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THIRD EUROPEAN MARINE SCIENCE AND TECHNOLOGY CONFERENCE

Lisbon, 23-27 May 1998

PROJECT SYNOPSES
Volume IV: Advanced Systems

EUROPEAN COMMISSION

Edith CRESSON, Member of the Commission
responsible for research, innovation, education, training and youth.

DG XII/D-03 - Marine Science and Technology

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European Commission

THIRD EUROPEAN MARINE SCIENCE AND TECHNOLOGY CONFERENCE

Lisbon, 23-27 May 1998

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PROJECT SYNOPSES

Volume IV: Advanced Systems

EUR 18220 EN

Published by the EUROPEAN COMMISSION
Directorate-General XII – Science, Research and Development

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Cataloguing data can be found at the end of this publication.

Luxembourg: Office for Official Publications of the European Communities, 1998

ISBN 92-828-2896-4 (Volumes I to VI)
ISBN 92-828-3037-3 (Volume IV)

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Printed in Italy

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.

[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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TECHNOLOGICAL SOLUTIONS IN THE "GEOSTAR" BENTHIC OBSERVATORY

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SUMMARY

GEOSTAR is a scientific and technological project (MAST-III) aimed at the design, manufacturing and test of an autonomous benthic observatory able to perform continuous long-term (up to 1 year), geophysical, geochemical and oceanographic measurements at seafloors (down to 4,000 m). The first demonstration mission of GEOSTAR is planned in Adriatic Sea during Summer 1998.

The present paper describes the main objectives of the project together with the complete system as results of the design and manufacturing phase.

1. INTRODUCTION

The purpose of GEOSTAR project is the realisation of a benthic observatory able to perform long-term, continuous and integrated geophysical, geochemical and environmental measurements at the seafloors. GEOSTAR approach is in accordance with the scientific and technological recommendations expressed by Thiel et al. (1994), by the European Committee for Ocean and Polar Sciences Workshop (ECOPS, 1994) and the International Workshop on "Multidisciplinary observatories on the deep sea floor" (Montagner and Lancelot, 1995). Moreover the realisation of the prototype follows feasibility studies for Abyssal Benthic Laboratories (Berta et al., 1995) and Long-term Ocean Monitoring System (MAST2-CT940082) promoted by EU in the early 90s.

In order to overcome limitations of the methods presently used for deep sea research (such as free-fall benthic landers, instrument packages deployed by manned submersibles or deep sea ROVs), GEOSTAR concept has been based on three key elements:

- surface assisted deployment and recovery by means of a tool capable to handle heavy packages;
- communication with land based centre during the mission;

_ extension of the capabilities of the bottom station, thus enabling multidisciplinarity and long-term autonomy of in-situ operation.

GEOSTAR observatory prototype hosts sensors for geophysical, geochemical and oceanographic researches in deep sea. It is conceived to be a node of existing and future monitoring networks, making possible their extension offshore.

Although the project will end with a short term demonstration mission in shallow water, the prototype has been designed with reference to a longer scientific mission in deep water (4000 m).

The prototype consists of a deployment tool, i.e. the Mobile Docker, and of a Bottom Station including communication systems, sensor packages, and acquisition and control systems.

2. RESULTS

BOTTOM STATION

The Bottom Station (BS) is a stand-alone autonomous unit conceived as an open system, able to host other sensors with minor modifications, and designed in a modular way following the current standardisation in the design of the interfaces. Specific requirements of the various scientific packages such as mounting constraints and possible interference have been fulfilled in the design phase of the BS. Particular attention has been devoted to the minimisation power consumption. Besides the inclusion of low-power devices, suitable strategies and solutions both hardware and software were adopted such as the monitoring of battery current supplied, the capability to put in stand-by all those devices that are not continuously working, the capability to switch off any malfunctioning device. A general sketch of the BS is give in figure 1.

The function of the BS frame is to support and protect the scientific payload and auxiliary systems. Due to the long exposure to aggressive environment and to foreseeable critical conditions of stresses and solicitations, structural elements are made of Aluminium alloys of the 5000 series with high resistance to corrosion and proper mechanical properties. This material fits the specific requirement of some scientific packages (non-magnetic materials to avoid interference on the magnetometers measurements) and contributes to lower the weight of BS.

The resulting technical characteristics of the BS are:

- dimensions: base 3500 x 3500 mm overall, height 2900 mm;
- weight in air: approx. 2700 kg (frame + scientific payload).

pressure vessels have been designed and manufactured in Titanium alloy to include the electronic control system and the battery pack. The pressure rating for the vessels is 4000 meters, while the internal pressure will be maintained to 1 bar to allow the normal functioning of electronic hardware and batteries not pressure compensated.
An energy requirement of max. 25 kWh has been calculated for the 3 month test mission and a rechargeable Silver-Zinc pack and a primary Lithium pack have been selected.

