	Envelope Detection	

Table 1 APRI Hardware Specification.

The A/D board operates under the control of dedicated software that facilitates configuration and initialisation of the APRI and handles the recording of data to disk.

This software allows the operator to set the following parameters prior to commencing data acquisition:

- Pulse Repetition Rate (2, 4, 8, 16, 32, 64, 128, 256, 512 Hz)
- Profiling Range (0.64, 1.28, 2.56 m)
- Channel inhibition (On/Off for each channel)
- Pulse duration (16, 32, 64 μs)
- Transmission level (0dB to -21 dB in 3dB steps)
- Data Decimation (2^n samples n= 0, 1, 2, ... 8)

The requirement of sampling the backscattered signal at 75 kHz makes serious demands on the performances of the host PC. The Microsoft Windows and Windows95 operating systems cannot achieve such high speeds compared to MS-DOS largely due to the non-deterministic timing of Window's message queue, which results in unpredictable delays occurring when running normal Windows programs. Windows also uses a considerable amount of memory and high disk activity at random times. Therefore in order to achieve maximum data acquisition, processing and storage rates APRI control software has been written to run under MS-DOS with the data written directly to memory prior to being written to disk.

Data are collected in frames. Each frame comprising a finite set of A/D samples. A trigger starts each frame and is used to synchronise the transmitted pulses.

At the time of writing ensemble averaging and processing of the APRI data is undertaken offline due to the limited number of clock cycles between successive pings. This regime although allowing the evaluation of a variety of processing algorithms to be tested on raw data files, does however mean that the data rates that the APRI must handle are extremely high. For example, at a pulse repetition rate of 471 Hz (the maximum Pulse Repetition Rate) with all channels being logged, the data rate on the PCI bus is:

$$7 \text{ channels x } 128 \text{ samples x } 477 \text{ Hz x } 2 \text{ bytes} \approx 855 \text{ kB/s}$$

The availability and addressing of RAM memory on the logging PC presently places a constraint on the size of file that can be acquired by the APRI to a maximum file size of 64 Mb, or 75 seconds recording time. A time series of this length, whilst adequate for the controlled experimental conditions of the tank trials, is of limited use for discerning transport processes in the natural environment. Effort is therefore presently being directed at providing on line averaging of the data as well as direct transcription of the data to hard disk in order to reduce data rates and extend the duration of APRI data sets.

3 APRI Tank Trials

Initial trials of the TRIDISMA Acoustic Prototype Research Instrument were undertaken in the Oscillating Wave Tunnel, shown below, located at Delft Hydraulics' DeVoorst Laboratories in September and October 1997.

Figure 1 Oscillating Wave Tunnel Measurement Section with APRI and reference instrumentation installed. The tunnel is basically a U tube with a piston in one leg and the other open. A sediment bed is placed in the channel linking the two legs over which measurements can be made.

The tunnel measurement section has a rectangular cross section with nominal dimensions of 1.1m x 0.3m x 14m with a measurement region located at the midpoint of the flume. Wave periods of between 5 and 15 seconds can be simulated with either a sinusoidal or non-sinusoidal form, in the range indicated in figure 2. The maximum orbital velocity that can be produced is 2m/s. A series of re-circulating pumps can, in addition, apply a small net current to the orbital motions induced by the piston of up to 0.65 m/s. No wave induced vertical velocities are produced by the tank other than those formed by the interaction of the flow with bedforms.

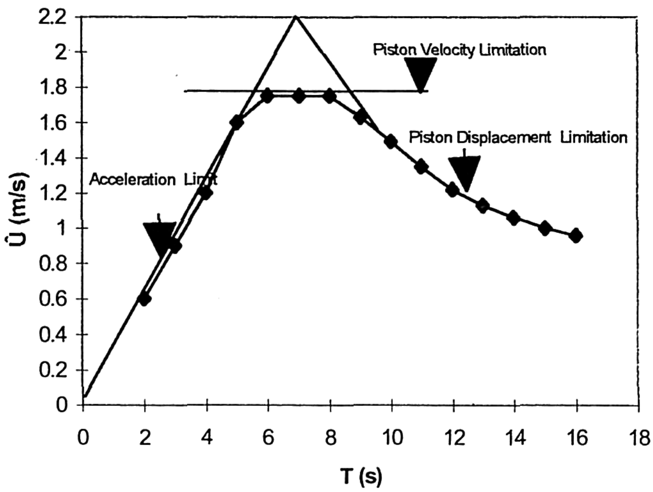


Figure 2 Range of experimental conditions capable of simulation in the Delft Oscillating Wave Tunnel.

The tank was filled with sieved dune sand with a median grain size (d_{50}) of 130 μm to a level of 0.30 m above the base leaving approximately 0.80 m for measurements.

The APRI was installed in one of the tank's removable steel inspection plates which are mounted on the tanks upper surface so as to both minimise disturbance to the flow and to reduce scour of the sediment bed. Each plate is 1.0 m in length and 0.02 m thick and fits via a compression seal onto the tank. In order to minimise reverberation and cross talk transducers were set into a 0.035m thick section of Perspex inset into the steel plate.

Given the narrow cross section of the tank transducers were configured linearly with provision for two additional 2 MHz correlation transducers provided to allow different separations to be investigated for assessment of the correlation technique. Data thus refers only to the axial flow component of the tank. Transducers were mounted with the their beams as close to vertical as possible, although an aperture inclined at 45°was also provided to take a second 0.5 MHz transducer to allow Doppler measurements to be collected in a forward looking mode. The transducer spacing used for the correlation measurements were set to 0.04, 0.10, 0.15 and 0.29 m

Figure 3 Transducer arrangement for APRI tank trials. Transducers are grouped according to measurement technique they perform From left to right: 500 kHz (45°) Doppler/Backscatter, 4MHz, 2 MHz, 1 MHz Backscatter, 500 kHz Doppler/Backscatter (0°), four 2 MHz Correlation. The net current in the tank is from right to left.

Over a three-week period a series of 12 separate experimental conditions where investigated including both the wave, current and wave/current conditions outlined in table 2. Velocity and concentration data were collected by both the APRI and conventional reference instruments including a laser Doppler anemometer and an optical backscatter sensor and synchronised with the APRI data by means of a TTL output from the APRI. Output from the TTL line was logged at 25 Hz as a simple voltage by the Delft AUKEPC data logging system together with signals from both the reference instrumentation and the wave generator.

The reference measurements comprised

- OPCON — Optical Concentration meter giving time dependent measurements of concentration in the range 0.1-40 g/l.
- PUMPED SAMPLES — Time averaged measurements of concentration and particle size
- LDA — 2 component velocity measurements using laser Doppler anemometer operating outside the tank
- ADV — 3 component velocity measurements using Acoustic Doppler
- Wave Generator — Synchronisation of position within wave cycle

Time lapse video records of each experimental run were also collected using standard video equipment.

Analysis of samples collected under the different experimental conditions using a pump sampling array have been undertaken to determine both the mass concentration and particle size at known heights above the sediment bed (ranges from the APRI).

Samples of the sediment suspension for each experimental condition investigated were collected by means of a series of peristaltic pumps connected to a sampling inlet array. Samples of the suspension were collected at a constant flow rate of 0.5 l/s over a period of several minutes from which mass concentrations were determined using both calibrated sand settling towers and conventional gravimetric techniques. Particle size variations with height have also been investigated by means of a Coulter LS130 Laser Particle Sizer. From the size distribution determined for each sample arithmetic statistics have been calculated based on a simple interpolation scheme. These data form the basis for evaluation of the APRI acoustic backscatter system's performance.

Each experimental run involved collecting data for a period of approximately 3-5 minutes with the APRI sampling initiated when conditions were judged to be steady. Measurements of the height of the reference instrumentation together with the height of the bed in the measurement section were recorded at the start, mid point and end of each run. Each run was repeated several times with the reference sensors at a different height each time to allow profiles of concentration and velocity to be built up.

During "current only" conditions a turbulence grid comprising small concrete blocks was used to promote sediment entrainment to a point where sufficient scatterers were present in suspension to allow acoustic measurements to be made.

The bulk of the experimental work focussed on 4 wave/current conditions which had been subject to previous investigation and numerical modeling by the group at Delft and are indicated in bold in the table.

Experimental Condition	Wave Period (s)	Mean Flow (m/s)	Oscillatory Flow (m/s)
m14	0.00	0.25	0.00
m16	0.00	0.35	0.00
m18	0.00	0.45	0.00
m19	0.00	0.50	0.00
m99	0.00	0.63	0.00
m01	**4.00**	**0.25**	**1.10**
m31	7.20	0.25	0.95
m30	**7.20**	**0.25**	**1.50**
m33	**7.20**	**0.45**	**1.10**
m02	**12.00**	**0.25**	**1.10**
m80	16.00	0.00	0.80
m82	16.00	0.35	0.80

Table 2 Experimental conditions investigated during the APRI Trials

In order to provide a further test of the validity of APRI measurements conditions in the Oscillating Wave Tunnel have been simulated for the 4 wave/current cases of interest using a 1 dimensional time dependent model of flow velocity and sediment concentration. This model allows simulation of the development of the sheet flow layer and allows computation of the instantaneous sediment flux over the course of a wave cycle.

The formulation of the problem in the model is;

$$\frac{\partial U}{\partial t} = -\frac{1}{\rho}\frac{\partial p}{\partial x} + \frac{\partial}{\partial z}\left[\nu_z \frac{\partial U}{\partial z}\right]$$

$$\frac{\partial C}{\partial t} = \frac{\partial C}{\partial z}\left[W_S C + \varepsilon_S \frac{\partial C}{\partial z}\right]$$

$$\nu_z = (\kappa z)^2 \left|\frac{\partial U}{\partial z}\right|$$

Where U is the current velocity, z the height above the bed and C the sediment concentration. The term W_S represents the fall velocity of the sediment particles whilst ε_S describes the sediment diffusivity, κ is the von Karman constant and ρ the fluid density.

Under the conditions of wave/current interaction the sheet flow layer which develops close to the bed acts as a roughness element which reduces the velocities above this layer. This effect is parameterised in the model through the use of an Eddy Viscosity reduction factor β, the value of which has been found by experimental studies. Inclusion of this term allows the thickness of the sheet flow layer to be estimated with reasonable accuracy although concentrations in this layer are not simulated correctly as other factors such as grain-grain interaction as become more than simple turbulent diffusion.

4 APRI Backscatter Profiles of Sediment Size and Concentration.

Over the past decade multiple frequency acoustic backscatter systems have been validated by a number of laboratory, field and theoretical studies showing how the size and concentration of sediment can be obtained from backscattered signals. However, the inversion of the backscattered acoustic field to obtain scatterer concentration and size information becomes problematic near the seabed where the attenuation due to sediment scattering becomes significant.

Firstly, the form function and cross-section of the sediment particles are subject to some uncertainty due to the irregular shape of the sediments. In regions where the concentration gradient is steep errors arising from these uncertainties rapidly accumulate and lead to over or under estimation of the concentration profile.

Further problems arise from the stability of the inversion algorithms used in this near bed region where disturbances caused by unstable wave and sediment movements are rapid in both time and space.

Several inversion schemes have been proposed for obtaining the size and concentration of the suspended sediment. Hay and Sheng (1992) utilised an inversion algorithm that compared the ratio of backscattered signal strengths at different frequencies to precalculated theoretical form function ratios in order to determine the sediment size. Crawford and Hay (1993) took this approach further by using the difference of measured signals instead of the ratio, again obtaining the size profile of the scatterers by comparison with theoretical differences in the form functions.

Both methods are inherently similar but are optimal for different signal levels. The problems with inversion arise when the concentration of sediment is high and increases rapidly. This occurs, as Thorne et al. (1997) pointed out, because of the manner in which the inversion is computed with the initial estimate of concentration in each range step ignoring the attenuation due to scattering in that step. The attenuation is then accounted for by using this initial estimate of concentration to provide improved concentration estimation. The problem with this calculation is that it is unbounded and relatively small errors can rapidly accumulate.

To prevent this from occurring, the signal arising from the bed echo has been used as a constraint in the ratio algorithm developed by Thorne et al. (1995), and has been shown to produce improved results. However, there are uncertainties in the magnitude of the bed echo because the acoustic reflection properties of seabed can change significantly during the measurement period.

A new inversion algorithm based on the attenuation of sediment has been developed in the TRIDISMA project, where a local linear predictor is used to implicitly include the contribution of attenuation at each step. This introduces a higher stability in the proposed algorithm than in the conventional inversion algorithm, especially in the high concentration. The computational time is also reduced significantly because iteration is no longer required for the attenuation estimation.

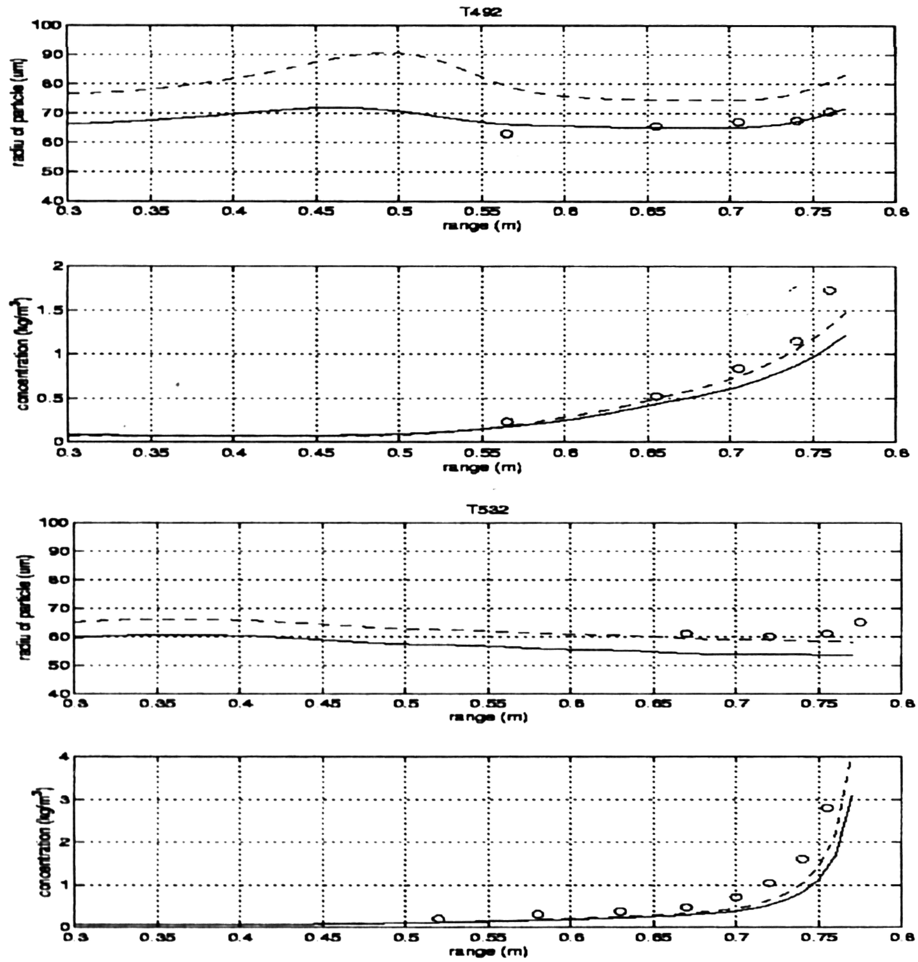
In order to assess the performance of the inversion scheme the variations in mean size and concentration with range for different wave states were investigated by averaging 1 minute long data samples. Figure 4 shows the results for the mean-radius and concentration of the suspended sand obtained using the proposed inversion technique and data from the oscillating wave tunnel. The first case is for a simple current with an average velocity of 0.63 m/s. The lower plot ~~in figure 4~~ shows the results of data collected under wave and current conditions in the tunnel with waves of 12s period and an instantaneous peak current of 1.1m/s with a net current of 0.25m/s superimposed. In this case data has been averaged over 5 wave cycles. Range is measured from the surface of the transducer and the sand bed occurs at 0.78 m.

There is generally a good agreement between the results of pumped sample concentrations and sizes and the inversion results. It is however noted that on a number of occasions the inversion results based on only two frequencies give a better agreement with pumped sample results than does the three-frequency inversion scheme. It is suggested that the simple high pass filter model used by Sheng and Hay (1988) to model the form function and the acoustic cross-section and utilised in the present inversion algorithm fails to account for the uncertainties in these terms. Further potential sources of error must also include errors in the acoustic calibration of APRI which will also affects the inversion results, particularly when comparing between transducers.

In addition to obtain average profiles data have been inverted using a 0.25-second ensemble average in order to visualise the variation in sediment concentration with height as a function of a wave cycle. An example of such a time history is shown in Figures 5 for the wave conditions corresponding to periods of 12 seconds and a net current of 0.25 m/s.

The results show a high degree of structure coherent from wave to wave whilst spikes in data appear to be mainly caused by errors in the size estimation of the sediment scatterers

Figure 4 Results for the mean-radius and concentration of the suspended sand for a) current only and b) wave/current conditions. The solid line represents 3 frequency inversion results and the dashed line the results obtained from inversion of the 2 and 4 MHz data. The circles correspond to reference measurements obtained from analysis of pumped samples.



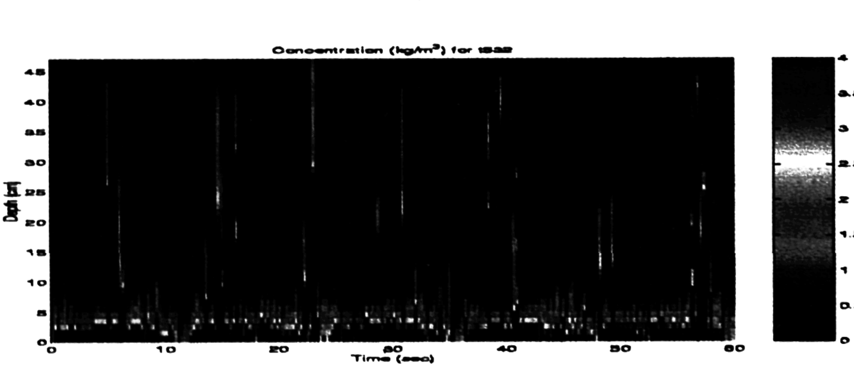


Figure 5.60 second time series of sediment concentration profiles. Wave period 12s

5 APRI Coherent Doppler and Acoustic Correlation Profiling of 3 Dimensional Flow Velocity

5.1 Coherent Doppler

The TRIDISMA APRI was developed to resolve profiles of horizontal and vertical profiles of velocity using two independent measurement systems. Vertical components of velocity are determined by coherent Doppler techniques whilst horizontal velocity components are derived from correlation of backscatter time series recorded by a series of transducers with a well-defined geometry and collimated beams.

The Doppler velocity profiles are derived from a system transmitting pulses of sound at 526 kHz, with a selectable pulse length of either 15 or 60 microseconds, at pulse repetition frequencies selected from 235 or 470 Hz. The received signal backscattered from particles within the transducers field of view is processed to give the in phase and quadrature components compared to the transmitted signal. These components are digitised at 75.2 kHz but can be decimated by a factor of 2, 4 ... etc. to give sampling frequencies of 37.6, 18.8 kHz ...etc. The system thus measures the phase shift of the backscattered sound from pulse to pulse due to the radial movement of the scatterers in the beam of the transducer, in bins whose length is defined by the digitisation frequency and the transmitted pulse length.

The velocity of the scatterers in each bin along the beam being determined from this phase shift. This gives a system where velocity profiles are available in the direction of the sound beam, at a spatial resolution determined by the pulse length and digitisation frequency, and a temporal resolution given by the pulse repetition frequency. In order to reduce errors in velocity estimation, the velocities in each bin are normally determined by averaging over 16 or 32 pulses. Phase shifts of +/- pi are readily determined, and these correspond to +/- 0.31 metres/second velocity with this transmission frequency and when using a pulse repetition frequency of 470 Hz. Velocities above these are aliased and must be de-aliased by tracking, or other methods.

The inclined Doppler transducer was mounted looking into the flow to enable measurements to be obtained of the vertical profile of horizontal currents. These measurements are valid if the vertical component of flow is only a small fraction of the horizontal flow. Data close to the transducer is lost due to the transducer being unable to resolve Doppler shifts shortly after the transmit pulse due to ringing in the transducer.

Figures 6 and 7 show Doppler data collected in the Delft Oscillating Wave Tunnel for a steady current and for a steady current with waves of 12-second period superimposed. A further comparison of the mean velocity profile obtained from the 500 kHz coherent Doppler is presented in figure 8. Preliminary analysis of these data sets show the APRI Doppler to perform well under both steady and oscillatory current conditions and be capable of providing information on boundary layer structure with a high temporal and spatial resolution. Comparison of results from 60 second averages of Doppler data show close agreement with independent reference measurements lending further support to this claim.

Figure 6 Horizontal velocities (mm/s) recorded by the Doppler mounted at 45° for conditions of current only. NB The top of tank is located at the lower edge of the figure.

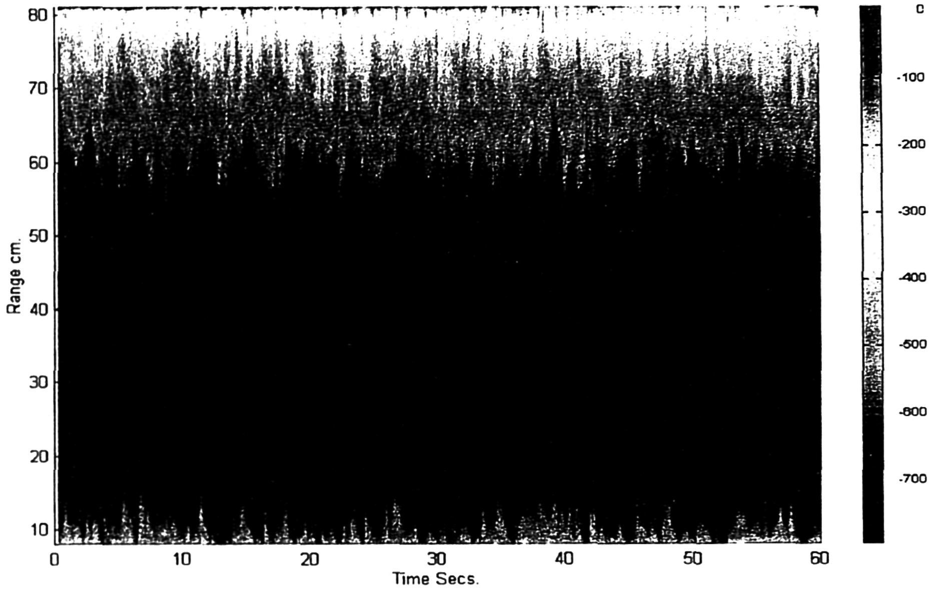
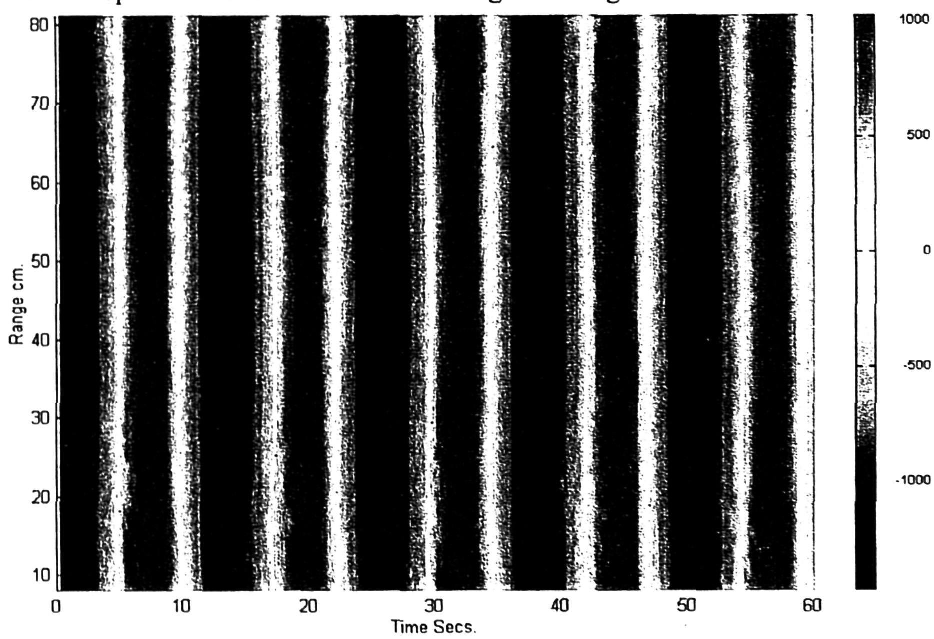


Figure 7 Horizontal velocities (mm/s) recorded by the Doppler mounted at 45° for conditions of a wave of 12 seconds period superimposed on a net current of 0.25 m/s. NB The top of tank is located at the lower edge of the figure.

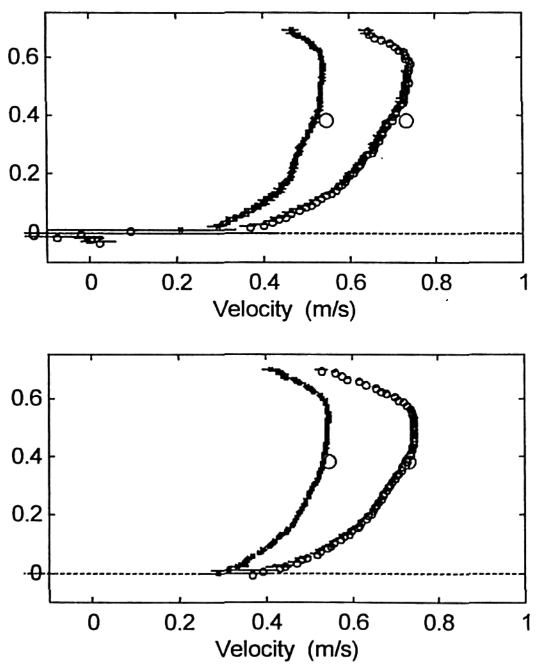


5.2 Acoustic Correlation Methods

This approach uses the coherence in the suspended sediment field to measure vertical profiles of the horizontal current velocity. The technique uses two horizontally separated transducers which are directed vertically downwards towards the bed. As suspended sediments advect below the transceivers profiles of the backscattered time series signal are recorded.

If the suspended field has a coherence distance greater than the separation of the transducers similar structures will be recorded in the backscattered signal at each transceiver. Cross-correlation analysis can then be applied to the two time series of the backscattered signals and the time for the suspension structures to advect beneath the transceivers measured. The velocity is then calculated from the ratio of the separation of the transducers and the advection time.

Figure 8 Comparison of mean and standard deviation of velocity profiles determined by Acoustic Correlation (upper) and coherent Doppler techniques (lower) under current only conditions at two different flow speeds. Circles correspond to the mean axial velocities determined by the laser Doppler Anemometer.



Preliminary results of the comparison of correlation with the APRI Doppler and velocity reference instrumentation have been undertaken and initial results are encouraging, although there is a slight and, as yet, unexplained deviation from the mean values of order 5%.

Figure 8 shows however the marked increase in the standard deviation of velocities as the bed is approached. The inference from this that in the near bed region the flow structure is not coherent over the transducer separation distance. However it is also possible that locally generated small scale structures blur the acoustic picture. Further work will be directed at resolving this issue.

7 Conclusions

An acoustic prototype research instrument (APRI) which couples acoustic backscatter, coherent Doppler and acoustic correlation has been constructed and successfully trialed in an oscillating wave tunnel with encouraging results. Both wave/current and steady current conditions have been studied and the system is shown to yield realistic estimations of both size and concentration profiles of suspended sand at upwards of 4 Hz using a new and stable inversion algorithm. Velocity profiles determined by means of coherent Doppler techniques are likewise showing detailed information on flow structure with centimetric spatial resolution and temporal resolution of better than 10 Hz and show good agreement with conventional instrumentation. Preliminary analysis of cross correlation estimators for velocity determination are within 5% of both Doppler and reference instrument estimates. However, in the near bed region the link between the length scale of bursting phenomenon and the advection field requires further study in order to reduce standard deviations to acceptable levels.

We consider that TRIDISMA is, for the most part, yielding results, which will in due course provide new insights into the transport process.

8 Acknowledgements

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9 References

1. S.D.Kaminga, A.P.Plaisant, C.Camporeale, D.Nijvelt and H.Østergaard-Madsen. Velocity and refraction index profiling by acoustic remote sensing. Marine Science and Technologies Second MAST days and Euromar Market 2 879-893, 1995.
2 P.J.Hardcastle, A high resolution coherent acoustic Dopller current profiler fro measurement of near bed turbulent flow, Proc IEEE Oceans 95, 2 1361-1366, 1995.
3 A.E.Hay and Jinyu Sheng, Vertical profiles of suspended sand concentration and size from multifrequency acoustic backscatter, J. Geophysical Research, 97 (C10) 15661-15677, 1992.
4 A.M.Crawford and A.E.Hay, Determining suspended sand size and concentration from multifrequency acoustic backscatter, J. Acoust. Soc. Am. 94 (6) 3312-3324, 1993.
5. P.D.Thorne and P.Hardcastle, Acoustic measurements of suspended sediments in turbulent currents and comparison with in-situ samples, J. Acoust. Soc. Am. 101 (5) 2603-2614, 1997.
6. P.D.Thorne, G.P.Holdaway and P.J.Hardcastle, Constraining acoustic backscatter estimates of suspended sediment concentration profiles using the bed echo, J.Acoust. Soc. Am. 98 (4), 2280-2288, 1995.
7. Jinyu Sheng and A.E. Hay, An examination of the spherical scatter approximation in aqueous suspensions of sand, J. Acoust. Soc. Am. 83(2) 598-610, 1988.

TITLE: Sonar Technology for Monitoring and Assessment of Benthic Communities (**BioSonar**)

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Sonar Technology for Monitoring and Assessment of

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SUMMARY

The optimal hydroacoustic monitoring strategy of benthic communities ranging over several square kilometers or more requires careful design of the transect grid to be used. Among other factors, prior knowledge about the local structure of the spatial variation of e.g. common mussels can enhance the results drastically, when the end goal is to produce interpolated distribution maps. In this paper we present results based on field campaigns in the Mediterranean near Cartagena in the south east of Spain, and near Copenhagen in Øresund between Sweden and Denmark. Methods applied include calculation of semivariograms and kriged maps for echo sounder measurements, application of a range of filters on side scan sonar images as well as feature space analyses comprising Bayesian discriminant analyses and non-parametric classification. We conclude on this particular issue that a two-step approach can be recommended for mapping of larger-scale areas, and that the use of proper ground truthing is crucial for the quality of the resulting maps.

INTRODUCTION

Monitoring of typical benthic communities and quantification of their living conditions is an important tool for establishing and maintaining knowledge about the marine environments. The health of benthic communities is closely influenced by environmental impacts due to human activities in coastal areas, and many benthic communities have central roles in their ecosystems. In Northern Europe this applies to e.g. common mussels (*Mytilus edulis*) and in the Mediterranean to e.g. neptune grass (*Posidonia oceanica*). The neptune grass meadows and the common mussel beds play vital roles in favouring biological diversity in the marine ecosystems. Benthic communities are good environmental impact indicators as they respond in well-understood ways, and are important for the sustainability of their ecosystems. The priorities for protection of the environment are strengthened in these years, and the demands for information at higher resolution scales are continually rising. Thus, it is vital to develop methods and technology dedicated to deliver high-resolution information on the health of the environment, in particular the difficult observable conditions at sea.

State of the art is based on traditional methods of sampling and on evolving remote sensing methods. To use hydroacoustic recordings for estimation of benthic communities geostatistical methods can be applied. These methods are expected to give the best results, if they rely on structural models of the spatial objects to be estimated.

The first preliminary steps have been taken at the construction of the Fixed Link between Sweden and Denmark. Hydroacoustic monitoring of mussel distributions was applied in baseline studies of the environmental programme of the construction (Madsen, 1995; Sørensen 1994), and has been recommended for surveillance of mussel beds. Cross validation of distribution maps estimated from hydroacoustic records by geostatistical methods showed a satisfactory concordance with video recordings of the sea bottom. The results from these studies give expectations of a successful outcome of the BioSonar project.

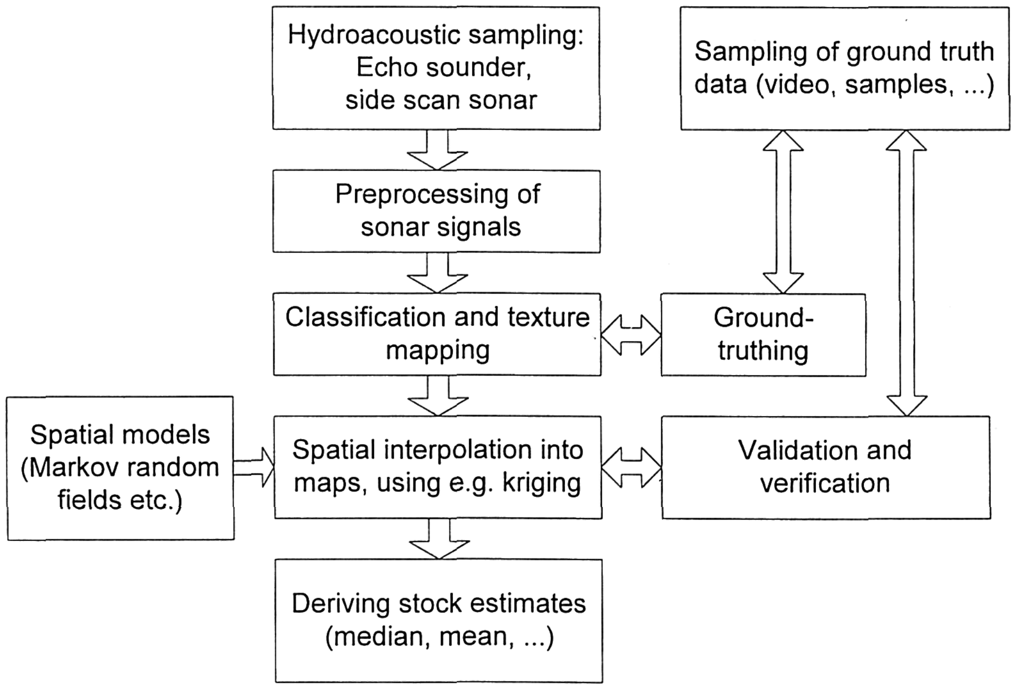

Figure 1 An outline of the data flow in BioSonar.

In BioSonar a range of methodologies are tested. They have a range of common properties, as outlined in Figure 1. The data processing spanning from the hydroacoustic sampling to the resulting maps basically consists of five steps, as depicted in the main column in Figure 1. The methodologies differ due to different contents within each process. For example, one methodology will rely solely on RoxAnn echo sounder measurements, and will thus differ in the sampling process from methodologies aiming at integration (data fusion) of both RoxAnn and side scan sonar signals. Another example is that kriging is applied to create maps from echo sounder signals whereas mosaicking is applied to create maps from side scan sonar images. The methodologies will also differ with respect to the ground truth data applied, and will depend on the nature and extent of the spatial models applied in the geostatistical estimation algorithms.

The BioSonar project is concerning how to produce reliable and precise distribution maps for benthic communities at optimised costs, that is applying as many automated processes as possible. One of the major challenges of our approach is that there are several chains in the process that need refurbishment and elaboration in order to produce the resulting distribution maps. The overall goals of the BioSonar project can be summarised as

- **Quantitative detection** (ability to detect benthic communities, and to characterise their density with satisfactory precision)
- **Reliability** (reproducible or traceable, objective methods)
- **Automation** (computerisation of all possible work processes)
- **Change detection** (methods for monitoring changes in benthic communities).

Thus, it is an objective to assess the precision by quantitative methods and to automate the processes as far as possible. The aim is to attain a degree of automation where the overall processing from the gathering of raw data till the preparation of the distribution maps to be inserted in GIS systems is computerised. Some of these processes are likely to require subjective decisions to be carried out during the use of computer programs, and might therefore not be totally objective or independent of the operator.

It should be underlined that the participants are approaching the project from an end user point of view. That is, it is the resulting maps of the distributions or intensities of the benthic communities in question that have been focused upon. The properties of the map generation process from measurements to the final maps are studied with the views of biologists, mathematicians and statisticians, but without considerations about instrument refinements or underwater acoustical elaborations.

BioSonar can partly be described as a project dealing with the transfer of space remote sensing techniques to acoustic applications within aquatic biology, along with the proper use of ground truthing to validate the extracted information. A long term goal of the project would be to develop techniques and procedures that would enable the detection and discrimination of, e.g *Caulerpa taxifolia* from other species, as this species has become an environmental problem due to its competition with other species in the Mediterranean.

WORK PLAN

The BioSonar project consists of an initial, analytical phase, which was finalised at 8 months, and two subsequent, intertwined phases of creating and testing methods for data analysis and data processing and different lines of data flow.

The purpose of the initial phase was to create a set of building blocks for the practical work to be carried out. The building blocks comprise

- an inventory of suitable statistical models and methods,
- selection of hydroacoustic instrumentation,
- preparation of sampling designs, and
- selection of end user tools: GIS systems.

Hydroacoustic instrumentation

For use in the project an echo sounder add-on device called RoxAnn and a side scan sonar add-on device called EOSCAN were selected. These are quite different hydroacoustic measurement devices, which gives a good span of available technology and provides a basis for subsequent data fusion analyses.

The RoxAnn device, consisting of a head amplifier, a parallel receiver and a software package, records time-integrated parts of the first and the second backscattered echo from the sea floor, called E1 and E2 respectively. The existence of a relation between sea floor morphology and E1 (sea floor roughness) and E2 (sea floor hardness) is detailed on an empirical basis in Chivers, Emerson & Burns (1990) and Chivers & Burns (1992). A theoretical justification for the said interpretation of E1 and E2 is given by Heald & Pace (1996). In the BioSonar field campaigns an echo sounder frequency at 200 kHz has been used. Preprocessing of the RoxAnn data before analysis includes among others triangulation to account for discreteness of the geographical co-ordinate locking from the connected DGPS device.

The GeoAcoustics side scan sonar device is operated at 100 kHz and 500 kHz and produces grey level scale data in a proprietary format, which can be postprocessed to yield data in a readable format and to ensure proper georeferencing of the recorded pixels.

FIELD CAMPAIGNS

The field campaigns were executed in the Mediterranean near Cartagena in the southeast part of Spain, and near Copenhagen in Øresund between Sweden and Denmark. The design of the field campaigns were based on three types of sea bed areas:

- Test areas (two areas have been selected at sizes between 1 - 2 km²)
- Bottom type areas (3 - 6 areas at a of size 100 x 100 metres)
- Footprint areas (one for each campaign having a size of 60 x 60 metres)

The purpose of the test areas was to collect mixed data representing real world cases. Thus, the intention was that the two test areas should be different e.g, one representing a density gradient of mussels and one centered on a mussel bed. Or, as in the Meditteranean case one test area representing an area of healthy neptune grass meadows (Cabo de Palos), and one representing an area of degraded meadows (Mazarron).

The purpose of the bottom type areas was to map practically uniform sea floor areas with solitary populations. The data from the so-called bottom type areas were used as references when the test areas are studied, and in preliminary studies of discriminating power of data.

The footprint area measurements were introduced to assess repeatability and to assess drift from day to day, i.e. as a possible calibration means. The repeatability should be assessed both for raw signals and for estimated areas.

In Øresund two test areas were applied, one located on the southern edge of Drogden and the other on the northwestern edge of Flinterenden. 3 bottom type areas were used in the Øresund, namely sand (100% coverage), a mussel bed (100% coverage), and solitary eelgrass (*Zostera marina*) meadow at approx. 100% coverage. The areas of the Øresund field campaigns are depicted in Figure 2.

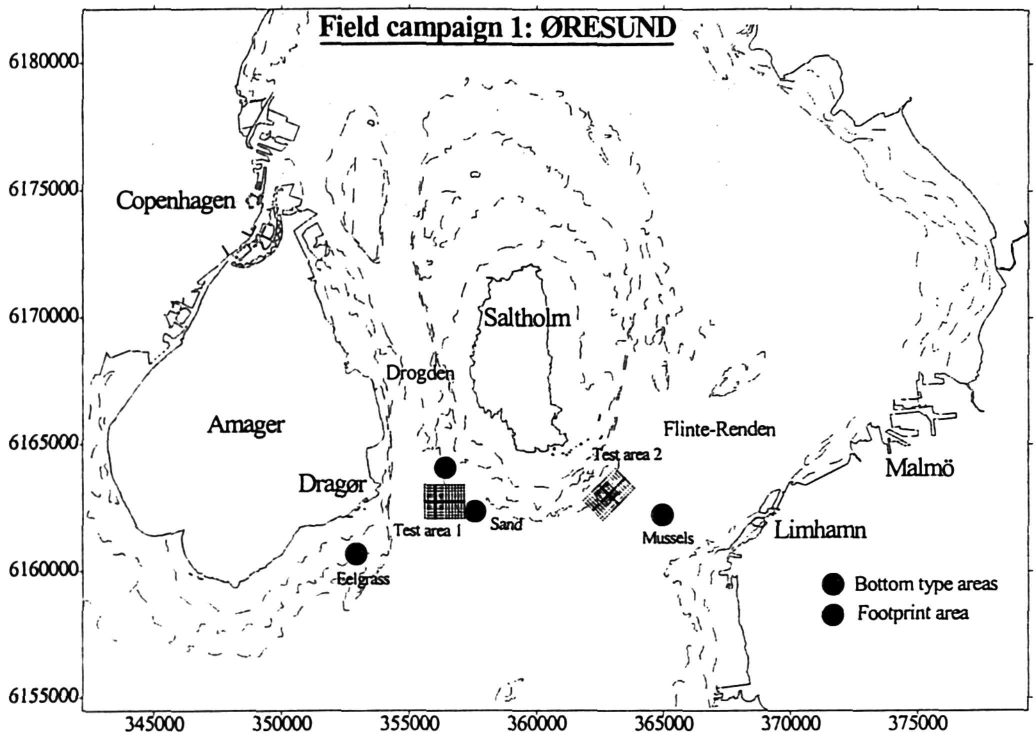

Figure 2. Geographical map including test areas etc. in Øresund. Coordinates are UTM 33 Northings and Eastings.

To study the spatial properties of the hydroacoustic measurements at various scales in the test areas a basic orthogonal transect grid having a distance between transects at 120 metres were applied. This basic transect grid were supplemented by a more dense zone consisting of a cross having 7 transects interspaced by 20 metres in each of the orthogonal directions, and a fringe on the cross consisting of 4 transects separated by distances at 60 metres. This is illustrated for test area 1 in Øresund in Figure 3.

In the Meditteranean area two test areas were devised too, and five bottom type areas, namely Sand (Cabo de Palos), Mud (Mazarron), Neptune grass (*Posidonia oceanica*) meadow 100% coverage (Cabo de Palos), degraded posidonia meadow (Mazarron) and a Cymodocea meadow 100% coverage (Cabo de Palos).

Ground truthing was prepared using video and still photos. Vertical video recordings were taken at fixed stations, primarily at intersection points in the transect grids. The video camera was mounted with a measuring tape. Still images were prepared for fixed stations by means of a photo-sampler. The images cover approx. 2 m² of the sea bottom.

A range of exogeneous and supplying variables were measured, including depth, temperature and salinity profiles, chlorophyll (when feasible), Seston and Transparency. Sea state was opted for, but the measurements were not implemented.

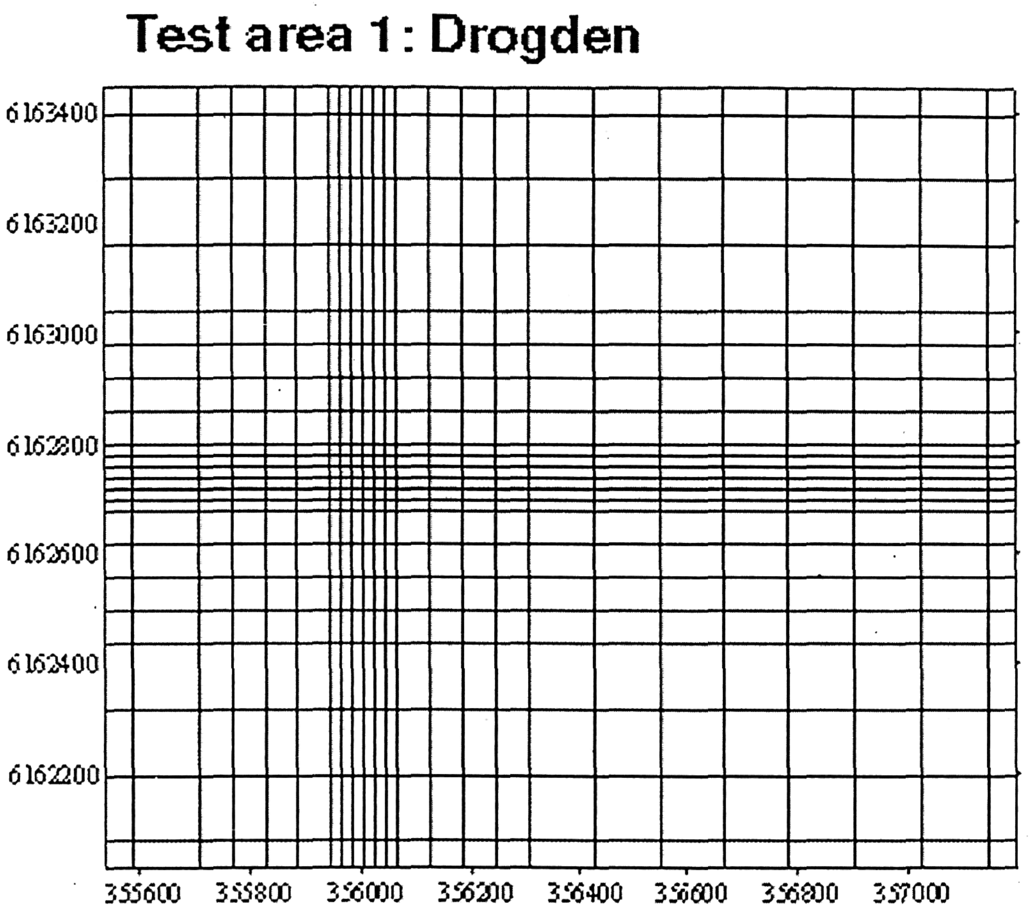

Figure 3. Test area 1, Øresund, with the dense cross positioned at a place presumed to have a potential gradient in mussel density. Coordinates are UTM 33 Northings and Eastings.

The field campaigns in the Meditteranean and in Øresund have been carried out in the two consecutive years 1996 and 1997. The areas in Øresund were surveyed in weeks 42 and 43 (last half of October) in 1996 and 1997, and the Meditteranean areas were surveyed in weeks 46 and 47 in 1996 and weeks 47 and 48 in 1997, i.e. the change detection analyses will not be affected by elementary seasonal variations.

RESULTS AND DISCUSSION

In the following we will try to describe the achievements made in the BioSonar project until now. Results from the initial phase comprised, among others, an inventory of spatial and geostatistical models, consisting of

- traditional spatial statistical models assuming 2. order stationarity,
- use of image descriptions based on Markov random fields,
- use of Bayesian discriminant analysis for classifications in feature space, as well as
- a non-parametric method based on so-called deformable templates;
- Boolean models with seeds as potential models for the spatial patterns

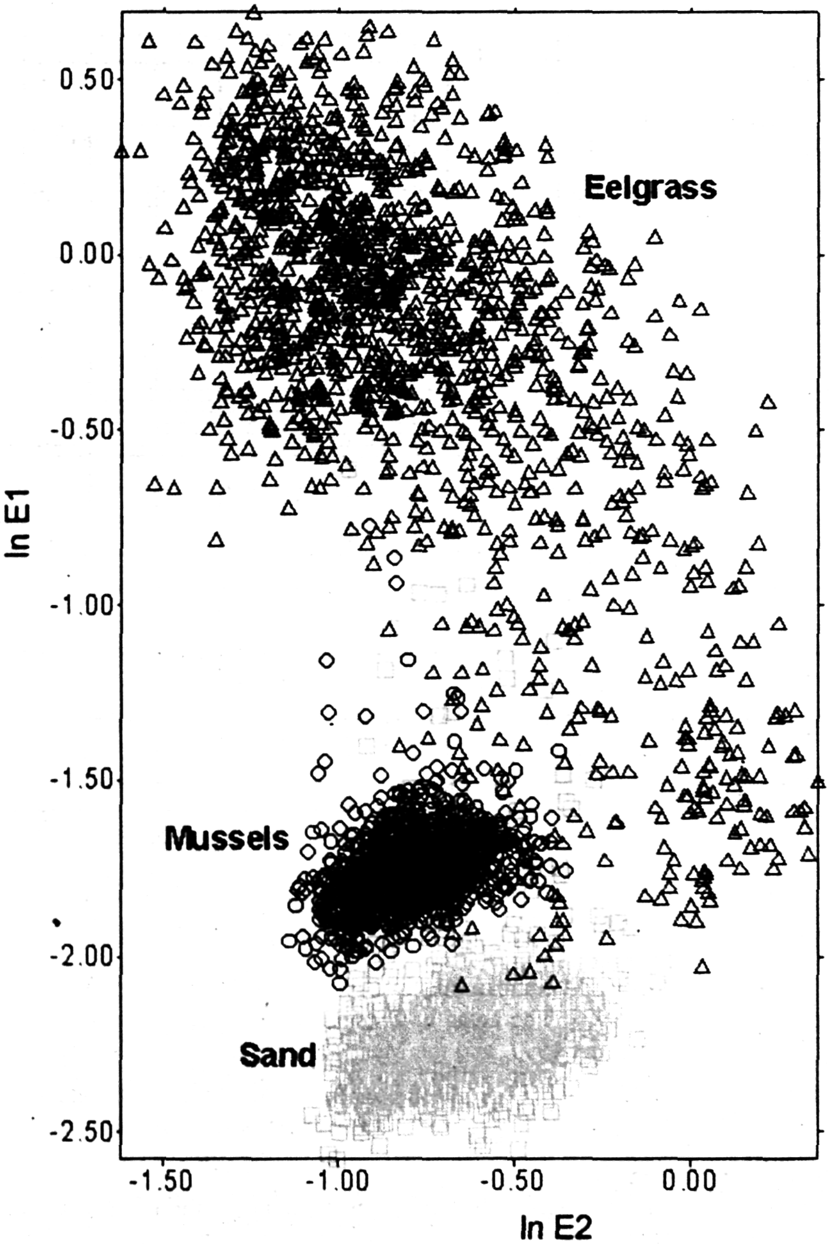

Figure 4. The RoxAnn observations made in the bottom type areas sand, eelgrass and mussels in the 1996 field campaign in Øresund depicted in the logarithmic feature space.

A description of the inventory of statistical models is given in the first annual report of the project, MAS3-CT95-0026 (1997).

The GIS systems Intergraph and MapInfo were chosen as end user tools.

Results achieved from data analyses

Initial data analyses focused on the possibility to distinguish hydroacoustic measurements in feature space. The feature space is the 2D space consisting of the logarithms of E1 and E2, i.e. vectors y = (lnE2, lnE1)', and is illustrated for the observations made in the bottom type areas in the 1996 field campaign in Øresund in Figure 4.

Bayesian discriminant analysis assuming Gaussian distributions of the bottom type observations were used to study the possibilities for segmentation of the sea floor in four distinct classes, being sand, mussel, eelgrass and other / reject class. The results yielded high resubstitution rates at 96% - 99% for the three bottom type classes, which establishes that the basic features of the observations can indeed be used to segment the sea floor in the said classes.

Use of the non-parametric method called deformable templates, which might be argued is more realistic as it does not rely on assumptions of a certain shape of distribution of the observations in feature space, achieved similar high resubstitution rates, depicted in Table 1.

Table 1. Confusion matrix for classified RoxAnn data bottom type areas from the 1996 field campaign in Øresund, using Deformable Templates. The average accuracy rate is 96.9%.

	Classification classes			
True bottom type	Mussel	Zostera	Sand	Total
Mussel	98.56%	0.54%	0.00%	100%
Zostera	1.25%	96.84%	0.51%	100%
Sand	2.89%	0.83%	95.39%	100%

Furthermore, a very high degree of overlap was found between test area 2 in Øresund known to consist merely of one large mussel bed and the mussel bottom type area, as depicted in Figure 5. As can be seen, the observations from the two mussel bed locations overlap substantially.

Exogeneous variables

Concentrations of suspended matter were practically constant during the field campaigns and have thus had no influence on the measurements. In some cases changes in salinity affected signals from the side scan sonar. Long-term effects on benthic communities from changes in salinity do not lie within the scope of the BioSonar project. However, depth is an exogeneous variable that has been given some attention.

A series of analyses of variance employing a range of models including the two factors bottom type and depth and their interactions were conducted. The overall impression of the results is that, based on the data sets from the 1996 field campaign, the RoxAnn measurements and the water depth is not very strongly dependent, E2 being slightly more dependent on depth than E1.

Studies of the footprint areas yielded very similar signatures for a transect at a length of 60 metres, having an average standard deviation for lnE1 at 0.36 and for lnE2 at 0.60, reflecting that the footprint signatures for E2 seems to be more noisy than the signatures for E1.

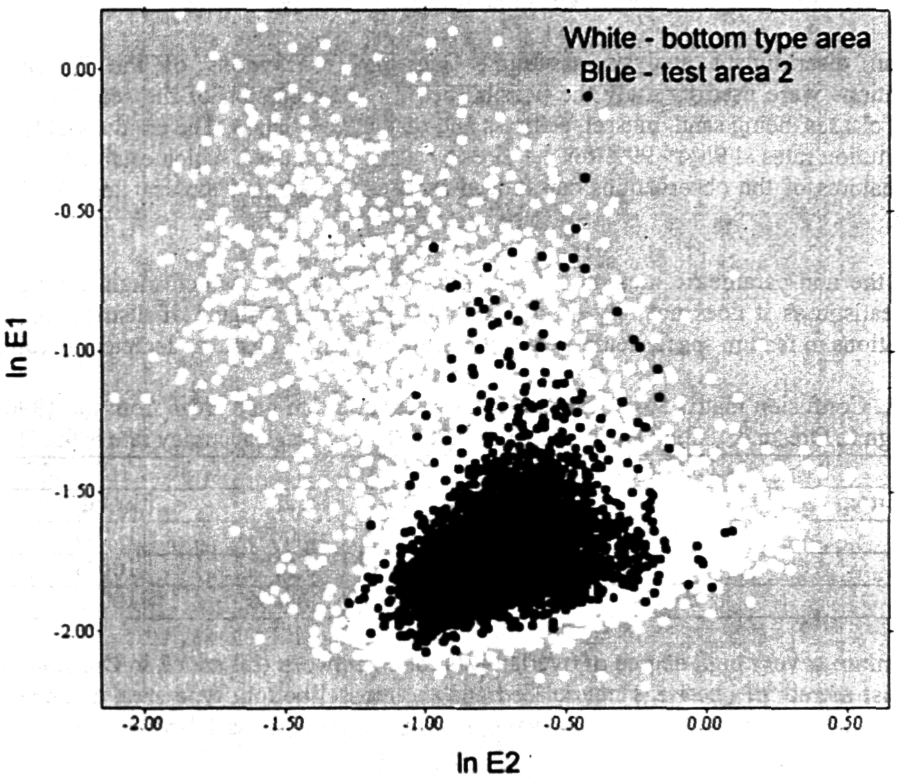

Figure 5. Overlap in 2D-feature space between mussel bottom type area and test area 2 in the 1996 field campaign in Øresund.

Ground truthing

The still photos and video recordings made in intersection grid points respectively along every 5th transect were used to check the classifications in feature space of the RoxAnn measurements. The inspection of ground truthing proved crucial to the success of data processing when it was found that a part of the Eelgrass bottom type in Øresund actually consisted of sand beds, which resolved some obstacles in the classification analyses.

Aerial flight photos available for the Eelgrass bottom type have been used for verification as well, with good results.

Classification of side scan sonar images

Classifications based on an edge-preserving filter have been tested on a range of recorded side scan sonar images. An example of a resulting confusion matrix is given in Table 2.

Table 2. Confusion matrix for classified bottom type areas from the 1996 field campaign in Øresund. The average accuracy is 40.8%.

	Classification classes			
True bottom type	Mussel	Zostera	Sand	Total
Mussel	58.52%	10.24%	31.24%	100%
Zostera	43.88%	11.23%	44.90%	100%
Sand	37.41%	9.93%	52.66%	100%

In general, the results are not satisfactory due to a high confusion degree between classes visible in the confusion matrices. This problem is not due to the methodology in itself, because the same one was applied in the still photo location with a much better result (the High Diversity Area Training Set was able to recognize most of the species, especially in the Palos Test Area).

The problem appearing in the Bottom Types processed, as a whole is the mosaicking effect. In the present case, two image registers were mosaicked to comprise the whole Bottom Type. These registers have always the same direction and, even so, the errors introduced are numerous. Among them, a different acoustic response given by the same Bottom Type in the different registers used has to be highlighted. As the classification procedure is based on the Digital Values of each pixel, these errors imply that the same Bottom Type is separated in different classes. As the Training Set has to comprise all the classes to be found in each Bottom Type, the confusion leads to a misclassification. In conclusion, a textural image analysis is recommended, to be added to the classification only based on digital numbers that gives satisfactory results when processing independent, not mosaicked scenes.

Spatial variability

For the RoxAnn observations, exhibiting the necessary segmentation or discrimination abilities in feature space, the spatial variabilities were analysed using the geostatistical technique of calculating and interpreting semivariograms, see e.g. Isaaks & Srivastava 1989. A property of the semivariograms is that they provide information on the range of the spatial correlation of data, R. Locations at a distance greater than R from each other can be assumed to be uncorrelated. To be able to interpolate distribution maps by e.g. kriging it is a requirement that the distance between transects is less than R to ensure a basic correlation structure in the interpolated maps.

The ranges, R, derived from the analyses carried out at present vary between 25 and 80 metres, yielding prescriptions for a second-step sampling of the studied areas at transect distances no greater than 40 metres, i.e. using R/2 as a rule of thumb. In conclusion, a recommendation of a two-step approach seems likely to be the outcome for larger-scale areas. In this approach the first step will be to study a localised subset region of the area in question, considered to be typical or representative for the local properties of the whole area. Subsequently, from the geostatistical analysis of the data thus obtained, it can be inferred what the proper transect distance for a survey of the whole area should be.

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REFERENCES

Chivers, R.C., Emerson, N. and Burns, D.R. (1990): New Acoustic Processing for Underway Surveying. The Hydrographic Journal Vol 56, April 1990, pp. 9-17.

Chivers, R. & Burns, D. (1992): Acoustic surveying of the sea bed. Acoustics Bulletin, Vol. 17(1), pp. 5-9.

Conradsen, K. (1984): En introduktion til statistik, 1a, 1b, 2a og 2b (In Danish). Institute of Mathematical Statistics and Operations Research, Technical University of Denmark, Lyngby.

Cressie, N.A.C (1991): Statistics for Spatial Data. John Wiley & Sons Inc., New York.

Heald, G.J. & Pace, N.G. (1996): An analysis of the 1st and 2nd backscatter for seabed classification. Proceedings of the 3rd European Conference on Underwater Acoustics, 24-28. June 1996, Vol II, pp. 649-654.

Isaaks, E.H. & Srivastava, R.M. (1989): An Introduction to Applied Geostatistics. Oxford University Press, New York. 561 pp.

Madsen, K.N. (1995): Supplementary mapping of the mussel beds along the alignment in the Øresund. Report from VKI to Øresundskonsortiet A/S, 27 pages.

MAS3-CT95-0026 (1997): Scientific Report for Year 1 of the BioSonar Project. Report prepared by the BioSonar project partners to the MAST Office of the European Union.

Sørensen, P.S. and Madsen, K.N. (1994): The Statistical Basis for Use of RoxAnn Measurements to Prepare Distribution Maps. Report from VKI to Øresundskonsortiet A/S, 53 pages.

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THE REMOTE DETECTION OF SEDIMENT INSTABILITY ON THE EUROPEAN CONTINENTAL MARGIN AND SLOPE

POSEIDON

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SUMMARY

The aim of POSEIDON was to develop deep water, high resolution instruments and methods for use in seafloor stability studies. The project began on 1 February 1996 and ran for two years. The main results are: a 6,000 m rated 240 kHz multibeam sonar for centimetre-scale seafloor bathymetric and sidescan measurements; a 2,000 m rated seafloor lander for in-situ seismo-acoustic and geotechnical measurements; and a slope stability database for integrating, displaying and interpreting marine survey datasets. Ways to improve slope stability hazard mapping were investigated and laboratory scale model studies of slope stability mechanisms were carried out.

INTRODUCTION

Slope stability is a potential hazard to oil, pipeline and cable companies as they extend their activities beyond the continental shelf. There is abundant evidence for giant submarine landslides in the recent geological record, mostly originating at the shelf edge where the seafloor falls away more steeply (Masson, Kenyon & Weaver, 1996). One example is the Storegga Slide off the coast of Norway where an estimated 5,600 km^3 of sediment was involved (Bugge, Belderson & Kenyon, 1988; Evans, King, Kenyon, Brett & Wallis, 1996). Of course, not all submarine landslides are as large as Storegga, but even small slips and slumps represent a potential threat. Unfortunately, our knowledge of marine sediment stability is still quite limited; much more work is needed before we can reliably predict when and where slope failure will occur. In particular, making detailed, quantitative measurements of sediment properties presents a major challenge.

Detailed slope stability studies require high resolution instruments that can operate in water depths up to 2,000 m and deeper. Both in-situ and remote measurements are needed to quantify, map and monitor the physical properties of the seafloor and the upper 200 - 300 m of the sediment column. Perhaps the most important considerations for future studies are the design of integrated surveys, and the bringing together of disparate datasets for unified interpretations.

While seismo-acoustic methods offer an efficient means of imaging the seafloor and sub-bottom, geotechnical drilling and seafloor measurements are the only reliable way of determining the sediment engineering properties at present. However, there is an increasing interest in the possible use of seismo-acoustic methods for predicting sediment geotechnical properties (e.g. Hovem, Richardson & Stoll, 1991). The knowledge gained from seafloor studies involving integrated remote and in-situ datasets would help towards achieving this goal.

The general aim of POSEIDON is to consolidate and to promote expertise within the European Union for slope stability hazard assessment. The specific objectives were:

1. To develop new and existing deep water instruments and techniques for detailed seafloor and sub-bottom sediment characterisation;
2. To create a slope stability database tool for improved interpretation of geophysical and geological datasets in terms of hazard assessment;
3. To perform laboratory scale model studies of slope failure mechanisms and their acoustic responses so that we may better recognise real life slope failure indicators.

INSTRUMENT DEVELOPMENT

Sonars

Bathymetric soundings and sidescan imaging are needed to identify seafloor features, such as pock marks or faults, that may indicate an unstable slope. Two instruments have been developed in POSEIDON that fit into this category, the Seabat, and the Scatterometer.

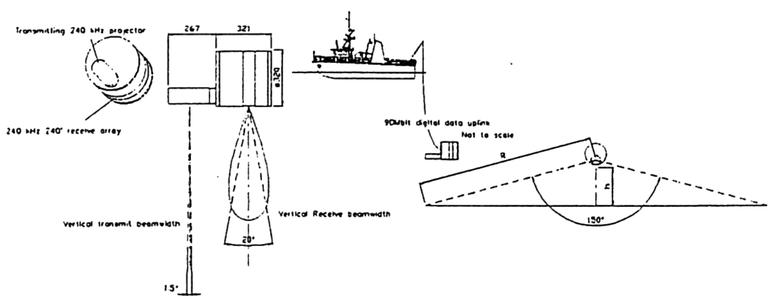

Figure 1. The deep water, 240 kHz, Seabat multibeam sonar.

The Seabat (RESON A/S) is a 240 kHz multibeam sonar rated to 1,500 m. The development work was conducted in collaboration with the Technical University of Denmark who modelled the deep water behaviour of the transducer housing. The deep water Seabat is capable of operating simultaneously as a bathymetric sounder and as a geological sidescan. The Seabat has a maximum range of about 300 – 500 m (*R* in Figure 1) and gives a 150° swath. This is achieved by using two crossed arrays. The receiver is a 240° forward looking array of 160 single ceramic elements with a horizontal beamwidth of 1.5° and a vertical beamwidth of 20°. The transmitter has a horizontal directivity of 150° and a vertical directivity of about 1.5 °. The system became commercially available in Autumn 1997 as a direct result of the POSEIDON investment.

The Scatterometer (Southampton Oceanography Centre) is a parametric array capable of projecting a narrow beam of sound at the seafloor in the frequency range. The beam width is about 3°, approximately constant over the frequency range 3.5 - 15 kHz. The beam can be steered electronically so that backscatter strength can be investigated as a function of grazing angle (Figure 2). The Scatterometer can also be used as a high resolution sediment profiler, and has possibilities for generating surface waves that can be detected by seafloor instruments. The array itself is hexagonal, about 60 cm wide, and is made up of 721 individual piezoelectric elements (Somers, Flewellen & Huggett, 1994). The major development during POSEIDON was the adaptation of the array to act as both source and receiver. In the previous version, a separate end-fire array was deployed from the deep towed vehicle on which it was mounted (TOBI; Flewellen, Millard & Rouse, 1993), but proved to be too fragile during se trials. The use of a single array makes this instrument much more robust and easier to fit to deep towed vehicles.

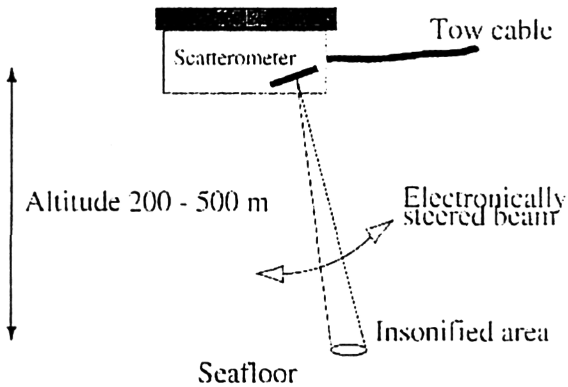

Figure 2. Mode of operation of the Scatterometer when mounted on a deep towed vehicle such as TOBI.

The Seabat and Scatterometer will enable very high resolution bathymetry and backscatter strength measurements to be conducted on particular areas of interest, such as within a pock mark or on a single bottom type. The information obtained could be used to calibrate lower-resolution sidescan sonar images in terms of sediment properties, and could even be used, for example, to detect the initial stages of gas build-up and escape at the seafloor.

Seismics

Seismic methods are important for defining sub-bottom geological structures. They are also increasingly being used to extract sediment physical properties through the use of inversion schemes and models (amplitude variation with offset analysis, for example). Seafloor receivers offer better signal to noise ratios over surface towed systems and potentially higher resolution. They can be deployed in a grid pattern over the survey area and used in conjunction with a range of acoustic source types.

The High Frequency Ocean Bottom Hydrophone system (HF-OBH; GEOMAR) offers higher resolution over traditional ocean bottom receivers. They are rated to full ocean depth (6,000 m) and can be configured to receive signal frequencies in the range 0.1 – 4,500 Hz. This frequency range is particularly useful for determining the detailed compressional wave velocity distribution of the uppermost 200 - 300 m of deep ocean sediments. It enables high-resolution seismic images of sediment structure to be obtained. A previous version has already been successfully used to map gas bearing sediment layers on the Norwegian continental margin (Mienert, Posewang & Baumann, 1997; Posewang, 1997). The development work in POSEIDON involved upgrading the HF-OBH's recording system so that the sampling rate and record duration could be configured to suit the particular sub-bottom target. The system is shown in Figure 3.

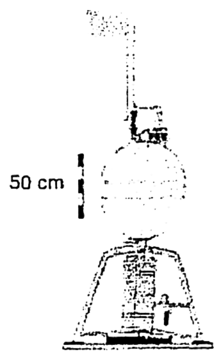

Figure 3. The high frequency ocean bottom hydrophone system (HF-OBH).

In-situ

While geotechnical drilling is the most conclusive way of demonstrating sub-bottom sediment properties (e.g. Pelletier, Doyle & Dutt, 1997), in-situ measurements in seafloor sediments still have their value. Indeed, field data and theory show that sediment elastic properties vary most rapidly in the 10 m below seafloor (Stoll, 1989). Accurately measured seabed properties are important for input to seismic inversion schemes for extracting deeper sediment information.

Two in-situ instruments have featured in POSEIDON: the SAPPA (Sediment Acoustic and Physical Properties Apparatus) which has been developed entirely within the project; and the Geotechnical Module, an existing instrument to which new sensors have been added.

The SAPPA (Southampton Oceanography Centre) is a seabed lander for deploying a range of geophysical and geotechnical sensors. By making both geophysical and geotechnical measurements at the same time, it will enable investigations into the inter-relationships among sediment properties under in-situ conditions. At present it is designed to operate in water depths up to 2,000 m on a load-bearing, conducting cable and will eventually be able to work to 6,000 m using acoustic telemetry for command and control. Shallow water sea trials have demonstrated that S-wave data can be quickly acquired once it is on the seafloor, and several sites can be visited by lifting it off the bottom and moving to a nearby location. The system successfully acquired horizontally and vertically polarised shear wave datasets during a recent environmental study in the Arabian Gulf (Kenyon et al, 1997).

Figure 4 shows how the SAPPA probes will be used to acquire "crosshole" P-wave data in the upper 1 m of sediment. While the P-wave measurement system has not yet been tested at sea, it gave broadband signals in the frequency range 1 - 10 kHz in the SOC water tank. The S-waves signals are recorded using a three-component geophone (orthogonal x, y & z axes); example S-wave data are shown in Figure 5 for wave propagation through beach sand.

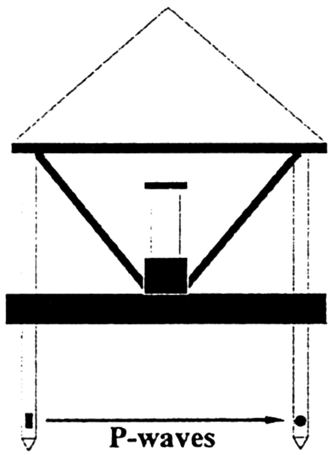

Figure 4. P-wave measurements with SAPPA – "crosshole" mode.

The Geotechnical Module (IFREMER) is also a seabed lander and was designed to make shear strength measurements on deep ocean soft sediments. It can visit several stations by lifting off and moving close to the seabed while suspended by cable from the operating vessel. Its probes can penetrate to a sub-bottom depth of 2 m and it can take a single 2 m long push core for each deployment. During POSEIDON it has been furnished with new temperature and pore pressure sensors that will enable seafloor stability conditions to be accurately determined.

DATA INTEGRATION

Slope stability database

An ideal slope stability study would comprise regional sidescan sonar, bathymetry and seismic surveys followed by more detailed sidescan, bathymetry and seismics on areas of interest, including geological sampling and in-situ measurements. Given that these measurement techniques, some of which were discussed above, cannot all be carried out at the same time, the coregistration of datasets becomes an important issue, especially for the high resolution work where features a few centimetres in size become significant. While methods to improve the absolute position fixing of datasets in geographic coordinates were not addressed in POSEIDON, we have concentrated instead on creating a database tool for integrating these disparate datasets. This will enable the maximum amount of information to be extracted from the available data, and should provide new insights into datasets not previously interpreted in a unified fashion.

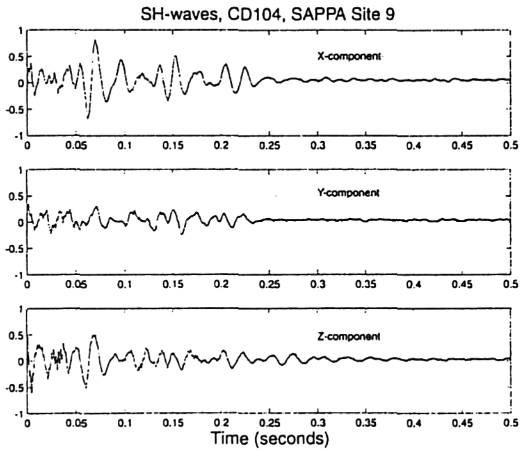

Figure 5. Example three-component SH-wave data recorded by the SAPPA on a sandy seafloor.

The POSEIDON database system (DBS), created by the Vriji Universiteit Brussel, has automated a number of mapping and spatial analysis tasks. It enables the manipulation of very large bathymetric and attribute datasets and allows spatial query, spatial analysis and modelling. The analysis and modelling software comprises: an Intergraph Modular Geographical Information System (GIS) Environment and Bentley MicroStation GeoGraphics for spatial and graphic data analysis; and an Oracle relational database for data storage and queries.

Storegga example

It is difficult to demonstrate the capability of a DBS without reference to a specific example. The original intention of the project was to use the technologies developed in POSEIDON-I (funded) to conduct a dedicated marine experiment in POSEIDON-II (not funded). The Storegga Slide was chosen as a focus for our studies because of the active interest in that area by offshore industries. A small region, approximately 10 nm by 10 nm (see Figure 6) was chosen on the northern edge of the slide scar for detailed studies. This target area contains three areas of interest:

1. Undisturbed sediments with gas escape features (pock marks);
2. Disturbed sediments of the actual slide;
3. The transition zone between the above.

Despite the lack of new data, we were able to carry out a preliminary study using existing datasets provided by GEOMAR. These were processed by IFREMER and used by the Free University of Brussels to populate the POSEIDON DBS.

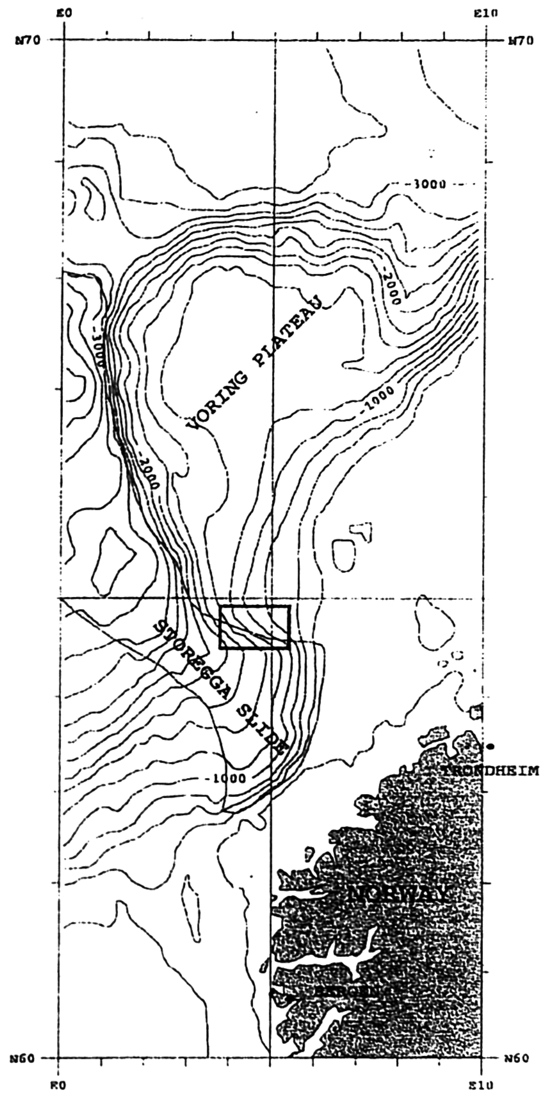

Figure 6. Location of the Storegga Slide and the POSEIDON study area.

Graphic information

The graphic components largely consist of bathymetric information and sidescan sonar acoustic backscatter strength images. At present, only a small amount of bathymetric data for Storegga is available giving only partial coverage. The digital file of existing data is in vector digital format, containing isobaths with attached depths in 10 metre intervals and basic graphic objects such as points, lines, polygons and text. The spatial database was constructed using the following steps:

- Feature creation;
- Define feature level and symbols;

- Feature associations.

Three feature types were recognised, summarised in Table 1. Colour, line weight and line style were assigned to give a level structure, which minimises the redundancy of redrawing and optimises design work and production (Van Biesen, Peirlinckx, Cisneros and Yamba, 1997). Each feature was then assigned to associated attribute tables (e.g. location and geotechnical properties from in-situ or laboratory core measurements). Table 2 gives the information used to set up the POSEIDON DBS map projection and coordinate system. The latter were chosen to provide a consistent format and to define the level of precision for the data.

Feature name	**Feature type**
depth_contour	*Line*
depth_contour_value	*Label*
sampling_site	*Point or area centroid*

Table 1. Definition of Storegga graphic features.

Norwegian Sea Etop5, NGDC & GEBCO data	N 55°00′ W 20°00′	N 70°00′ E 20°00′
Storegga Slide NGDC & GEBCO data	N 62°00′ E 2°00′	N66°00′ E 7°00′
Storegga Slide Northern Scarp Hydrosweep data	N 64°35′ E 3°50′	N 64°45′ E 4°30′

Table 2. Geographical coordinates and origins of bathymetric datasets for the digital terrain models.

Relational database

All non-graphic data or descriptive attribute information, such as core log information, sedimentological descriptions, or in-situ geotechnical data are stored using Oracle database management system (DBMS) software. The DBMS enables the definition, creation and maintenance of the database, and gives controlled access to the information stored in it. One important process associated with the DBMS is the normalisation of attributes; that is, the process of converting complex relations into a larger number of simpler relations that satisfy the relational rules. It involves the study of the type, format, dimensions and interrelations of the data to be stored. It simplifies tables and eliminates ambiguous and repeated groups of attributes, and avoids anomalies.

The relevant slope stability parameters were defined according to current knowledge of slope stability processes and available core data. The following variables were identified:

1. Geophysical parameters: resistivity; compressional and shear wave velocity and attenuation; seafloor temperature; seafloor pressure as a function of time; sediment depth; etc.
2. Geotechnical parameters for fine grained sediments: sediment shear strength and compressibility; acoustic velocity; porosity and pore pressure; temperature; permeability; consolidation; cohesion; granulometry; heterogeneities; mineralogy; colour; sediment density; magnetic susceptibility; calcimetry; datation; X-radiography, etc.

Each data type is accompanied by basic information on time and spatial location recorded in latitude and longitude coordinates, or in the coordinates of one of the standard cartographic projections, or in arbitrary rectilinear coordinates with a local origin. Other information stored includes sediment depth, description of the laboratory analysis programme and documentation (published or unpublished). At present, we only have data from one core station (M26/3-529), so the work we have been able to do with regard to normalisation, etc. has been severely limited.

The sediment attributes are stored as rows in tables. Each row contains one or more columns for which a value can be entered. The tables were defined using Structured Query Language (SQL) and Data Definition Language (DDL). SQL*Loader was used to load data from external files.

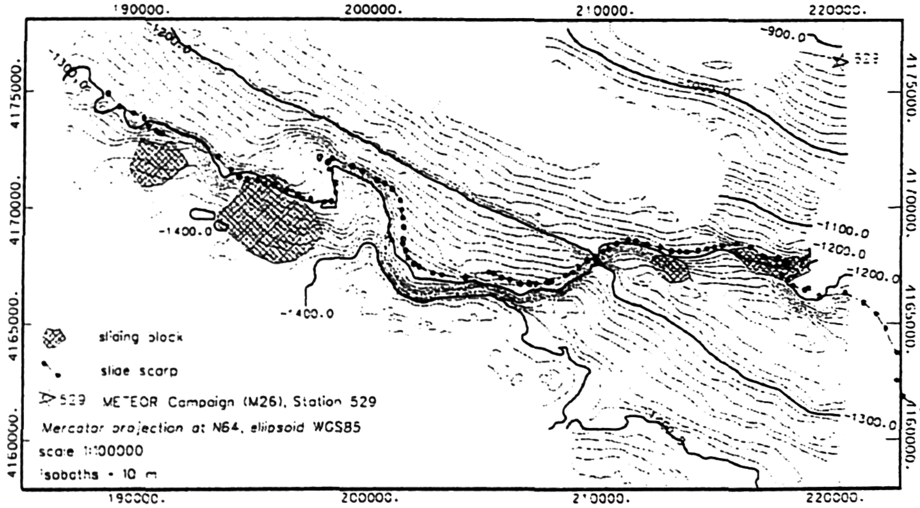

Figure 7. Digital terrain model of POSEIDON study area showing location of slide blocks, as recognised from the POSEIDON DBS 3D visualisation (not shown).

All the records are viewed and manipulated with SQL. The GIS part of the POSEIDON DBS allows two methods for accessing the data: direct access through the GIS software tools that contain a menu of database queries; and access through the relational DBMS which allows researchers to access data by station attribute and data type.

Slope stability modelling

It was not possible to allocate geotechnical zones in the Storegga study area because of the lack of data. However, it was possible to produce a three dimensional (3D), digital terrain model (DTM) and depth surface models using the POSEIDON DBS tools. These models take some time to produce, but are a necessary step for slope stability analyses. The 3D visualisation of the data enabled slide scarps and sliding blocks to be recognised, illustrated in two dimensions in Figure 7.

The general procedure was as follows:

1. Input 2D bathymetry and set up coordinate system;
2. Edit and complex contours;
3. Convert 2D contours to 3D contour map and tag contours with water depth values;
4. Build DTM or TIN (Triangulated Irregular Network);
5. Display and analyse the DTM.

In view of the limitations of the POSEIDON Storegga dataset, IFREMER conducted an investigation into how existing hazard modelling procedures may be improved drawing on their experience in other submarine slide areas, such as the Nice Slide.

They found that traditional continuum mechanics method cannot be applied in practise to the study of slope stability mainly because of the inherent heterogeneity of marine sediment physical properties. Other factors include the presence of fluids under pressure within the sediment pores and the complex geometry of natural slides. Although complex sediment characteristics can be handled fairly well in terrestrial environments using finite element modelling, this is not the case for submarine environments where data are hard to acquire and are often sparsely distributed.

In practice, different levels of accuracy can be obtained for describing sediment geometric and physical characteristics depending on the scale of investigation. At a very local scale, a limit equilibrium method such as the method of slices (Puech & Dendani, 1993) may be appropriate, whereas at a more regional scale (several hundreds of metres to several kilometres) the amount of data usually does not allow more than a crude interpolation of the physical characteristics of the sediments over the survey area.

To investigate the global stability of an area, the slope gradient will generally be the determining factor along with the cohesion of the sediment. The most useful method is the infinite slope approximation (e.g. Skempton, 1964), whenever it is geometrically applicable. In a complex gullied or irregular topography, only local stability can be assessed (that is at the scale of a scarp or a canyon wall). To better constrain the infinite slope stability model, a good understanding is needed of the deep structure of the area, such as the identification of possible slip surfaces, as well as the identification of possible fluid circulation patterns or of any source of excess pore pressure through the porous medium.

It is clear that we need to improve the way in which we assess in-situ parameters, our understanding of fluid circulation (e.g. gas), and how excess pore pressure affects sediment strength properties. Without these advancements, there is no way of progressing beyond the rather crude infinite slope method.

LABORATORY EXPERIMENTS

Gassy sediments and slope stability

Gas hydrates comprise a mixtures of frozen water and gas that forms under specific temperature and pressure conditions (Kvenvolden, 1993; Soloviev & Ginsburg, 1994). They are most often identified by the presence of a "Bottom Simulating Reflector" (BSR) on seismic records, caused by the acoustic impedance contrast between the accumulated gas and the overlying hydrated sediments. The pressure and temperature phase boundary tends to mirror the seabed, and so BSRs may cut across geological horizons. They tend to form in seafloor sediments in water depths of 300 to 500 m, that is, they occur on the continental shelf edge and upper slope where many submarine landslides originate (e.g. Miles, 1995). At Storegga, seismic profiles show that the main slide plane occurs at the same sub-surface depth as a BSR in adjacent undisturbed sediments. The decomposition of the hydrate at its base will tend to generate excess pore fluid pressures in the sediments and will lead to a reduction in shear strength. This is one possible mechanism for the sliding. Other features recognised at Storegga include gas chimneys on seismic sections, and pock marks on sidescan sonar images indicating the presence of free gas.

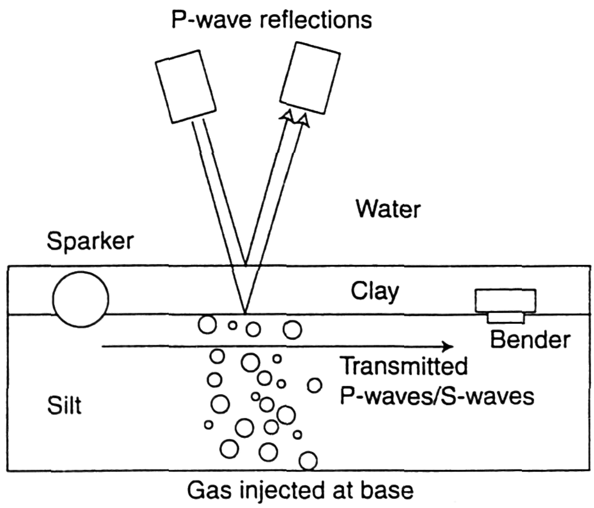

Figure 8. Experimental setup showing components referred to in text.