Specific devices

As the seismometer and magnetometers installing requirements do not allow a direct mounting on the BS frame, actuation systems have been designed and manufactured specifically for these two sensors based on an acoustic release commanded from the surface.
One of the most stringent requirements of the scientific payload is relative to the sensitivity of the magnetometers: any source of magnetic fields, like electric currents or any source of deformation for the magnetic flux, must be kept as far as possible from the instruments. On the other hand, all the instrumentation must be protected against any possible impact, therefore must be kept inside the BS frame shape. The solution adopted is to support the two

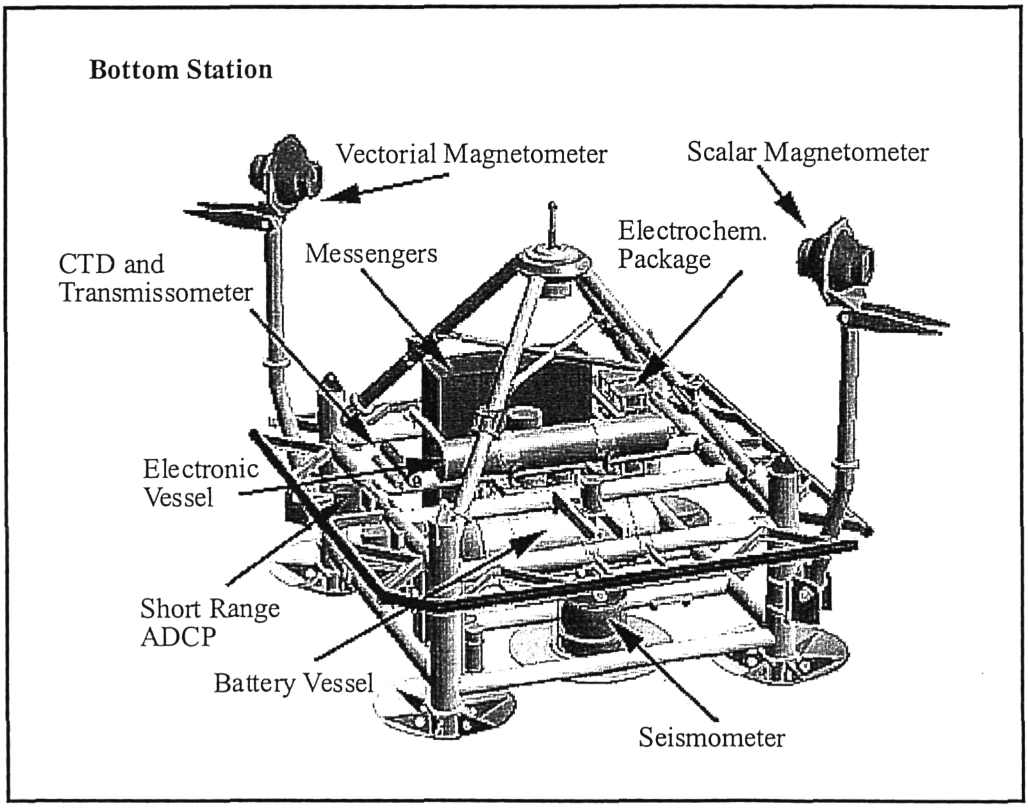

Figure 1 - Bottom Station

magnetometers with swinging arms to be operated after touchdown. The resulting distance between the instruments and any noise source located inside the BS frame (> 2.6 meters) and the distance between the two magnetometers (> 5 meters) are sufficient to minimise disturbances.

Another active device will be used to drop the seismometer, in order to decouple it from the station and to put it in direct contact with the seafloor.

Status sensors

The BS is equipped with a set of sensors devoted to the monitoring of the status and integrity of the Station most critical parts during the phases of the Mission (deployment, autonomous operation, recovery, etc.).

The Status Sensors adopted for the BS are listed in table 1.

Sensor	*Range*	*Notes*
X-Y Tilt meter	+/- 10 _	Mounted inside electronic vessel
Temperature sensor	-40 to +110 _C	Managed by a dedicated electronic board. One mounted inside electronic vessel, one inside battery vessel
Leak detection	on-off	Managed by a dedicated electronic board. One mounted inside electronic vessel, one inside battery vessel
Battery voltage	0 to 32 V	Managed by a dedicated electronic board mounted inside battery vessel. Monitors battery status.
Battery current	0 to 8 A	Managed by a dedicated electronic board mounted inside battery vessel. Monitors current consumption
Echo Sounder	0.3 - 30 m	Mounted on the BS frame, downward looking. Used to guide final approach to seabed and to determine touchdown.

Table 1 - Status sensors of the Bottom Station

Data Acquisition and Control System Units

The Data Acquisition and Control System is based on 2 low power units. The first unit is devoted to the Mission & Power Control, while the second one is devoted to the Data Acquisition from the scientific packages. A 9600 baud serial link connects the two units. For each unit a low pówer state is foreseen during periods in which there are no activities in progress (control, data acquisition or communication).