Water tank experiments

The aim was to investigate how we might recognise slope failure indicators on seismo-acoustic records before, during and after a slide event, with particular reference to gassy sediments. The experiments were carried out at CNRS-Marseilles using a specially designed acquisition set-up (CPE-Lyon). Two sediment models were used: a one layer silt model, and a two layer clay/silt model. Gas (air) was injected at the base of the models and changes in acoustic signals were monitored; changes in surface morphology were also logged using video cameras. Broadband seismic signals were generated by a mini-sparker planted in the sediment. S-wave signals (< 1 kHz) were detected using bender elements buried within the sediment. Separate P-wave (500 kHz) transducers were used to measure reflections from the water/clay and clay/silt interfaces. Both active and passive experiments were performed.

The results are still being analysed, but they indicate that it is possible to monitor gas build-up and migration using seismo-acoustic methods.

CONCLUSIONS

The main achievements of POSEIDON to date are:

1. A commercially available deep water, high resolution, multibeam sonar;
2. A new seabed lander for in-situ geoacoustic and geotechnical measurements;
3. A dedicated database management tool for integrating, manipulating, modelling and interpreting slope stability datasets;

We intend to use the knowledge gained from the laboratory tank experimental results, and from the Storegga historical data analysis during future marine slope stability experiments.

REFERENCES

Bugge, T., Belderson, R.H. & Kenyon, N.H., 1988. The Storegga Slide, Phil. Trans. R. Soc. Lond., A 325, 357-388.

Evans, D., King, E.L., Kenyon, N.H., Brett, C. & Wallis, D., 1996. Evidence for long-term instability in the Storegga Slide region off western Norway, Marine Geology, 130, 281-292.

Flewellen, C. G., Millard, N. & Rouse, I. 1993. TOBI, a vehicle for deep ocean survey. Electronics and Communication Engineering Journal, April , 85 - 93.

Hovem, J. M., Richardson, M. D. & Stoll, R. D. (Eds.), 1991, Shear waves in marine sediments. Kluwer Academic, Dordrecht, pp. 593.

Kenyon, N. H. et al, 1997, Geological processes in the Strait of Hormuz, Arabian Gulf: a contribution to the Scheherezade Programme. RRS Charles Darwin Cruise 104 Leg 2. Southampton Oceanography Centre Cruise Report, 11, pp. 61.

Kvenvolden, K. A., 1993, A primer of gas hydrates. In: Howell, D. G. (ed.) The future of energy gases. U.S.G.S. Prof. Paper 1570, 279-291.

Masson, D. G., Kenyon, N. H. & Weaver, P. P. E., 1996, Slides, debris flows and turbidity currents. In, C. P. Summerhayes & S. A. Thorpe (Eds.), Oceanography, an illustrated guide. Manson. 136–151.

Mienert, J., Posewang, J. & Baumann, M., 1997. Geophysical signature of gas hydrates along the north-eastern Atlantic Margin: possible hydrate-bound margin instabilities and possible transfer of methane from oceanosphere to atmosphere. Geol. Soc. Lond. Spec. Publ. (in press).

Miles, P.R., 1995. Potential distribution of methane hydrate beneath the European continental margins. Geophysical Research Letters, 22(23):3179-3182.

Pelletier, J. H., Doyle, E. H. & Dutt, R. N.,1997, Deepwater geotechnical investigations in the Gulf of Mexico. Underwater Technology, Vol. 22 No. 2, 63-73.

Posewang, J., 1997. Nachweis von Gashydraten und freiem Gas in den Sedimenten des Nordwesteuropaeischen Kontinentalabhangs mit hochaufloesenden reflexionsseismischen Methoden und HF-OBS Daten. Berichte aus dem Sonderforschungsbereich 313, PhD Thesis University of Kiel, Germany pp 137.

Puech, A., Dendani, H., 1993, Modélisation des instabilités des pentes sous-marines. In: Projet Mers Profondes. CLAROM.

Skempton, A.W., 1964, The long-term stability of slopes, Géotechnique, 14, 77-101

Soloviev, V. A. & Ginsburg, G. D., 1994, Formation of submarine gas hydrates. Bull. Geol. Soc. Denmark, 41, 86-94.

Somers, M. L., Flewellen, C. G. & Huggett, Q. J., 1994, The IOSDL acoustic scatterometer for sediment studies, in "Proceedings of the Second European Conference on Underwater Acoustics", L. Bjørnø (ed.), Lyngby (DK), July 4 - 8, Volume 1, pp497-502, Office for Official Publications of the European Communities, L-2985 Luxembourg.

Stoll, R. D.,1989, Sediment Acoustics. Lecture notes in earth sciences, S. Battacharji, G. M. Friedman, H. J. Neugebauer & A. Seilacher (Eds.), No. 26, Springer Verlag, pp. 154.

Van Biesen, L., Peirlinckx, L., Cisneros, Z. et Yamba, P., 1997. Impact of measurement science in sustainable management. In: New Measurements-Challenges and Visions, XIV IMEKO WORLD CONGRESS, Tampere, Finland, 1-6 June 1997, vol.Xa, 97-102. In: New Measurements-Challenges and Visions, XIV IMEKO WORLD CONGRESS, Tampere, Finland, 1-6 June 1997, vol. Xa, 97-102.

TITLE: **HOLOMAR:**
High resolution *in situ* HOLOgraphic recording and analysis of MARine organisms and particles

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HIGH RESOLUTION *IN SITU* HOLOGRAPHIC RECORDING AND ANALYSIS OF MARINE ORGANISMS AND PARTICLES (HOLOMAR)

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SUMMARY

The overall objective of HOLOMAR is to develop, construct and evaluate a fully-functioning, prototype, underwater holographic camera (holo-camera) and associated replay system for large-volume holographic recording and analysis of marine organisms (marine plankton and seston) within the upper water column. The camera will allow holograms of partially overlapping volumes to be simultaneously recorded with either an in-line (object in transmission) or an off-axis (object in reflection) holographic geometry. A dedicated, hologram replay facility will be developed in parallel with the holo-camera, containing fully-automated image analysis and data extraction facilities which will allow species identification and measurement of local concentration of a variety of marine organisms. The overriding benefit of developing this unique method of high resolution, 3D imaging is that it allows the non-intrusive, *in-situ*, recording of living organisms and inanimate particles in their natural environment. Furthermore, it affords the ability to gain knowledge of the behaviour of marine biological communities, their relationship with each other and with the particles with which they interact. This has hitherto been difficult or impossible to obtain. The holo-camera will be capable of either ship deployment or attachment to a fixed buoy and will be appropriately automated and controllable from the ship. The use of the entire system will be demonstrated and evaluated in a series of laboratory, tank, dockside and, if possible, sea trials.

INTRODUCTION

It is widely accepted that the earth's climate is intimately connected with and dependent upon processes occurring in the seas and oceans. Some of these processes are strictly chemical and physical but the underlying controlling forces are nearly always biotic involving the growth, death and sedimentation of the diverse array of plants and animals of the sea. An important goal in oceanographic research is to improve our understanding of the relationships between biotic and abiotic particles of the ocean. The accuracy of the conceptual and mathematical modelling of these bio-geochemical processes suffers at present from inadequate data. This is largely due to an absence of appropriate techniques for making the necessary observations.

Appropriate models cannot be developed unless, and until, reliable and accurate, non-invasive techniques are available to study the spatial relationships of marine organisms. Up until now a wide variety of optical and electronic techniques have been employed with varying degrees of success, in the laboratory and in the field, to obtain the necessary data. Because of the unique advantages offered by holography for high-resolution imaging of large water volumes, this technique offers great promise to marine biologists chemists, geologists and bio-geochemical modellers.

Holographic recording and replay has many advantages over conventional imaging techniques for underwater visual inspection and mensuration. The high resolution and three-dimensional nature of holography, allows optical recreation of an underwater scene in the laboratory. The images, so produced, are life size, fully three-dimensional and located in the real-image space in front of the observer. In this form the images can be directly interrogated by measuring microscopy or video to extract information at any point in a given plane of the image; for example, the dimensions, shape, identity and relative position of the particles can be determined. Individual "optical sections" of the image can be isolated and viewed independently. Holography provides significant advantages (e.g. high resolution, freedom from parallax and perspective effects and wide dynamic range), over other visual inspection techniques. Both off-axis (Fresnel) and in-line (Fraunhofer) recording geometries can be applied depending on the nature of the scene of interest.

The essence of our work is *in-situ* recording of holograms of aquatic systems using a pulsed laser together with their subsequently replay, in air, in the real image mode of reconstruction. This ability to select planes of the image for interrogation ("optical sectioning") is crucial in evaluating the real image; and it is this that sets holography apart from standard photography or stereo photogrammetry. This process of interrogation of an accurate real image holographic reproduction by direct optical measurement is known, by analogy with photogrammetry, as HOLOGRAMMETRY. It is worth pointing out, that the use of holography as a means of high-resolution imaging and mensuration has been exploited in only a few areas of science and technology and that our application represents a significant advance on the state-of-the-art.

METHODOLOGY OF HOLOGRAPHIC RECORDING AND REPLAY

Holograms of aquatic systems will be recorded *in-situ* using a pulsed laser. The holo-camera will allow holograms to be recorded, simultaneously, with either an in-line (object in transmission) or an off-axis (object in reflection) holographic geometry. Although the recording of the holograms take place in water, replay of the image is carried out in air, in the laboratory, to provide identification and measurement of the organisms and particles.

In-line holography

A single laser beam is directed through the sample volume towards the holographic plate and records the optical interference between light diffracted by the object and the undiffracted portion of the illuminating beam (Figure 1). No spatially separate reference beam is used. The replayed hologram simultaneously forms two images located on the optic axis; which for a collimated beam are at equal distances on either side of the holographic plate. The organisms will be illuminated in transmission and the scene needs to have an overall transparency of about 95% in order that speckle noise does not seriously degrade the image quality. This criterion sets an upper limit on the recordable particle concentration at around 40 particles per cubic centimetre (for particles of around 20 μm diameter and a recorded volume 1 m deep). A further factor of in-line holography is the need to balance the size of the particles to be recorded with the object-to-hologram distance. The upper limit for good size measurement is about 1 mm at a recording distance of 1 metre; however particles down to around 5 μm dimensions can be identified and measured with ease.

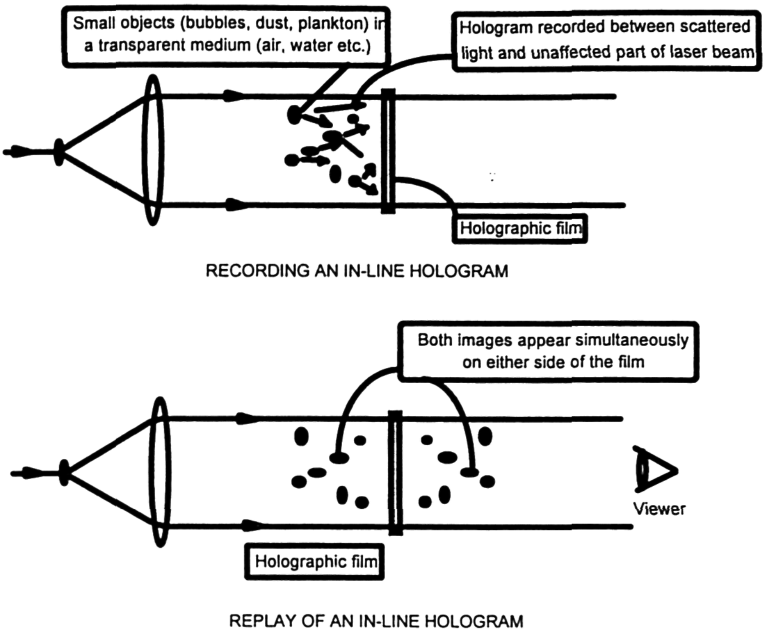


Figure 1: Recording and replay of an in-line hologram

Off-axis holography

Off-axis holography utilises a two-beam geometry: one beam illuminates the scene and the other directly illuminates the holographic film (Figure 2). It is the interference between diffuse light reflected from the scene and the spatially separate reference beam that meets the plate at a finite interference angle. On replay, the real and virtual images are spatially separated which makes their interrogation easier. Consequently, off-axis holography is usually applied to, primarily, opaque subjects of large volume, making it better suited to recording of more dense aggregates of marine particles. The scene can be front, side or back illuminated (or some combination of all three). The virtual image (Figure 3) is observed as if viewing the scene through a window and from the point of view of precision measurement is not the best way to interrogate the data. For data extraction and measurement, real image reconstruction (Figure 4) presents an image that floats in the real space in front of the observer. It is this mode which allows interrogation of the image by precision translation of a video camera or measuring microscope through the image. Although there is no real upper limit to the size of particles that can be recorded (this is determined by available energy and coherence of the laser) this sets a practical lower limit to the resolution that can be recorded to about 100 μm.

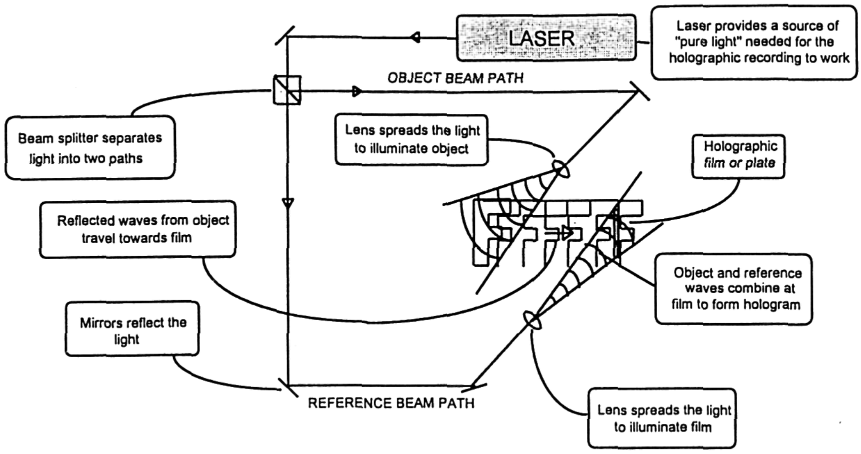


Fig 2: Recording an off-axis hologram

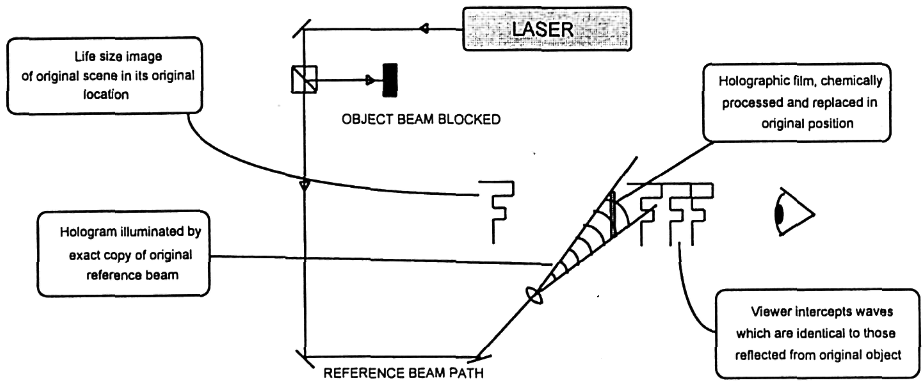


Fig 3: Replay of the virtual image from an off-axis hologram

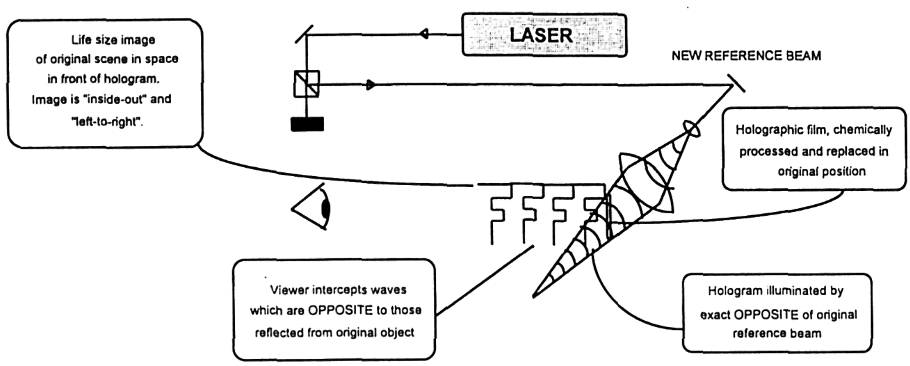


Fig. 4: Replay of the holographic real image from an off-axis hologram

Comparison between in-line and off-axis holography

Clearly both schemes overlap in their characteristics and the circumstances in which they would be used. These basic techniques have been evaluated for recording of plankton, and compared and contrasted in order to ascertain which method was most applicable for a given situation. Each method has different features and advantages (see Table 1) depending on the situation in which recording is carried out.

	IN-LINE HOLOGRAPHY	OFF-AXIS HOLOGRAPHY
Hologram replay	Fraunhofer real image	Fresnel real image
Subject dimensions	1 μm to 1 mm	> 100 μm
Subject transparency	95% unobscured field needed	High reflectivity needed
Illumination	Single beam, back-lit	Twin beam, variable angles
Particle concentration	Low concentrations	Medium/high concentrations
Optical density of medium	Contributes to background noise	Contributes to background noise
Refractive index mismatch	Not critical	Significant for finite field angles

Table 1: Comparison of in-line vs. off-axis hologrammetry.

Methodology of hologram replay

The holograms will be replayed, in the laboratory, in air, using the real image mode of reconstruction. The image is located in the space between the hologram plate and the observer. In real image replay, the holograms are reconstructed using the conjugate of the reference beam (in practice, a collimated beam is often used in recording and replay). The images, so produced, are life size, fully three-dimensional, retain all the parallax and perspective information of the original scene and are located in real-space in front of the observer. Although the image is also "pseudoscopic" (reversed left-to-right and back-to-front), for purposes of dimensional measurement this is unimportant. Holograms will be replayed in either in-line or off-axis modes, in the same facility, depending on how they were recorded. A video camera (with or without lens, as appropriate) is mounted on a computer-controlled x-y-z micropositioner. The camera is translated through the image to extract information regarding shape, identity, dimensions and relative position. Automation of the replay together with image analysis and data extraction facilities allows species identification and measurement of local concentration of a variety of marine organisms.

PROPOSED SYSTEM CONFIGURATION AND SPECIFICATION

The complete HOLOMAR system will consist of two parts: the holo-camera for recording of the holograms and an associated reconstruction facility for the replay and analysis of the holograms.

The HoloCamera

A holographic camera (holo-camera) will be produced comprising laser, holographic optics, and watertight housing and support frame and will be designed to meet the following specification:

- The holograms will be recorded using a pulsed laser.
- The laser will be a frequency-doubled Nd-YAG laser of (up to) 650 mJ output energy, 6 to 8 ns duration and output wavelength of 532 nm.
- The holo-camera will allow simultaneous recording of overlapping volumes of water using both in-line and off-axis holograms.
- By using both geometries to simultaneously record overlapping volumes of water the camera will exploit the distinct advantages of each method.
- The in-line method can produce images of organisms with sizes in the range 5 to 250 μm (at concentrations up to a few thousand per cubic centimetre at the smallest sizes).
- The off-axis method is better for larger organisms from about 100 μm upwards at much higher concentration levels.
- Sample volumes of up to about 10^5 cm^3 (100 ℓ) will be recorded in the field (depending on configuration).
- Stand-off distances will be up to one metre from the subject scene (depending on configuration).
- The holo-camera will be equipped with data handling capacity to allow the use of other sensors (such as temperature, salinity and pressure) to provide detailed knowledge of the ambient conditions in which the holograms were recorded.
- The prototype system will be designed to operate down to about 100 m depth
- The holo-camera will be capable of either ship deployment or attachment to a fixed buoy and will be remotely automated and controllable.
- The prototype will be a tethered camera and will not be deployed from an ROV.

Figure 5 shows a preliminary holo-camera layout and configuration (both plan and 3d views). The general beam paths for the in-line and off-axis geometries are shown together with the essential optical components. The laser beam is generated from the laser head and is split into two paths at the first beam splitter (BS1). The transmitted path forms the illuminating beam for the off-axis mode. The reflected path continues and, following beam steering and expansion optics, is collimated to form the reference beam for the off-axis mode and also the illuminating/reference beam for the in-line method. Some of this beam is tapped off at a second beam splitter (BS2) to provide the off-axis reference (at an angle of 60° to the normal to the plate). Two arms protrude from the front of the camera to provide the in-line optics. Two high quality optical windows in these arms allow the in-line beam to pass into the water and back through to the plate holder. The optical window for the off-axis mode is recessed into the housing and allows return of the reflected/scattered light from the object volume. Smaller windows in the angled walls provide the object illumination for the off-axis mode. As can be seen the off-axis mode captures a larger scene volume than does the in-line but a significant degree of overlap between the two is provided. Both plate holders will be of the same design and will hold 40 to 50 plates each. The entire camera is enclosed in a pressurised, water-tight housing and the overall dimensions are expected to be about 800 mm diameter by 1200 mm. The centre-line of the in-line optics will be of the order of 250 mm to 400 mm from the front face of the housing. The 3d view gives a better indication of the optical path layout and the direction of the illumination beams.

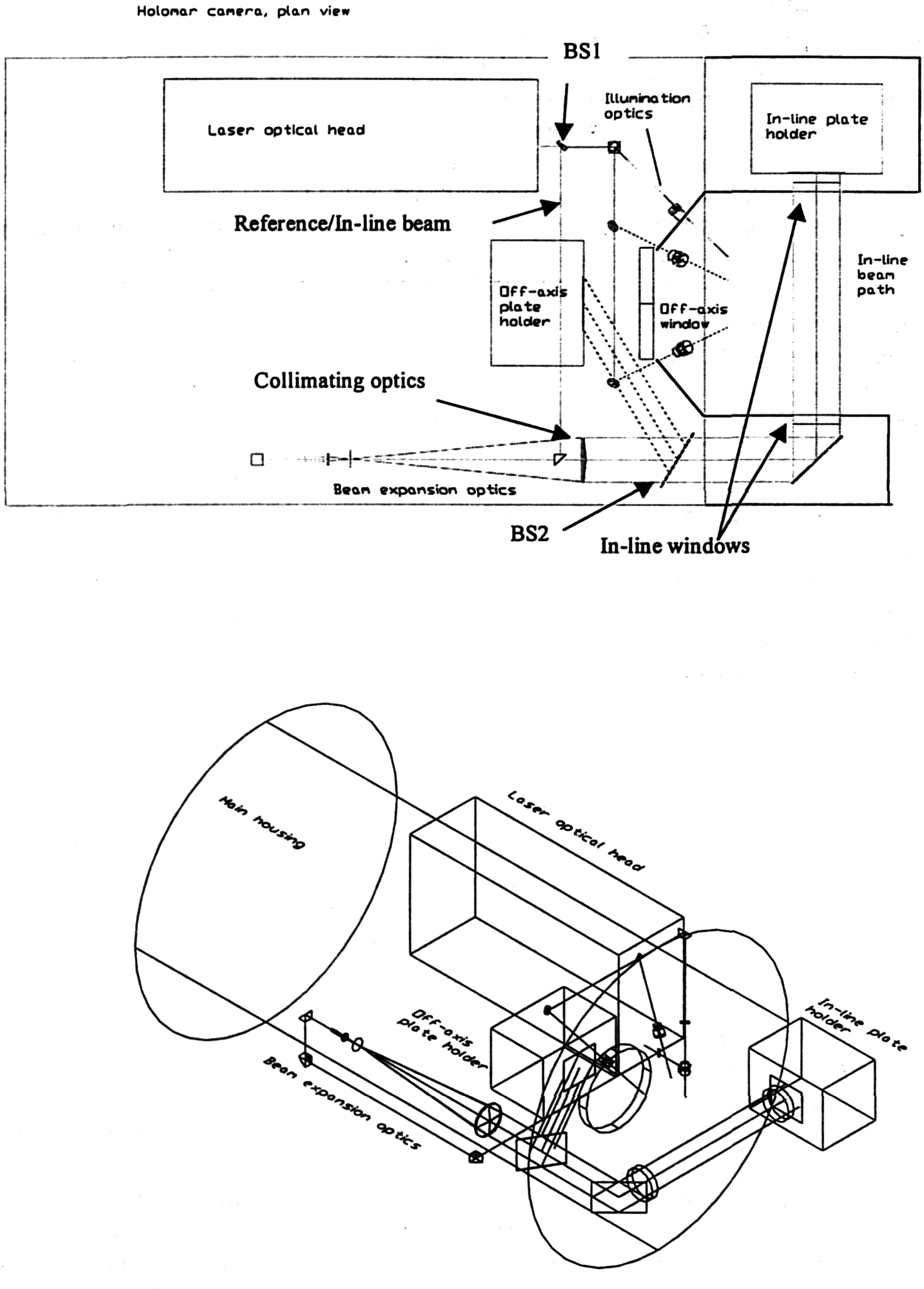


Fig 5: Plan and 3d views of the proposed HoloCamera configuration

The Replay System

Although the objects to be recorded are located in water, replay of the image is carried out in the laboratory in air, using the real-image mode of reconstruction. Precision replay of the holograms will be accomplished in a dedicated reconstruction facility. The replay system will comprise laser, reconstruction optics and image acquisition and analysis instrumentation and will be designed to meet the following specification:

- The images are located in the space between the observer and the holographic film and are life size, fully three-dimensional and retain all the parallax and perspective information of the original.
- The required resolution is 5 μm for a range of organism dimensions up to 150 μm for in-line holograms and a lower limit of 100 μm for off-axis holograms.
- The replay system will incorporate precision control (2 μm steps in xyz axes) of an observation video camera (within the image volume) to allow precise dimensional measurement of the relative location of the organisms
- Precision control of the orientation (< 0.5° in each rotational axis) and optimisation of the resolution and brightness of the hologram.
- Specially developed image processing algorithms will enable identification of individual species at family or genus level and measurement of size and automatic extraction information regarding shape, dimensions, relative position and measurement of local concentration and distribution of a variety of marine organisms.
- Data acquisition methods will allow measurement of distribution and local concentrations of marine organisms and particles in a variety of subsea systems from the holograms, such as, local interactions between meso-zooplankton and micro-zooplankton, phyto-plankton and seston.

Figure 6 shows a schematic of the replay system for in-line and off axis modes. The laser beam is a HeCd emitting at 442 nm. Beam steering and collimating optics provide the reconstruction beam. The plates are mounted on a holder which allows motorised translation and rotation in all six degrees of freedom. The plates can be orientated at the exact recording angle (60°) for the off-axis mode or normal to the reconstruction beam for the in-line case. The video camera (CCD or newvicon) is mounted on precision micropositioners and can be translated through the real image volume to a 2 μm precision.

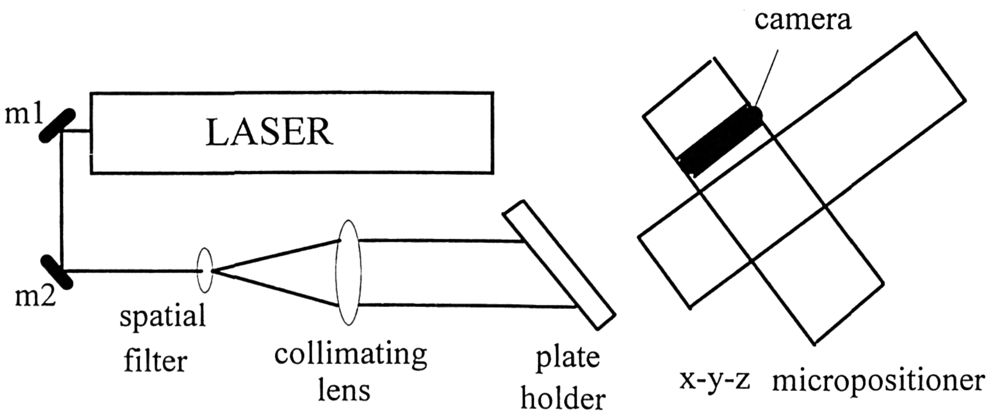


Fig 6: Schematic of holographic replay facility

DETAILED DESCRIPTION OF WORK PACKAGES

Work Package 1: The holographic laser and associated facilities

Co-ordinator: Quantel

Objective: Development of a pulsed frequency-doubled Nd-YAG laser suitable for recording high-resolution in-line and off-axis holograms in a subsea environment.

This aspect of programme is concerned with the laser, its selection, design and development for subsea use and implementation within the prototype holo-camera. Laser technology is now sufficiently advanced to permit development of a miniaturised holographic source, of very narrow spectral band emission, that can operate in industrial environment, with low purchase, operating and maintenance costs. To date, this new source does not exist on the market. To fill this need, QL will develop a new narrow band Nd-YAG laser specifically for holographic applications in hostile environments.

Laser design strategies

The coherence length requirement (about 1 m) for holography requires that the laser operate in a single longitudinal mode (SLM) and single transverse mode. Although several techniques exist by which SLM operation can be obtained in solid-state lasers our choice of passive Q-Switching of a Nd-YAG rod lies with using a thick saturable absorber as the best design strategy for this project. SLM operation is a result of both slow bleaching of the absorber, and spatial hole burning in the bulk of the crystal. Over 95% of the shots will be SLM, without any etalon or cavity length control. The result is a simple, small and sturdy laser, utilising a minimum of electronic components and is ideally suited to field use. Both QL and Aberdeen have experience in the design of such lasers. Frequency doubling of the YAG crystal produces an emission wavelength of 532 nm that corresponds closely to the oft-quoted peak transmission window of seawater. The inherently short pulse duration of the frequency-doubled Nd-YAG laser (<10 ns) increases the maximum recordable particle velocity and enhances freedom from external vibration. Fast repetition rates (up to 1 Hz) allow easy optical alignment.

The layout and mechanical assembly will be designed to meet operational and storage requirements associated with underwater deployment. The power supply will be based on existing, compact and efficient power sub-assemblies, modified to accommodate specific operation requirements in terms of line voltage, cooling and space. The control electronics will be microprocessor based and will provide the necessary laser control signals, and will exchange information with the master processor driving the full holo-camera. Further considerations include cooling and temperature regulation in water and air; the ability of the laser to operate at pressure; and, safe use of the laser in an "industrial" environment.

Specification

A prototype laser adapted for underwater recording of holograms will be developed to meet the following specification:

PARAMETER	SPECIFICATION	COMMENT
Generic type:	Frequency-doubled Nd-YAG	
Wavelength:	532 nm	
Energy:	650 mJ (at 532 nm)	Only 50 mJ needed for in-line
Repetition rate	Single shot & 1 Hz	1 Hz for alignment
Pulse duration	6 - 8 ns	Sub nanosecond NOT required
Coherence length	≥ 1 m (at 532 nm)	Critical for off-axis holograms
Beam diameter	9 mm	
Divergence	≤ 0.6 mrad	
Shot-to-shot variability	≤ ± 5%	

Work Package 2: Holographic optics and film transport

<u>Co-ordinator</u>: *Aberdeen Univ.*
<u>Participants</u>: *Brunel, Nemko, Holo3*

<u>Objective</u>: Development of the holographic camera optics to enable in-line and off-axis holograms to be recorded of marine organisms and particles; and, development of a precision film holder and transport mechanism for holographic film.

The holo-camera optics represents a challenge of engineering to design and build a robust, field instrument that can provide precision recording of holograms. The camera needs to provide stable optical paths and yet still include sufficient operational variability in the parameters.

Design features

Two holographic geometries (in-line and off-axis) will be used in recording. The holo-camera optics will incorporate both schemes into a single head to allow simultaneous recording of partially overlapping water volumes. The holo-camera will offer the ability to vary laser energy and object and reference beam ratios and to alter beam path length as required (particularly in the off-axis configuration). The whole holo-camera will be enclosed in a light- and watertight, pressurised enclosure but still allows the emulsion to be easily changed when the instrument is recovered at sea. A subsidiary light shutter, linked to the laser fire control will be required to eliminate any film fog between exposures and to ensure fail-safe operation of the laser. Spatial filtering of the beams is necessary to ensure good illumination of the subject and film.

To enable large numbers of holograms (50-100) to be recorded in a single "dive", an automatic plate holder and transport mechanism will be developed. The choice of holographic emulsion type is limited by the lack of suitable holographic emulsions. Birenheide are the only EU supplier, Kodak are often used in the US; in the former Soviet Union workers use their own range of emulsions, but quality control is variable and they tend not to respond well to pulsed lasers. A programme of evaluation of different available emulsions, together with appropriate wet chemical processing is mandatory.

The laser light passes through an observation window into the water and the light reflected from or transmitted through the subject returns back through the window to the film holder.

Due to the pressure differential between the housing and the water, the optical performance of the window may alter and produce lens-like properties which could degrade the quality of the holograms. An investigation of the behaviour of such windows will be undertaken and, if appropriate, means of minimisation or correction for any implicit distortions should be included. Monitoring of the extent of the deformation will lead to the implementation of procedures to minimise or correct for these shape changes. Holography and/or speckle interferometry will be used in evaluation, techniques of pressure balancing will be used in construction.

Holo-cameras have been built before but have been mainly lab-based and used in interferometric applications where constraints on emulsion support are not severe. Never has a holo-camera, with this specification, been built for subsea use. The design of such a holo-camera presents some significant engineering challenges but requires no new technology.

Work Package 3: Holo-camera housing, submersible support frame and laser control system

Co-ordinator: NEMKO
Participants: SOC

Objective: Design and construction of a water-tight pressure housing and control system to enable the system to operate at water depths of 100 m; design and construction of a suitable support frame to allow ship based deployment of the system.

The third major challenge is to package the holo-camera in a fully watertight and light-tight pressurised housing that will allow operation in the sea down to about 100 m. The housing will have one or more optical windows to allow transmission of laser light into the water for subject illumination and return of the laser light to illuminate the holographic plates. During deployment, the holo-camera will be mounted on a rigid submersible support frame to preserve the spatial location of the laser and optics, whilst allowing the system to be operated from a ship via a derrick. In addition it must act as a support for the other sensor systems and all associated cabling. The control system is probably best located on the frame as the local high-voltage for the laser should not be taken over long cables from the surface. A microprocessor-based system will interface the laser and camera control to the other sensors attached to the platform. Initial design is based on an existing successful support frame.

Work Package 4: Hologram replay

Co-ordinator: Brunel Univ.
Participants: Aberdeen, Holo3

Objective: Design and construction of a holographic replay and measuring machine dedicated to the analysis of underwater holograms.

A crucial aspect of high resolution imaging by holography is the replay of the hologram. No matter how well the hologram is recorded, imprecise replay will remove all the advantages afforded by the technique. A scanning and measuring reconstruction facility will be constructed to cover a replayed image volume of 1000×200×200 mm^3. The design will incorporate the ability to replay in-line and off-axis holograms using the same laser, beam-expander and film transport system. Each of the mounting stages will be under computer control via an RS232 interface.

It should be noted that minimisation of optical aberrations, especially in the off-axis geometry, requires replay at a shorter wavelength than that used in recording (actually the recording wavelength divided by the refractive index of water). For holographic recording with Nd-YAG (doubled), replay would be at 400 nm. Replaying in the deep blue region of the spectrum is difficult because fewer suitable lasers are available and reduced sensitivity of photo-detectors. For in-line holograms, choice of replay laser is not a crucial factor. However, for in-air replay of off-axis holograms recorded in water the replay laser should have a wavelength near to that of the original recording laser divided by the refractive index of water. For recordings carried out using a frequency-doubled Nd-YAG laser (532 nm), optimum replay is at 400 nm and the choice of the replay laser is more challenging. The 406 or 413 nm lines of the krypton-ion laser are two possibilities, as are the less ideal 442 nm line of the He-Cd laser or the 458 nm line of the argon-ion laser.

Precision replay of holograms is a difficult task. All replay conditions must be matched precisely to recording conditions. A misalignment of the hologram by 0.5° in any plane can reduce resolution by as much as 50%. The design and construction of such a facility again, requires no new technology, but does require novel and precision engineering. Optimisation of the hologram will be made easier by the image processing concepts in WP5.

Work Package 5: Image processing and automated information extraction

Co-ordinator: *Genoa Univ.*
Participants: *Udine Univ.*

Objective: Development of image processing methodology and techniques to allow automated identification and classification of plankton species from the replayed holograms.

Image processing of holographic images is a unique feature of this work. No other research group has specialised in the particular problems associated with optimising, analysing and identifying specific features from real image replay of holograms. In general, the images taken from holograms must be processed to extract useful features and properties such that it is possible to (i) derive 3D co-ordinate information relating to location of the organism, and (ii) identify and classify different types of marine organism. The latter task could be accomplished in different ways depending on the analysis desired, i.e. whether it is necessary to identify each single species or a distribution of species. It is also necessary that the hologram orientation with respect to the replay be beam be optimised such that the projected image displays maximum resolution and brightness.

A particular difficulty revolves around the fact that the hologram itself presents a 3D image in real space. Although moving a video camera or ground glass screen through the image volume allows planes of the subject to be isolated, light from other points of the scene will form out-of-focus images at the selected plane. Image processing algorithms are being developed to extract or eliminate this extraneous information while retaining and allowing the identification of the marine organism. Initial work shows that images possess noise whose effect can be reduced by some frequency-like filtering, but also that different species of plankton can be identified and isolated. After noise reduction, a segmentation technique can be applied to extract features useful to classify each single species. Region-based and edge-based methods will be used to identify the main object in the image and then features would be extracted and sent to a classification system. Starting from a few 2D images of the 3D scene, 3D information may be recovered by using different approaches. Amongst the wide range of alternative approaches, are some robust methods that operate on images taken with the same video camera and are probably suited for application to holograms of biological species, where an extremely high precision is not required. Taking into account these considerations and the characteristics of the replay machine, the development of a 3D-information extractor based on one of these approaches will be undertaken. The use of reference markers present in the acquired holograms will allow exploitation of *a priori* information to recover absolute co-ordinates with respect to a reference system fixed into the real world. Automated optimisation of the replay conditions will be incorporated to allow the hologram to be aligned such that maximum resolution and brightness from the image are obtained.

For classification of species of marine organism, the use of a neural network-based algorithm fed by different features will be developed. Neural nets have the important characteristic of evolving naturally to find the best discriminatory hyper-surface between the classes and, also, they are trained by examples. Due to the large amount of data available, it would be the optimum solution to classify the plankton. However, classical statistical algorithms will be investigated as a basis to develop a robust classifier.

Conceptually, this is the most challenging aspect of the programme. Many alternative strategies and algorithms need to be explored before reaching the favoured solution. This is the control centre for the replay and dictates the quality and usefulness of the final result.

Work Package 6: System integration

Co-ordinator: Aberdeen Univ.
Participants: All

Objective: Integration of holo-camera sub-system components; integration of hologram replay and data acquisition sub-systems, establishment of safe, healthy and reliable work practices for the operation of the laser, electrical supplies and associated facilities;

Prior to subsea deployment and evaluation of the complete holo-camera and associated replay facility, the sub-components making up each of these systems (holo-camera and replay) need to be integrated and their performance evaluated under stringent and rigorous conditions. Parameters to be considered include field operation of the laser, control system, durability of the housing and analysis of holograms. In some cases (e.g. the replay), evaluation will take place using existing laboratory recorded holograms. The complete holo-camera will operate in accordance with the recommendations incorporated within the European Standard EN 60825-1:1994 *Safety of Laser Products* and also in accordance with regulations covering the use of electrical systems offshore.

Work Package 7: System evaluation and hologram interpretation

Co-ordinator: Southampton Oceanography Centre
Participants: All

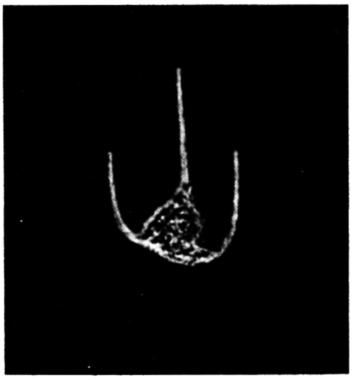
Objective: Verification and evaluation of the operation and application of the entire system; replay and image analysis by means of a series of laboratory, observation tank and dockside trials, aimed at providing real data from the marine environment.

The final aspect of the programme is the deployment and evaluation of the complete system in a series of laboratory, tank and dockside trials. This crucial aspect of the programme will see the holo-camera used in the field to record marine organisms and particles in their natural environment. Subsequent analysis of the holograms will take place using the replay machine and its associated processing system. Identification of organisms, measurement of their location and concentrations will be carried out to demonstrate the viability of the technique.

PREVIOUS WORK IN HOLOGRAPHY OF MARINE ORGANISMS

We show here a series of holograms recorded in our preliminary studies of holography of marine organisms. These initial holograms were recorded in the laboratory using both in-line and off axis techniques and utilised a pulsed ruby laser (λ = 694 nm). The organisms were cultured and fixed samples in observation tanks of 100 ℓ volume.

The in-line holograms show dinoflagellates of around 250 μm in length. The off-axis holograms show images of zooplankton. Both these off-axis images were taken from the same hologram. The animals were approximately 250 mm from the hologram plane and were about 10 mm apart in the water volume. They are of the order of 1 mm long. The out of focus image of one organism can be seen in the photograph of the other.



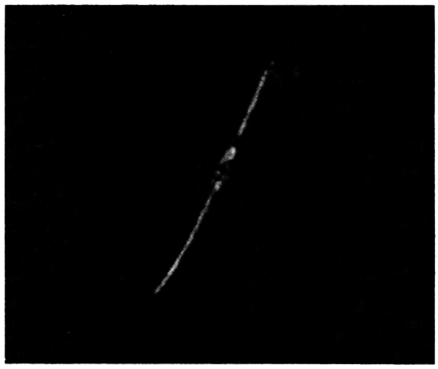


Fig 7: Photographs taken from real-image reconstructions of in-line holograms of dinoflagellates

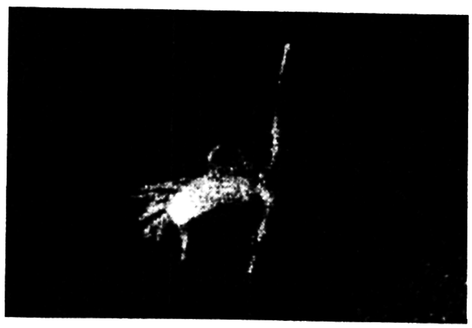


Fig. 8: Photographs taken from real-image reconstructions of off-axis holograms of zooplankton

III.1.4. Marine biotechnology

TITLE: Bioactive Marine Natural Products in the Fields of Antitumoral, Antiviral and Immunomodulatory Activity.

CONTRACT NO.: MAS3-CT95-0032

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BIOACTIVE MARINE SUBSTANCES IN THE FIELDS OF ANTITUMORAL, ANTIVIRAL AND IMMUNOMODULATORY ACTIVITY

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SUMMARY

The purpose of the project, which runs from 1996 to 1998, is to propose new substances to the European pharmaceutical industry for the treatment of cancer or infectious diseases (antiviral or immunostimulating substances) or the suppression of immune reactions after organ transplantations. These new substances are being searched for in marine invertebrate organisms, especially sponges and ascidians. This report describes the first results obtained.

1. INTRODUCTION

The purpose of the project is to propose new substances for the treatment of cancer or infectious diseases (antiviral or immunostimulatory substances) or the suppression of immune reactions after organ transplantations.

This choice of marine organisms for the discovery of such substances was based on the great originality of the molecules that they contain, particularly in the case of certain classes of invertebrates. The number of unstudied species is still considerable. Moreover, the main interest of previous studies has been essentially chemical. Thus, the therapeutic potential is still largely unexplored. The present results in this field of investigation are related to antitumor activity, and several marine substances have undergone clinical experimentation (didemnin B, girodazole, dolastatine 10, bryostatine 1, ecteinascidine 743, etc.). The other two fields are related and complementary and have been explored less.

Some phyla of invertebrates are more likely to provide such substances, especially those which are fixed and cannot escape, have no external physical protection (e.g. shells) against predators, and need to develop chemical means of defence in order to survive. This is especially the case of sponges and ascidians.
Sponges, which constitute the branch of marine organisms responsible for the greatest number of original and often interesting molecules (girodazole, discodermolide, manoalide, mycalamides, avarol, etc.), were given major priority in our considerations. Ascidians (Tunicates), a branch including fewer species, constituted a second priority group because of the exceptional frequency of nitrogenous metabolites and the great number of molecules of pharmacological interest (didemnin B, bistramides, eudistomins, ecteinascidines, patellamide D, etc.).
As these two phyla are both made up of filtering organisms, it has been suggested that several of the metabolites considered could come from associated microorganisms. This is an important advantage in the perspective of biotechnological production of interesting molecules.

Three climatically or geographically varied sampling zones have been included in our choice:

- Two very widely separated tropical zones: the Vanuatu and the Djibouti coasts. This choice was made because considerable interspecies competition exists in tropical seas. The probability of the presence of chemical defence substances (cytotoxins and thus potentially antitumoral substances) is therefore high. Some species of New Caledonia, collected before the beginning of the program, were also investigated.

- A warm temperate zone: Ustica Island (north of Sicily) and along the coasts of Pantelleria Island (south of Sicily). Mediterranean organisms have already been studied several times, particularly by two of our teams (the Dipartimento di Chimica della Sostanze Naturali, Napoli; and SMAB, Nantes). Samples from Ustica Island were also collected just before the beginning of the program.

- A cool temperate zone: the French Atlantic coast. This zone has already been partially explored relative to antitumoral and antibacterial aspects, notably by SMAB, Nantes. Current research shows fewer favorable antitumoral results than for the study of species in warmer waters. Antiviral and immunomodulatory activities have not been searched for in organisms in this zone. The influence of climate on this type of property has not yet been determined. The initial plan was to explore this zone only if the number of collected species in other zones proved insufficient.

As opportunities allow, some species from other geographical origins are to be explored selectively, as well as other pharmacological properties.

2. COLLECTION OF ORGANISMS

For the reasons mentioned above, this section does not include the collection of species from New Caledonia (2 sponges only, studied for antitumoral activity) and Ustica (55 samples corresponding to 36 different species of sponges).

The first expedition to obtain sponge and ascidian samples in the Vanuatu was conducted in June and July 1996. Ninety sponges and 7 ascidians were gathered on 7 sites (Efaté, Emae, Epi, Malekula, Maskelyme, Santo and Tongoa) at depths of -5 to -60 m. For each specimen, from 0.5 to 4 kg were collected. Sponges were identified by Dr. J. Hooper, Museum of Queensland, Brisbane, and ascidians by Dr. F. Monniot, MNHN[1], Paris. One species of ascidian remains to be identified. About 40% of the collected sponges and two of the collected ascidians were identified as new species.
A second sampling cruise in the Vanuatu was conducted in May-June 1997, after most of the pharmacological screening was completed, in order to collect interesting species in greater amounts. Relevant organisms were collected again at the same spots as in 1996. Thirty-one sponges and 2 tunicates were sampled in amounts between 5 and 13 kg, except for 3 sponges that were found in smaller quantities (1 to 2 kg).

The collection on the coast of Pantelleria was performed in September 1996. A total of 40 specimens were collected by scuba diving from 6 different stations. About half of the collected specimens were identified on the spot as species already collected in previous expeditions. The remaining specimens were combined into 6 homogeneous groups, in amounts ranging from 0.2 to 1.5 kg. Taxonomic identification of the organisms is in progress.

In the context of the Auracea 96 mission, the first collection of specimens in Djibouti was co-organized with the Ardoukoba Association (Y. Zeau) which ensured the logistics and filming (photography and video). Dives were performed in two areas. The first, in the Muscha archipelago (October 10-22, 1996), involved 23 diving points, particularly Grand Signal Island, the northern part of the reef and Dankali Bank. The second (October 24-November 4, 1996) concerned all the Ghoubet Kharab.
Around 100 samples were collected (from 0.2 to 20 kg) by scuba divers at depths ranging from -1.5 to -38 m. Seventy species (9 ascidians, 61 sponges) gathered in amounts exceeding 1 kg will be studied for their bioactive substances. The other samples will be identified zoologically to improve our knowledge of the marine fauna of Djibouti.
Out of the 80 samples selected for the screening, 30 (25/67 sponges, 4/12 ascidians and 1/1 cnidarian) have now been identified by Prof. C. and Dr.F. Monniot, MNHN (Paris) for the ascidians and by Prof. I. Micha (University of Tel Aviv) for the sponges. One of the collected ascidians was identified as a new species. For many sponges, only the genus has been determined thus far, and it is not yet clear whether there are new species among them.

To date, collections have been made only selectively along the Atlantic coast.

3. PHARMACOLOGICAL SCREENINGS

These screenings have been performed from aqueous (H_2O), ethanolic (EtOH) and glycolipidic extracts from the different species. Glycolipidic extracts were obtained either through extraction by

[1] MNHN : Muséum National d'Histoire Naturelle de Paris

$CHCl_3$-MeOH (9:1) of the residue of the previous extraction by EtOH [(GL) extracts] or through extraction of the raw material by the same solvent and isolation of the glycolipidic fraction by liquid chromatography (GL extracts).
The extracts which have been or will be subjected to screening are:
- From Ustica samples: EtOH extracts;
- From Pantelleria samples: H_2O, EtOH and (GL) extracts;
- From Vanuatu samples: H_2O and EtOH extracts;
- From Djibouti samples: H_2O, EtOH and GL extracts. GL extracts have not been yet subjected to pharmacological screening.

A. SCREENING FOR CYTOTOXIC AND ANTIPROLIFERATIVE ACTIVITY

Tests have been performed on the non-small-cell lung cancer cell line NSCLC-N6 and its clones C15, C65, C92 & C98 developed at the University of Nantes. Only mildly active extracts have been selected (IC_{50} between 3 et 30 µg/ml). Except for Ustica (an earlier study), the priority (1 to 3) of studying has been determined from the differences of activity observed on the different clones. Priorities have not yet been determined for Djibouti active extracts.

The total number of positive results is reported in Tables I (sponges) and II (ascidians). Among them, 24 samples of sponges (25%) and 1 sample of ascidian (6%) have been selected to date from Pantelleria and Vanuatu with the priority 1.

B. SCREENING FOR ANTIVIRAL ACTIVITY

Extracts have been evaluated for their potential antiviral activity against 3 viruses: HIV-1, herpesvirus 1 and dengue virus (only for Vanuatu samples):

- Assessment of the anti-HIV effect of the extracts was based on evaluation of inhibition of HIV-1 replication by counting the syncytia on MT4 cells.
- The HSV-1 strains were cultured on Vero cells. Anti-HSV activity was assessed by studying cell viability at MOI 0.01 ID_{50}/cells of infection.
- Extracts of Vanuatu organisms were tested on one of 4 dengue serotypes at the Institut Pasteur in Noumea. The cytotoxicity dose 50% will be determined in pork epithelial kidney cells, and then the minimal concentration inhibiting virus replication. The interpretation method used consists in observing lysed areas due to viral proliferation.

The present results are reported in Tables I (sponges) and II (ascidians). For anti-HSV-1 activity, 5 samples of sponges (3%) and 2 samples of ascidians (13%) have been selected with the priority 1. For anti-HIV-1 activity, no extracts have been selected at this level. No priority has been determined for antidengue activity.

Table I. Antiproliferative ant antiviral activities from samples and extracts of sponges: % of positive results* (priority 3, at least).

	Collected samples	Antiprolifer. activity	Anti-HSV-I activity	Anti-HIV-1 activity	Anti-dengue activity
<u>Samples :</u>	223	45% (211)	8% (165)	12% (149)	3% (90)
- Ustica	60	27% (55)	4% (23)	0% (24)	
- Pantelleria	6	100% (6)	0% (6)	0% (6)	

- Vanuatu	90	53% (90)	4% (76)	12% (75)	3% (90)
- Djibouti	67	43% (60)	16% (60)	22% (44)	
Extracts		**33% (333)**	**5% (269)**	**9% (188)**	**2% (175)**
- H2O ext.		31% (150)	2% (118)	12% (74)	2% (89)
- EtOH ext.		35% (177)	8% (145)	6% (108)	2% (86)
- (GL) ext.		33% (6)	0% (6)	33% (6)	

* In parentheses, the number of samples of sponges from which at least one extract has been tested or the number of extracts tested to date.

Table II. Antiproliferative ant antiviral activities from samples and extracts of ascidians: % of positive results* (priority 3, at least).

	Collected samples	Antiprolifer. activity	Anti-HSV-I activity	Anti-HIV-1 activity	Anti-dengue activity
Samples :	19	**26% (15)**	**33% (15)**	**0% (10)**	**0% (7)**
- Vanuatu	7	28% (7)	0% (7)	0% (7)	0% (7)
- Djibouti	12	25% (8)	62% (8)	0% (3)	
Extracts :		**16% (18)**	**22% (22)**	**0% (16)**	**0% (14)**
- H2O ext.		7% (15)	0% (11)	0% (8)	0% (7)
- EtOH ext.		15% (13)	45% (11)	0% (8)	0% (7)

* In parentheses, the number of samples of ascidians from which at least one extract has been tested or the number of extracts tested to date.

C. SCREENING FOR IMMUNOMODULATORY ACTIVITY

The immunological test panel includes the following systems:

- Preliminary tests: selection of extracts for immunomodulatory activity
    - LAL test (*Limulus* Amebocytes Lysat) on bacterial endotoxins (LPS)
    - Cytotoxicity against L929-cells in the MTT assay
- Immunological assays, Stage I: search for an immunomodulatory activity
    - Effect on T cells (LTT test)
    - Effect on phagocytosis (on macrophages isolated from murine bone marrow)
- Immunological assays, Stage II: search for an immunostimulating activity
    - Release of cytokines :
        * Release of IL-2 from murine spleen lymphocytes
        * Release of TNF-a from murine bone-marrow lymphocytes
    - Activity of natural-killer-cells (from murine spleen) against YAC1-cells
    - Induction of cytotoxic macrophages (from murine bone marrow) against P815 cells

or,

- Immunological assays, Stage II': search for an immunosuppressive activity
    - Immunosuppression of activated lymphocytes or macrophages

The present results, which concern immunostimulatory activity, are reported in Tables III (sponges) and IV (ascidians). Five samples of sponges (3%) have been selected to date with priority 1. No ascidian extracts have been selected.

D. SCREENING FOR ANTI-BACTERIAL AND ANTI-YEAST ACTIVITIES.

These tests were not initially planned in the program and were performed independently on extracts from the Vanuatu and from ethanolic extracts from Djibouti. The potential antibiotic effect of the Vanuatu extracts was determined on 4 bacteria (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and 1 yeast (*Candida albicans*). Extracts from Djibouti were tested on 3 bacteria (*Staphylococcus aureus, Enterococcus hirae* and *Escherichia coli*) and 2 *yeasts (Candida albicans* and *Saccharomyces chevalieri*). Results are given in Tables III (sponges) and IV (ascidians). All ascidian extracts were inactive.

Table III. Immunostimulant and antibiotic activities from samples and extracts of sponges: % of positive results* (priority 3, at least).

	Collected samples	Immunostim. activity	Anti-bacterial activity	Anti-yeast activity
Samples :	**223**	**8% (175)**	**21% (149)**	**10% (149)**
- Ustica	60	31% (19)		
- Pantelleria	6	0% (6)		
- Vanuatu	90	8% (89)	21% (90)	12% (90)
- Djibouti	67	3% (61)	22% (59)	6% (59)
Extracts :		**5% (299)**	**14% (239)**	**6% (239)**
- H2O ext.		0% (151)	4% (90)	6% (90)
- EtOH ext.		10% (138)	20% (149)	7% (149)
- (GL) ext.		0% (6)		

* In parentheses, the number of samples of sponges from which at least one extract has been tested or the number of extracts tested to date.

Table IV. Immunostimulant and antibiotic activities from samples and extracts of ascidians: % of positive results* (priority 3, at least).

	Collected samples	Immunostim. activity	Anti-bacterial activity	Anti-yeast activity
Samples :	**19**	**0% (12)**	**0% (12)**	**0% (12)**
- Vanuatu	7	0% (7)	0% (7)	0% (7)
- Djibouti	12	0% (5)	0% (5)	0% (5)
Extracts :		**0% (19)**	**0% (19)**	**0% (19)**
- H2O ext.		0% (12)	0% (7)	0% (7)
- EtOH ext.		0% (7)	0% (12)	0% (12)

* In parentheses, the number of samples of ascidians from which at least one extract has been tested or the number of extracts tested to date.

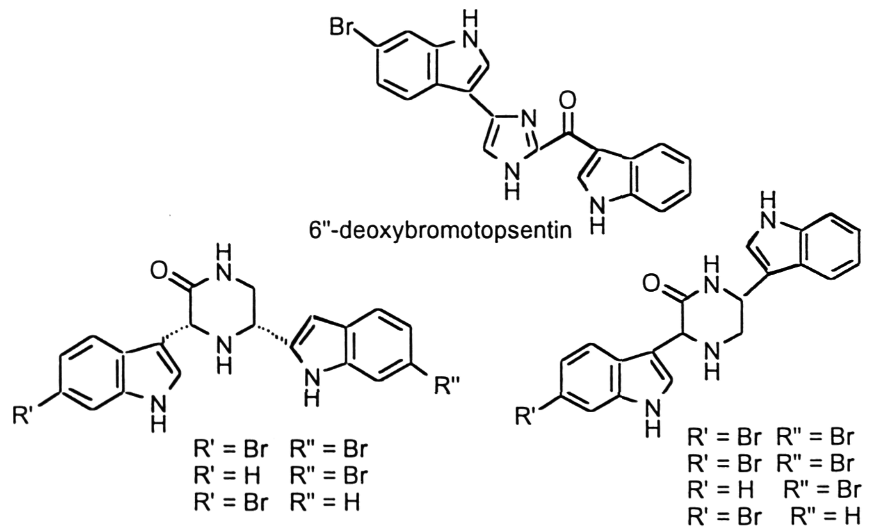
4. IDENTIFICATION OR SYNTHESIS, AND EVALUATION OF POTENTIALLY ANTITUMORAL SUBSTANCES

A. A SELECTION OF BIOACTIVE SUBSTANCES ISOLATED AND CHARACTERIZED TO DATE

• ***Raphisia lacazei,*** an Ustica sponge

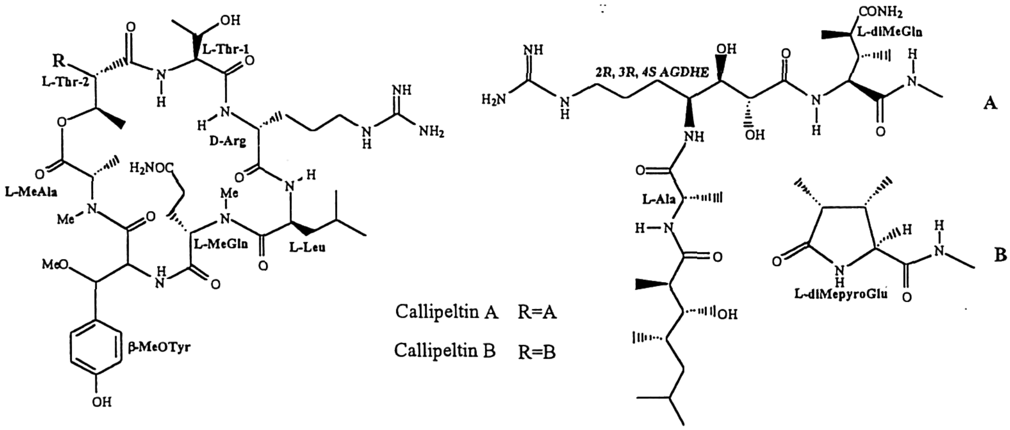
The crude ethanol extract of *Raphisia lacazei*, a sponge collected in Ustica and never investigated before, showed moderate activity in the antiproliferative test. Chromatographic separation eventually led to the isolation of the pure active components. After spectroscopic investigation, 4 of these components proved to be known indole alkaloids: topsentin A, B1 and B2 and 4,5 dihydro-6"deoxytopsentin. In addition, 8 new indole alkaloids were isolated in small amounts. Complete spectroscopic investigation by 2D NMR techniques allowed assessment of their structures. One was structurally correlated with the major topsentins, while the other seven correlated with the dihydrohamacantin skeleton. Topsentins B1 and B2, the two compounds isolated in largest amounts, showed more interesting activity and were subjected to pharmacological tests *in vitro* and *in vivo.*

new indole alkaloids from the Mediterranean sponge *Raphisia lacazei*:



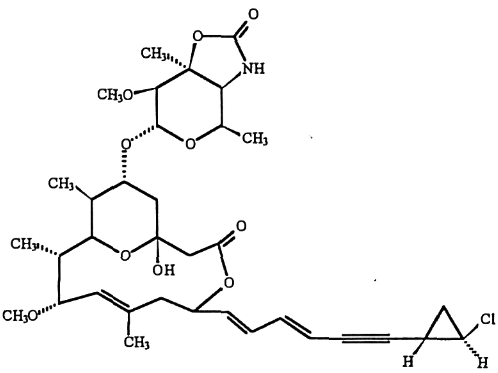
• ***Callipelta*** **sp.,** a New Caledonian sponge.

The crude ethanol extract of *Callipelta* sp. showed antifungal, antiviral and cytotoxic activities. The isolation work on the sponge extracts afforded two kinds of bioactive metabolites: a) three new peptides: callipeltin A (active against HIV, CD_{50} 0.29 μg/ml), B and C (active as an antifungal against *C. albicans*) (D'Auria 1996, Zampella 1996); and b) three new antiproliferative macrolides: callipeltoside A, B and C (Zampella 1996 & 1997). Structural elucidation of these compounds was obtained through analysis of their MS and NMR spectral data, chemical degradation and derivatization. In particular, the major callipeltin A possesses a cyclodecadepsipeptidal structure containing (together with conventional aminoacids) three novel residues not previously described from natural sources: β-methoxy-Tyr, 3,4-dimethyl-Gln and 4-amino-7-guanidino-2,3-dihydroxyheptanoic acid (AGDHE). Callipeltin B, a cyclooctadepsipeptide, differs from callipeltin A by having a 2,3-dimethylpyroglutamic acid as the N-terminal residue, while callipeltin C is simply the acyclic derivative of callipeltin A. The cytotoxic callipeltosides represent a new class of marine glycosylated 14-membered macrolides with a dienyne chlorocyclopropane side chain. Their mode of action involves a cell-cyclodependent effect, with cell blokade in G1 phase.



Callipeltin A R=A

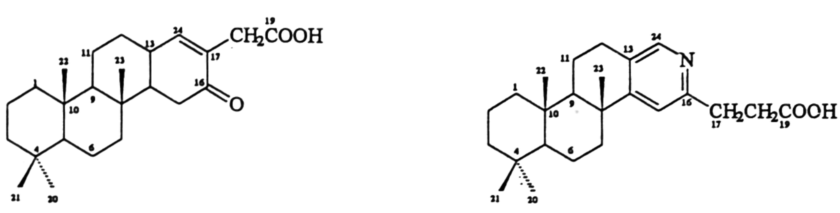
Callipeltin B R=B



Callipeltoside C

- ***Petrosaspongia nigra,*** a New Caledonian sponge.

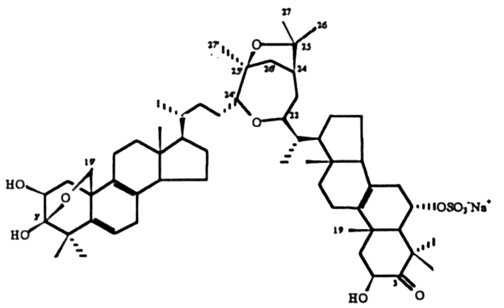
Preliminary *in vitro* cytotoxic tests (P388 and KB cell lines) showed that the apolar extracts of this organism inhibit 100% of cell growth at 10 μg/ml. The dichloromethane extract contained 13 new sesterterpenoids together with two known sesterterpene lactones. All the compounds were able to inhibit cell growth, indicating that these derivatives are responsible for the observed cytotoxicity of the apolar extracts of the organism. From a chemical point of view, these compounds can be divided into three classes: a) ten new sesterterpene lactones structurally related to the two known compounds already isolated by New Zealand researchers from another specimen of *P. nigra.*; b) three new sesterterpenes related to luffolide (a compound isolated for the first time from *Luffariella* sp., showing potent anti-inflammatory activity); and c) two new nor-sesterterpenes, one of which is a terpene-alkaloid (Gomez Paloma 1997).



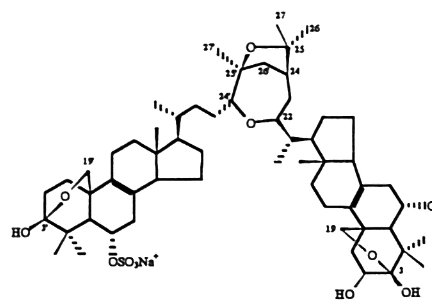
Selected sesterterpenes isolated from *P. nigra*

- *Crella* sp., a Vanuatu sponge.

The ethanolic extract of this sponge showed antiproliferative activity in preliminary tests on NSCLC-N6 (IC_{50}: 9.8 µg/ml). Kupchan solvent partition of the crude methanolic extract obtained from the lyophilized sponge, followed by chromatographic fractionation of the active chloroform and butanol fractions (IC_{50} <3.3 µg/ml and 6.2 µg/ml, respectively against NSCL-N6 cells), led to the isolation of a series of new dimeric mono- and disulfated steroidal derivatives named crellastatins (two examples are shown below). These compounds represent the first example of through-the-chain dimeric natural steroids. They possess a number of unusual structural features such as the 3α-hydroxy-3,19-epoxy function in both tetracyclic nuclei and the linkage between the side chain, made up of two ethereal bridges 22-24' and 25-25' and by a carbon-carbon bond between positions 24-26'. All the compounds tested so far proved highly cytotoxic against NSCLC-N6 cell lines. Study of the *in vivo* activity of the major component R1697C1 on NSCLC tumors grafted into the nude mouse is now in progress. The structures proposed for the different crellastatins isolated from *Crella* sp. were derived through extensive 2D-NMR experiments.



R1697 C4

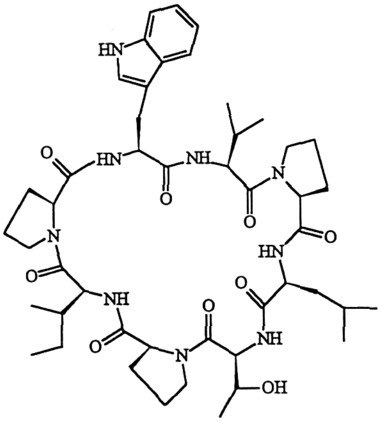


R1697 D4

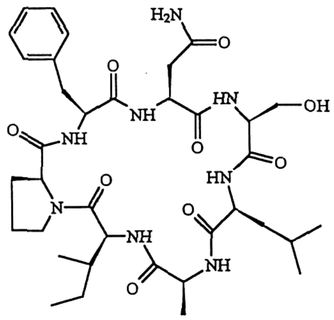
- ***Axinella carteri***, a Vanuatu sponge

The usual extraction, Kupchan solvent partition procedure and chromatographic fractionation, monitored by antiproliferative tests on NSCLC-N6 cell, afforded nine bioactive cyclopeptides. Structural work by MS and 2D-NMR techniques led to the identification of five known compounds already described in the literature. Two representative examples of this class of compounds, phakellistatin 10 (R1652-C6) and stylostatin 1 (R1652-C1), are reported below.
The four remaining compounds appear to be new bioactive metabolites. Mass spectral data and 2D NMR techniques allowed us to establish the aminoacid composition of the major axinellin A (2x L-Phe, L-Asn, L-Ile, L-Thr and 2xL-Pro). The aminoacid sequence in axinellin A is currently under investigation through MS-MS fragmentation analysis and interpretation of NOESY and HMBC spectra. The same MS and NMR spectral analysis was applied to axinellin B, indicating the presence of L-Trp, L-Phe, L-Val, 3x L-Pro, L-Leu and L-Thr. Chemical and structural work on axinellins C and D, which has just started, has revealed the presence of an

N-methyl-aminoacid residue together with the usual proteinic aminoacids. The complete structural characterization of all the new metabolites is currently in progress.



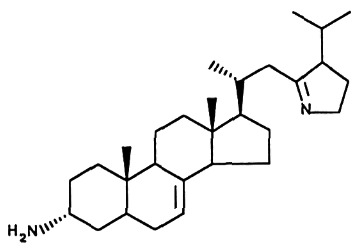
PHAKELLISTATIN 10 (R1652C6)
G.R.Pettit *J.Nat.Prod*, 1995, *58*, 961-965



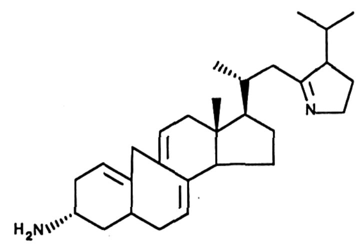
STYLOSTATIN 1 (R1652C 1)
G.R.Pettit *J.Org.Chem.*, 1992, *57*, 7217-7220

- ***Corticium*** sp., a Vanuatu sponge

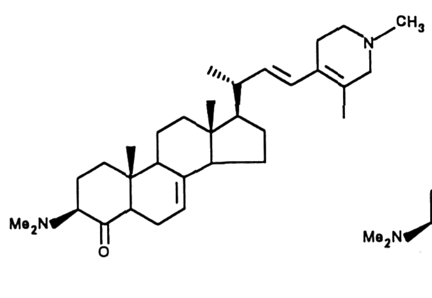
Ethanolic extracts of this sponge exhibited a consistent inhibitory effect on KB cell lines at 10mg/ml. The usual extraction procedures and chromatographic separation of fractions with antiproliferative activity on NSCLC-N6 and L16 cell lines led to the isolation of bioactive steroidal alkaloids. Although these molecules are known metabolites of certain land plants, they have been previously reported in marine organisms only in a sponge of the genus *Plakina*. These new steroidal alkaloids possess a structure related to plakinamine A and B isolated from *Plakina* sp. A few representative examples are reported below.



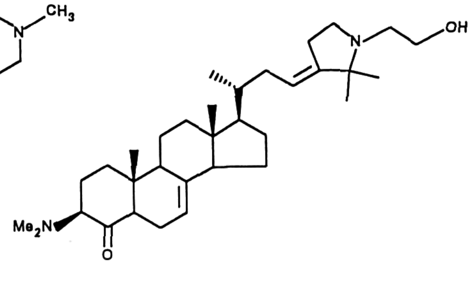
R1718-C1



R1718-C2



R1718-C3



R1718-C4

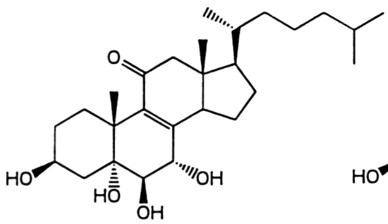
• *Clavelina lepadiformis*, a Corsican ascidian

Lepadin A was isolated from a Corsican specimen of this ascidian. This substance was previously identified from a North Sea sample (Steffan 1991). X-ray analysis elucidated the structure which had not previously been completely determined on the basis of NMR experiments alone. Pharmacological studies of this substance are in progress.

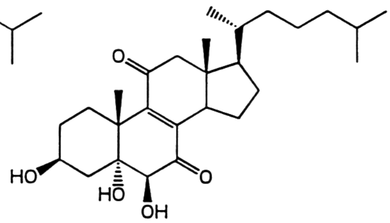
B. SYNTHESIS AND PHARMACOLOGICAL EVALUATION OF BIOACTIVE SUBSTANCES

• **Incrustasterol A (INA) and B (INB).**

Incrustasterol A and B (INA and INB) are two novel polyoxygenated steroids isolated from the marine sponge *Dysidea incrustans*, showing cytotoxicity against human tumor cell lines (Casapullo 1995). The synthesis of incrustasterol A and B has been accomplished starting from 7-dehydro-cholesterol in 11 and 12 steps respectively (Izzo 1996). The total yields were 1.6% and 0.9% respectively.



INA



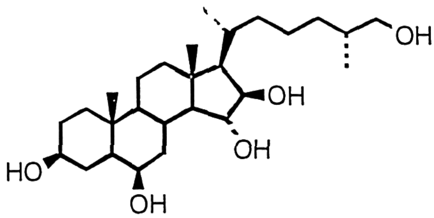
INB

• **(25*R*)-5α-cholestane-3β,6β,15α,16β,26-pentol, a cytostatic starfish steroid.**

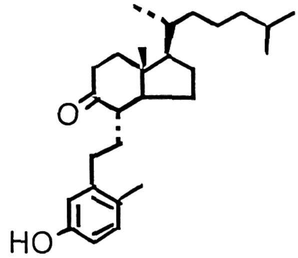
The title compound was previously isolated from an Antarctic starfish belonging to the *Echinasteridae* family and reported to have a cytostatic effect on human bronchopulmunary non-small-cell lung carcinoma cells (NSCLC-N6) (Iorizzi 1996). To allow *in vivo* studies on NSCLC tumors grafted into the nude mouse, the compound was synthesized from commercially available diosgenin. The synthesis (Izzo, submitted) was performed in 13 steps and in an 8% total yield. The compound is being subjected to pharmacological evaluation *in vitro* and *in vivo*.

• **Calicoferol E, a marine 9,10-secosteroid.**

Calicoferol E is a member of a family of biologically active 9,10-secosteroids isolated from an undescribed gorgonian of the genus *Muricella* (Seo 1995). In the context of synthesis and pharmacological evaluation of degraded marine steroids (De Riccardis 1995), calicoferol E has now been synthesized (De Riccardis, submitted). The synthesis has been performed by means of a convergent strategy in which the aromatic portion was synthesized in 6 steps and in a total yield of 6%, while the bicyclic fragment was synthesized in 5 steps starting from vitamin D_3 and in a total yield of 25%. The two fragments were connected by radical coupling leading to the target molecule in 2 steps and in a total yield of 14%. The compound shows cytotoxic activity on NSCLC-N6 clones C65 (<3.3 μg/ml) and C98 (5.8 μg/ml).



(25*R*)-5α-cholestane-3β,6β,15α,16β,26-pentol



Calicoferol E

5. IDENTIFICATION OR SYNTHESIS, AND EVALUATION OF POTENTIALLY ANTIVIRAL SUBSTANCES

To date, no major antiviral compounds have been identified from the collected samples.

- *Halicortex* sp., an Ustica sponge

From the ethanolic antiherpetic extract of *Halicortex* sp., a pure compound was isolated with the molecular formula $C_{22}H_{20}N_7O_3Br$, which possesses slight anti-HSV-1 activity (therapeutic index > 3.5). Structural elucidation is in progress.

- **Sample 2151**, a Vanuatu sponge

The aqueous extract from the sponge 2151 possesses cytotoxic (IC_{50} = 6.3 µg/ml on NSCLC-N6 L16) and antiherpetic activities (therapeutic index = 7). A peptide, constituted of serine, valine, alanine, glycine, leucine isoleucine, proline, threonine and two other as yet unidentified amino acids, was isolated (probably MW: 732). This product has a therapeutic index above 22 on herpes simplex type 1 virus and is not cytotoxic. Structural elucidation is in progress.

- ***Clavelina moluccensis***, a Djibouti ascidian

Lepadiformin and four related products were isolated from the cytotoxic and antiherpetic ethanolic extract of *Clavelina moluccensis*. Lepadiformin was previously identified as the cytotoxic compound of *C. lepadiformis,* an ascidian collected off the coast of Tunisia (Biard 1994). One of the four products (CM1) was identified, and structural elucidation of the other three (CM3, CM4 and CM5) is in progress. On herpes simplex virus type I, CM1 possesses a weak activity (therapeutic index: 2.5) which cannot account for the activity of the extract (therapeutic index > 20). Lepadiformin is not active, and CM4 is too toxic. Lepadiformin and CM1 are not active on dengue. Other pharmacological investigations are in progress.

6. SYNTHESIS, AND EVALUATION OF POTENTIALLY ANTI-INFLAMMATORY SUBSTANCES

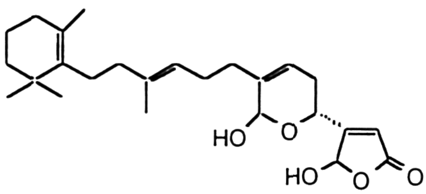
- **Analogs of the anti-inflammatory sponge sesterterpenes manoalide and cacospongionolide B.**

Manoalide and cacospongionolide B are two sponge metabolites exhibiting many biological activities, among which the most important is anti-inflammatory activity. Several syntheses of manoalide have been published, and attempts have also been made to synthesize the pyranofuranone moiety, which is considered to be a potential pharmacophoric group of these compounds.

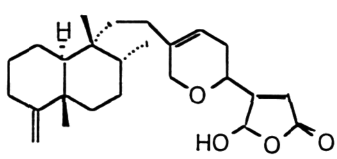
We have now developed a new general strategy (Soriente 1996) for the synthesis of the pyranofuranone moiety, which has allowed the preparation of 5 analogs of manoalide and 5 analogs of cacospongionolide B (De Rosa, submitted).

The anti-inflammatory activity of the compounds has been evaluated at the Department of Pharmacology of the University of Valencia (Alcaraz, Paya, Garcia Pastor). The two series of compounds were tested for their inhibitory effects on secretory phospholipase A_2 belonging to groups I, II and III and showed similar enhanced activities in both series of analogs relative to the increasingly hydrophobic character of the side chain. The IC_{50} of the most active compounds, FCA and FMA, was similar to that of manoalide. FMA was tested in carrageenan paw edema in mice and showed an activity similar to that of the reference anti-inflammatory drug indomethacin.

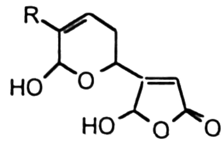
In addition, MCA showed antiviral activity on HIV at 1 μg/ml and a delay at 0.1 μg/ml. MCA, MMA, BCA and MMA showed weak cytotoxic activity on NSCLC-N6 cells. BCA showed weak antiangiogenic activity but with undesirable side effects.

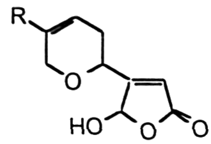


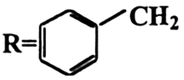
Manoalide

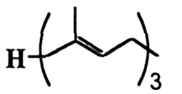


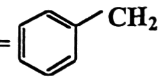
Cacospongionolide B

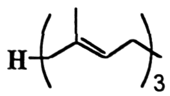




MA; R=H
MMA; R=CH_3
CMA; R=$CH_3CH{=}CHCH_2$
BMA; R= 

FMA; R= 

CA; R=H
MCA; R=CH_3
CCA; R=$CH_3CH{=}CHCH_2$
BCA; R= 

FCA; R= 

7. CONCLUSION

The search for new substances of pharmacological interest from sponges and ascidians of southern Melanesia, eastern Africa, and the Mediterranean or eastern Atlantic coast is in progress. About 250 species have been collected and investigated for antitumoral, antiviral or immunomodulatory activities. About 78 substances have already been identified (48 new ones), and the synthesis of 16 marine active compounds or derivatives has been performed. Only some examples of these results are included in this report. Although no substance has yet proved of sufficient interest for therapeutical applications, many promising leads are being explored or remain to be explored.

8. REFERENCES

Biard J.F., Guyot S., Roussakis C., Verbist J.F., Vercauteren J., Weber J.F., Boukef K. 1994. *Tetrahedron Lett.* **35**, 2691.

Casapullo A., Minale L., Zollo F., Roussakis C., Verbist J.F., 1995, *Tetrahedron Lett.* **36**, 2669.

D'Auria M.V., Zampella A., Gomez Paloma L., Minale L., Debitus C., Roussakis C., Le Bert V., 1996, *Tetrahedron*, **52**, 9589.

De Riccardis F., Spinella A., Izzo I., Giordano A., Sodano G., 1995, *Tetrahedron Lett.* **36**, 4303.

De Riccardis F., Della Sala G., Izzo I., Sodano G., submitted for publication.

De Rosa M., Giordano S., Scettri A., Sodano G., Soriente A., Garcia Pastor P., Alcaraz M., Paya M., submitted for publication.

Gomez Paloma L., Randazzo A., Minale L., Debitus C., 1997, *Tetrahedron* **53**, 3243

Iorizzi M., De Marino S., Minale L., Zollo F., Le Bert V., Roussakis C., 1996, *Tetrahedron* **52**, 10997.

Izzo I., De Riccardis, F., Massa A., Sodano G., 1996, *Tetrahedron Lett.* **37**, 4775.

Izzo I., De Riccardis F., Sodano G., submitted for publication.

Seo Y., Shin J., Song J., 1995, *J. Nat. Prod.* **58**, 1291.

Soriente A., De Rosa M., Scettri A., Sodano G., 1996, *Tetrahedron Lett.* **37**, 8007.

Steffan B., 1991, *Tetrahedron* **47**(41), 8729.

Zampella A., D'Auria M.V., Gomes Paloma L., Capsullo A., Minale L., Debitus C., Henin Y. 1996, *J. Am. Chem. Soc.* 118, 6202.

Zampella A., D'Auria M.V., Minale L., Debitus C., Roussakis C. 1996, *J. Am. Chem. Soc.* **118**, 11085.

Zampella A., D'Auria M.V., Minale L., Debitus C., 1997, *Tetrahedron* **53**, 3243.

TITLE: Microorganisms in deep sea vents and marine hot springs as sources of potentially valuable chemicals

CONTRACT N°: MAS3-CT9595-0034

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MICROORGANISMS IN DEEP SEA VENTS AND MARINE HOT SPRINGS AS SOURCES OF POTENTIALLY VALUABLE CHEMICALS.

D. Prieur and partners

CNRS, Station Biologique, 29682 Roscoff, France

SUMMARY

The objective of the project is to isolate thermophilic microorganisms from selected hot deep and shallow marine environments and to screen them for selected biomolecule production: enzymes, lipids, polymers, compatible solutes, antibiotics and other pharmaceutical drugs. Various cultur methods (including mixed microbial consortia and pressurized cultures) will be employed in order to obtain a large variety of organims. Those producing interesting substances will be then characterized both taxonomically and physiologically, in order to allow (eventually) patenting, and improvement of the compound production. Depending of the properties found, further cooperations could be developped with specialized laboratories and/or industrial companies, in order to characterize the molecule (s) selected, to take patents and to develop industrial applications.

INTRODUCTION:

Marine microorganisms and particularly Prokaryotes have not been intensiveley screened for potential biotechnological properties. Similarly, if the anaerobic hyperthermophiles (from both terrestrial and marine origins) have been intensively studied, aerobic thermophiles received a lower interest.

For these reasons, we decided to set up a collection of marine aerobic thermophiles from various geographic locations in the Atlantic and the Mediterranean Sea. This collection which consists of nearly 500 isolates is unique.

Among the aerobic thermophiles, the more frequent organisms are heterotrophic, and so normally easy to grow in high densities. In addition most of the known isolates belong to the genera *Thermus* and *Bacillus* (although novel genera cannot be excluded for recent isolates), for which genetic tools exist. For all these reasons, aerobic thermophiles possessing interesting biotechnological properties should be used in industrial processes withou major problems to be overcome.

While the screening processes are in progress, we are particularly interested in iniating contacts with industrial partners, to discuss about commercial interest of screened molecules or who would like to suggest new directions for the screening of our strain collection.

WORKPLAN

An overview of the total work plan is given in figure 1. Two years after the beginning of the project, several encouraging results have been obtained. However, inorder to keep open the possibilty of patenting, no information can be published early. Thus the screening processes and general procedures will be described in this paper.

1.1. A unique collection of thermophilic organisms:

A collection of 595 thermophilic aerobic strains isolated from samples collected in different geographic areas (table 1, figure 2 to 4) has been established. A preliminary set of data characterizing the strains has been entered into a data base, then distributed among the partners to allow selection of strains particularly adapted to a specific screening.

1.2. Screening protocols:

1.2.1. Polysaccharide degradation:

A pre-screening protocol has been developped and successfully tested by partner 2 (ICETEC, DR. J. Kristjansson).

Minimal agar media with NACL (1% w/v) and a solution of vitamin B, containing different polysaccharides was used for the prescreening of strains (strains isolated by ICETEC have been used for this purpose). The polysaccharides were alginate (1%w/v), Carragenan (1%w/v), carboxyl methyl cellulose (CMC) (1%w/v), chitosan (1%w/v), locust bean gum (LBG) (0.5%w/v) and agar (1%w/v). Detailed media composition is given in annex 4.

The strains were all precultivated on media 166 with 1% NaCl, and then incubated on the different polysaccharides for 5 days at 60, 65 or 72°C, after which they have been scored as +, ++, and +++ indicating growth, good growth and very good growth respectively.

The results of the prescreening tests are given in table 2.

Polysaccharide	+	++	+++	Total
Alginate	66	**18**	**6**	90
Carragenan	102	**25**	**1**	132
CMC	**12**	0	0	12
Chitosan	134	**28**	**1**	163
LBG	165	75	**31**	271
Agar	**32**	**1**	0	33

Table 2: Gowth on different polysaccharides. A total of 310 strains were tested on minimal media with 1% NaCl and a polysaccharide as sole carbon source. The bold numbers indicate strains which will be tested further on the corresponding polysaccharide.