Data Storage Units

Data logging system is based on 2 low power units. Each of these units manages the operation of an hard disk; for the purposes of the first test mission (1.5 Gbytes max. data storage requirements), two hard disks of 1 Gbyte each have been selected. These units are interfaced to the Mission & Power Control Unit, by means of a serial link having a baud rate of 57.600. Each Data Storage Unit is normally in low power mode, and is waken up by the Control Unit when a Data Record transfer is to be initiated.

Data Storage Units are able to answer on request to general checks commands sent by the Control Unit, such as check of the amount of available mass memory and check of internal status.

MOBILE DOCKER

The Mobile Docker (MD) is basically a special, simplified version of a Remote Operated Vehicle ROV, capable to deploy at sea floor heavy payloads and subsequently recover them as a shipborn procedure. A general sketch of MD is shown in Fig. 2. By means of cameras, the MD is capable to locate the BS in the pre-determined installation area or find it for retrieval. The MD is equipped with two thrusters ensuring mobility on the horizontal plane while the winch of the used vessel regulates the descent/ascent. The thrusters with up to 700 N maximum thrust each, can be used independently in both senses of revolution and magnitude of thrust. The thrusters are controlled through a man operated joystick placed at the shipborn MD operator console. Power is directly supplied via cable allowing continuous testing during the mission. The material for the outer frame of the structure includes Al Mg Si 1 - 80x80x5 square tubes. The total height of the MD is about 2700 mm. The housing system consists of three basic components: Docking Cone (MD), Latch Device (MD) and the pin (BS).

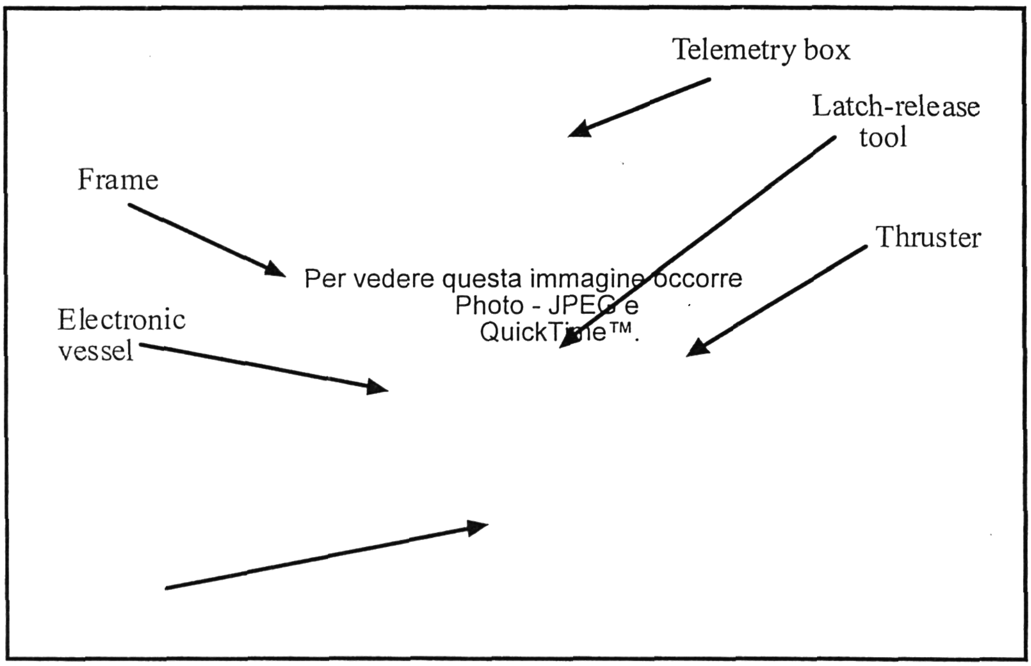

Figure 2 - Mobile Docker

The funnel shaped Docking Cone has the function to make easier the mechanical connection to the BS.

The Latch Device (stainless steel) is positioned on the top of the MD. It consists of a spring-leaver mechanism including sensors for position checks and the electrical linear actuator. The linear actuator is operated from the vessel allowing to repeat the redocking procedure as often as needed. Figure 3 shows the BS-MD system during docking.

COMMUNICATION SYSTEM

The Communication System is based on the integration of two different communication devices, each devoted to different tasks.

The first one consists of a set of releasable capsules, called Messengers, capable to transfer data by satellite telemetry once arrived at sea surface. Two types of Messengers are used:

- Expendable Messengers, released periodically (depending on the mission duration) or under particular conditions (i.e., in case of failure detection in the BS); they can store up to 32 Kbytes of data and transfer them through satellite telemetry;
- Storage Messengers, released on external request (e.g., by operators on a ship) and storing 1 Mbyte of data.