This protocol for pre-screening will be repeated on strains isolated by other partners.

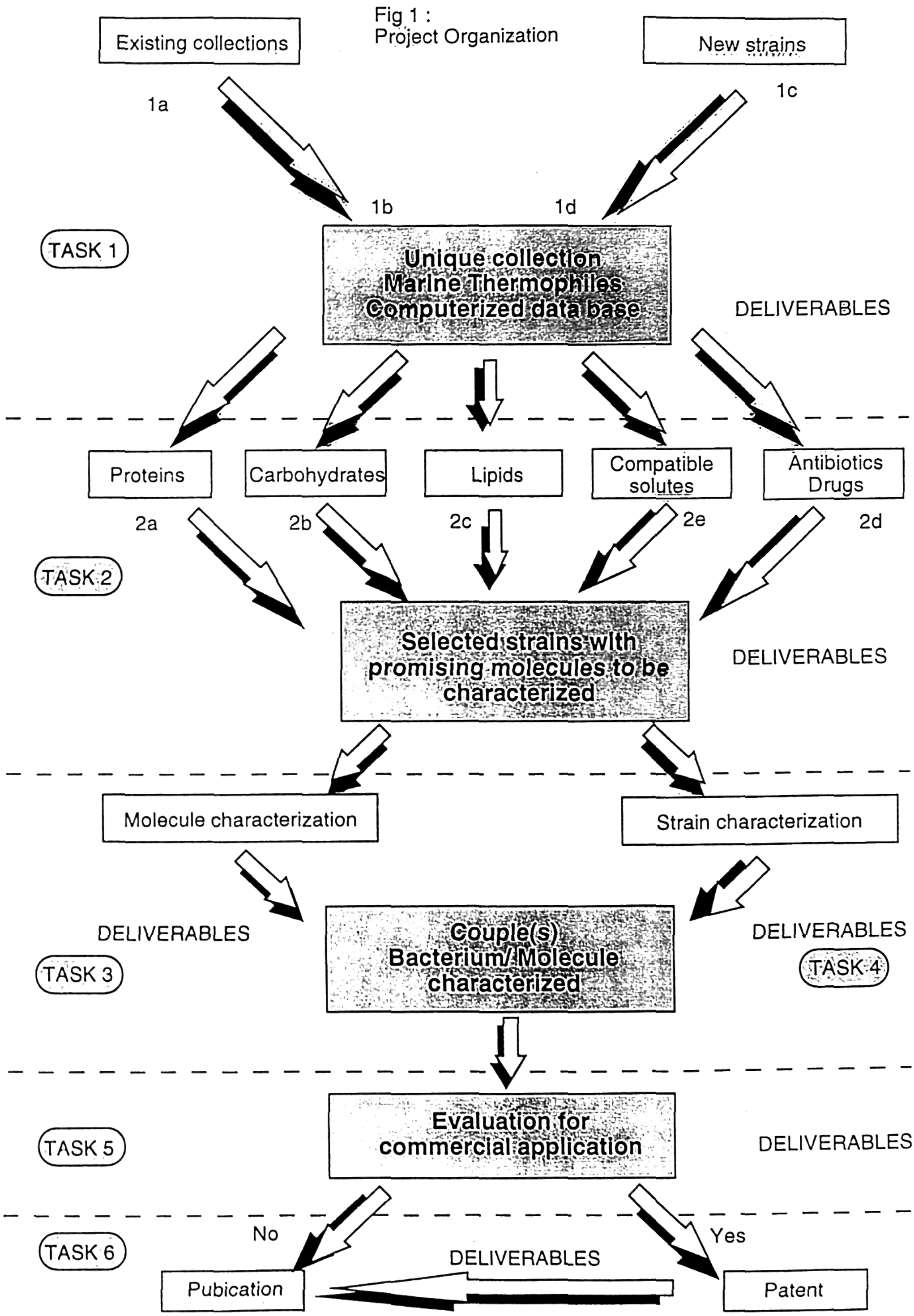


Fig 1 :
Project Organization
Existing collections
New strains
1a
1c
1b
1d
TASK 1
Unique collection
Marine Thermophiles
Computerized data base
DELIVERABLES
Proteins
Carbohydrates
Lipids
Compatible solutes
Antibiotics Drugs
2a
2b
2c
2e
2d
TASK 2
Selected strains with promising molecules to be characterized
DELIVERABLES
Molecule characterization
Strain characterization
DELIVERABLES
TASK 3
Couple(s) Bacterium/Molecule characterized
DELIVERABLES
TASK 4
TASK 5
Evaluation for commercial application
DELIVERABLES
TASK 6
No
Yes
DELIVERABLES
Pubication
Patent

Partner	Site/Cruise	Number of strains	Total of strains
1) CNRS.Roscoff	Gyaynaut Microsmoke Mar Biolau	32 25 71 18	146
2) Icetec.Reykjavic.	Iceland: Oxarfjorour : Hrisey : Reykjanes : Berserkseyri : Lysuholl : Akraos : Saltfactory : Blue Lagoon	121 4 36 27 3 35 49 32	307
3)University of Coimbra. Coimbra	Azore Stufe di Nerone	9 4	13
4).CNR-ICMIB. Arco Felice.	Fumosa Ischia-Sorceto Ishia-Castaromana Ishia-Castiglione Ischia-Maronti	1 6 1 3 3	14
5) University of Messina. Messina	Stromboli Panarea Lipari Vulcano	5 2 6 51	64
6). CAMR. Portown Down.	Italy France Thailand North sea	42 6 1 2	51

Table 1: The collection of marine aerobic Thermophiles.

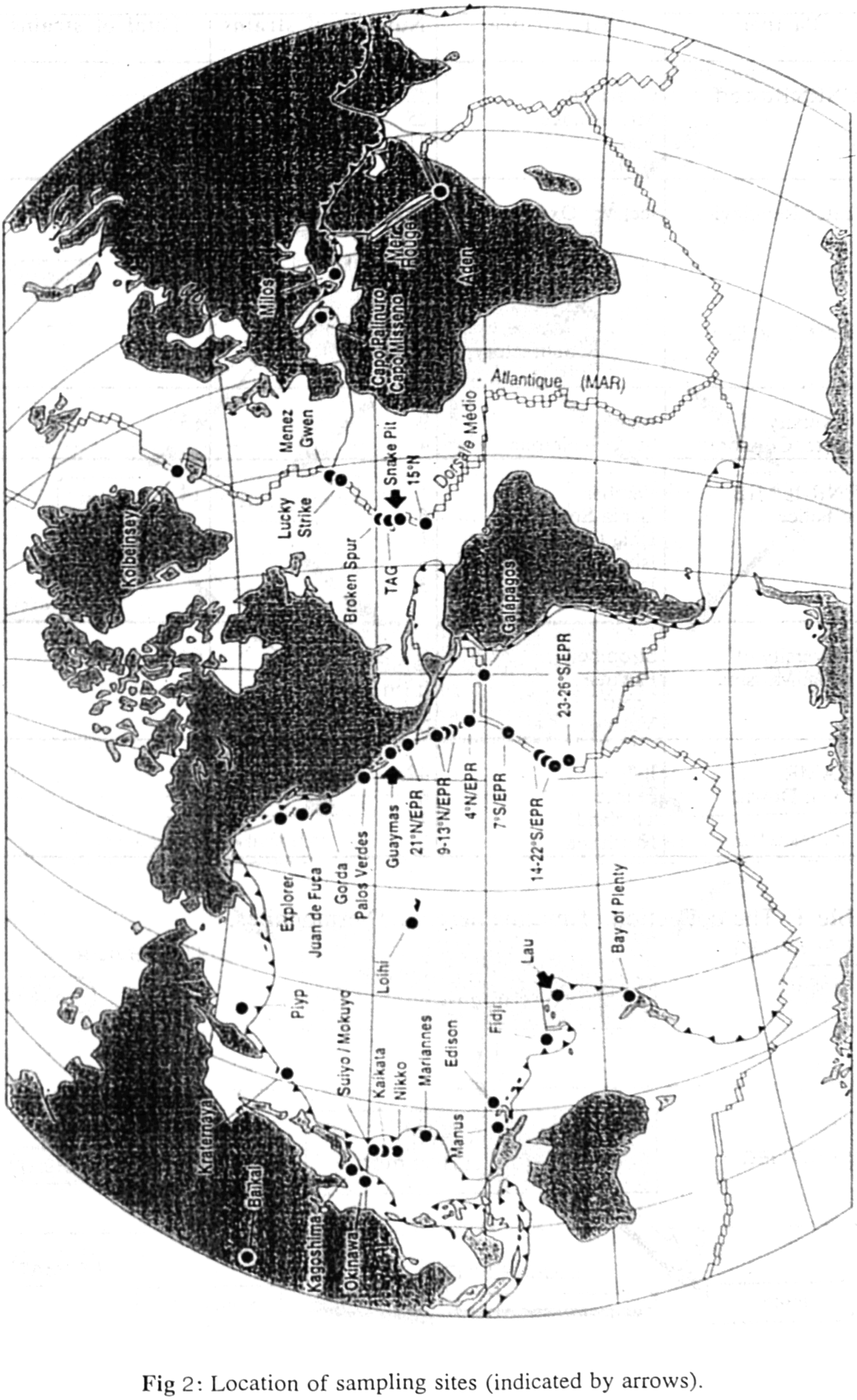
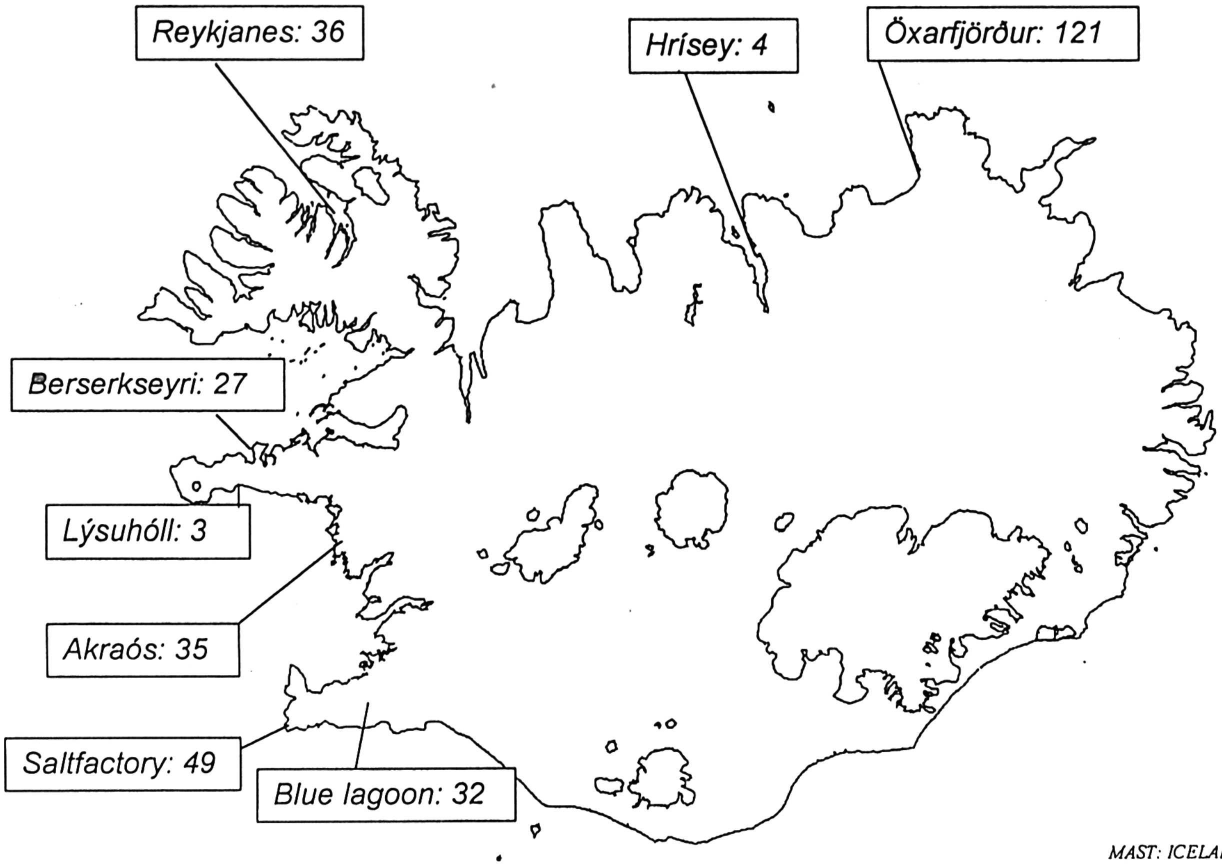


Fig 2: Location of sampling sites (indicated by arrows).

Fig 3: Location of sampling sites in the Eolian Islands (Italy).

Areas for sampling of thermophilic halotolerant microorganisms



MAST: ICELAND

Fig 4: Location of sampling sites along the Icelandic coast.

1.2.2. Polysaccharide production:

Microorganism and culture conditions:

Microorganism was grown in 1 liter batch culture. Culture medium contained 6 g liter- 1 of glucose or sucrose and 1 g liter- 1 of Yeast extract plus marine salt solution. The glucose medium supplemented with 20 g liter-1 of agar was used for plates preparation.

Prescreening protocols:

Exopolysaccharides:

The analysis could be done on all strains able to growth on sugar medium or on strains showing on agar plates mucous colonies. microorganism was grown in 1 liter batch culture. Culture medium contained 6 g liter-1 of glucose or sucrose or other sugars and 1 g liter- 1 of Yeast extract plus salt marine solution. Cells were harvested by cultural broth centrifugation in a stationary phase of growth (1 liter, 9800 x g, 20 min.). The liquid phase was treated with 1 volume of cold ethanol added dropwise under stirring. Alcoholic solution was kept at - 18°C overnight and then centrifuged at 15300 x g for 30 min. The pellet was dried and send to us.

Lipopolysaccharides:

The analysis must be done only on Gram negative cells. Gram negative cells, growth in standard medium, were collected at late exponential growth phase, washed once with distilled water and lyophilized.

Production of EPS:

Microbial growth and EPS production were monitored quantitatively both for batch and fermentative cultures by sampling 10 ml of culture broth at 0, 8,16, 24 and 48 h; biomass production was followed turbidimetrically reading the absorbance at 540 nm; EPS production was tested on cell free cultural broth with phenol-sulfuric acid mediod using glucose as standard (1).

Isolation and purification of EPS:

Cells were harvested by cultural broth centrifugation in a stationary phase of growth (1 liter, 9800 x g, 20 min.). The liquid phase was treated with 1 volume of cold ethanol added dropwise under stirring. Alcoholic solution was kept at -1 8° C overnight and then centrifuged at 15300 x g for 30 min. The pellet was dissolved in hot water (1/10 initial volume). The same procedure was repeated again. The final water solution was dialyzed against tap water (48 h) and distilled water (20 h), than freeze dried and weighted. The sample was tested for carbohydrate, protein and nucleic acid contents.

Polysaccharide was purified by Gel Chromatography (Sephadex G-50; 2.SxSO cm) using H_20/Pyridine/AcOH (500:5:2, by vol) as eluant, fractions were collected with a flux of 6 ml h- 1 (5 ml each fraction), followed by anion exchange chromatography (Sepharose DEAE CL,6B; 1.5x40 cm) eluted with 0. 1 liter of H_20 and 1 liter of a NaCl gradient from 0 to 1 M with a flux of 12 ml h- 1, the volume of each fraction was 10 ml. Fractions were tested for carbohydrate qualitatively by spot test on TI-C sprayed with a-naphthol and quantitatively by Dubois method (1). The α-naphtol positive fractions were pulled, exhaustively dialyzed against water, freeze-dried and weighted. This material was used for all analytic work.

Colorimetric assay:

Carbohydrate content was performed according to Dubois method (1) reading absorbance at 490 nm and using glucose for calibration curve. Total protein content was estimated by using Bradford reagent (Bio-Rad) and bovine serum albumin as a standard. Nucleic acid content was tested spectrophotometrically reading the absorbance at 260 nm. Pyruvate was detected after polysaccharide hydrolysis (100°C, 3h) using a solution of 0.5% w/v of 2,4-dinitrophenylhydrazine in 2M HCl (4). Sulfate presence was identified by Silvestri method (2).

Molecular weight estimation:

Molecular weight was estimated by: 1) gel filtration on Sepharose CL,6B column (1 x80 cm) using H_2O/Pyridine/AcOH (500:5:2, by vol.) as eluant. Fractions were collected at 3.7 ml h- 1 and tested by spot test on TLC sprayed with a-naphthol. 2) Density gradient centrifugation using a sucrose gradient from 0 to 50% w/v at 130000 x g for 16h. Centrifuge tubes were fractionated in 0.2 ml fractions diluted with water, dialyzed against water for 72 h and tested for carbohydrate presence as reported above. In both experiments 10 mg of EPS and a mixture of Dextran for calibration curves (10 mg each of.. T-700, mol. wt. 670000; T-400, 4 10000 and T- 150, 154000) were used.

Sugar analysis:

Hydrolysis of EPS was performed with 2M trifluoroacetic acid (TFA) at 120°C for 2 h. Sugar mixture was identified by TLC and HPAE-PAD using sugar standards for identification and calibration curves. TLC was developed with the following solvent system: a) acetone/ButOH/H_2O (8:2:2, by vol.) for neutral sugars; b) ButOH/H_2O/AcOH (3: 1: 1, by vol.) for acidic sugars; c) ButOH/EtOH/H_2O (5:3:2, by vol.) for oligosaccharides. Sugars were visualized by spraying TLC with a-naphthol. HPAE-PAD Dionex equipped with Carbopac PA 1 column was eluted isocraticary with: a) 15 mM NAOH for neutral sugars, b) buffer 100 mM NaOH and 150 mM NaOAc for acidic sugars.

Methylation analysis:

Methylation of the polysaccharides was carried out according to the methods described earlier (3, 4). The methylated material (0.5 mg) was hydrolysed with 2 M TFA at 120°C for 2 h and then transformed in partially methylated alditol acetates by reduction with NaBH4, followed by acetylation with Ac20/Pyridine (1: 1, by vol) at 120! C for 3 h. Unambiguous identification of sugars was obtained by GLC and GC-MS using sugar standards. GLC runs were performed on a Hewlett-Packard 5890A instrument, fitted with a FID detector and equipped with a HP-5-V column and N2 flux of 100 ml min-1. The temperature program used was: 170°C (1 mn), from 170°C to 180°C at 1°C min-l, 180°C (1 min), from 180°C to 210°C at 4°C min-1. GC-MS was performed on a Hewlett-Packard 5890-5970 instrument equipped with a HP-5-MS column and with a N2 flux of 50 ml min- 1; the temperature program used was: 170°C (1 min), from 170°C to 250°C at 3°C min-1.

Absolute configuration:

The absolute configuration of the sugars was performed as described by Leontein et al. (5) using optically active (+)-2-butanol by GLC of their acetylated-(+)-2-butyl glycosides. For GLC runs the same instrument and conditions described in methylation analysis were used. Retention times were determined by comparison of the sample with authentic references.

Spectroscopic analysis:

Infrared spectrum of polymer (KBr tablet, 100 mg) was recorded at room temperature using a FT-IR BIO-RAD spectrometer. Ultraviolet spectra of EPS were obtained reading the absorbance of aqueous solutions (3 mg ml-1) from 350 to 210 nm on a Varian DMS-90 instrument. Optical rotation value was obtained on a Perkin-Elmer 243 B polarimeter at 25° C in water. NMR spectra were obtained on a Bruker AMX-500 (500.13 MHz for 1H and 125.75 MHz for 13C) at 70°C. Prior to analysis EPS samples were exchanged twice in D20 with intermediate lyophilization and then dissolved in 500 MI D20 to a final concentration of 40 mg ml- 1. Chemical shifts were reported in ppm relative to sodium 2,2,3,3,-d4-(trimethylsilyl)propanoate for 1H and CDC13 for 13C NMR. The 1JH- 1,C- 1 values were determined by HMQC inverse detected experiments.

Culture conditions for lipopolysaccharides:

Gram negative cells were collected at late exponential growth phase, washed once with distilled water and lyophilized.

Lipopolysaccharide extraction and analysis:

Cells (1 g lyophilized cells) were extracted with hot phenol-water (67°C under stirring). LPS were hydrolysed with 2% Acetic acid at 80° C for 2 hr. 0-antigen fraction was obtained by partition beetwen chloroform-water phase. Water-phase analysis was performed as described in polysaccharide protocols. Organic- phase was done according to fatty acid analysis as described in lipid protocols.

1.2.3. Lipid analysis:

Growth conditions for preparing biomass:

There is considerable evidence that the lipid composition is markedly affected by growth condition. The bacteria for comparative studies are grown under standardized conditions of pH, temperature, medium composition and phase of growth. The cells will be harvested at the beginning of the stationary growth phase. It will be better to avoid in the culture medium material containing fatty acids.

Lipid extraction:

The dried cells (200 mg salt-free at least) will be extracted with $CHCl_3$-MeOH (1/1 v/v) at room temperature for 3 days.

Thin layer chromatography (TLC):

The total lipid extract is chromatographed usually on TLC developed with $CHCl_3/MeOH/H_2O$ (65/25:4 by vol.) in a single development. The lipids are detected by charring at 150° C for 10 min using plates sprayed with 50% (v/v) methanolic-H_2S0_4, 1 % (w/v) cerium sulphate- 1 M H_2S0_4. Phospholipids are detected by Zinzadze reagent, aminolipids by spraying with 0.2% (w/v) solution of nihydrin in butanol saturated with water followed by heating and glycolipids can be detected by spraying plates with 1 -naphthol reagent followed by heating. In some case the TLC will be run in double development: the first direction $CHCl_3/MeOH/H_20$ and the second one $CHCl_3/MeOH/AcCOOH/H_20$ (80/12/1514 by vol.).

Non polar compound analysis:

The total lipid extract is treated with n-hexane that will extract the neutral lipids which are analysed on TLC developed with petroleum ether/ethyl ether (96:4, v/v) or with n-hexadecane/ethyl acetate (98:2 v/v) and visualysed under UV lamp or by iodine exposition.

Hydrolysis of complex lipids:

Strong acid methanolysis: methanol/HCl (9: 1) overnight at ca. 80°C.The mixture is dried and extracted with $CHCl_3/MeOH$ (1: 1, v/v) and analysed by TLC. Alkaline mild hydrolysis: 10 mg of pure compound with 10 mg of Na_2C0_3 anhydrous and 3 ml of MeOH at room temperawm for 1 day. 'Me niixwm is filtered and neutralysed with HCl in MeOH and then dried. The dried mixture is extracted in $CHCl_3$ and the remaining is dissolved in water.

TLC of hydrolysed lipids:

The solvent-soluble part of the hydrolysis mixture (both acid and alkaline) was chromatographed on TLC developed with $CHCl_3/MeOH$ (9/1, v/v); n-hexane/ ethylacetate (75/25, v/v); n-hexane/ethylacetate (8/2, v/v) for archaeal core lipids. For fatty acid methyl esters petroleum ether/ ethylic ether (96/4, v/v). The unsaturated fatty acids are analyzed in n-hexane/ethylacetate (9/1, v/v) on TLC impregnated with $AgNO_3$. The spots are visualised by iodine exposition or by carbonisation as before reported.

Results:

The first screening permits to know the type and the number of complex lipids present in the isolate with a realtive Rf. For neutral lipids, it is possible to know if there are spots UV-absorbent. After hydrolysis it is possible to possible to discriminate between archaea and bacteria isolates. Thee specific TLC permits to know the type of core lipids present if the microorganism taken in consideration is an Archaea. For fatty acid the specific TLC permits to know the presence of saturated, monosaturated and polyunsaturated fatty acids. The first screening for core, lipids could be carried out also in the niixture of microorganisms in order to ascertain the presence of bacteria alone or archaea alone or both type of microorganisms.

Further analysis:

If the amount of lipid extract is enough, it will be possible to purify the more abundant lipids and charaterize by spectroscopy approach (^{1}H and ^{13}C NMR, FAB-MS). The purification will be done by Silica-gel column chromatography or semipreparative TLC and/or combination of both chromatographies. If from the first screening it could be hypothysize the presence of new type of lipids, more biomass will be prepared for detailed chemical studies. The fatty acids will be analysed by GC and CG-MS in the conventional way.

1.2.4. Antimicrobial activity:

All the strains of the collection will be checked for anti-microbial activity against a range of targer organisms including *Staphylococcus aureus*, *Steptococcus pyogenes*, *Mycobacterium sp.* and *Pseudomonas aeruginosa*.

The isolates are grown on their isolation media and then overlayed with soft agar containing the target organism. The trypticase soft agar is teamed until molten and then held at 55°C until required. The target organism (0,5 ml from a broth culture) is added to sterile glass tubes and 3,5 ml of trypticase soy agar is added. The contents are mixed and poured onto the surface of the agar plate with the marine isolate on. The agar overlay is allowed to set and the plates are then incubated at the optimum temperature for the target organism. A control plate with no bacterial growth is overlayed with the target organism to ensure a lawn of bacteria is produced. The plates are examined for zones of inhibition around the thermophilic isolate indicating it produces an anti-microbial compound.

1.2.5. Compatible solutes:

Compatible solutes are extracted from cells during the mid-logarithmic phase of growth using boiling 80% ethanol for 10 min. The cell debris are centrifuged and the supernatant placed in roun-bottom evaporation flasks. The cell pellet was resuspended in 80% ethanol and extracted once more at 4°C overnight. The suspension was centrifuged and the supernatant of the second extract combined with the first and evaporated to dryness in a rotary evaporator. The extract is washed once with chloroform to remove apolar components and used for NMR analysis.

All NMR spectra are acquired on a bruker AMX500 spectrometer. ^{13}C-NMR spectra are recorded at 121.77 Mhz using a 5 mm carbon selective probe head. Typically, spectra were acquired with a repetition delay of 5 s and a pulse width of 7 μs corresponding to 90°C flip angle. Proton decoupling is applied during the acquisition time only, using the wideband alternating-phase low-power technique for zero-residue splitting sequence. Chemical shifts are referenced to the resonance of external methanol designated at 49,3 ppm.

^{31}P-NMR spectra are recorded at 204,45 Mhz, with or without proton broadband decoupling, with a repetition delay of 5 s and a pulse width of 16 μs corresponding to 60° flip angle. Chemical shifts were referenced with respect to external 85% H_3PO_4.

^{1}H-NMR spectra are acquired with water presaturation, 6 μs pulse width (corresponding to a 60° flip angle) and a repetition delay of 10 s. Formate was added as a concentration standard. Proton chemical shifts are relative to 3-(trimethylsilyl) propanesulfonic acid (sodium salt). Quantification of the organic solutes are based on ^{1}H- and/or ^{31}P-NMR spectra of ethanol extracts. Phase-sensitive nuclear Overhauser effect spectroscopy and proton-homonuclear shift correlation spectroscopy are performed by using standard Bruker pulse programs. Spectra are acquired over a 5-Khz bandwidth, collecting 4,096 (t2), 512 (t1) data points. ^{1}H-^{13}C heteronuclear multiple quantum coherence spectra are acquired by collecting 4,096 (t2), 256 (t1) data points; 3,5 ms are used for evolution of 1JCH.

A preliminary screening carried out on 7 coastal vent strains and 3 deep-sea strains, indicated that all strains accumulated primilarily trehalose and β-mannosylglycerate, but a few also accumulated very low levels of glycine betaine.

CONCLUSIONS:

After one year, a unique collection of almost 600 aerobic thermophilic marine organisms has been established. During the second year of the project, the strains have been distributed in all the partner laboratories, each of them being specialized in a paricular screning task. In parallel, a preliminary phenotypic characterization of the strains has been carried out in order ot estimate the diversity among the isolates of the collection.

During the second year of the project, the screening protocols have been initiated. Although not completed alraedy (and some results must been confirmed), very encouraging results have been obtained and should lead to at least scientifically interesting molecules in the next future. Characterization of these molecules, and producing strains, evaluation of their commercial interest are presently under progress.

TITLE: Marine bacterial genes and isolates as sources for novel biotechnological products (project **MARGENES**)

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MARINE BACTERIAL GENES AND ISOLATES AS SOURCES FOR NOVEL BIOTECHNOLOGICAL PRODUCTS

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SUMMARY

The overall objective of the research project is the development and application of a new molecular strategy to turn the hidden biodiversity of marine bacteria into novel biotechnological products. This strategy is based on an integrated molecular approach using bacterial DNA and RNA obtained directly from the marine environment to circumvent the need for cultivation since only a very small fraction, usually less than 1%, of the marine bacteria can be cultured. The molecular approaches used are gene cloning and expression of marine bacterial genes, estimation of the structure of bacterial communities that carry the genes in situ, and molecular characterization of bacterial isolates. This integrated approach will be applied to a set of diverse marine habitats to obtain information about i) the diversity of microbial genes in marine habitats and their carrying bacteria, and ii) the expression systems necessary to make the genetic information available for biotechnological products such as enzymes and antibiotics. The diversity of the microbial community will be determined with rapid molecular profiling techniques (5S rRNA analysis and denaturing gradient gel electrophoresis of 16S rDNA) that are able to give an quantitative overview about the abundant species in the community. A set of different cloning vector will be used to generate polygenomic clone libraries directly from the marine habitat. Expression of these genes will be attempted by different expression vectors followed by automated activity measurements in an industrial high-through-put unit. These activity determinations and molecular analysis in combination with microbiological and biotechnological studies will result in: i) commercial products, i. e. a variety of novel psychrophilic and mesophilic enzymes, and ii) development of an intelligent strategy for the assessment of microbial diversity in any natural environment and its biotechnological potential, i. e. how and where to look with what molecular approaches for which products.

1. INTRODUCTION

The overall objective of the research project is the development and application of a new molecular strategy to turn the hidden biodiversity of marine bacteria into novel biotechnological products. This strategy is based on an integrated molecular approach using gene cloning and expression of marine bacterial genes, estimation of the organismal structure of the gene carrying bacterial communities *in situ*, and molecular characterization of bacterial isolates. This integrated approach will be applied to a set of diverse marine habitats to achieve the following objectives:

- Assessment of the diversity of microbial genes in marine habitats.

- Determination of the organismal structure of the marine bacterial communities containing the genes.
- Identification of bacterial strains isolated from the selected habitats.
- Evaluation of cloning and expression strategies for genes from marine bacterial communities that make genetic information available for biotechnological products.

The following final objectives will be achieved with these molecular analysis of marine genes and bacteria in combination with microbiological and biotechnological studies:

- Provision of production strains for commercial products, i. e. a variety of novel psychrophilic and mesophilic enzymes and antibiotics.
- Development of an intelligent strategy for the assessment of microbial diversity in any natural environment and its biotechnological potential, i. e. how and where to look with what molecular approaches for which products.

Our current understanding of the evolution of life is that it started in the ocean and was for at least the first two billion years purely microbial. The large size of the marine environment, the variability of the habitats and the enormous amount of microorganisms observed per volume of seawater as units of evolution are additional multiplicators of biological diversity. These basic facts of our biological understanding indicate that phylogenetic and metabolic diversity of marine microorganisms should be greatest among all forms of live (Woese 1987). This was demonstrated recently by molecular analysis of DNA obtained directly from marine microbial communities, using comparative 16S rDNA sequence analysis (Giovannoni et al. 1990, DeLong et. al. 1992, Fuhrman et al. 1992, McInerney et al. 1995). These communities comprised an enormous diversity of bacteria, including completely new phyla for the domain *Bacteria* and new lines of decent for the *Archaea*. This genetic diversity of very conserved homologous genes represents only the tip of the iceberg in terms of genetic diversity of marine bacteria that should be reflected in many novel biochemical pathways comprised of an almost indefinite number of new enzymes.

It is likely that marine prokaryotes will also be a reservoir of useful natural products and isolation and screening has revealed a number of interesting novel structures from marine-derived bacteria (Faulkner 1993). Many natural products from marine organisms represent new classes of chemical structures previously unknown and points towards a diversity of metabolism paralleling that of terrestrial microbial populations (Austin 1989).

Many of the antibiotics and therapeutic agents isolated from marine bacteria were from the readily culturable fraction of the population in shallow waters. Few studies have analyzed isolates from deep water samples. It is evident from a wide range of studies using molecular methods that the vast majority of the bacterial population in natural environments has yet to be cultured (Amann et al. 1990, Ward et. al. 1992). Therefore, it is a well known dilemma of marine microbiology that marine bacteria are very difficult to grow in the laboratory, e.g. from open ocean seawater samples only about 1‰ of the cells detectable by microscopic methods can be cultured (Jannasch and Jones 1959, Austin 1989). This means that the classical way to do biotechnolgy, i. e. the growth of the relevant microorganism to obtain the product, is blocked. An additional problem with marine bacteria is their well know difficulty to express the relevant function in the laboratory because the conditions they encounter in the test tube is so different from their natural environment.Whilst few developments have occurred in the problems of isolation and culture there have been significant and notable advancements in the molecular analysis of marine bacterial diversity *in situ* (Fuhrman et al. 1993, Mullins et al.1995).

2. WORKPLAN

A DIRECT MOLECULAR ANALYSIS OF DNA/RNA FROM MARINE BACTERIA

We will solve the basic dilemma in marine biotechnology by looking at the genetic information of marine bacteria directly, i. e. without any cultivation, in three molecular ways:

i) Cloning and expressing the genes.
ii) Profiling and sequencing rRNA or its genes from the whole microbial community.
iii) Molecular identification of isolated marine bacteria.

The molecular analysis of genes, communities and isolates will be complemented by a functional analysis of the obtained clones and isolates in a high-through-put unit to select for novel biomolecules such as enzymes and antibiotics (Fig. 1).

i) Cloning and expressing of marine microbial genes

Analysis of genetic diversity requires the detection of diversity within genes and this can be achieved directly by analysis of DNA isolated from water without the need to culture specific components of the aquatic biomass. Many studies have amplified 16S rRNA sequences from aquatic environments in an attempt to demonstrate the inadequacies of existing culturing techniques to detect diversity (Amann et al. 1990). The complexity of DNA extracted from the total bacterial biomass in soil was determined by reassociation and showed that genetic diversity was about 200 times that of a population of isolated bacteria from the same sample (Torsvik et al. 1990). This method was capable of detecting differences in genetic diversity of soils but is not suitable for multiple determinations and expression of gene functions.

To retrieve as much genetic information as possible we will clone prokaryotic DNA obtained from a variety of carefully selected microbial communities by using a universal non-selective cloning strategy. This approach will result in the generation of polygenomic libraries that contain functional genes for the investigation of metabolic diversity. Such libraries can be made from sample DNA and then screened for diversity using signatures molecules such as 16S rRNA. Functional expression of genes cloned in the library can be determined by screening clones for enzymatic and bioactive activity using a range of expression vectors. In addition these shotgun libraries can be analyzed using probes and primers specific for antibiotic pathways and conserved sequences from families of degradative enzyme genes. The major task for this phase is the evaluation of genetic diversity in sample DNA and then within the genomic libraries created by shotgun cloning of DNA fragments of different sizes. Therefore, a range of methods will be applied to detect diversity and this will be further evaluated by analysis of clones capable of expression. Since the bottleneck in the clone analysis is the shear number of clones to be screened, about several 10^5, an industrial high-through-put unit will be used for the screening.

ii) Profiling and sequencing rRNA or its genes from the whole microbial community

The direct "fishing-for-marine-genes" approach will be complemented by two other molecular approaches to obtain information from the same marine samples on the taxonomic structure of

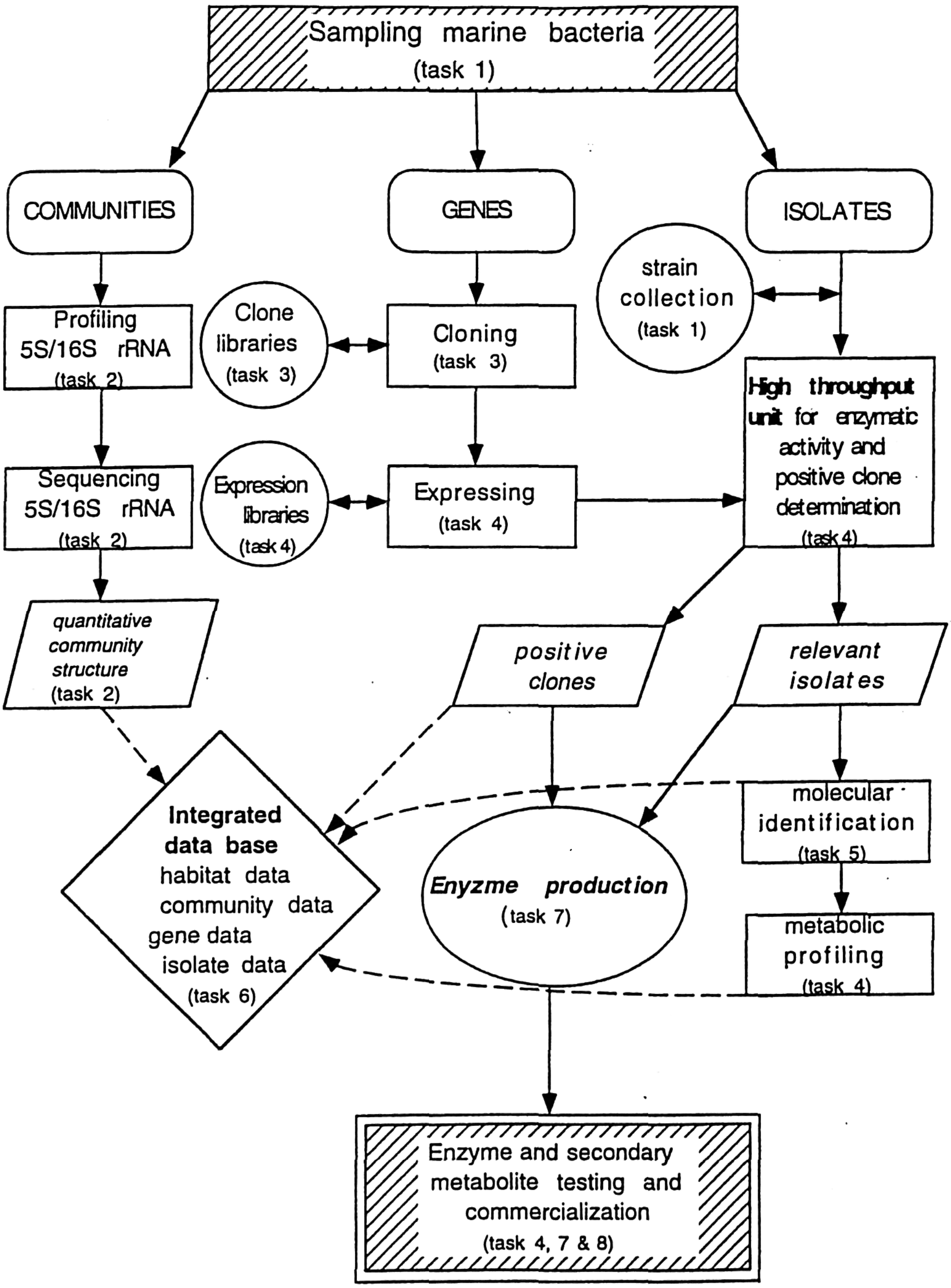

Fig. 1. Integrated molecular approach for the biotechnological exploitation of marine bacteria consisting of: i) cloning and expressing the genes, (middle part - task 3,4); ii) profiling and sequencing rRNA or its genes from the whole microbial community (left side - task 2); iii) molecular identification and activity determination of isolated marine bacteria (right side - task 4); and iv) detection of novel enzymes and secondary metabolites (bottom - task 7,8).

the respective microbial communities. Determination of the bacterial community structure will be done by rapid molecular profiling techniques, such as 5S rRNA profiling (Höfle and Brettar 1995) and 16S rDNA gradient gel electrophoresis (DGGE) including sequencing of the abundant rRNA (Muyzer et al. 1993). Analysis of PCR products by DGGE will allow analysis of the diversity of PCR-amplified fragments obtained from the extracted DNA. Genus and family-specific 16S rRNA primers are available (Amann et al. 1995) for analysis of diversity within bacterial taxonomic groups. In this way we aim to determine the diversity within the sample DNA and in addition detect diversity of gene sequences cloned within several gene banks. The analysis of 5S rRNA gives vital information about the abundant components of the population which are active (Höfle and Brettar 1995).

A major drawback in the use of *in situ* molecular detection techniques relates to concerns about PCR bias and whether predicted taxonomic diversity data truly represent active components in the population. The use of RT-PCR coupled with an accurate RNA extraction technique may overcome this problem and present a method for investigating active components *in situ*. In addition problems of multiple copies of 16S rRNA genes with slightly different gene sequences will be avoided as only one operon is transcribed.

iii) Molecular identification of isolated marine bacteria

The determination of the taxonomic position of bacterial isolates will consist of a two step genotyping procedure: 1st LMW RNA profiling or DNA fingerprinting of all relevant isolates to genotypically group them at the species level (Höfle and Brettar 1996, Ziemke et al. 1997), and 2nd 16S rDNA sequencing of the genotypes that could not be identified to the species level by LMW RNA profiling (Woese 1987). All these molecular data will go into an integrated data base that will be used to reduce genotypic redundancy and help identify the most promising clones or isolates for the more detailed analysis of antibiotics or enzymes and improvement of strains for production.

B DISCOVERY OF NOVEL ANTIBIOTICS AND ENZYMES

Based on the results from the first round of activity screening of the clones and isolates with the high-through-put unit the most promising clones and isolates will be selected for medium size up-scaling of their production. This decision has to be balanced in terms of numbers and amount of substance needed for the specific analysis needed. This decision will be assisted by the integrated data base. Growth of interesting *E. coli* clones for enzymes and antibiotics will be facilitated by the fact that they only need standard growth condition to obtain sufficient biomass. Also, these clones can be readily screened for novel genetic information because the cloned marine genes already contain the genetic markers for sequencing the relevant genes.

Isolated bacterial strains need more attention to optimize their growth conditions and also larger amounts of enzymes are needed to obtain structural information, since the genes containing the sequence information for the proteins are not marked. Therefore, it is very important for these selected isolates to have their taxonomic and phylogenetic position. Since only those strains with a certain degree of novelty are promising for novel products and worth the effort of further analysis.

3. CONCLUSIONS

Central to this research project is the integrated use of state-of-the-art molecular biology for the assessment of genetic resources form natural environments and its biotechnological exploitation. To our knowledge, gene cloning and expression of genes and gene clusters has not been applied to marine microbial communities before. Also, quantification of gene abundance with independent molecular methods will make the assessment of the environmental polygenomic clone and expression libraries possible. The corroborating data on marine habitats and isolation strategies will give the project a unique dimension in marine biotechnology.

The polygenomic clone and expression libraries from marine microbial communities will result in the end in *Escherichia coli* clones the are ideally suited for multiple screening at an industrial scale. These *E. coli* clones have the advantage of low background and standard growth conditions for these screens due to their uniform microbiological nature. This will improve the detection and later production of novel biotechnological products, such as enzyymes and antibiotics, by orders of magnitude.

REFERENCES

Amann, R. I., Krumholz, L. and Stahl, D. A. (1990). Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology. J. Bacteriol. 172:762-770.

Amann, R.I., Ludwig, W. and Schleifer, K.H. (1995) Phylogenetic indicators and *in situ* detection of individual cells without cultivation. Microbiol. Rev. 59: 143-169.

Austin, B. (1989). Marine Microbiology. Cambridge Univ. Press, New York

DeLong, E.F. (1992). Archaea in coastal marine bacterioplankton. Proc. Natl. Acad. Sci. 89: 5685-5689.

Faulkner, D. J. (1993). Marine natural products. Nat. Prod. Res. 10:269-309.

Fuhrman, J.A., McCallum, K. and Davis, A.A. (1992). Novel major archaebacterial group from marine plankton. Nature 356: 148-149

Fuhrman, J.A. McCallum, K. and Davis, A.A. (1993). Phylogenetic diversity of subsurface marine microbial communities from the Atlantic and Pacific Oceans. Appl. Environ. Microbiol. 59: 1294-1302

Giovannoni, S.J., Brigtschgi, T.B., Moyer, C.L. and Field, G.K. (1990). Genetic diversity in Sargasso Sea bacterioplankton. Nature 345: 60-63

Höfle, M.G. and Brettar, I. (1995). Taxonomic diversity and metabolic activity of microbial communities in the water column of the Centratl Baltic. Limnol. Ocenaogr. 40, 868-874

Höfle, M.G. and Brettar, I. (1996). Genotyping of heterotrophic bacteria from the Central Baltic using Low-Molecular-Weight RNA profiles. Appl. Environ. Microbiol. 62, 1382-1390

Jannasch, H.W. and Jones, G.E. (1959). Bacterial populations in seawater as determined by different methods of enumeration. Limnol. Oceanogr. 4: 128-139

McInerney, J.O., Wilkinson, M., Patching, J.W., Embley, T.M. and Powell, R. (1995). Recovery and phylogenetic analysis of novel Archaeal rRNA seqüences from a deep-sea deposit feeder. Appl. Envrion. Microbiol. 61: 1646-1648

Mullins, T.D., Britschgi, T.B., Krest, R.L. and Giovannoni, S.J. (1995). Genetic comparisons revealed the same unknown bacterial lineages in Atlantic and Pacific bacterioplankton communities. Limnol. Oceanogr. 40: 148-158

Muyzer, G., De Waal, E. C. and Uitterlinden, A.G. (1993). Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. Appl. Environ. Microbiol. 59:695-700.

Torsvik, V., Goksoyr, J. and Daae, F. L. (1990). High diversity in DNA of soil bacteria. Appl. Environ.Microbiol. 56:782-787.

Ward, D.M., Bateson, M.M., Weller, R. and Ruff-Roberts, A.L. (1992). Ribosomal RNA analysis of microorganisms as they occur in nature. Adv. Microb. Ecol. 12: 220-286

Woese, C.R. (1987). Bacterial evolution. Microbiol. Rev. 51: 221-271

Ziemke, F., Brettar, I. and Höfle M.G. (1997). Stability and diversity of the genetic structure of a *Shewanella putrefaciens* population in the water column of the central Baltic. Aquat. Microb. Ecol. 13: 63-74

TITLE: **BIOLOGY OF SPONGE NATURAL PRODUCTS** (project **SYMBIOSPONGE**)

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SUMMARY

The biological and chemical aspects of selected sponge natural products (secondary metabolites) of interest for human use will be studied to obtain understanding of:
(1) the cellular origin and possible microsymbiont involvement, and
(2) the ecological significance of sponge secondary metabolites, and
(3) patterns in these processes enabling rationalization of exploration for and exploitation of sponge secondary metabolites.
The results will have a direct bearing on policy decisions concerning industrial production of sponge secondary metabolites which are too difficult or too costly to synthesize. A major deliverable of the proposed research will be the formulation of a standard protocol of research steps needed as a basis for such policy decisions. The project is scheduled to start on April 1, 1998.

INTRODUCTION

Marine sponges are a source for a dazzling variety of natural products with bioactive properties (Sarma et al., 1993). The bioactivity may be effective against viruses or prokaryotes, but often also affects eukaryote cells, including human cancer cells; such bioactive compounds have yielded an interest from the pharmaceutical industry (Munro, et al., 1994). In other cases bioactivity has been found against a variety of marine, freshwater or terrestrial pests. Currently, several large scale programs are in progress in which sponges are collected and screened (cf. Munro et al., 1994 for a review). So far the practice is that sponges are collected randomly, not directed by other objectives than search for bioactivity of any kind. Such collecting programs are yielding a confusing array of organic molecules of very different affinities (Blunt & Munro, 1997). Patterns in the distribution of these molecules may exist (cf. Braekman et al., 1992; Andersen et al, 1996; Van Soest et al., 1997), - in particular in the secondary metabolites, smaller byproducts of the primary metabolism -, but have been insufficiently demonstrated. It appears as if many sponges have their own particular type of - toxic - molecule. Morphologically similar sponges may have similar molecules, but apparently many exceptions exist. Reasons for this apparent lack of consistency may be manifold. In the case of involvement of symbiotic microorganisms (Fusetani & Matsunaga, 1993; Faulkner et al., 1994) related sponges - and even the same sponge species - may have very different bioactive molecules. In other cases, misidentification or casual treatment of the source sponge may be the cause of an apparent lack of consistency. In general, so far very little attention has been focussed on the source organisms, the sponges.

It is obvious that any attempt at industrial production of a given sponge secondary metabolite - other than production of an artificially synthesized analogue - is hampered severely by the lack of consistency and the uncertainties surrounding the source organism. Knowledge about the identity of the sponge source, the natural function of the secondary metabolite, the variability of the amounts produced and the parameters governing this (Uriz et al., 1996), the biosynthetic relationships between various molecular derivatives, the cellular origin of them (Garson, 1994), and possible involvement of microsymbionts (Faulkner et al., 1994) is vital for being succesful in attempts at mariculture, mass culture of sponge cells or microsymbionts. Random, undirected collection of large amounts of sponges is wasteful with regard to research funds as well as with regard to the environment.

We have taken it upon ourselves to set up guidelines for a more rational approach towards the discovery and potential exploitation of sponge natural products. We will do that by performing a detailed model-project on a selected group of sponges (Haplosclerida s.l. and Halichondrida s.l.), consisting of combined chemical-, chemosystematic-, ecological- and microbiological observations, pattern analysis, decision making, and hypothesis-testing. This will lead to a series of recommended research steps for future large scale attempts at mass production of sponge natural products.

The research will be performed in three distinct phases (see Figs. 1 and 2):
Phase I: the exploratory phase in which patterns and correlations will be recognized concerning systematics, secondary metabolite types, sponge cell and microsymbiont spatial distribution and large scale environmental parameters. This phase will also be used for trials of various techniques and experiments of Phase II. The phase will be closed off by the selection of 3-4 target sponge species for in-depth studies.
Phase II: the experimental phase in which 3-4 cases of suspected sponge cell and microsymbiont involvement in bioactive molecule production will be submitted to

rigorous tests (ecology, cell observations and culture, localized extractions). The phase will be closed off by the pooling of all data from the various disciplines leading to a corroboration or refutation of the assumed origins of the secondary metabolites.
Phase III: Formulation of research protocols for future research into the possible industrial production of a given secondary metabolite.

MATERIALS AND METHODS

Haplosclerid and Halichondrid sponges will be collected using SCUBA (shallow water) and/or dredges (deep water). Specimens will be photographed upon collection. Various types of fixations of material will be made immediately after removal from the water (see Fig. 1). Voucher specimens will be studied for identity and phylogeny using routine morphological as well as molecular (28S rDNA) characters (Fig.3).

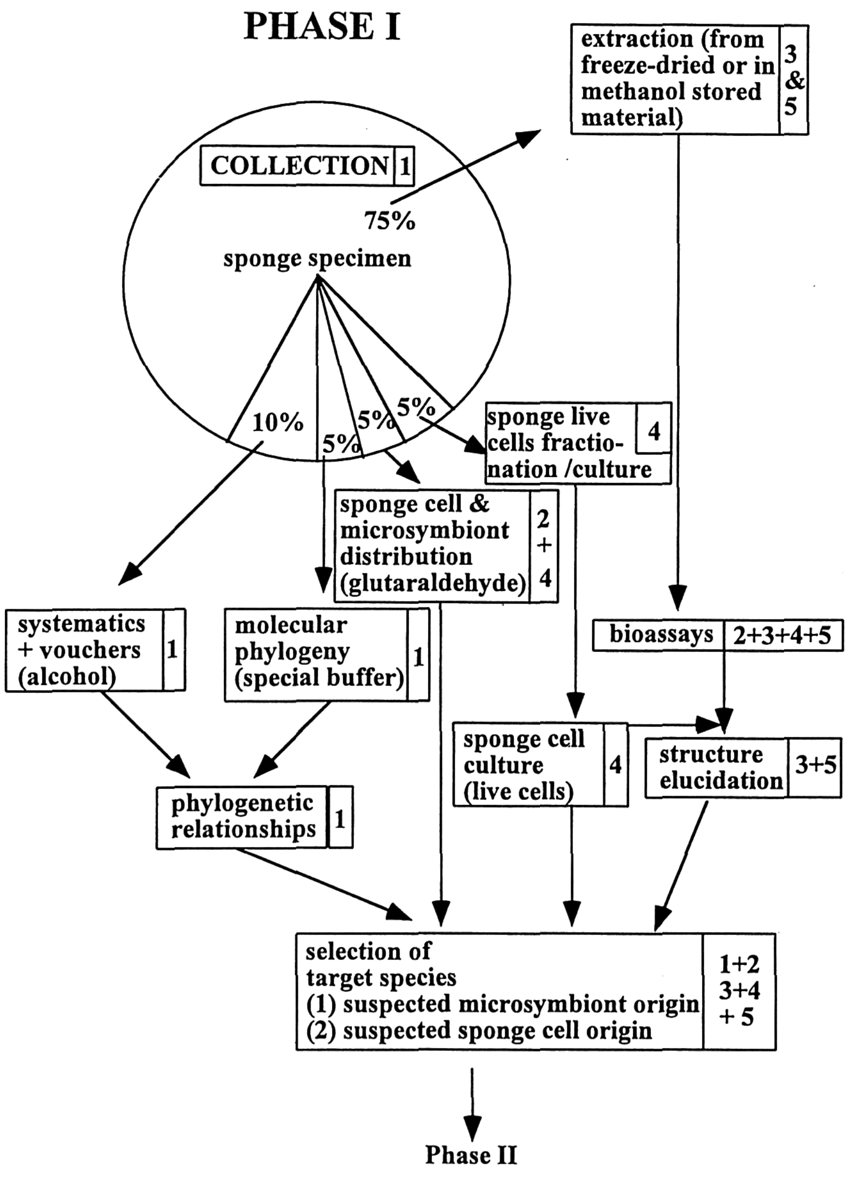


Fig. 1. Structure of the SYMBIOSPONGE project: Phase I treatment of collected material (numbers 1-5 alongside the various project activities indicate involvement of the various partners)

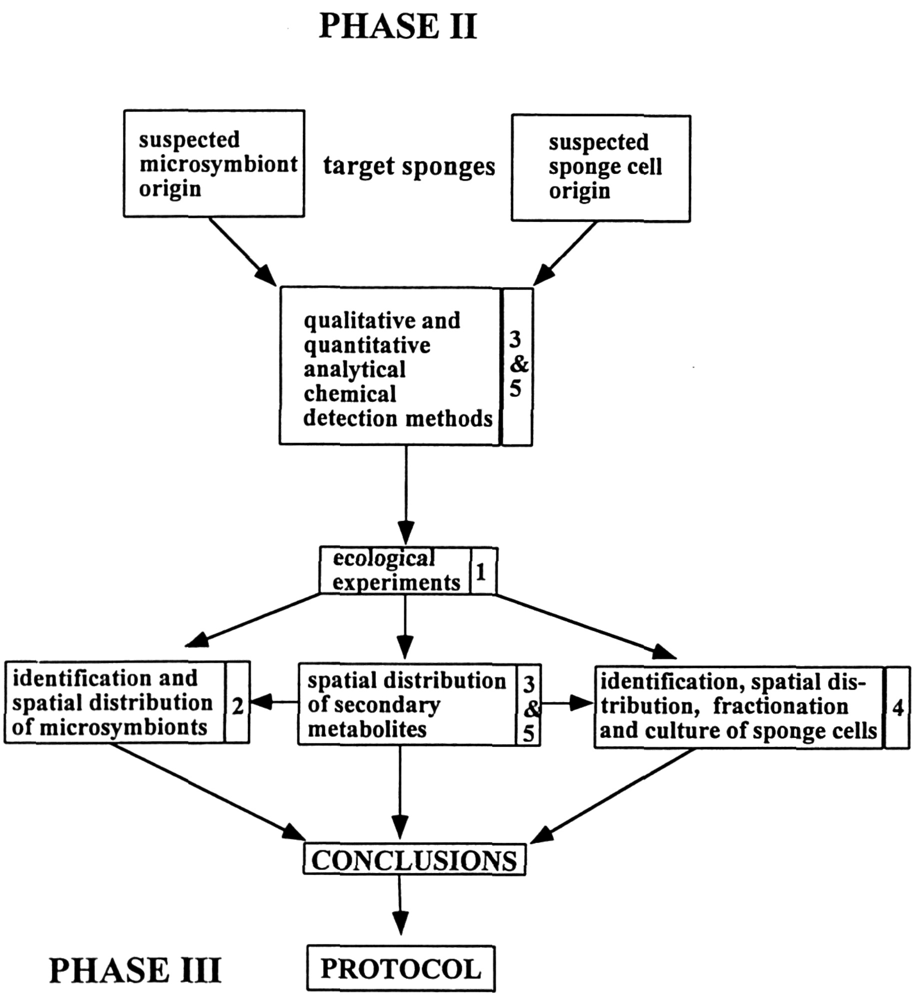


Fig. 2. Structure of the SYMBIOSPONGE project: Phase II experiments and treatment of selected sponges (numbers 1-5 alongside the various project activities indicate involvement of the various partners)

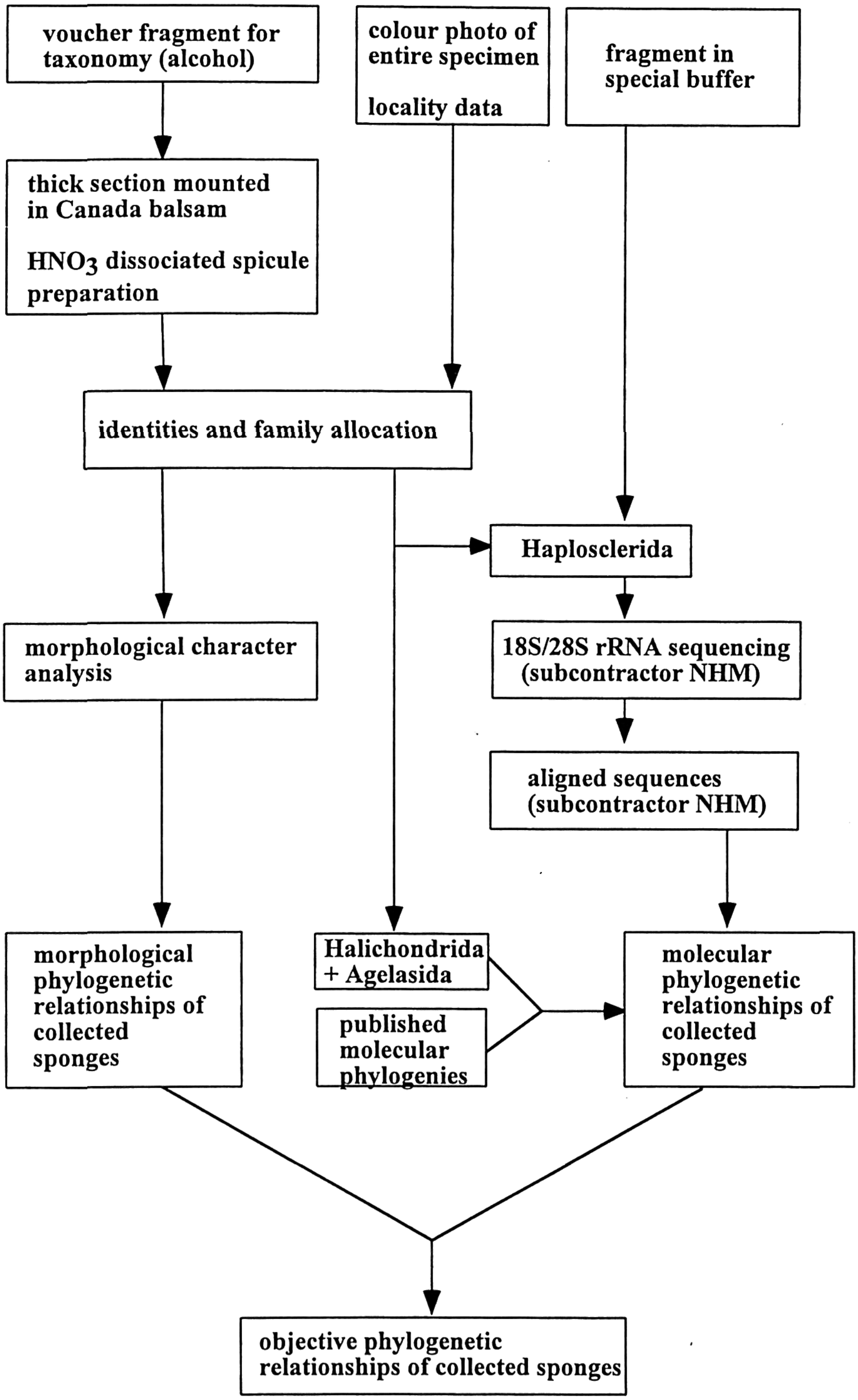


Fig. 3. Process of identification and classification of sponges collected in Phase I of the SYMBIOSPONGE poject.

Collected sponges preserved in methanol or as freeze-dried material will be extracted with methanol and dichloromethane (Fig. 4). The primary extracts will then be tested for their biotoxicity using an invertebrate bioassay organism, the *Artemia* toxicity test, and several prokaryote and eukaryote bioassay organisms (bacteria, fungi, yeast).

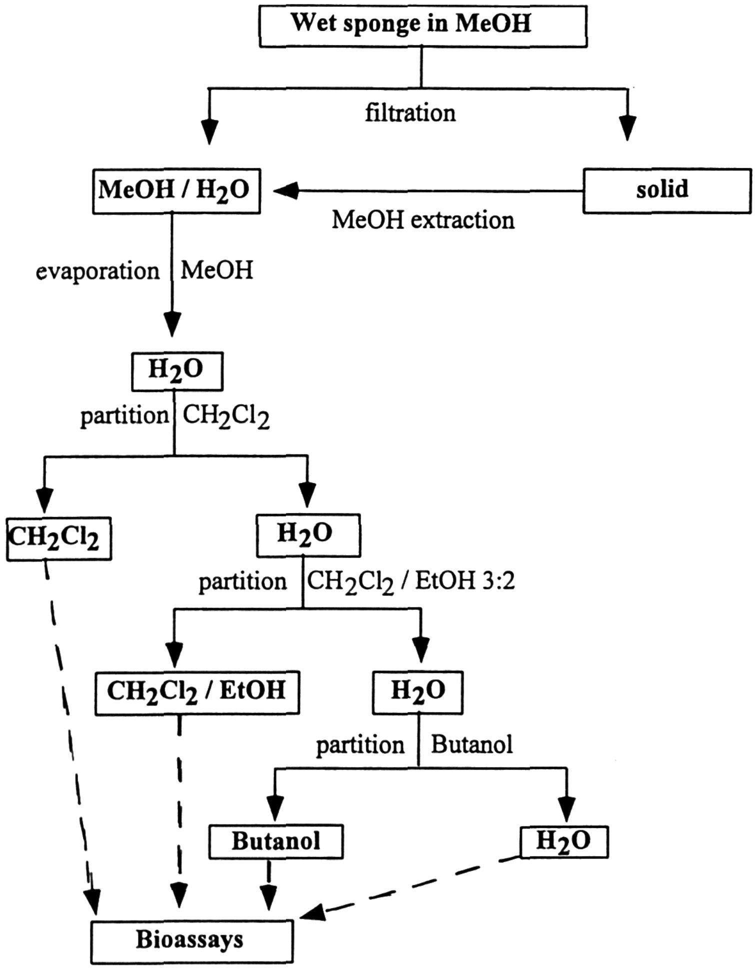


Fig. 4. Chemical extraction of collected sponges for bioassay experiments during Phase I and II of the SYMBIOSPONGE project.

Cytological analyses will consist of two different approaches, one using glutaraldehyde-fixed material (Fig. 5), the other using live sponges (Figs. 6-7): (1) sponges will be fixed in glutaraldehyde (Fig. 5),

(a) for microsymbiont detection; thick sections will be stained with suitable fluorochromes and viewed by fluorescence microscopy and confocal scanning light microscopy. If microsymbionts are present, populations will be characterized by different parameters. Microsymbionts will be further identified by fluorescence in situ hybridization using rRNA-targeted oligonucleotides as probes;

(b) for sponge cell spatial distribution, samples will be postfixed in 1% osmium tetroxyde and thin sections will be studied by Transmission Electron Microscopy (TEM).

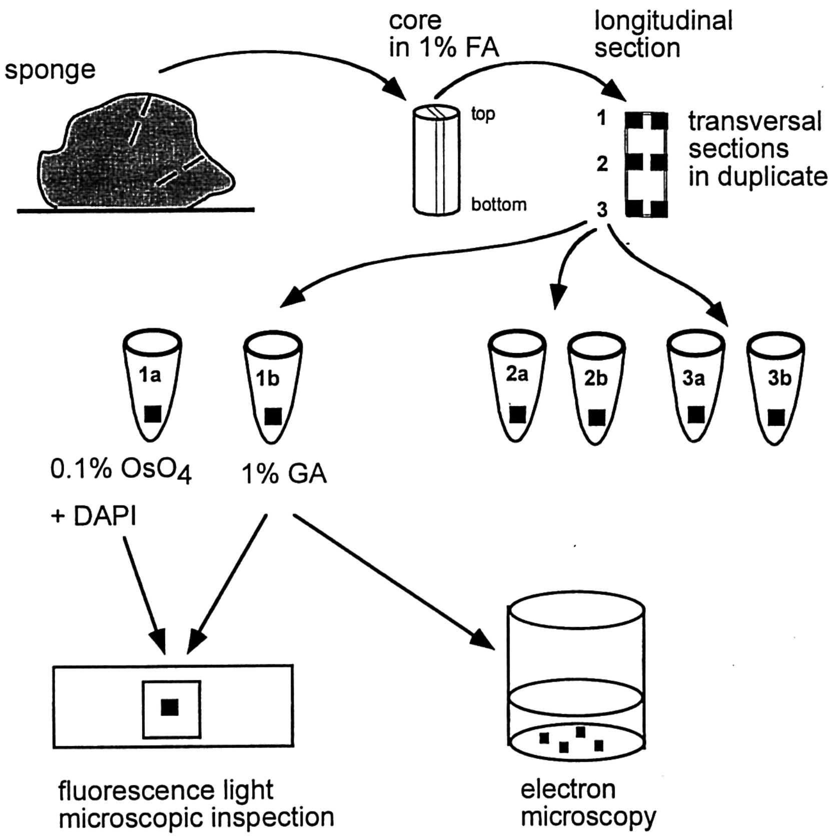
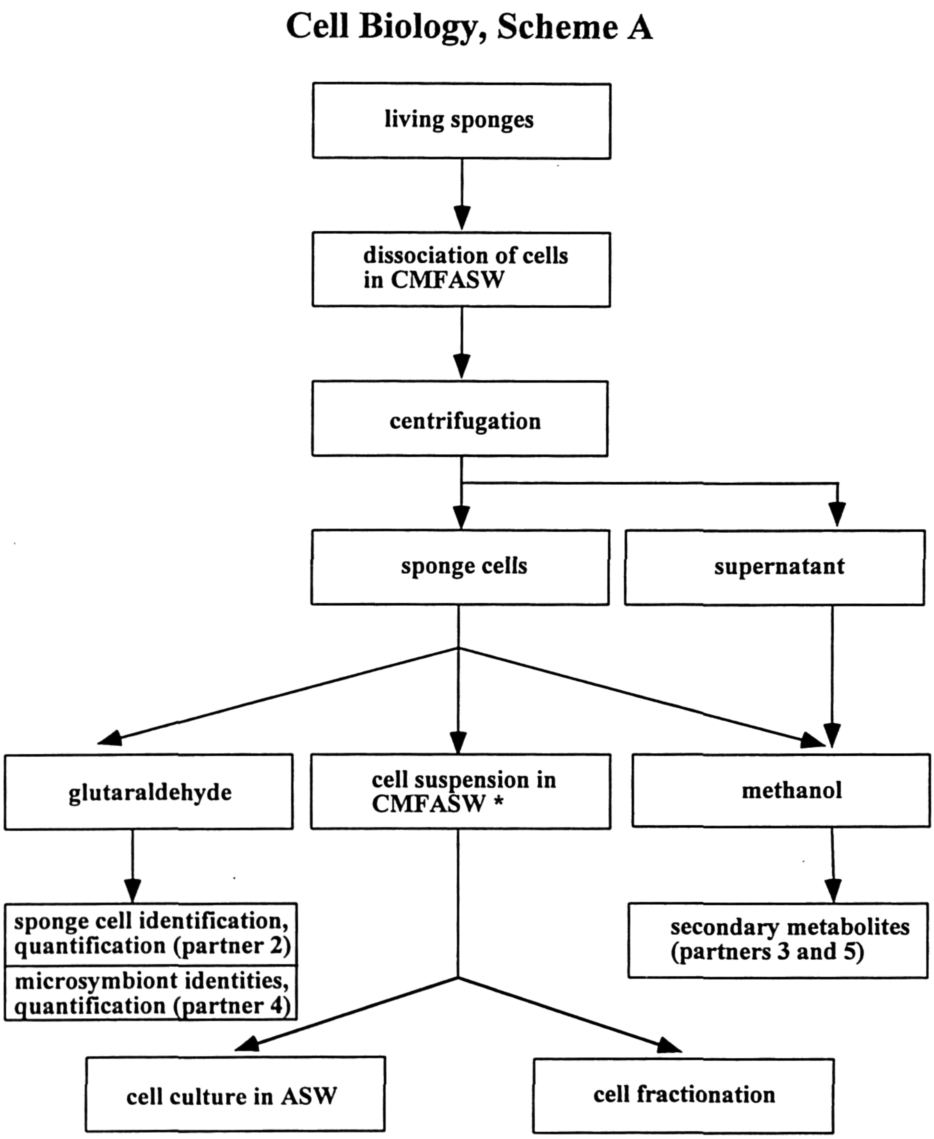


Fig. 5. SYMBIOSPONGE project treatment of fixed microsymbiont and sponge cell material for in situ observations.

(2) Live sponges will be dissociated into single-cell suspensions. Recognition of secondary metabolite production will be realized using two advanced techniques:

(a) cell fractionation into pure cell populations using continuous or discontinuous Percoll gradients (Fig. 6);



*) if necessary transfer to partner 4's laboratory

Fig. 6. SYMBIOSPONGE project treatment of sponge cells for fractionation.

(b) symbiont-free sponge cultures, initiated either from pure cell populations or from dissociated sponge cell suspensions (Fig. 7).

Cell Biology, Scheme B

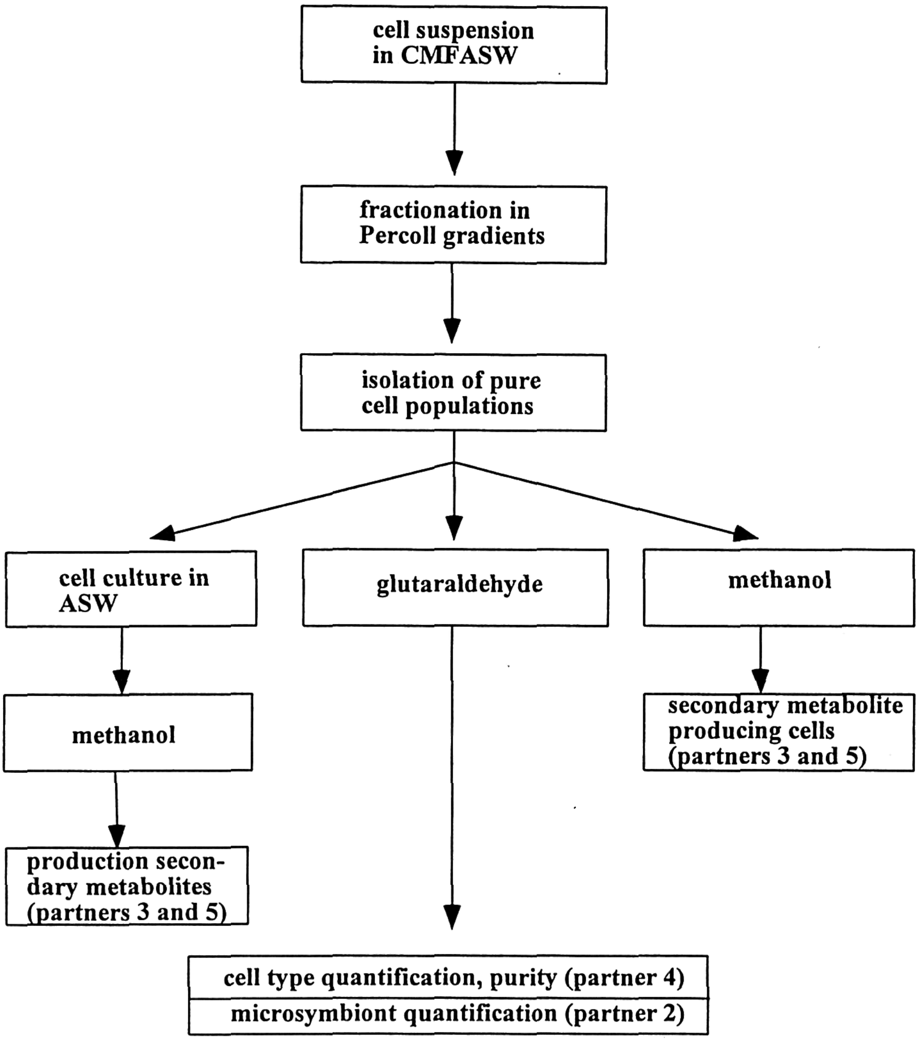


Fig. 7. SYMBIOSPONGE project treatment of sponge cells for cell culture.

Experimental observations will be made in situ using various types of manipulations (caging, artificial standard lesions, confrontation with substrate competitors, crude extract assays with substrate competitors and potential predators).

DELIVERABLES

All data and observations collected during the project, both in Phase I and Phase II, will be pooled. The combined results of all research items will enable us to draw conclusions on the successfulness of the various methodologies employed in this study and will allow more general statements about their applicability for similar or other cases of sponge natural products. These conclusions will be laid down in a series of recommended research steps which will allow user groups to reach a firm basis for decisions on industrial production of promising secondary metabolites.

Detailed recommendations will be made concerning

- Collection and specimen handling (voucher deposition; ecological and geographic considerations; population strength of a potential target species; biological conservation policies)
- Chemosystematics (validity and usefulness for directing collection programs)
- Bioassays (usefulness of crude extract assays; assay-guided chemical fractionation methods)
- Chemistry (fractionation-, isolation-, and structure elucidation techniques; quantitative analytical techniques for simple detection and analytical techniques for within sponge spatial distribution of a given metabolite)
- Microsymbiont involvement (analytical techniques for qualitative and quantitative within sponge spatial distribution; techniques for microsymbiont identification)
- Sponge cell involvement (survey of cell fractionation approaches; methods for symbiont-free sponge cell culture)
- Variability of secondary metabolites in the host organisms (ecological background; sponge manipulations)

These recommended research steps will lead to a decision pathway directed towards industrial production of interesting sponge metabolites.

REFERENCES

Andersen, R., Van Soest, R.W.M. and Kong, F., 1996. 3-Alkylpiperidine alkaloids isolated from marine sponges in the order Haplosclerida. In: S.W. Pelletier (ed.). Alkaloids: Chemical & Biological Perspectives, 10, 301-356 (Pergamon, New York).

Braekman, J.C., Stoller, C., Daloze, D., and Van Soest, R., 1992. Chemotaxonomy of Agelas. *Biochemical Systematics and Ecology*, **20** 417-431.

Blunt, J.S. and Munro, H.M.G., 1997. MarineLit. A marine literature database maintained by the Marine Chemistry Group, University of Canterbury (Christchurch, New Zealand).

Faulkner, D.J., Unson, M.D., and Bewley, C.A., 1994. The chemistry of some sponges and their symbionts. *Pure and Applied Chemistry*, **66** (10/11), 1983-1990.

Fusetani, N. and Matsunaga, S., 1993. Bioactive sponge peptides. *Chemical Reviews*, **93**, 1793-1806.

Garson, M.J., 1994. The biosynthesis of sponge secondary metabolites: why it is important. In: Van Soest, R.W.M., Van Kempen, T.M.G., and Braekman, J.C. (eds.). Sponges in time and space, 427-440 (Balkema, Rotterdam).

Munro, H.M.G., Blunt, J.W., Lake, R.J., Litaudon, M., Battershill, C.N., and Page M.J., 1994. From seabed to sickbed: what are the prospects ? In: Van Soest, R.W.M., Van Kempen, T.M.G., and Braekman, J.C. (eds.). Sponges in time and space, 473-484 (Balkema, Rotterdam).

Sarma, A.S., Daum, T, and Mueller, W.E.G., 1993. Secondary metabolites from marine sponges. (Mosby, Berlin)

Uriz, M.J., Becerro, M.A., Tut, J.M., and Turon, X., 1996. Location of toxicity within the Mediterranean sponge Crambe crambe (Demospongiae: Poecilosclerida). *Marine Biology*, **124**, 583-590.

Van Soest, R.W.M., Fusetani, N., and Andersen, R., 1997. Straight chain acetylenes as chemotaxonomic markers of the marine Haplosclerida. In: Watanabe, Y. and Fusetani, N. (eds). Sponge Sciences, 3-30 (Springer, Tokyo)

TITLE: Marine Cyanobacteria as a source for bioactive (apoptosis-modifying) compounds with potential as cell biology reagents and drugs.

CONTRACT NO: MAS3-CT97-0156

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MARINE CYANOBACTERIA AS A SOURCE FOR BIOACTIVE (apoptosis-modifying) COMPOUNDS WITH POTENTIAL AS CELL BIOLOGY REAGENTS AND DRUGS

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1. INTRODUCTION

Apoptosis is presently one of the most intensely studied biological phenomena, both within basic and applied bio-sciences. Hence, drugs that can affect these processes are eagerly searched for both to help research (marine and general) and as potential new drugs in conditions of increased apoptosis (degenerative disorders, HIV) and decreased apoptosis (cancer, certain autoimmune diseases).

In this respect, the most promising presently available compounds originate from marine micro-organisms, but little is done in Europe (compared to Japan, Australia and North America including Hawaii) to systematically detect new such compounds or to improve (by hemisynthesis) the pharmacological properties of the existing ones.

Particular impetus to launch the present project comes from a) the enormous biodiversity of the marine environment suggesting that it is the richest source of bio-active compounds, b) conviction that marine microbes like cyanobacteria represent an underexploited resource for drug development, c) promising findings by the collaborating partners of the proposed network of novel apoptosis-inducing bio-active substances from marine microbes, d) the obvious biological relevance for the marine ecosystem functioning of identifying apoptosis modulating compounds, and e) the importance for efforts to preserve the marine biodiversity of successfully demonstrating novel useful compounds from the marine environment.

The present partners - representing European microbiologists, cell biologists and chemists will jointly exploit marine cyanobacteria as a source for useful bio-active compounds.

Particular emphasis will be given to discover and develop more precise chemical tools (directed against specific enzymes) to control the cell signalling reactions leading to apoptotic (programmed) cell death.

2. OBJECTIVES

The two main objectives of the project are (I): to search for novel apoptosis-modifying compounds from marine cyanobacteria and (II): to hemisynthetically modify natural products in order to enhance their usefulness as biological tools or drugs.

This will be obtained through:

(•) **Sampling and culturing of cyanobacteria** Sampling will be from coastal areas (Baltic sea, Iberian coast, Norwegian fjords) as well as from more extreme polar regions, including Spitsbergen and certain areas of the Antarctica. Cultures (including already existing cultures from partners′ own collections) will be initial small-scaled cultured and screened for bioactivity. Cultures producing bioactive substances will be cultured axenic and further be large scaled.

(• •) **Extraction, partial purification and initial screening for bio-activity**. Extracts will be partially purified and tested for ability to elicit apoptotic cell death in a number of normal and malignant cell types, for effects on the cytoskeleton, and for effects on isolated protein kinases and phosphatases known to affect cell function and viability. Such a systematic survey has not been done before, but extrapolation of data from preliminary screening of scattered samples indicate a huge potential for discovery of new activities.

(• • •) **Isolation, structure elucidation, and detailed probing of the mechanism of selected bio-active compounds**. This will involve scale-up of the initial amount of relevant sample, and of extraction and initial purification procedures, followed by final purification and structure elucidation. The purified compounds will be thoroughly characterised with respect to action on cells and isolated cell signalling enzymes (key kinases and phosphatases), and for degradation and metabolism.

(• • • •) **Hemisynthetic modification of natural compounds , and detailed probing of the effects of chemical modification on biological function and availability**. This will serve to characterise important functional groups of the natural compounds, but mostly to enhance their usefulness as cell biology tools or drugs (increasing bioavailability, potency, specificity). In the first phase hemisynthesis will be performed for nodularin and certain novel marine microcystins, which are already available in the network.

3. WORKPLAN

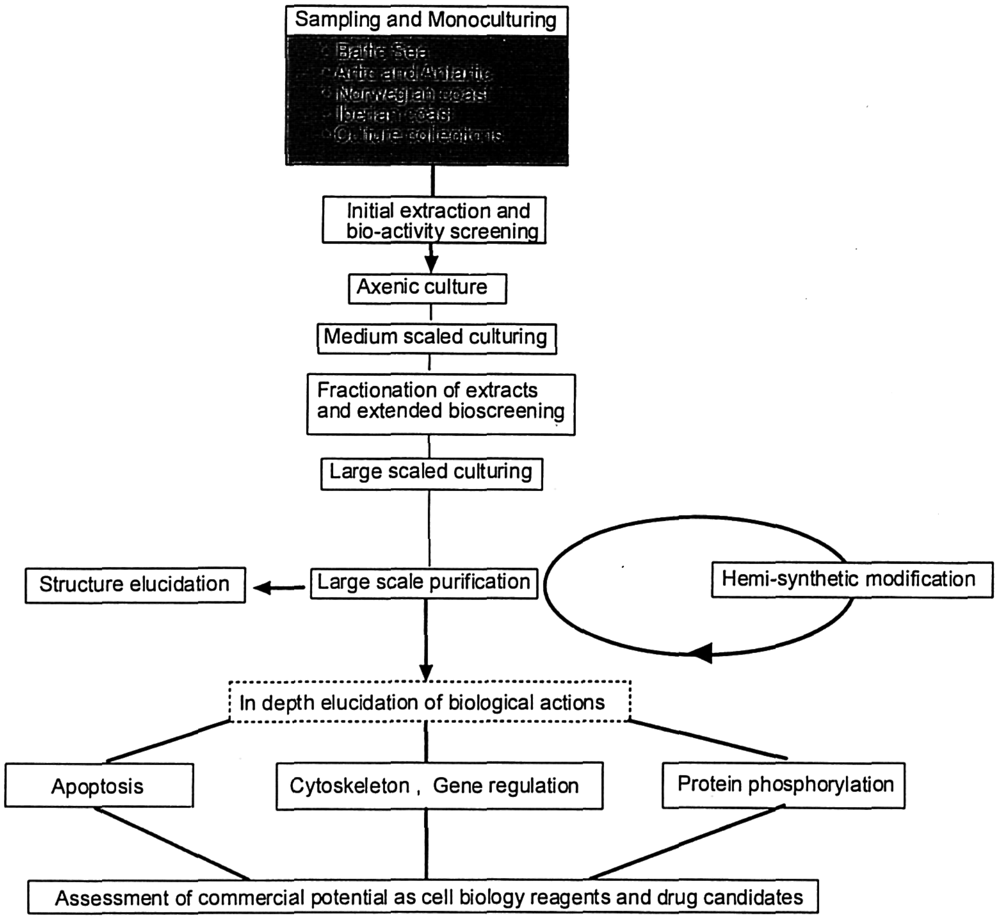
The project is divided into the following 3 tasks:

Task 1 Sampling and culturing of cyanobacteria.

Task 2 Screening and in-depth bio-assay of cyanobacterially-derived substances with respect to apoptogenic and protein phosphorylation modifying properties.

Task 3 Isolation, structure elucidation, hemisynthesis and exploitation of compounds.

The work plan:



III.1.5. Submarine geotechnics

TITLE: Integrated System for Analysis and Characterization of the Seafloor (ISACS)

CONTRACT NO: Mast 3-CT95-0046

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INTEGRATED SYSTEM FOR ANALYSIS AND CHARACTERIZATION OF THE SEAFLOOR

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SUMMARY

The ISACS[1] project started 1st March 1996 and runs for 36 months. The first half of the poject has been completed generally according to plans and expectations. The objective of ISACS is to prove the feasibility of the analysis and characterization of the seafloor by the exploitation and by a suitable integration of data gathered from commercially available sonar equipment.

1. Introduction

In several industrial and scientific applications, a detailed knowledge of the seafloor upper strata characteristics and properties is of paramount importance, especially in shallow water inside the continental shelf. However, a standard technology or routinely surveying these properties and making them accessible to the end-users has still to be developed. The ISACS project has aim to investigate methods and techniques for processing data gathered with existing sonar equipment and to come to an automated quantitative and qualitative characterization of the seafloor.

The ISACS project is divided into five tasks: Field data acquisition, data fusion and preprocessing, data inversion and seafloor charcterization, volume image processing and test of the concept. The project involves eight partners from five different countries, and has a total budget of 2 millions ECU, of which 1.4 million is founding from the European Union.