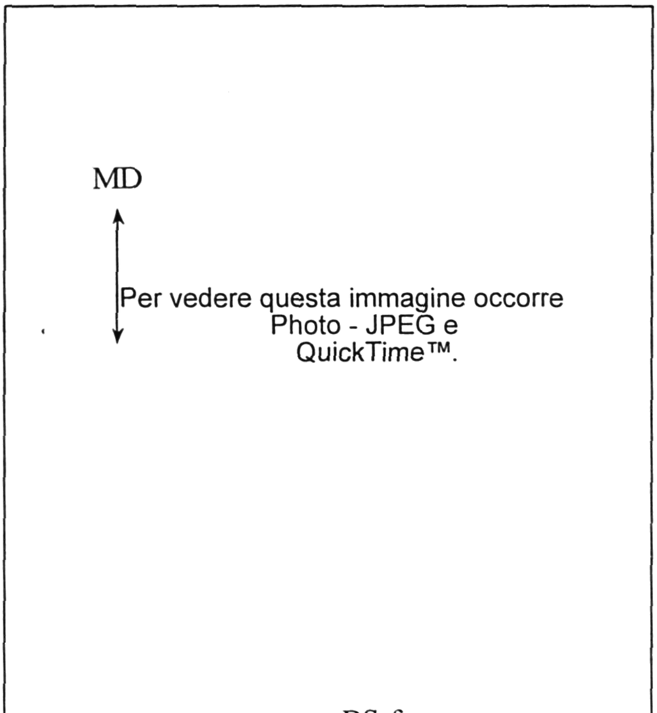

Figure 3 - Layout of the BS docking by MD

The second mean of communication is an Acoustic Telemetry System used as a standard serial link between the BS and the ship when the station is no longer connected to the Active Docker.

A standard interface for data transmission via cable is also available to enable real-time connection of the BS to external units or directly to shore stations.

SCIENTIFIC PAYLOAD

The configuration and specification of the scientific payload were based on scientific requirements for geophysical, geochemical and oceanographic measurements in the sea floor. Some of the sensors selected are standard packages available on the market, eventually modified, others are originally developed within the GEOSTAR project.

The scientific payload consists of a three-component broad-band seismometer, scalar Overhauser and two-axis fluxgate magnetometers, a Conductivity, Temperature and Depth sensor (CTD), a transmissometer, an electrochemical sensor package for pH, Eh, H_2 and H_2S measurements, a short range Acoustic Doppler Current Profiler (ADCP).

The three-axial seismometer is a Guralp broad-band CMG3-T sensor, modified and integrated in a self-contained unit (17" glass sphere) to prevent water intrusion and to guarantee stable pressure conditions. Capabilities include: automatic fine levelling, up to +/- 30_; high accuracy clock with an absolute error $< 2 \times 10^{-3}$ s per sample; frequency range 0.0027 to 50 Hz. The absolute position and the orientation of the seismometer will be known with an accuracy ≤ 100 m and $\leq |1°|$ respectively. The high-accuracy clock of the seismometer provides time reference for the whole station, allowing precise time correlation among all the scientific measurements.

The scalar Overhauser magnetometer is a modified version of the model GSM-19L by GEM Systems, to work properly in the extreme conditions of GEOSTAR experiment; it is based on the Overhauser effect and characterised by a very small power consumption. Accuracy is about 1 nT for a magnetic field of 45000-50000 nT.

The X-Y magnetometer is developed by the Istituto Nazionale di Geofisica (ING). The sensor is vertically suspended to support maximum tilt of $\pm 15^{0}$. Accuracy in H $(=(X^2+Y^2)^{1/2})$ is around 2 nT. The further presence of motion sensors allows to measures tilt and heading of the sensor together with magnetic field. Minimum data acquisition is 1 data series/min where 1 data series stands for total field and magnetic components X, Y properly averaging higher frequency (5-10 Hz) acquired data. Each magnetometer is placed in a 17'' benthosphere and is kept at around 50 cm over the seafloor.

The geochemical monitoring system includes sensor packages for continuous and long-term monitoring of the main geochemical parameters of sea water, such as temperature, salinity, pressure, turbidity, pH, Eh, H_2, H_2S. Conductivity (salinity) and temperature measurements are performed by a high accuracy and stability CTD (SBE-16 SEACAT, Sea-Bird Electronics, Inc.). Sea water turbidity is monitored by a 0.25-m-pathlength transmissometer of type Alphatracka Mk II (Chelsea Instruments Ltd.). Measurements of pH, Eh, H_2, H_2S are made by an innovative electrode package, developed by the University of Newcastle upon Tyne inside the project. Measurement of pH are made by a special ion-selective electrode which is suitable for use at high pressure. The sensors for the detection and measurement of gaseous species are facilitated by special amperometric sensors. The amperometric sensors include self-cleaning and self-calibration facilities.
The oceanographic sensor is a short-range ADCP (Acoustic Doppler Current Profiler), a 300 kHz RDI Workhorse Monitor modified for operating down to 6000 m. The ADCP is mounted on the BS frame at about 1.5 m over the seafloor, without obstacles above, which would cause interference to the acoustic beams.

DATA MANAGEMENT

Data management strategy can be subdivided into five basic steps:

- local data management
- data transfer
- data management at shore
- data processing
- data dissemination

Local Data Management includes all operations that are carried out automatically by the BS electronics, according to a mission strategy programmed by the operator before deployment. Operations carried out are: acquisition of data from the scientific packages, data pre-processing to obtain reduced data (such as average, minimum and maximum values) and a first level of validation (to detect significant events, such as major failures to the sensors that could require modification to the mission strategy), periodic generation of the data structures, storage of data structures.
Periodic data transfer is made possible by the release of Messengers, thus allowing to check the status of the station and get a partial but significant idea of the acquired data. This transfer is uni-directional and may occur periodically (at pre-programmed times) or on detection of major events or failures. Messengers with limited capacity are used for the transmission of Summary Messages, data structures containing a set of reduced but significant data such as statistics for each measurement, occurrence of anomalous situations, status of the station

devices (batteries, electronics, attitude sensors, internal temperature etc.); up to one month of summary data (30 Summary Messages) can be stored in a single capsule.

Data capsule with a larger capacity are used as a limited back-up of data records stored inside the station. A data transfer through Acoustic Link ensures direct bi-directional interface between the operator and the station during mission, is possible. Due to the limited data rate obtainable and the limited energy available in the station, this link is used only to test the station status, request the transmission of Summary Messages and shut down the sensors (before recovery).

All data collected are stored in the BS mass memory and will be completely available after the recovery.

After station recovery, a second level of quality checks is performed, mainly aimed at the verification of the number and quality of data records, presence of gaps, alarms etc.

Finally, data dissemination is performed through a data bank in which data are grouped for scientific field with international exchange format. The access to the databank can be either via Internet or FTP address.

3. CONCLUSION

A summarising block diagram of all the GEOSTAR sub-systems is shown in Fig. 4. GEOSTAR represents the "founder" of a new generation of deep-sea observatories based on the permanent link with a land based centre, the assisted deployment/recovery from the surface and the multidisciplinarity of the collected data.

Both the prototype and the individual scientific modules as well as the technological solutions applied, can be exploited in applications different from the original ones (e.g., pollution studies, tsunamis forecasting, etc.).

Moreover GEOSTAR, being a modular research tool, foresees the possibility of including both additional sensors packages and other satellite apparatuses, and to operate as a node of future offshore monitoring networks. Therefore it responds also to the expectancies of a wider scientific community, including biologists and environmental engineers. The utilisation of the prototype in multidisciplinary experiments brings to share facilities and lower costs, and helps for further progresses in the deep sea research.