The five tasks in the ISACS project and how these are related to the partners are shown in Figure 1. The project partners are:

- Norwegian university of science and technology, NTNU, Norway
- University of Genova, DIST, Italy
- University of Algarve, UALG, Portugal
- Royal Institute of Technology, KTH, Sweden
- National Defense Research Establishment, FOA, Sweden
- SACLANT Undersea Research Centre, SACLANTCEN, Italy
- Fugro Consultants International BV, The Netherlands
- Kongsberg Simrad AS, Norway

[1] Integrated System for Analysis and Characterization of the Seafloor

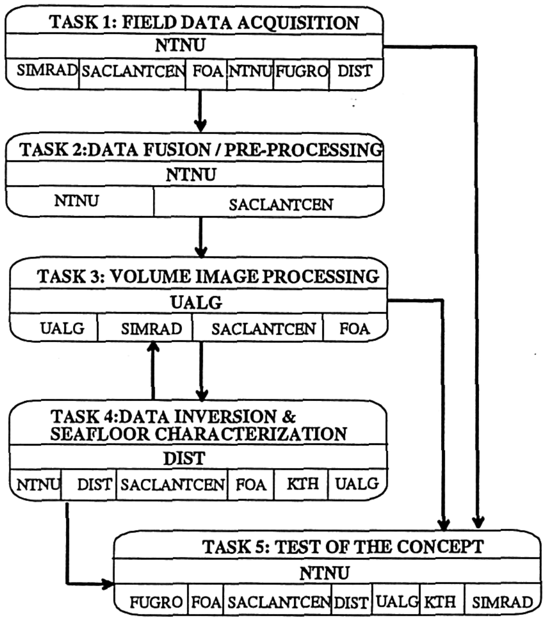

Figure 1*; Illustration of the relations between the different tasks of ISACS and the different partners. First line in each `group' is the task name, second line gives the responsible partner, and third line indicates the involved partners.*

2. Objectives

The objective of ISACS is to prove the feasibility of the analysis and characterization of the seafloor by the exploitation and by a suitable integration of data gathered from commercially available sonar equipment.

The project will treat acoustic backscattered data acquired by instruments like multibeam echosounders, sidescan sonars and bottom penetrating parametric sonars (a special sonar generating low frequency sound waves from two higher frequencies transmitted simultaneously). An integration of such data is to design signal processing and 3D image processing tools for extracting the major features from the received data. The geoacoustic properties will be determined from the acoustic backscattered data and algorithms are being developed for both characterization of the seafloor and to detect and possibly identify buried or semi-buried objects. The geoacoustic properties will be complemented and compared with geotechnical and in situ measurements, in order to verify our results.

The obtained seafloor characterization will be of benefit to several categories of end-users. In particular will these results be useful for geotechnical engineering, offshore industry, environmental research and monitoring, archeological research and identification of natural deposits (i.e. sand banks).

The different end-users have different needs, but it is mainly a question of resolution and penetration depth. The main seafloor properties of interest may be:

1. Bathymetry
2. Sound speed
3. Density
4. Attenuation of compressional wave
5. Bottom surface roughness
6. Sediment inhomogeneities

In addition to the estimated quantities above, 2D and 3D images will be generated for the surveyed area. These images will include a representation of the surveyed data and the estimated parameters, including visualization of objects.

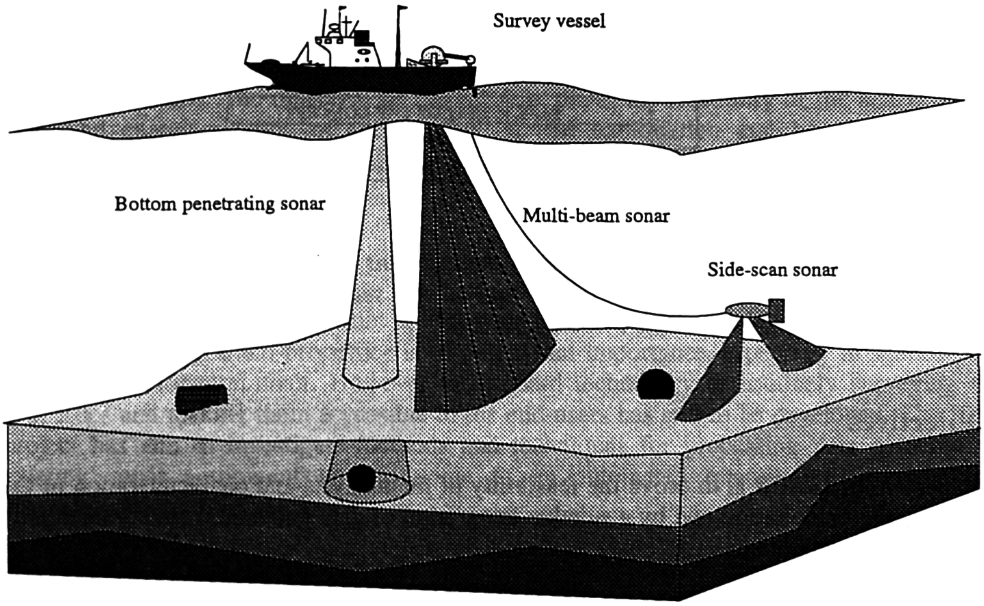

Figure 2; *A typical configuration of a survey for data collection in the ISACS project.*

3. Data gathering

Data has so fare been gathered at three regions in Europe; Mediterranean, Baltic Sea and the North Sea. The data gathering has been done in two steps: First a set of preliminary studies were performed at the different locations. Secondly main sea trials were done in each of the three regions.

Different acquisition systems have been used during the surveys. In general data has been collected using multibeam echosounders, side-scan sonar and bottom penetrating sonar. The first two instruments will characterize the seafloor surface, while the bottom penetrating sonar is used for characterization of the sediment volume as well as it also provides

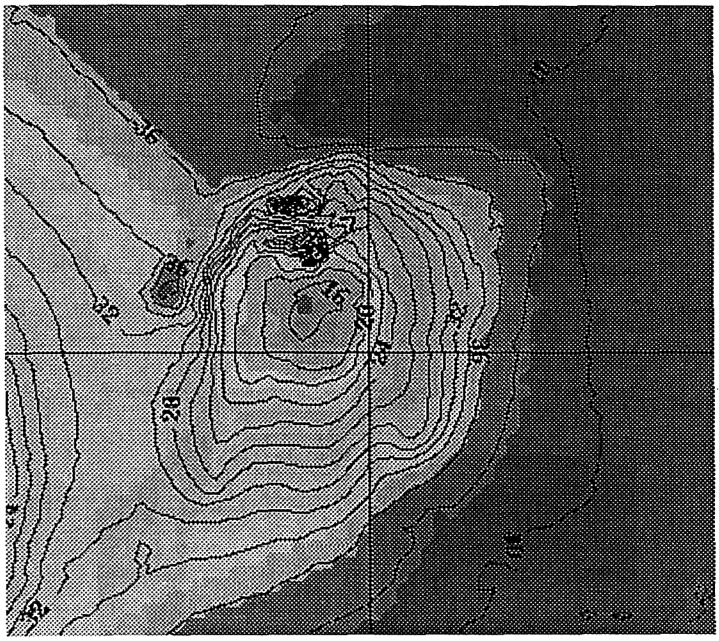

Figure 3; Bathymetric map of a small seamount made with data from a multibeam echosounder. The data is acquired outside Stockholm (From the Baltic survey).

information about the seafloor surface. Figure 2 shows a typical survey configuration for data gathering within the ISACS project.

Figure 3 shows a part of a bathymetric map generated from the multibeam echosounder EM1000. The EM1000 is operating at a frequency of 95kHz and the data was gathered outside Stocholm in the Baltic Sea. The map has 2 meter countour levels and shows a small seamount on the seafloor. The size of the picture is approximately 350 x 350 m.

Data gathered from the same area, but with the bottom penetrating sonar (Topas) is shown in Figure 4. The data is taken as a line from left to right (North -South) in Figure 3. The data shows a vertical slize of the seamount. The data is obtained using a Ricker pulse with center frequency 5kHz and a bandwidth of 5kHz. The ping repitition rate was 400 ms and the survey speed was 5 knots. The depth is indecated by lines at each 10 ms.

4. Data fusion /preprocessing

The data fusion and preprocessing part of ISACS can be divided into two sub tasks.

The first task was to provide a device independent representation of the acoustic data. By choosing the ADAM format, provided by Saclantcen, one has a data format that is suitable for storage of hydro acoustic data from a survey with different recording instruments (Bergem, 1996; Canepa and Bergem, 1996). The general idea behind the ADAM format has been to store all the necessary cruise information in one file header, and to be flexible enough

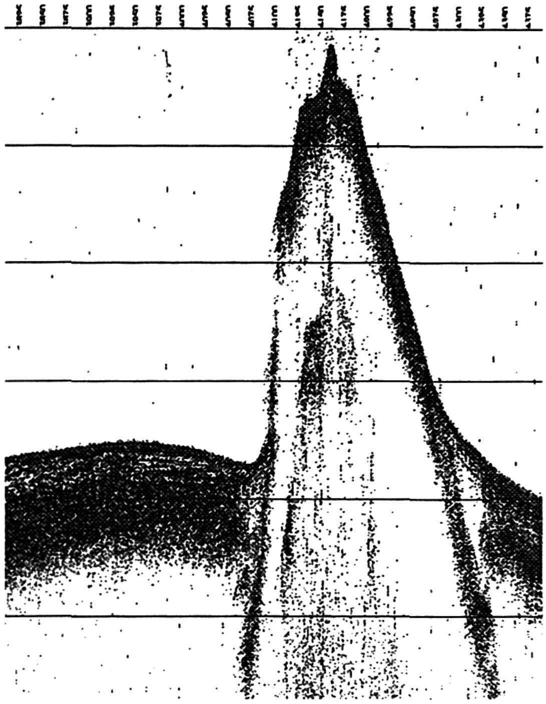

Figure 4; Vertical slize of the seafloor at the same position as in Figure 3. The data is gathered by the bottom penetrating sonar (Topas). The data is captured in a line from north to south (left to right) in the middle of Figure 3.

to have a file format that suits all the different instruments. At the same time the format is easy to implement and it provides easy access to and storage of the aquired data.

The second task in this part of the project is to determine the exact bathymetry of the surveyed area. The multibeam map in Figure 3 is made using standard procedures for map generation. This representation of the bathymetry smoths the data and information is lost. The bathymetry data will be used for correcting of grazing angles so that the true grazing angle is available for the determination of the backscatter strength as function of angle. Within ISACS we are developing a system that can handle these problems (Allnor, 1996). A software is being developed that can load a certain set of swath bathymetry data (like the one in Figure 3) and determines a true bottom map in terms of a triangular representation. The algorithm ensure that a minimum number of data points are used for the map and that they are located where they are needed.

An example of a bottom map from the Mediterranean is shown in Figure 5. The map is made using a triangular based data representation. Figure 6, left plot shows the same data as in Figure 5 but now as contour map, while the right plot shows the distribution of nodes used for generation of both the contour map and the 3D map. Note that the nodes are located closer in regions with changing bathymetry and vice versa.

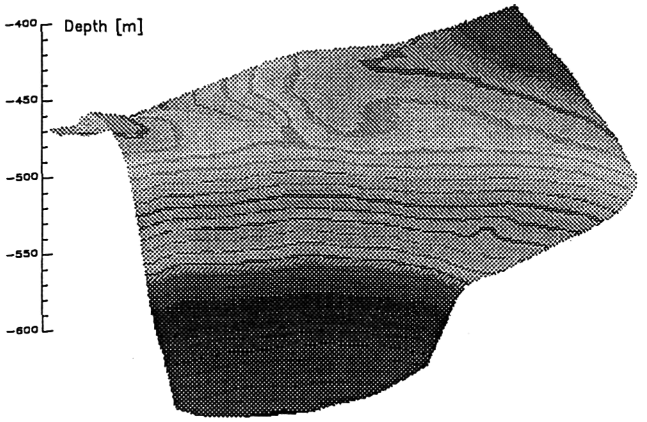

Figure 5; Bathymetry data from the Mediterranean survey.

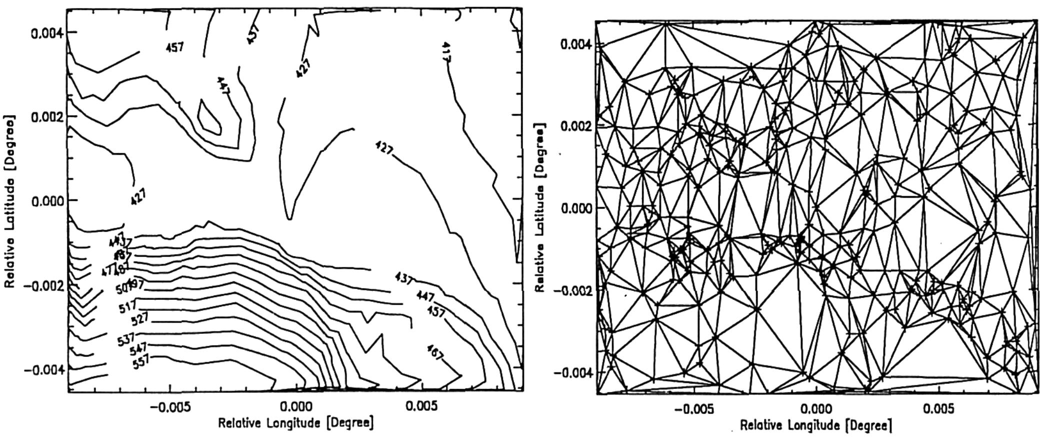

Figure 6; Left: A contour map of the data in Figure 5 Right: Nodes distribution from the triangulation algorithm.

5. Volume image processing

The 3D volume image processing task consists of segmentation of the aquired data and visualization of these data together with the belonging geoacoustical properties.

The segmentation is done to divide the 2D or 3D acoustic image into regions with similar geoacoustical properties i.e. regions of homogeneous backscattering strength as function of grazing angle. These regions are represented as surfaces when the data is from side-scan sonar or multibeam sonar and volumes when the data is acquired by the bottom penetrating sonar. A 3D data segmentation method has been developed (Loke and du Buff, 1998) and the method is now beeing tested.

The visualization involves a lot of challenges and is rather ambitious since it involves the integration of 2D surface data, 3D volume data and the results from the estimation of seafloor parameters (Task 4). At UALG one has choosen to use the VRML (Virtual Reality Modelling Language) to represent the volume data. Using WRML the data can be viewed using a standard WWW browser (Nikolov et al.,1997).

6. Data inversion and seafloor characterization

Two different approaches are used in order to extract surface roughness and geoacoustic parameters of the seafloor surface, namely model based inversion and classification based on predefined classes. Seafloor characterization involves also determination of responses from objects on or within the seabed

A numerical time-series model, BORIS[2] is developed at SACLANTCEN for determination of the acoustic backscattering from seafloor surface and volume inhomogeneities (Bergem et al. 1997; Pouliquen et al. 1997). For a chosen geometrical setup, the acoustic pressure field is determined at the receiver. The approach is based on the Kirchhoff approximation for the seafloor interface backscattering response and on small perturbation theory for the sediment volume response.

Figure 7 shows how the narrow beam of the bottom penetrating sonar illuminates a sandy seafloor. The figure shows aslo three different realizations of the time series response from a "homogenous" sandy seafloor .

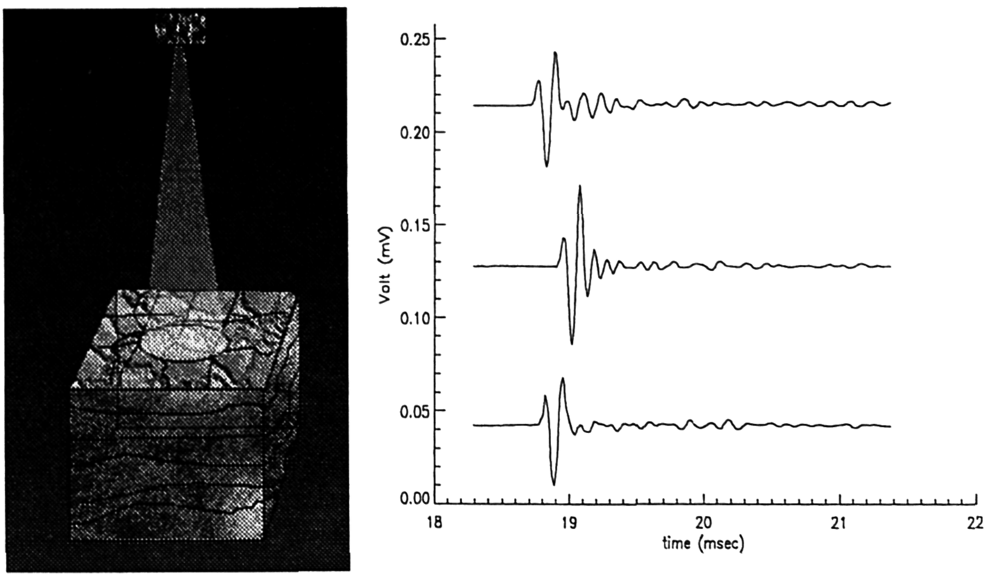

Figure 7; *Left: A schematic view of the Topas beam hitting the seafloor in the BORIS model. Right: Three time responses from a "homogenous" sandy seafloor using the BORIS model.*

[2] BOttom Response from Inhomogenities and Surface. BORIS has partly been financed by ISACS

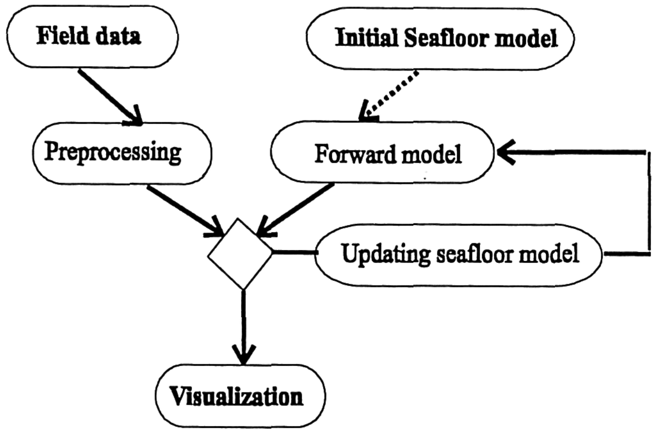

Figure 8; An inversion scheme for determination of seafloor properties.

An model based inversion code has developed at DIST for extraction of geoacoustic parameters, surface roughness and volume inhomogeneities (Caiti et al, 1997). The inversion follows a flowchart as described in Figure 8. The inversion algorithm is now being tested on synthetic data created with the BORIS model and real data gathered by the bottom penetrating sonar. The next step will be to include data from the multibeam echosounder in the inversion scheme to get a best possible description of the seafloor surface.

So far the BORIS model has been used as forward model in the inversion algorithm. Work will continue within this field and one will also look into the composite scattering models (Jackson et al., 1986) in particular for the inversion of smaller grazing angle scattering data.

Two different approaches are investigated for discrimination/classification of the seafloor. One approach is model-based and will utilize the BORIS model for generating a library of predefined classes that can be used for classification by comparison with recorded time series data through suitable algorithms, essentially looking at only one or a few time series at a time (Berntsen et al., 1997). The other approach is statistically based and will likely use several time series at a time in the classification. This approach will also be used as a first step in the segmentation of acquired multibeam and side-scan sonar data.

The classification and characterization of the seabed content includes modeling of back scattering from buried objects. Within ISACS models are being developed for scattering from objects in fluid media and in stratified fluid-elastic environments.

An analytical approach has been developed for the study of responses from a buried rigid sphere that is illuminated at angles below the critical (Hovem, 1997). In this case the wave that hits the object is inhomogeneous. The angular response from the object in such case is quite different from the angular distribution from a sphere that is illuminated only by waves above the critical angle.

A numerical model for scattering from objects in a horizontal layered fluid-elastic media is developed at KTH. Here one is working on a BIE- method (Boundary Integral Equation) for representation of buried objects (Karassalo and Matson, 1997). This approach can handle

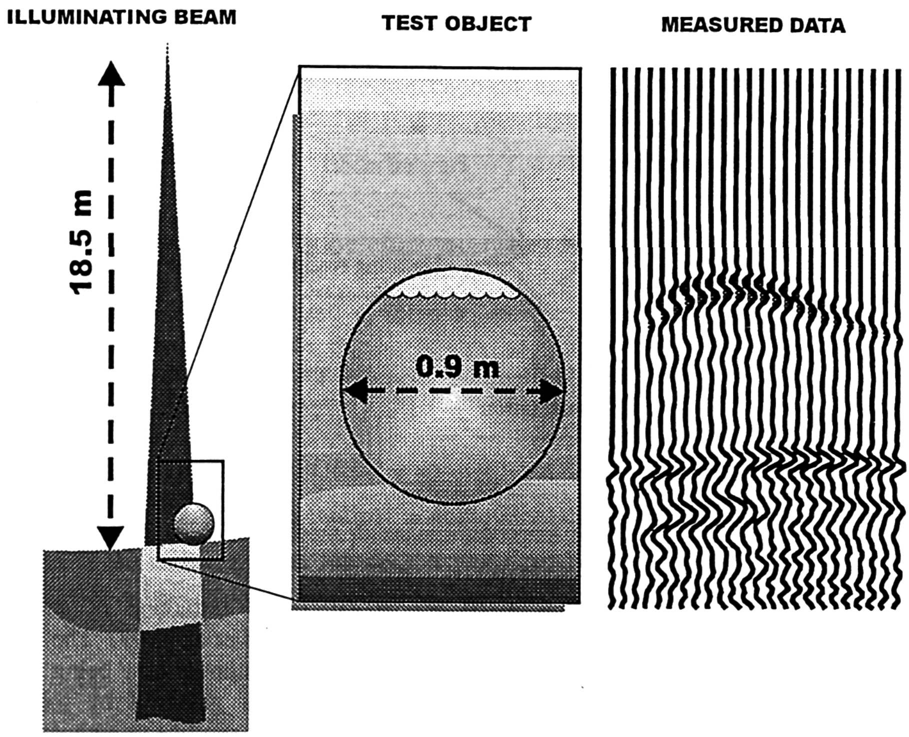

Figure 9; Survey scretch and time response of looking at a waterfilled sphere with the bottom penetrating sonar (Topas).

scatterers of irregular shapes and can therefore be applied to more general objects than the sphere. Work on extending the model to shell-structured objects and porting the code to parallell computing environment is in progress.

At FOA there has been conducted work on the development of remote sensing techniques for detection of buried objects using the bottom penetrating sonar (Topas). The Topas is a parametric source with a narrow beam that gives a small footprint on the seafloor. This system is therefore well suited for detection and separation of objects on and within the seafloor. The tests suggest that the bottom penetrating sonar is at least as good as the standard bottom-penetrating echo sounders (e.g. sparkers). Figure 9 shows a response from a waterfilled sphere that has been illuminated by the bottom penetrating sonar (Topas). The data were captured during our survey in the Baltic Sea.

7. Conclusions

The first half of the ISACS project has been completed according to plans and expectations. Data has been gathered in three different regions of Europe. A numerical model for generation of time responses from surfaces and volume inhomogeneities has partly been developed within ISACS. An model based inversion scheme for determination of seafloor properties is developed and is now being tested both on synthetic and real field data. A new

3D image segmentation method has been developed for dense grided bottom penetrating sonar data. The ISACS project has made good progress in its work on determination of responses from buried objects.

The next half of the ISACS project will be devoted to data processing and the integration of the different approaches and finally the concept test (Task 5, in Figure 1). The gathered data from the different locations will first be processed using the different algorithms that are specific for the acquiring instrument. The result from this processing will be used as input for integrating the different instruments and approaches to a final representation of the investigated site.

The concept test will mainly be to compare the obtained geoacoustic and geotechnical gathered data and to assess the feasibility of the ISACS concept, including recommendations for its exploitation in other more commercially orientated research and development.

References

Allnor, R., 1996, Building topography maps from swath bathymetry data: Concepts and considerations, in anual report MAST3-ISACS

Bergem ,O., Pouliquen,E., Canepa, G., and Pace,N. G., 1997, Time-evolution modeling of seafloor scatter. Part II: Experimental verification., SACLANTCEN Report SM-327

Bergem, O., 1996,"ADAM file format spesification, Ver 2" in Management report MAST3-ISACS 1996.

Berntsen, B., Bergem, O., Pouliquen, E. and Pace, N. P., 1997, Preliminary studies of seafloor discrimination using the BORIS model , SACLANTCEN report,in preparation

Caiti,A., Bergem,O., Pouliquen, E., Biancheri, A. and Scaricaciottoli, S., 1997, Seabed characterization by inversion of parametric sonar data: selection of the cost function, in Proc. Conf. High Frequency in Shallow Water Acoustics, Pace N. G. et al. (Eds), SACLANTCEN Report CP 45

Canepa, G. and Bergem,O., "Small C-routines to read the ADAM data files", in Management report:MAST3-ISACS, 1996.

Hovem, J. M., 1997 "Detection of buried objects in the seabed - A simple model" SACLANTCEN Report in preparation

Jackson, D.R. et al. , 1986, Application of the composite roughness model to high-frequency bottom scattering., J. Acoust. Soc. Am., 79(5)

Loke and du Buff, 1998 ,3D data segmentation by means of orientation-adaptive boundary refinement in an octree, in preparation

Karasalo, I., and Mattsson, J., 1997, Numerical modeling of acoustic scattering by smooth inclusions in a layered fluid-solid medium, iin Proc. Conf. High Frequency in Shallow Water Acoustics, Pace N. G. et al. (Eds), SACLANTCEN Report CP 45

Nikolov, S.G., Loke, E. and du Buf, J.M.H., 1997, Interactive 3D visualization of sonar data using VRML. Presented at the Int. Workshop on Synthetic-Natural Hybrid Coding and Three Dimensional Imaging, Rhodes, Greece.

Pouliquen, E., Bergem, O. and Pace, N. P., 1997, Time-evolution modeling of seafloor scatter, Part I: Theory, SACLANTCEN Report SM-328

TITLE : Characterization and observation of the sea-floor with a new multibeam front-scan sonar system (project **COSMOS**)

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CHARACTERIZATION and OBSERVATION of the SEAFLOOR with a NEW MULTIBEAM FRONT-SCAN SONAR SYSTEM

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SUMMARY

The Shared Cost Action project COSMOS, an investigation for a new surveying tool to produce bottom characterization, bathymetry and imaging, started on 1 October 1997 and runs for 36 months. The main aims of project COSMOS are described and the work plan is outlined.

1. INTRODUCTION

1.1 CONTEXT

For some years, there has been a growing interest for techniques to achieve sea-floor characterization. Studies intend to define parameters that depend on the interaction of acoustic waves with specific structures of the sea bottom. For instance, the echographic response with respect to the grazing angle is investigated as this dependence gives pertinent results (e.g. [1-3]). Hence, a systematic measurement of this dependency is essential for sea-floor characterization. A lot of work is running in order to derive sea-floor characterization from sidescan sonars and multibeam echo-sounders data [1-12]. With both systems, each ping insonifies the sea-floor in the across-track direction. A complete coverage is obtained after merging data corresponding to each scanned line into strips that are eventually mosaicked. Algorithms based on segmentation, incidence correction, spectral and texture analysis are investigated. However, because of the usual operating conditions, each individual patch of the seafloor is seen under a single incidence angle so that the systematic local angular dependency of the target strength is not available.

Sidescan sonars and multibeam echo-sounders provide images of the backscattered acoustic intensity, and enable to build maps of the relief [e.g. 13-14, also huge literature on the topic]. The strongly anisotropic way these system gather data influences the spatial distribution and the quality of the resulting information. They cannot deliver images of the nadir area. The blind sector extends roughly up to 10° on each side. In addition, only the multibeam echo-sounders deliver bathymetry samples of this area by processing specular echoes, which leads to classical artifacts [15-16]. An auto-calibration method has been also investigated for bathymetry data gathered with a multibeam echo-sounder [17], but the geometry of the setup yielded poor results (lack of redundancy, too narrow along-track angular aperture).

Recent efforts are supported in Europe and abroad for studying sonar systems based on synthetic aperture techniques (e.g. see [18]). Other systems based on improved athwartships multibeam technologies are also developed [19]. However, the data acquisition is still performed with a side-looking geometry, carrying the same drawbacks as side-scan sonar systems with respect to the relative lack of data redundancy and blindness in the nadir area.

On the other hand, several forward looking systems are proposed by different manufacturers ([20-21]). These systems are primarily devoted to object detection or obstacle avoidance. For this purpose, the transmitted beam is designed as a solid angle that is wide in the azimuth plane and narrow in site, i.e. the total aperture in the vertical plane is typically less than 15°. Images of the sea-floor are built using low grazing beam angles. Such a geometry does not provide a sampling of the seafloor over a large range of incidence angles, so that these systems are not suited for seabed characterization. They do not deliver bathymetry data of the front area either.

1.2 OBJECTIVES AND EXPECTED ACHIEVEMENTS

The general objective of project COSMOS is to make a significant progress in the acoustical methods for observing and analyzing large areas of the sea-floor by means of a solution that is meant to be complementary with the above mentioned techniques. The project is based on an original forward looking sonar that features a very large aperture in the vertical plane (see Figure) and an interferometric capability. The aim is to demonstrate - with data acquired at sea - that this geometry brings definite scientific and operational assets for the characterization of the seabed, mapping the relief and imaging the sea-floor. Such a system should fulfill the need in marine research programs for a fast, non destructive, cost effective technique to determine the nature of the sea-floor.

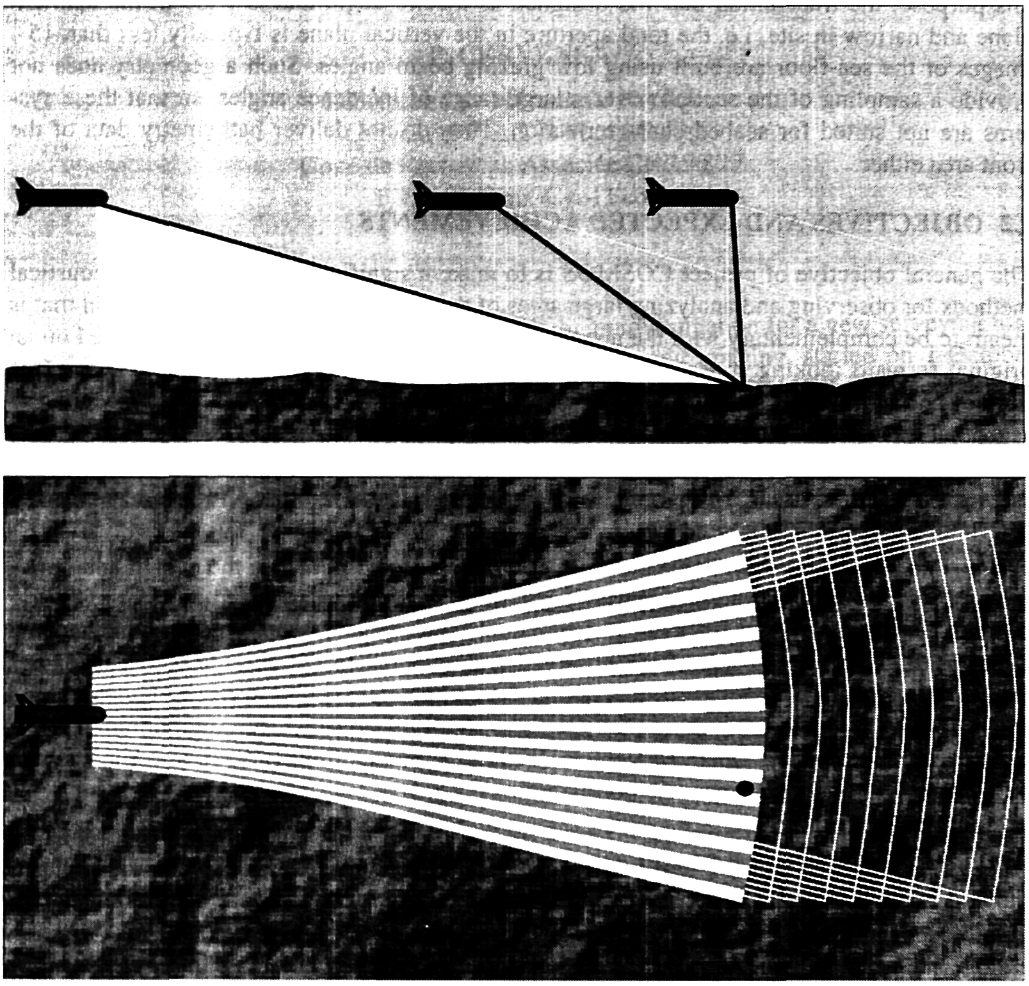
There is no such system available on shelf, so that the project includes the design and building of a prototype sonar whose dimensions and range capabilities are sized by the chosen frequency, i.e. approximately 100 kHz. The maximal slant range is several hundred meters, and the typical across-track width of the observed area is equal to the altitude of the antennae. Hence, this system can be used for surveying the continental shelf as well as the deep ocean floor, depending on the mounting platform, e.g. ship-hull, towed fish, Autonomous Underwater Vehicle or Remote Operated Vehicle. Within the scope of this project, experiments will be carried with a ship-hull mounted configuration.

With the proposed geometry, most parts of the sector that is scanned during each ping are repeatedly insonified (several tens times) under different incidence angles while the sonar is moving forward. This overlap provides the unique capability to measure the systematic angular dependence of the target strength over a wide domain ($\approx 75°$). Several classes of processing schemes that takes advantage of the multiplicity of data available on every elementary patch of the surveyed sea-floor will be investigated to achieve seafloor characterization.

The project addresses also new techniques that take advantage of the high data redundancy to derive an accurate bathymetry of the band centered on the survey track. Two approaches will be investigated and compared : 1) an interferometric method that features a self-calibration capability ; 2) an image based method derived from the stereo photogrammetry technique that is currently used with radar systems. Foreseen as leading to an attractive operational benefit, the feasibility to implement the in-line preview of the relief ahead of the platform will be assessed (using the interferometric scheme).

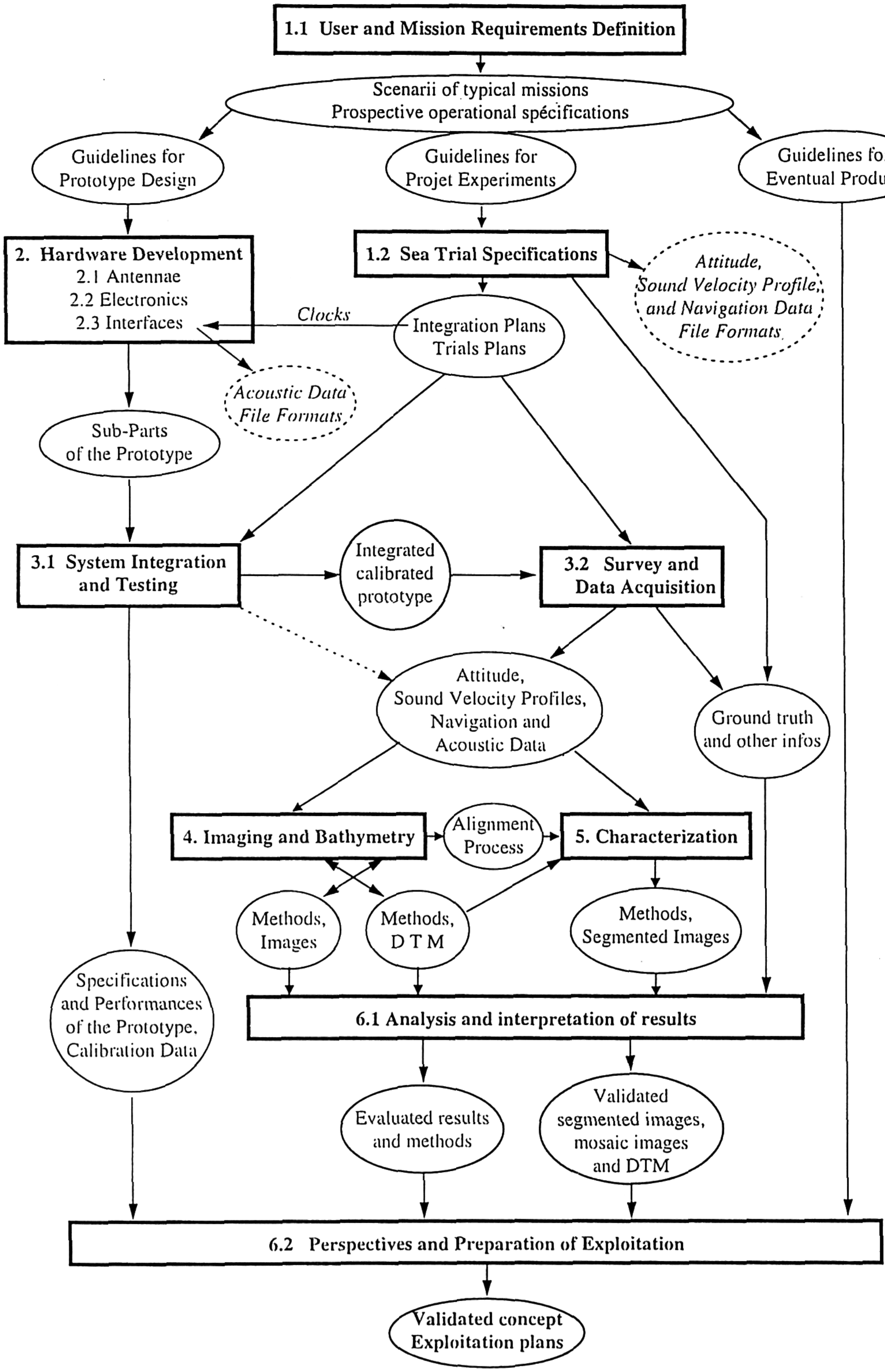
About imagery of the backscattered echoes, a non-coherent processing of data issued from successive, overlapping snapshots is expected to produce high quality mosaic images of the nadir area, where classical systems such as multi-beam echo-sounders and side-scan sonar systems are blind.

Together with these technical and scientific issues, the industrial and economic potential outcomes of this new tool will be analyzed.



Schematic representation of the COSMOS beams pattern

2. WORKPLAN


1.1 User and Mission Requirements Definition
Scenarii of typical missions
Prospective operational spécifications
Guidelines for Prototype Design
Guidelines for Projet Experiments
Guidelines for Eventual Product
2. Hardware Development
2.1 Antennae
2.2 Electronics
2.3 Interfaces
1.2 Sea Trial Specifications
Attitude, Sound Velocity Profile, and Navigation Data File Formats
Clocks
Integration Plans
Trials Plans
Acoustic Data File Formats
Sub-Parts of the Prototype
3.1 System Integration and Testing
Integrated calibrated prototype
3.2 Survey and Data Acquisition
Attitude, Sound Velocity Profiles, Navigation and Acoustic Data
Ground truth and other infos
4. Imaging and Bathymetry
Alignment Process
5. Characterization
Methods, Images
Methods, D T M
Methods, Segmented Images
Specifications and Performances of the Prototype, Calibration Data
6.1 Analysis and interpretation of results
Evaluated results and methods
Validated segmented images, mosaic images and DTM
6.2 Perspectives and Preparation of Exploitation
Validated concept
Exploitation plans

TASK 1 - PROJECT DEFINITIONS

The first part of this task concern the definition of user and mission requirements, including the prospective operational specifications. Several scenarii of typical missions are prepared. For example, operating simultaneously a COSMOS system and a multibeam echo-sounder will allow to deliver bathymetric data of the whole covered area, including at nadir of the platform (ROV, AUV, ...) track, with an even accuracy. Taking into account the constraints related to the navigation, scientist users give their specification to reach these goals. User requirements for accuracy of bathymetry, survey speeds, and accuracy of platform roll/pitch/yaw and heave measurements are examined. About seabed characterization, the prospective levels of classification are defined by reference to the scenarii. Specific requirements related to pipeline burial and cable laying are assessed. This task includes also the definition of the requirements for interfacing to standard sensor packages for platform motion. The requirements within the potential user community for transfer of data to standard visualization and analysis packages is also assessed.

The second issue addressed in this task is the sea trial specifications. The goal is to prepare the sea trials and data collection, to choose the locations of the field operation, and to gather available information concerning these areas. The definition of the sea trials methodology and infrastructure includes for example : the way to refer clocks, attitude sensors and antennae to each other ; the file formats concerning attitude, navigation, sound velocity profiles. Sea trial areas (for calibration, evaluation of the system and comparison processes) are selected according to data availability (seismic data, cores, images and bathymetry) by using a wide scientific data base.

TASK 2 - HARDWARE DEVELOPMENT

With a central frequency close to 100 kHz, experience showed that a technology of antennae based on composite ceramics is cost effective, provides a good efficiency, and allows versatile antenna geometries. The design aims at limiting the number of elements and the size of the antennae in order to facilitate the eventual implementation on a small platform. The total aperture encompasses a large solid angle : the center of the extreme beams formed at receive will be 25° apart in azimuth ; In the vertical plane, the coverage starts from near nadir (5° in order to avoid diaphony with backward echoes) to about 80°. Both transmitting and receiving antennae are mounted along an horizontal axis that is perpendicular to the platform track. The shape of the transmitter is part of a torus whose cross section features a non constant curvature : This geometry allows to control the transmit beam pattern (e.g. by maximizing the energy sent at the largest range), and gives an active surface that is large enough to obtain a sufficient source level. The receiving antenna consists of two parallel rows of phased arrays in order to perform beamforming (resolution better than 2° in azimuth) and interferometry (baseline $\geq$ 2 wavelengths).

The electronic design of both transmitter and receiver is mostly based on digital techniques, but for the front-ends. The transmitter delivers a large power (several kW). The transmitted signal is frequency modulated (3 kHz - 8 ms) in order to apply a pulse compression technique. It increases the effective transmitted power and improves the signal / noise ratio at receive. The receiving system performs first amplification with time varying gain, base-band demodulation, anti-aliasing filters and digitization. Then, beamforming is achieved in-line according to several azimuth angles, followed next by a pulse compression stage. The resulting complex acoustic data are stored in buffers.

The main output of the system consist of digital complex values. The prospective spatial sampling is about 20 cm in slant range and 1° in azimuth, i.e. about 256k complex values per second of echoes recording. The recording of this amount of data will be managed with resources that are currently available and whose complexity and cost are reasonable with respect to the size of this project (removable hard disks). File format will be chosen to be compatible with on-shelf software packages. System controls include items such as : Display of images that are refreshed after each ping ; Monitoring and recording of time varying gain ; Clock synchronization.