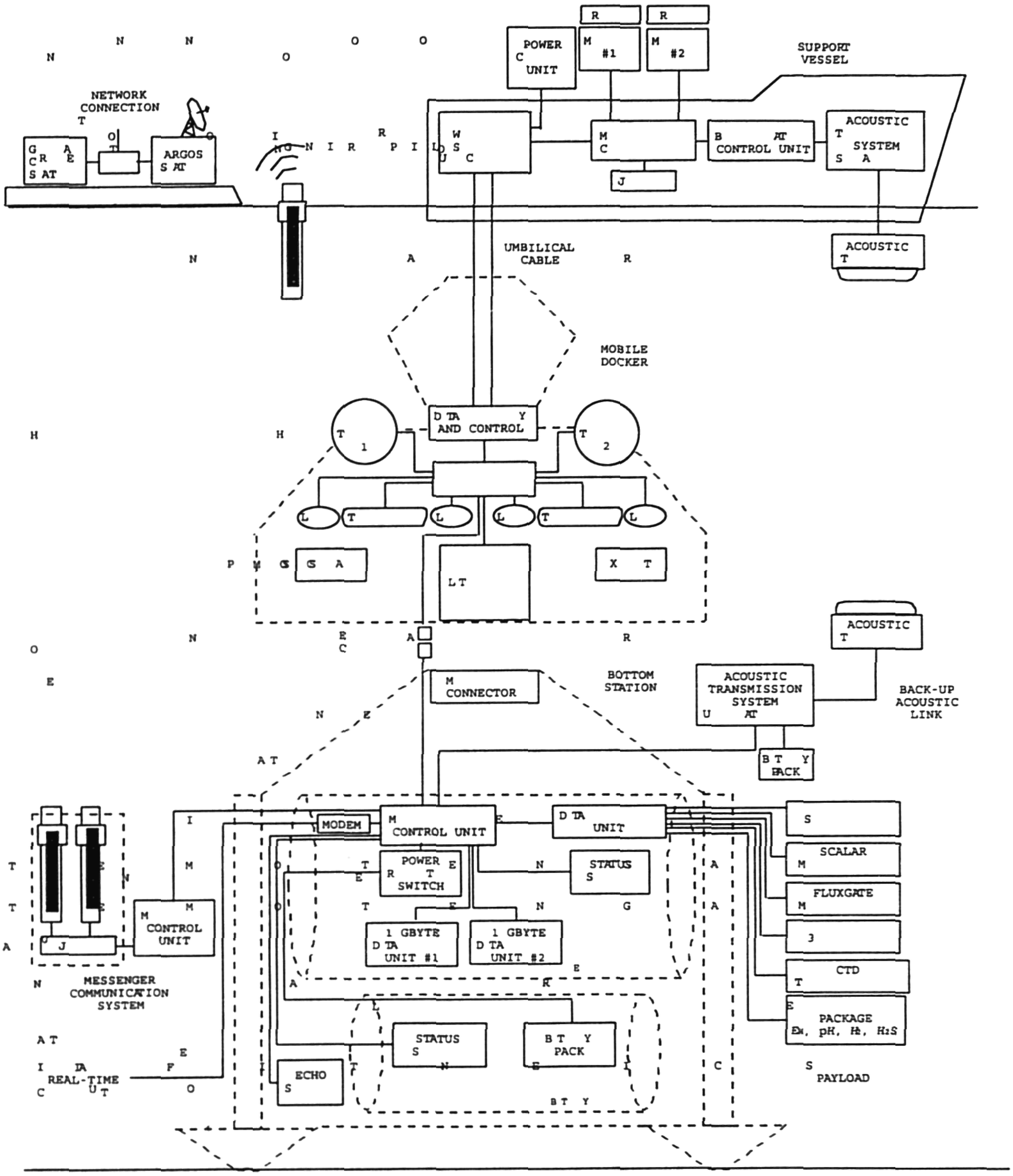

Fig. 4 - Block diagram of the GEOSTAR sub-systems

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TITLE: Autonomous Lander Instrument Platforms For Oceanographic Research.
(ALIPOR)

CONTRACT NO: MAS3-CT95-0010

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ALIPOR- AUTONOMOUS LANDER INSTRUMENT PLATFORMS FOR OCEANOGRAPHIC RESEARCH.

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1. SUMMARY.

ALIPOR is concerned with design, development and operation of lander instrument platforms capable of being deployed on the sea floor for periods of 8h to 1year taking measurements, sampling and conducting experiments autonomously at the deep-sea water-sediment interface. The aim is to develop a European fleet of lander vehicles capable of operating as a co-ordinated fleet in joint research programmes. Sea trials have shown that simultaneous operation of 12 landers from a single ship is possible achieving scientific sub-sea time equivalent to more than 5 times the ship time available. Landers are shown to be cost-effective means of conducting research in the deep sea.

2. INTRODUCTION.

The oceanic and atmospheric sciences have been revolutionised in recent decades by the use of satellites in earth orbit as instrument platforms that can continuously scan large areas of the earth's surface (Priede 1983). Synoptic imagery is possible of dynamic features such as gyres and frontal regions and unbiased sampling is possible for years at a time independent of any requirement for surface ships or personnel in the field. The maximum depth of penetration of solar radiation in the sea however is no more than 1000m and optical remote sensing rarely provides data extending more than 1m beneath the surface. For deep sea research there is therefore no panacea enabling a single instrument to sample on large temporal and spatial scales. A surface ship is always necessary and low frequency side scan sonars have a maximum range of about 10km on either side of the track of the vessel. Sonars providing high resolution imagery of sea floor require a transducer towed near the bottom and sample a swath width of no more than a few 100m. Optical imagery of the sea floor rarely extends over an area of more than a few square metres per image. ROVs and manned submersibles have penetrated to the maximum depths of the oceans but require a mother ship on station and duration of a mission is rarely more than 12h.

Multiple autonomous platforms placed on the sea floor that can function for extended periods of time without intervention from a surface vessel are recognised as the chief means of making progress in research in the deep sea sampling on temporal and spatial scales dictated by the dimensions of the environment.

The term "Lander" has come to be used for such platforms that descend to the sea floor, carry out a series of tasks autonomously for periods of days to years storing data on board, and then ascend to the surface at the end of the mission for recovery by a surface ship. A typical lander comprises a chassis, buoyancy module, recovery module and ballast with a release mechanism. One or more scientific payloads can be attached, depending on the size of the lander, for monitoring processes in the surface layers of the sediment and the bottom water. The lander is usually ballasted to be negatively buoyant so that it descends to the sea floor at a rate of 0.5 to 1.0 $m.s^{-1}$ which gives a relatively soft landing so as to not disturb the sediment. At the end of the mission, steel ballast weights, are shed either by time release or acoustic command from the surface. The lander then ascends by virtue of positive buoyancy to retrieved by the surface ship. A recovery module is usually equipped with radio beacons, strobe lights and visual markers to aid location. Landers have evolved from conventional oceanographic instrument moorings such as current meter or sediment trap arrays but are characterised by an instrument package standing or suspended close to the sea floor.

3. THE ALIPOR PROGRAMME.

It is recognised that in order to carry out large scale research in the deep sea, the resources of single institutes and countries are likely to be insufficient. A number of groups throughout Europe have developed deep-sea lander technologies and it is important that these systems should be able to work together in joint programmes and also avoid mutual interference. The aim of the ALIPOR programme is to create a compatible European fleet of lander vehicles and to gain direct experience of operation of fleets of landers from one or more ships.

A complementary development is a compact lander designed for use from small vessels with insufficient winch capacity to sample full ocean depth. Numerous marine laboratories in Europe are based on islands or coastlines near deep-water but have no means otherwise of sampling the deep sea floor. The compact lander development will serve to broaden the foundations of deep-sea research in Europe.

ALIPOR has 9 partners from 6 European countries:

The University of Aberdeen (Partner 1) is concerned with systems for low frequency (8-12 kHz) acoustic telemetry between the surface and lander, and development of high frequency (77 kHz) sonar systems for following movements of fish across the sea floor (Bagley & Priede 1997, Bradley *et al.* 1997).

Partner 2 , the NIOZ Laboratory at Texel in the Netherlands operates a fleet of lander vehicles and is contributing to two aspects of the ALIPOR programme: Firstly the design of pH and conductivity probes that are inserted into the sediment by the lander to study geochemical processes and secondly a large lander system to monitor the water layers between 0 and 3 metres above the sediment.

Partner 3 the Institute of Marine Biology , Crete (IMBC) in Greece is developing a compact lander suitable for use from small ships.

Partner 4. the GEOMAR Institute at Kiel in Germany is also a major lander operator. Under the ALIPOR programme they are undertaking two particular developments.
A gas and water flux chamber lander has been designed to be placed over sea- bed vent sites. In contrast to typical free-fall landers this instrument is placed on the sea floor using a video controlled system on the end of an umbilical. The other major innovation is a nepheloid

layer chamber system designed to concentrate, by pumping, living particulate matter from the benthic boundary layer water and then to measure its biological activity.

Partner 5, the Martin Ryan Marine Research Institute in Galway Ireland is not concerned with lander technology *per se* but is developing a sediment profile imaging (SPI) system based on a digital camera and optical wedge driven into the sediment. This provides basic information for planning and interpretation of lander missions

Partner 6 the IFREMER Centre de Brest has considerable experience of Lander operations and contributes two major lander vehicles to the programme. The RAP II a respirometer chamber lander and the MAP, Module Autonome Pluridisciplinaire designed to measure physical processes in the benthic boundary water layers. IFREMER within the ALIPOR programme is designing an *in situ* chemical analyser for nitrate at ammonia, a moveable chamber design and developing data management and logging protocols on landers with multiple instruments.

Partner 7 is the Scottish Association for Marine Science Dunstaffnage Laboratory, Oban Scotland who are preparing a scientific payload concerned with measuring the effect of high water flow on biological activity in deep-sea sediments, the flow respirometer chamber FRC.

Partner 8, the Department of Analytical and Marine Chemistry at the Universities of Göteborg, Sweden operates a modular lander system and is concerned with two aspects of ALIPOR, benthic chambers and sediment probes.

Partner 9 the Alfred Wegener Institute, in Bremerhaven, Germany has addressed the problem of operation of oxygen sensors and their long-term stability under high pressure.

ALIPOR is a three year programme structured in the following way:

Year 1 (1996) Sea trials with existing technology
Year 2 (1997) Development of new technology.
Year 3. (1998) Sea Trials with New technology.

4. MULTI-LANDER OPERATIONS:

Scheduled within ALIPOR are two major cruises on board the *RRS Discovery* during which as many landers as possible are being operated simultaneously in the NE Atlantic Ocean.

The first ALIPOR cruise was undertaken during 27 July to 26 August 1996. The multi-lander cruises are the responsibility of the University of Aberdeen and are designed to directly test the compatibility and operational characteristics of the European fleet of deep-sea lander vehicles. 28 scientists and engineers participated in this cruise using the lander vehicles and equipment listed in Table 1.

The vessel sailed from Southampton and between 29 July and 23 August worked along a transect in the NE Atlantic between 49°26'N, 13°33'W (700m depth) in the east and 47°36'N, 19°51'W (4600m) in the west, a distance of approximately 300 nautical miles.

TABLE 1. LANDERS AND OTHER GEAR CARRIED ON THE 1ST ALIPOR MULTI-LANDER CRUISE. 27 JULY TO 26 AUGUST 1996.