TASK 3 - SYSTEM TEST AND DATA COLLECTION

The first part of this task consists of : Testing in tank the different subparts of the system ; Achieving the mechanical and electronical integration of the different subsystems in the perspective of the sea trials ; Performing a first sea trial (planed schedule : March 1999) that is primarily intended for the testing of the integration of all the required hardware and software, and for assessing the functional validity of the system, including calibration. The prototype will be ship-hull mounted. Already available attitude sensors and differential GPS receivers will be used.

Then, as defined in Task 1.2, the ship-hull mounted calibrated prototype will be used to collect data for post-processing purpose and database elaboration (planed schedule : October 1999). The characterization, mapping and imaging capabilities of COSMOS will be thoroughly investigated with these sets of data. All information that is required for the proper exploitation of data acquired with the prototype system, e.g. sound velocity profiles, attitude and navigation data, raw acoustic data, will be assembled and archived on CDROM.

TASK 4 - BATHYMETRY AND IMAGING

This task deals with building mosaic images of the acoustic intensity backscattered by the seafloor, mapping the relief, and supplying pertinent inputs for the characterization of the seabed. The common issue that is addressed in this task is to find the location of the areas on the seafloor from where originated the recorded data. The space-time correspondence between data acquired during successive pings will be derived, at several levels of accuracy.

Within a first step, the radiometric transforms to map data in a common reference system will be derived from the available information on the platform cinematic, beam angles, and assumed relief. According to these relations, the primary sectorial images will be converted into ground-projected gridded images, and next, be merged in mosaics. The ratio width / length of the resolution cell decreases when comparing the first images where a given area appears far ahead of the platform, with the last images where the platform comes close to nadir of the same area. Hence, it seems reasonable to expect that mosaic images thus obtained by non coherent synthesis will be easier to interpret, because the linear resolution is more homogeneous, and because the speckle effect is drastically reduced.

Another domain of investigation concerns bathymetry obtained by means of the interferometric technique, i.e. the differential phase measurement of signals received on the pair of receiving arrays. Data are derived from the temporal evolution of the elevation angle corresponding to incoming echoes within each beamformed direction. The accuracy of the correspondence between phase and geometric angles is altered by numerous space-time varying factors, e.g. celerity profile in the water column. A common practice is to build conversion tables with a calibration process. These tables are occasionally updated using empirical methods, or corrected with deterministic models. However, bathymetry profiles exhibit often remaining biases. We propose to study a technique that takes advantage of the data redundancy to update continuously the conversion tables. The devised technique is based on the statistical analysis of the mismatches between along-track profiles yielded by successive pings. The feasibility to implement this technique in-line will be also assessed.

The geometry of COSMOS opens a promising alternative method similar to the stereo-photogrammetry technique currently applied with data acquired by satellite to provide terrain-elevation measurements at high vertical and horizontal accuracy. Each ping provides an image that is well defined in terms of slant range and azimuth direction. During the platform motion, part of the same landscape is still viewed, but with a representation that changes in this coordinate system and that depends on the relief. Hence, mismatches between the projected images obtained with the a priori mapping are likely to remain because of the discrepancy between the assumed relief and the actual relief. Correlation and triangulation techniques performed on images that are not featureless are expected to give a fairly accurate measurement of these discrepancies, hence enabling to derive the actual relief. This method presents an advantage of paramount importance : whenever features on the seafloor can be properly tracked during several pings (i.e. with an along-track baseline that is large enough), the accurate 3-D positioning should be successfully achieved without demanding requirements with respect to the accuracy of the platform location. In addition, once each individual image has undergone these fine corrections, it is expected that the fusion process will deliver high quality mosaic images. Actually, the devised method is kind of a non coherent synthetic aperture technique with a self-focusing capability.

TASK 5 - CHARACTERIZATION OF THE SEABED

The aim of an automatic seabed characterization system is to "segment" the sea bottom into smaller regions, and to assign one of several sediment types to each region, in reasonable accordance with ground truth. The objective of this task is to assess the system potentialities in this domain, by studying thoroughly a few typical cases of seabed configurations. COSMOS is particularly well suited to deliver convenient data for this investigation, since any given patch of the seabed will be successively insonified from various incidence angles. Hence, the nature of the seabed does not need to be (at least partially) homogeneous along the swath, a condition that must be fulfilled with classical systems. Several classes of processing schemes will be investigated :

The backscattering strength dependence on angle will be exploited after correction for the local slopes derived from the bathymetry processing, and compensation for the propagation losses and for the geometry of the beam footprints. The method will be applied on data obtained on various sediment configurations, in order : 1) to establish typical average relations between backscattering strength and incidence angle, 2) to study their potentialities for automated identification; and 3) to use them in sonar image correction in order to remove the average angle influence on local reflectivity.

The signal statistics from one single beam may be studied both in the time and in the spectral domain. Considering the short wavelengths used by the system, there is evidence that structural parameters of the surface affect the time evolution of the amplitude of the backscattered echoes. Several amplitude analysis techniques (e.g. fractal properties, statistical feature selection, higher order spectra, Markov random fields) will be compared. The signals will also be analyzed with classical spectral analysis approaches. The respective influences of the beam grazing angle and azimuth direction will be checked. The methodology will be the same as in the previous sub-task : processing data from well-calibrated zones, definition of typical values for classifying descriptors, and exploitation for identification. The results will be compared with studies based on side-scan sonar data.

A statistical analysis of the phase derived by the interferometric process will be performed. The dependence on the sea-floor characteristics and on the grazing angle will be investigated. The second order statistics of the phase values may be a function of the height and correlation length of the bottom roughness.

Task 6 - System Evaluation and Perspectives

At the end of the project, the collected information will be interpreted and evaluated, in order to assess the validity of the new concept COSMOS. The next steps that are likely to be the continuation of this project will be prepared.

The system will be tested over well-known areas. Hence, the quality of mosaic images, bathymetry and characterization maps will be evaluated by comparison with the available data base and ground truth information. Statistical analysis of the sedimentary properties together with their geological interpretation will be performed and correlated with acoustical data collected by the system. The degree of correlation between the fine-scale variability, or texture, of the images, and the different seafloor types, will be assessed. Digital terrain models derived from the interferometric and photogrammetry techniques will be compared. After demonstrating the progress achieved with respect to other survey systems, the most proficient, cost-effective methods to obtain seabed characterization, bathymetry and images will be selected and proposed for applications.

Using an existing database of potential users, the potential of the system will be analyzed, and the likely response of the market to the new system be assessed. The possible benefits of the system when compared to those already on the market will be studied. Factors of interest will include the coverage rates, the accuracy of bathymetric information and the degree of portability for use on vessels of opportunity. Factors to be considered in determining the future direction of the project will be the nature of the results obtained, and the reaction of the user community to these results. Of considerable importance will be the suitability of the system for production, i.e. the amount of work needed on hardware and software development before it can be deemed to be a finished product. The above factors will be analyzed in order to produce a recommendation for the continuation of the project and to prepare exploitation.

As a whole, the expected outcome of this project is a validated concept for a new surveying tool. Examples of applications that are likely to be eventually considered are : Equipment of ROVs ; Bathymetry surveys ; Optimization of pipeline burial ; Preparation of the setting of off-shore equipment ; Monitoring of sedimentary processes ; Archaeological surveys ; Detection and monitoring of environmental hazards.

Part of this project share common interests with the MAST III project ISACS (Integrated System for Analysis and Characterization of the Seafloor) under contract n° MAS3-CT95-0046. "The objective of Isacs is to prove the feasibility of the analysis and characterization of the seafloor by the exploitation and by the suitable integration of data gathered from commercially available sonar equipment." Because COSMOS may become another available tool, the potential for a future "Concerted Action" with ISACS will be considered.

REFERENCES

[1] J.H. Clarke, Toward Remote Classification Using the Angular Response of Acoustic Backscattering : a Case Study from Multiple Overlapping GLORIA Data, *IEEE J. Ocean. Eng* **19** 1, 1994.

[2] C. de Moustier and D. Alexandrou, Angular Dependence of 12 kHz Seafloor Acoustic Backscatter, *J. Acoust. Soc. Am.* **90** 1, 522-531, 1991.

[3] N.P. Pace and H. Gao, Swath Seabed Classification, *IEEE J. Ocean. Eng.* **13** 2, 83-90, 1988.

[4] B. Chakraborty, R.A. Hagen and H.W. Schenke, Determining seabed backscatter parameters using Hydrosweep System, *Proc. 3de Eur. Conf. Underwater Acoustics* **I**, 99-104, 1996.

[5] S. Dugelay, X. Lurton and J.M. Augustin, A new method for seafloor characterization with multibeam echosounders : image segmentation using angular backscatterring, *Proc. 3de Eur. Conf. Underwater Acoustics* **I**, 439-444, 1996.

[6] Z.H. Michalopoulou and D. Alexandrou, On acoustic seafloor characterization, *Proc. 2de Eur. Conf. Underwater Acoustics* **II**, 931-936, 1994.

[7] R. Finndin, A. Bolinder and G. Shippey, Classification and mapping of side-scan sonar data, *Proc. 2nd Eur. Conf. On Underwater Acoustics* **II**, 683-688, 1994.

[8] L.M. Linnett, S.J. Clarke and D.R. Carmichael, The analysis of sidescan sonar images for seabed types and objects, *Proc. 2nd Eur. Conf. On Underwater Acoustics* **II**, 733-738, 1994.

[9] X. Lurton, S. Dugelay, J.M. Augustin, Analysis of multibeam echo-sounder signals from the deep seafloor., *Proc. Oceans 94* **III**, 213-218, 1994.

[10] R.P. Dziak, H. Matsumoto and C. Fox, Estimation of Seafloor Roughness spectral parameters from multi-beam sonar acoustic backscatter data : Axial Seamount, Juan de Fuca Ridge, *Geophysical Research Letters* **20** 17, 1863-1866, 1993.

[11] D. Alexandrou, D. Pantzartzis, A Methodology for Acoustic Sea-floor Classification, *IEEE J. Ocean. Eng.* **18** 2, 1993.

[12] T.B. Reed and D.M. Hussong, Digital Image Processing Techniques for Enhancement and Classification of SeaMARC II Side-Scan Sonar Imagery, *J. Geophys. Res.* **94** B6, 7469-7490, 1989.

[13] E. Hammerstad, S. Åsheim, K. Nilsen and H. Bodholt, Advances in multibeam echo sounder technology, *Proc. Oceans 93* **I**, 482-487, 1993.

[14] C. de Moustier, State of the Art in Swath Bathymetry survey systems, *Int. Hydro. Rev.* **65**, 25-54, 1988.

[15] H. Tonchia, Evaluation of a deep water wide swath echo sounder for hydrographic surveys, *Proc. Oceans 94* **III**, 225-230, 1994.

[16] C. de Moustier and M.C. Kleinrock, Bathymetric Artifacts in Sea Beam Data : How to Recognize Them and What Causes Them, *J. Geophys. Res.* **91**, 3407-3424, 1986.

[17] R. Schreiber, H.F. Wentzell, and F Ziese, Efficient seafloor mapping and sub-bottom profiling with Atlas Hydrosweep and Atlas Parasound, 103-110.

[18] ACID and SAMI European' MAST programs under contracts n° MAST-CT89-00002-CD and MAS2-CT93-0048.

[19] J.F. Denis and B. Ollivier, A New Generation Deep Water Multibeam Echo Sounder, *Proc. Oceans 94* **III**, 239-243, 1994.

[20] C.D. Loggins, Ahead-Look Sonars : Design Comparisons and Performance Considerations, *Int. Underwater Systems Design* **17** 4, 15-23, 1995.

[21] Anonymous, Directory of sonar suppliers and products, *Int. Underwater Systems Design* **18** 4, 18-23, 1996.

[22] G. Jin and D. Tang, Uncertainties of differential phase estimation associated with interferometric sonars, *IEEE J. Ocean. Eng.* **21**, 53-63, 1996.

[23] WEB site for project COSMOS : http://www.ccr.jussieu.fr/cosmos

TITLE: Grouted Offshore Piles for Alternating Loading (project **GOPAL**)

CONTRACT NO: MAS3-CT97-0119

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Objectives

Piling is the most prevalent foundation system for structures in the marine environment. The European offshore industry is continually seeking to reduce piling costs; innovative foundation systems are required. This research provides enabling technology to **increase pile capacity** for both static and alternating loads: installing jet grouted bulbs at the pile tip to increase end bearing and take advantage of increases in shaft resistance with time. These solutions will reduce pile length and foundation costs.

Contents of Project

Offshore structures are subject to environmental loads which induce alternating compression and tension forces at the foundation level. Foundation piles resist applied loads by friction along the shaft and end bearing of the tip on underlying soils. This project addresses both issues. Current design methods underestimate shaft friction in sand, neglecting long term increases in capacity. End bearing can be increased by enhancing existing onshore technology, **Jet Grouting**, to improve soil below the pile tip.

Pre competitive work required to develop these concepts includes:

- research on increase of shaft friction over time;
- basic geotechnical properties of jet grouted soils;
- modifications/extensions of existing jet grouting technology;
- onshore field tests to prove effectiveness.

Europe has a strong onshore soil improvement industry. After Japan, it is considered to be the world leader. The offshore sector is also significant, including national petroleum companies and major marine/coastal construction contractors. The objective of this project is to create synergy between these groups, transferring onshore technology to the marine environment. These methods will open new markets for European Jet Grouting Companies, increase Europe's competitiveness in offshore construction, and allow development of marginal hydrocarbon reserves.

This project is in the germinal stages. Current work is concentrating on definition of the potential market for the process, identification of main geotechnical and technological requirements, and initial design of the grouting equipment/field test. Results will be released to the engineering/scientific community as available.

TITLE: Very High Resolution Marine 3D Seismic method for detailed site investigation (VHR3D)

CONTRACT NO: MAS3-CT97-0121

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VERY HIGH RESOLUTION MARINE 3D SEISMIC METHOD FOR DETAILED SITE INVESTIGATION VHR3D

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Abstract

The Very High Resolution 3D Seismic Project (VHR3D) is aimed at a cost-effective detailed 3D reconnaissance of seabed sediment properties for geological, geotechnical and environmental site investigation purposes, based on very high resolution 3D seismic.

The project includes the development of a flexible 3D seismic method and the extraction of geotechnical information from the 3D seismic data leading to the creation of a 3D physical model of the subsurface based on the acquired information.

Basic processing steps will include navigation geometry, signal analysis, static corrections and binning. Imaging will be performed using stacking and migration techniques. A subset of the 3D data volume will be treated for seismo-acoustic attribute information (velocity, attenuation, impedance).

Empirical relationships between the acoustic parameters and geotechnical properties will be used to develop algorithms to invert the seismo-acoustic responses. Laboratory measurements will provide calibration data.

A practical guide of specifications will be set up for economic use of the VHR3D method, in order to assure optimum applicability of this method and to increase its competitiveness and market potential.

1. OBJECTIVES

1.1. Objectives of the project

The Very High Resolution 3D Seismic Project (VHR3D 1998-200) is aimed at a cost-effective detailed 3D reconnaissance of seabed sediment properties for geological, geotechnical and environmental site investigation purposes, based on very high resolution 3D seismic.

The main objective of the project is twofold :

- the development of a flexible 3D seismic method which allows optimal coverage of targets of different size;

- the extraction of geotechnical information from the 3D seismic data leading to the creation of a 3D physical model of the subsurface based on the acquired information.

The term Very High Resolution (VHR) seismic here refers to mean seismic source frequencies between 1 and 2 kHz (i.e. signal wavelengths of about 1 m), with an expected vertical resolution in the order of decimetres and a penetration down to about 100 m. The lateral resolution should therefore be of the same order of magnitude as the vertical resolution.

Because the acquisition of high-quality data is the basis on which much of the success of the project will depend, major effort will be placed on carefully tailoring the seismic acquisition system to address the site characteristics. Therefore two complementary systems will be developed :

- a compact rigid array allowing studies of geotechnical and environmental sites of limited area extent (100 x 100 m) in very shallow water (25 m) and with limited penetration (50 m),

- a dynamic flexible array for geotechnical / geological targets of small (250 x 250 m) to medium size (1 x 1 km) in water depths up to 100 m and allowing a deeper penetration (100 m).

The first system will yield a high manoeuvrability in difficult navigation areas, whereas the second system will be more appropriate to open sea areas. Both systems will be used for data acquisition in sites which have already been investigated.

The processing of the data will be, by necessity, of the same high standard as the acquisition. Data editing and basic processing steps related to navigation geometry, signal analysis, static corrections and binning are important as subsequent analysis will be fully dependent upon it. Imaging will be performed using stacking and migration techniques. A subset of the 3D data volume will be treated for seismo-acoustic attribute information, including velocity, attenuation and acoustic impedance.

The empirical relationships between the acoustic parameters and geotechnical properties will be used to develop algorithms to invert the seismo-acoustic responses. Laboratory measurements will provide calibration data and information to further develop the geo-acoustic models. This will help in the assessment of the validity of the inversion.

Ultimately this will make it possible to obtain a 3D model of the physical and geotechnical properties of the study site. A set of synthetic seismograms will be generated for comparison with the real dataset. Together with the reference to groundtruthing information, this will validate the feasibility of the geophysical methodology.

With the experience gained in this project, the definition of a practical guide of specifications can be set up for economic use of the VHR3D method. The applicability of this method to the existing commercial sector, as well as possible future developments relating to near-target studies, will be thoroughly investigated.

1.2. State of the art

1.2.1. Seismic acquisition and processing

Up to present, the principal acquisition techniques in VHR seismic have been based on 2D single-channel, or at best multichannel streamers. While this technique provides an image of the different sedimentary layers, as well as velocity information in the multichannel case, the resulting subsurface image remains inaccurate because the data by necessity are interpreted as if in a vertical plane along the direction of the ship's movement. Off-line events (e.g. related to dipping layers) decrease the resolution of the image and ultimately influence the accuracy of the calculated seismic attributes.

The use of a 3D field layout can overcome this problem. Whereas VHR 2D surveys are now in common use, most 3D seismic acquisition is restricted to the hydrocarbon exploration industry because it involves complex field and processing procedures, which have tended to make it costly and time-consuming. The adaptation of this 3D technique to VHR investigations is advancing very slowly, with several years of delay. The reason for this is not only the different economic markets, but also the severe technological problems related to a reduction in scale (downscaling) and the algorithmic treatment of the data.

In recent years, the acquisition of VHR shallow 3D data has been demonstrated to be feasible, in a modest and cost-effective way. Using a relatively simple 3D field system consisting of 12 short dual-channel streamers towed from a source-bearing catamaran, a small 3D data volume was acquired on the Scheldt river near Antwerp. Time slices obtained after stacking showed a remarkable resolution. However despite the high quality results, the data were not of the optimum due to a number of shortcomings in the field system. Redimensioning, adaptation and optimisation of the 3D acquisition method are therefore now required.

Up until now, the processing sequence of shallow seismic data has seemed to stagnate around the 1D assumption of the Common Mid Point (CMP). Indeed very few geophysicists attempt to go beyond Normal Move Out (NMO) and stack processing. The sea bed and its near subsurface are by nature 3-dimensional structures and should be treated as such.

This can be done by applying migration algorithms to the data. This imaging technique has the reputation of being difficult to use and requiring large computer resources. Its application for near subsurface seismic is in short supply, and implies challenging aspects related to the algorithmic treatment of the VHR data, taking into account both the resolution requirements and processing facilities available.

Velocity information, as needed for migration, is conventionally obtained using computation of NMO corrections of the reflection arrivals. In the case of shallow water and complex geology this approach may not be entirely reliable, and other methods can be explored in order to calculate velocities. The usage of tomographic inversion to produce a detailed velocity model is relatively recent, and has up to now only been tested in the hydrocarbon realm. Extending this technique to shallow data therefore represents a great challenge.

1.2.2. Geo-acoustic modelling

Many of the sea floor sediment properties that affect the acoustic response are of direct interest to geotechnical engineers (e.g. void ratio/porosity, density, grain size and distribution). Within the literature a large number of empirical relationships exist between the acoustic impedance, velocity and attenuation of acoustic waves and the physical and geotechnical properties of marine sediments. For example there is an excellent linear relationship between acoustic impedance and porosity, and given the impedance/velocity structure the bulk density of the material can easily be derived.

This inter-dependency between geotechnical and seismo-acoustic properties of marine sediments allows the extraction of geotechnical information from seismic data. The recent development of fast sampling, digital acquisition systems and the advance in processing techniques have made it increasingly possible to obtain significant information from the incoming seismic signal. The seismic attributes of most interest from this point of view are velocity, impedance and attenuation, and over the past few years scientists have been working towards a cost-effective methodology for the remote sensing of the physical properties of seabed sediments based on the inversion of these attributes.

However, as yet no system is available that can extract meaningful results consistently. The degree of correlation of the relationships can vary widely, ranging from excellent for acoustic impedance-porosity relations to poor for say some of the Atterberg limits. In practical terms, there still remains a large amount of research to be undertaken on determining the precise nature of the geo-acoustic relationships. Therefore further study is needed, and in particular studies to determine the standard deviations of these values; this will become increasingly important if commercial applications are to be realised.

To date in site investigation practice there has been a tendency to rely on information obtained from point sampling and attempt to extrapolate between these sample points. This may easily lead to misinterpretations which could ultimately prove very costly. Recently, seabed surface reflection responses recorded during continuous sub-bottom profiling have been inverted successfully to provide distribution maps of physical parameters.

The next stage is to extend this procedure to the subseabed layers to aid interpretation of the often complex Quaternary sequences. To this end there is a definite need for improved data quality and more detailed, 3D interpretation. Application of this methodology to the subsurface, using high-quality 3D seismic data, is of great importance, the major advantage being the cost effective production of a 3D physical model.

2. Work content

The work programme is structured into 6 major tasks, each task being under the responsability of one partner.

2.1 Task 1 : Seismic and positioning strategy (RCMG)

2.1.1 Seismic strategy

Practice has demonstrated that geophysical remote sensing of geological, geotechnical and environmental targets benefits from carefully tailoring the system to the site characteristics, in order to warrant optimal results. Very high resolution 3D seismic applied to such objectives hence not simply reduces to a mere downscaling of oilfield-type 3D seismic - from which the basic principles are borrowed - but it also calls for specific strategies.

In order to fully exploit the proposed 3D method a flexible approach is necessary. Therefore two complementary acquisition systems will be developed :

- Compact 3D acquisition system. The proposed acquisition system will involve a lightweight, lateral deployment of a high number (10 to 12) of short single- or dual-channel streamers towed from fixed points on the surface frame. Streamer spacing will be small (1 m). Remote-controlled modification of the wing span will allow convenient deployment and recovery, even on small vessels. A long axial multi-channel streamer will be used for velocity information. The platform itself will be configured to explore the possibility of hosting different sources and sensors.

- Flexible 3D acquisition system. The system will be capable of being dimensioned in a dynamic way for optimal coverage of larger geotechnical and geological sites (up to 1 x 1 km) in water depths up to 100 m and with deeper penetration (100 m). Investigation areas might include larger geotechnical sites (dams, artificial island sites, pipeline routes on the shelf, etc.) or sedimentary bodies of key geological importance. The system will consist of a multichannel array of longer streamers (for example 4 streamers, 6 channels each). By modifying the streamer spacing (up to 20 m) and the number of channels per streamer, the array will be adjustable to the size of the survey target. A long multi-channel streamer will also be used in order to obtain longer-offset data necessary for velocity calculations.

2.1.2 Positioning strategy

For near-shore surveys laser ranging positioning can be used. This method has already proven its reliability, and gives accuracy in the decimetre range. For offshore surveys GPS positioning can be applied using off-line trajectographic corrections involving a land-based GPS which provides a decimetre accuracy. Real-time D-GPS positioning allows accuracy in the meter range, and can be used for on-line navigation. Such positioning can also be used for larger-scale 3D surveys and 2D long-offset investigations which do not require decimetre accuracy. The possible use of other positioning systems (such as land beacons working in circular mode) will also be investigated.

Accurate relative positioning of the receivers compared to the source is also needed. Using a surface towed platform makes it possible to mount a positioning receiver directly on top of the source and in the immediate proximity of the very short streamers. This reduces the relative positioning error to a minimum. Former acquisition/positioning experience and the successful processing of acquired data has demonstrated the validity of such an approach.

When the positioning antenna is located on the vessel some control of the acquisition array will be necessary. The use of one or more built-in compasses will allow to register the streamer course, and will be used as input to model the drift of the streamers. Moreover, based on redundancy in first break picking throughout the receiver array, position monitoring can be performed within the processing of the seismic data themselves.

2.2 Task 2 : Data acquisition at sea (IFREMER)

The developed acquisition systems will be applied to a number of sites for validation of the different approaches (geological-geotechnical-environmental). One survey will be carried out for each approach, and different site options will be considered. The necessary vessels can be supplied by the different partners.

A preliminary choice of sites has been made with regard to existing and available geophysical / geotechnical data. These data will allow validation of the developed acquisition and processing methods, and enable geotechnical and physical modelling work to start at an early stage of the project, when 3D data will not yet be available. Possible site survey locations include:

- Zeebrugge harbour (North Sea) Toxic waste dumping site, marked by a strong lateral variation in facies and reflectors (locally strong attenuation, hyperbolae stacks, gas pockets, sandcover of varying thickness, pits, etc.). Analysis of dump structure, its burial or exposure. Site dimensions approx. 200 x 200 m, water depth less than 10 m.

- Mersey Docks and Harbour, Liverpool Bay (Irish Sea) High environmental interest related to accidental anthropogenic contamination, sludge and spoil dumping. Site dimensions 1 x 1 km, water depth less than 20 m.

- Monaco harbour (Mediterranean) The site contains a very complex 3D structure marked by a strong sloping paleomorphology, dipping bathymetry and highly variable geology, and is of high geotechnical interest (harbour extension, dike implementation ...). Site dimensions approx. 300 x 100 m, water depth 40 - 80 m.

- North Wales coast (Irish Sea) The complex Quaternary stratigraphy, marked by laterally and vertically highly variable glacial and post-glacial deposits including buried channels and infill sequences, is of high geotechnical and geological interest. Site dimensions 1 x 1 km, water depth less than 20 m.

- Rhone delta (Mediterranean) Highstand/lowstand sequences (late Quaternary, Holocene) marked by strong impedance contrasts. Specific targets such as incised valley fills, coarse grained deltas, retrogressive slides and mud waves require 3D imaging to reconstruct the climatic/glacio-eustatic and autocyclic controls. Target area dimension 200 x 600 m, water depth 100 m..

In addition to the dense 3D data set, a less dense network of longer-offset 2D seismic data will be acquired using a long multichannel streamer.

Although the surveys will preliminary be carried out in areas which have already been groundtruthed down to the depths of interest, new samples will be needed for physical and geophysical analysis in the lab in order to improve the empirical relationships and the geotechnical/seismic interpretation.

2.3 Task 3 : 3D data processing (ARMINES)

The processing sequence is separated into two different tasks : "3D data processing" and "Extraction of seismo-acoustical parameters". When possible, the processing will be done with standard available software. Because of the downscaling problem we are facing, some algorithms are not adapted or simply not available, and will be developed in this project.

For each source position, X, Y and Z coordinates will be obtained from the navigation data. From the streamer, built-in compass (streamer feathering) and boat motion, it is possible to calculate the X and Y offset variations of the hydrophones relative to the source position.

Correction for small-scale time variations e.g. due to tides, shot and receiver depths, and cable angles, will be very critical in VHR seismic to prevent destructive stacking of the data. These corrections can be greater than the predominant wavelengths of the data and make it difficult for automatic 'static' correction programs to produce valid results.

A detailed velocity model will be obtained by iterative reflection tomography using long offset 2D multichannel data. Initially, estimation of the velocity field is needed. For this initial model we will perform conventional velocity analysis using data from the long-offset multi-channel streamers. Some other methods such as Common Migrated Image analysis, or automatic 2D inversion using the Migration Based Travel Time (MBTT) method will be tested.

A first main step in the imaging process of shallow seismic data involves stacking of the NMO-corrected data. The results after stacking allow a first image control of the recorded data. The water bottom and its near subsurface are by nature 3-dimensional structures and should be treated as such. This requires migration processing, which removes the propagation distortion from the reflectivity.

2.4 Task 4 : Extraction of seismo acoustical parameters (OGS)

The basic seismo-acoustic properties to be determined include seismic velocity, acoustic impedance and attenuation. They are very important as they form a direct link with physical properties of the sediment, and provide a method for lateral extrapolation of the geotechnical properties measured on samples.

The velocity field connects the "seismic world" (time) with the "geological world" (depth). A reliable velocity model is not only indispensable to obtain correct geotechnical/physical parameters, but is also a necessary tool for correct depth imaging. The latter is particularly important for complex structures and heterogeneous media, in which case a time section may suggest apparent structures that are totally erroneous.

The method applied here will involve 3D tomographic inversion using a ray tracing algorithm based on the minimum time principle. This technique permits the inversion of reflected, transmitted and refracted arrivals, either jointly, or separately. The inversion of these travel times will be performed on data collected with appropriate offsets in order to reconstruct the local velocity distribution and reflector position at depth. The reliability of the velocity field thus obtained will be confirmed by 2-D pre-stack depth migration on selected lines.

Determination of the attenuation is of uppermost interest, not only for linking the seismic data to the geotechnical properties of the soil (e.g. compaction), but also to enhance the amplitude information of the seismic signal.

Acoustic impedance is a basic physical property. The estimation of reliable impedance values from seismic data demands very careful processing. In a first step the seismic traces are converted into a reflection coefficient series.

The resulting reflection coefficients allow computation of the acoustic impedance using inversion techniques. Velocity information from borehole cores (if available) can be used to calibrate the inversion. Different processing packages for inversion and attribute analysis already exist, and they will be adapted in order to allow application to the VHR 3D data.

2.5 Task 5 : Geotechnical / physical modelling (UWB)

The strong inter-dependence between the acoustic and geotechnical properties of a marine sediment is widely recognised and accepted. On this basis it will be possible to extract physical and geotechnical information from the seismo-acoustic responses of the 3D data through inversion processing, using algorithms developed from established empirical relations. Complementary laboratory tests on "calibration" cores will make it possible to determine the precise geoacoustic behaviour of the specific marine sediments under investigation.

The final objective, i.e. the production of a 3D physical / geotechnical model of the sediment parameters based primarily on the acoustic response, will be achieved with only a limited amount of groundtruthing.

The main physical factors that affect the acoustic response are well known, but their degree of correlation can vary widely. A particularly close relationship between acoustic impedance and porosity / void ratio has been identified. This and other empirical relationships will be used to produce geophysical-geotechnical algorithms for the inversion of seabed reflection signatures to provide sections of the physical properties (e.g. grain size, porosity, density).

Additional laboratory research is needed to provide calibration data which will make it possible to further develop the predictive geotechnical / physical property relations and in particular to determine the standard deviations of these values. This will include investigations of the influence of consolidation state on acoustic propagation including velocity and attenuation (a.o. measurements in geophysically-instrumented consolidation cells), relationships between velocity, density and physical properties, etc.

With access to the information from the seismic attribute studies, the inter-relationships between geotechnical and acoustic properties, and the improved geoacoustic models, it should be possible to produce a 3D model of the seabed sediment's physical properties (e.g. density, void ratio / porosity, moisture content, grain size). Given this information it is then theoretically possible to calculate order-of-magnitude estimates of geotechnical parameters such as shear strength and permeability.

2.6 Task 6 : Exploitation of results and market potential (HAL)

A study will be undertaken to investigate the required specifications of VHR3D surveys in relation to the experience gained in this project. Further the applicability of the different techniques developed in the course of the project (seismic acquisition, data processing, geo-acoustic modelling) to the current commercial market will be investigated. Possible future developments of the VHR3D method (a.o. related to near-target studies) will be also investigated.

3 The partnership

The work proposed within this project is divided into a series of tasks which involve seismic acquisition, site surveys, seismic processing, laboratory experimentation, and geoacoustic modelling. Because of the complementary nature of the different research teams, and the nature of the work involved, it will provide an opportunity to extend the existing collaboration with a view to realising the full commercial potential of the research developments.

3.1 IFREMER

IFREMER is France's oceanographical research institute. As such, it is responsible to the French Government for the promotion of advanced scientific research, technological and industrial developments related to oceanic resources and environmental protection, and particularly concerned with:

- management of large projects in cooperation with universities, research institutions and industry
- leadership of research and development within its own field of competence
- management of French oceanographic fleet and the development of new tools, techniques and land based facilities for the French oceanographic community
- international cooperation and promotion of French industry abroad. Therefore IFREMER is entitled to sign international cooperation agreements and acts as an industrial consultant to private firms

IFREMER will act as coordinator of the project. They will mainly be involved in « seismic and positioning strategy », « data acquisition » and « data processing ».

Specific contributions to the project include :

- Development of a flexible 3D acquisition method
- Site surveys with the flexible 3D field array
- Processing of the acquired 3D data volumes
- Geological interpretation and comparison with existing data
- Sampling and core analysis (Rhone site)
- Exploitation of the VHR3D method (specifications, future developments)

3.2 RCMG

RCMG has nearly 20 years of experience in high resolution reflection seismic and geophysical surveying in shallow waters : e.g. major research contributions to the MAST-I and MAST-II Programmes ("RESECUSED", "GISP", "STARFISH").

In the last decennium RCMG started to develop a VHR 3D seismic methodology building on developments in both the design of acquisition hardware (new seismic source - Centipede sparker; new field-layout - SEISCAT) and surface modelling software (Geofox). The horizontal and vertical resolution obtained with the developed system was never achieved previously. This research was carried out in the framework of the EEC Hydrocarbons Project 3639/85 "An integrated approach to stability evaluation of prospective offshore sites".

RCMG is well-equipped with an independant, mobile seismic acquisition system, a.o. consisting of a number of different high resolution seismic sources (Uniboom boomer, SEISTEC source, different sparkers, 15 in3 watergun, etc.) and receivers, with both analog and digital VHR acquisition. Standard and experimental 2D-processing of the digital seismic data (signal enhancement, stacking, migration, etc.) can be done on RCMG's industry standard seismic processing system.

RCMG will mainly be involved in « seismic and positioning strategy », « data acquisition » and « data processing ».

Specific contributions to the project include :

- Design and development of a compact 3D field array (including sea trials)
- Evaluation of the available positioning systems
- Site surveys with the compact 3D field array
- Quality control and pre-processing of the acquired data
- Geological interpretation and comparison with existing data
- Specifications of the VHR 3D seismic method

3.3. ARMINES

The Geophysics research group of Ecole des Mines was founded in 1989, and is constituted of 4 senior researchers. All members of the group have a wide experience in theoretical wave propagation and solving inverse problems leading to the development of imaging methods which can be applied to different kinds of seismic data.

The main activities in the three past years has been devoted to:

- Implementations of 2-D and 3-D migration and demigration algorithms using ray theory. This topic continues in close co-operation with several French oil compagnies and related institutions. Pascal Podvin, director of the laboratory, has been the coordinator of the EEC Joule II project "Fast asymptotic 3-D Green's functions with applications to seismic migration/inversion".
- Very High Resolution marine seismics with IFREMER as a partner. The results obtained have contributed in a significant manner to the development of cost-effective sophisticated processing algorithms (inspired from the oil industry).

Specific contributions to the project include :

- 3D acquisition requirements
- Assistance in site survey operations
- 3D data processing (geometry, preliminary processing, static corrections)
- Velocity analysis
- Stack and migration imaging
- Attenuation processing
- Exploitation of the VHR3D method (specifications and market potential)

3.4 OGS

The Osservatorio Geofisico Sperimentale (OGS), situated in Trieste, is a public body under the aegis of the Ministry of Universities and Scientific and Technological Research. Research in the field of Earth Sciences is performed by the Department of Geophysics of the

Lithosphere. This Department is well equipped for this research with powerful computer facilities and the geophysical exploration vessel OGS-EXPLORA.

The participation of OGS in this project is envisaged as a multidisciplinary "group participation" and involves four research groups of the Department of Geophysics of the Lithosphere with experience in different fields of geology and geophysics :

The GEMS (GEeophysical Modelling and Simulation) group is involved in the simulation of wave propagation in complex structures and media by full wave modelling techniques based on spectral methods. In addition, basic research on wave propagation is carried out.

The INTE (geophysical data INTErpretation) group is involved in planning, acquisition and interpretation of conventional multichannel seismic reflection data within the National Antarctic Program (PNRA). Recent research has focussed on HR seismic methods (multichannel and single channel) and active participation in the ODP to investigate relationships between lithology, the physical properties of sediments, and the seismic record.

The REDS (REsearch and Development in Seismics) group is involved in development of algorithms and software tools for seismic inversion. Recently a 2-D tomographic inversion package was completed for AGIP and OGS internal use. At present a joint project is going on with TNO (The Netherlands) for the EEC ESSI Programme.

The PROS (PROcessing Seismic data) group is involved in seismic data processing. The processing centre includes an industry-standard processing package installed in a network of workstations. Its activities include conventional HR site-surveys for the exploration industry, large volume regional processing, and VHR land 3D seismic in the EEC HIRES SESOM Programme.

Specific contributions to the project include :

- Preliminary processing and static corrections
- Tomographic inversion processing for velocity information
- Impedance and attenuation processing
- Laboratory measurements (consolidation tests, geotechnical parameters, ...)
- Seismic data interpretation and comparison with acoustic parameters
- Calculation of synthetic seismograms of the final 3D model
- Study of VHR seismic industry technology

3.5 UWB

The School of Ocean Sciences at UWB is a multidisciplinary department with wide-ranging expertise and facilities. Over the past two to three decades, the Geophysics Group within the School has established an international reputation in geophysical sensing of sea floor sediment properties. Advances in recent years have been in seismo-acoustic modelling of sediment behaviour, developing methodologies for in situ assessment of sediment properties and seismo-mechanical laboratory testing. In recognition of their research activities, the Marine Geophysics Group's commercial unit AUGER Geophysical Services has now been designated a Centre of Expertise by the Welsh Development Agency.

The Group's research activities are currently funded through two major defence related programmes: the US Navy's Coastal Benthic Boundary Layer Special Research Program, and the UK Defence Research Agency's Mine Countermeasures programme. While the former has provided the opportunity to collaborate in a series of multi-national, multi-disciplinary research experiments studying sea floor sediment properties and process interactions, the latter has provided an opportunity interact with a potential major end user of the research developments.

Specific contributions to the project include :

- Study of potential site locations
- Site surveys, including possible retrieval of gravity cores
- Seismic attribute analysis
- Laboratory analysis
- Creation of physical property sections
- Production of a 3D physical / geotechnical model
- Model validation via reference to groundtruth information
- Development of a marketing plan and study of future developments

3.6. HAL

HAL is a wholly UK owned independent company that provides a wide range of GeoScience Consultancy Services.

The company currently has an international client base of over 70 oil companies and government agencies with 15 full-time staff and over 80 associates. HAL is therefore very well-placed to act as a link between project partners and the petroleum exploration industry. The breadth of services offered and the large client-base mean that HAL will be able to ensure that the research is focused towards potentially commercial applications.

The market research and technical work will be undertaken by experts in the field used to working to tight deadlines in an efficient and cost effective manner. The company has a Positioning and Geodetic Survey Group which can provides consultancy in the area of positioning strategy. Through its contact with the industry, the company may obtain permission to use data from a well-site in southern North Sea.

Specific contributions to the project include :

- Selection of suitable survey sites
- Development of positioning strategy
- Seismic attributes analysis
- Acoustic impedance processing
- Development of a 3D physical / geotechnical model
- Review of the current VHR seismic industry technology

- Identification of potential market opportunities and marketing plan
- Future developments of the VHR 3D method

4. Application

The VHR 3D method presents a cost-effective method for obtaining detailed and precise, quantitative evaluations of the subsurface at the earliest stage of investigation. It allows engineers to plan targeted geotechnical sampling programmes in critical areas or model the behaviour of sea floor sediments to imposed conditions (dynamic loading, fluid flow, extraction of material, etc.).

This offers a wide range of technical, industrial and economic benefits to the offshore site investigation industry. Potential users of the method include the hydrocarbon industry (rig-site surveys, investigation of pipeline routes, etc.), coastal engineers (dam-site surveys, etc.), dredging and mineral extraction companies, and river and harbour authorities.

Owing to the flexibility of the acquisition strategy, the VHR 3D method can also be widely used for larger-scale geological and sedimentological site investigation purposes. Possible research topics include sediment and slope instability studies, the internal structure of sand banks and submarine dunes, shallow gas accumulation zones, the dynamics of deltas, estuaries and "swash" zones, and the evolution of sedimentary basins.

The VHR 3D method allows detailed and quantitative study of the sea floor sediment contamination (sludge dumps, waste accumulations). This is due to the fact that the physical properties of contaminated (e.g. by oil or gas) sediments differ significantly from those of natural sediments. This offers a vast area of applications in nearshore areas, such as harbours, bays, and estuaries, which are often subject to industrial activity and therefore extremely vulnerable to environmental contamination.

The VHR 3D method can be applied in detailed site survey investigations for borehole locations, carried out in the framework of major (European or other) drilling projects.

The possible extension of the VHR 3D method to deep-tow, near-target studies forms a serious scientific challenge. To this end, integration into and cooperation with on-going long-term deep seabed investigation programmes, both national and international, is of the highest importance.

The choice of a commercial partner, with a wide number of contacts in the VHR seismic industry, offers a good link between the academic and industrial world. This allows a better identification of future markets for VHR 3D seismic technology, which will result in optimum industrial exploitation from the project.

Finally, the development of cost-effective VHR 3D seismic methods is a serious challenge and requires long-term actions. Competition is at international level, especially involving North America. It therefore seems relevant to conduct this project at a European level.

5 Conclusion

The VHR 3D seismic method will allow for the better constraint of geotechnical models and thus the improvement of geotechnical, environmental and geological knowledge. In order to assure optimum application of the developed method and to increase its competitiveness, a specific task has been designated to investigate the market potential and possible exploitation of results. It mainly focuses on the following points :

- the development of an outline of specifications for the VHR 3D method,
- the study of current and potential future markets for the VHR3D method,
- potential future developments of the VHR 3D method, such as near-seabed (deep-tow) studies in deeper water environments.

The collaborative research proposed within this project will provide an opportunity to realising the full commercial potential of the research developments. IFREMER, RCMG, UWB and OGS have each pioneered the use of new technologies for marine soil investigation (VHR seismic reflection or refraction, S-wave studies, etc.). ARMINES has gained a good reputation in the oil industry by developing new, well-adapted processing algorithms taking into account the always increasing need of this industry. HAL provides a wide range of Geoscience Consultancy Services, with very close link to the oil industry.

All partners will collaborate for the dissemination of this new technology by the means of industrial or scientifical applications, as well as scientific publications.

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