Acronym	Name	Institute	Description
MAP	Module Autonome Pluridisciplinaire	IFREMER	Benthic Boundary Layer Flow.
LAFF	Large Abyssal Food Fall	Aberdeen	Dolphin carcass consumption
FFR 3	Free-Fall Respirometer 3	GEOMAR	Benthic chamber, Sediment Oxygen consumption
RAP II	Respirometre	IFREMER	Benthic chamber Sediment Oxygen consumption
Göteborg	Göteborg chamber and profiler lander	Göteborg	Benthic chamber Sediment Oxygen consumption, solute fluxes
AUDOS	Aberdeen University Deep Ocean Submersible II	Aberdeen	Photography and tracking of deep sea fish.
ATTIS 1	Acoustic telemetry and Transponder Interrogation System	Aberdeen	Acoustic Tracking of fish marked by ingestible transponders
ATTIS 2	Acoustic telemetry and Transponder Interrogation System	Aberdeen	Acoustic Tracking of fish marked by ingestible transponders
ATTIS 3	Acoustic telemetry and Transponder Interrogation System	Aberdeen	Acoustic Tracking of fish marked by ingestible transponders
ATTIS 4	Acoustic telemetry and Transponder Interrogation System	Aberdeen	Acoustic Tracking of fish marked by ingestible transponders
Fish Trap	Baited cage	Aberdeen	Capture of deep sea fishes.
SPI	Sediment Profile Imagery	Galway	Digital camera and sediment penetrating wedge. (Wire gear)

During 25 days in the working area 40 lander deployment and recovery operations were carried out which is equivalent to an average of one lander operation every 7.5h.The total amount of sub-sea time achieved during the course of the cruise was 3519h or more than 5 times the ship time expended. The usefulness of landers as a means of extending the capability of surface ships was clearly demonstrated.

Considerable experience was gained in handling of landers. A major problem is time taken to locate and recover a lander once it has surfaced. The average time required once the lander surfaced, to locate it, manoeuvre the ship alongside and successfully grapple it was 22 minutes. The time to bring the lander inboard and secured it so that the ship could get underway was 14 minutes. Thus the average total average recovery time was 36 minutes once the lander surfaced. The time taken for ascent of the vehicle from the sea floor, *ca.* 50-

90 minutes at abyssal depths must be added to these times. The *RRS Discovery* is a conventional ship with a bow thruster but no dynamic positioning capability.

It is clear from the experience of *RRS Discovery* cruise 222A that multi-lander operations are feasible. Furthermore during the cruise on 31 July 1996 a rendezvous was established with the Netherlands ship *Pelagia* and joint operations were undertaken deploying landers from both ships to form an array of 4 landers with 1 nautical mile spacing to obtain comparative measurements of sediment community oxygen consumption.

A second multi-lander cruise is scheduled for 24 August- 22 September 1998 cruise 236 of the *RRS Discovery* sailing from Lisbon and arriving in Southampton. Prior to this cruise the ship will be based at the Lisbon EXPO98 with a display of ALIPOR and other marine research. A larger fleet of second generation landers will be carried on this cruise. An important aspect of the multi-lander cruises is that engineers and technicians from different European countries are able to work together, compare techniques thus ensuring a rapid development and dissemination of knowledge in a working environment.

5. LANDER MISSION DURATION.

If experiments on the sea floor have a duration of months or years it is immediately obvious that an autonomous platform should be placed on the sea floor. However experiments of a few hours duration might be carried out either by suspending an instrument on a vertical (e.g. CTD) cable or by autonomous lander.

Assuming that more than one lander is available or the ship has other useful work to do a simple mathematical model indicates that for any experiment with a duration exceeding 8h on the sea floor, landers are to be preferred over conventional wire work from the point of view of efficient utilisation of ship time (Priede 1988). Efficiency of lander experiments has been greatly enhanced in recent years by the availability of GPS enabling ships to accurately return to lander deployment positions with a minimum of time required for searching acoustically or by VHF radio direction finding.

Maximum lander mission durations are 1 to 2 years limited by, power supply, memory capacity for storage of data, stability of sensor calibration and corrosion resistance of components.

6. LANDER SIZE.

For operation from most research ships maximum lander size is approximately 3m width length and height. But the smallest possible size is desirable. The weight of landers in use in Europe varies between 200kg and 2000kg including ballast. Experience from the first ALIPOR multi-lander cruise indicates a clear relationship between the weight of the lander and maximum sea state in which it is safe to deploy and recover the vehicle. The 2 tonne lander was restricted to sea state of Beaufort <3, whereas the landers weighing <500kg could still be handled in sea state 6 in the NE Atlantic.

It is evident that there are great benefits in minimising lander size and weight in order to ensure maximum flexibility in planning of operations at sea.

7. SEDIMENT INTERFACE CHAMBERS.

Several of the lander systems in the ALIPOR programme are concerned with the problem of measurement of oxygen and solute fluxes between the sediment and benthic boundary water. One approach to such measurements is to enclose a small volume of water in chamber that is clamped down against the sediment surface. Changes in the enclosed water are then measured over a suitable time period typically 12-72h depending on the flux rate being measured.

With a multiplicity of chamber designs there is considerable concern as to whether measurements from different laboratories are comparable. Benthic chambers vary in dimension from 15 to 80cm diameter and are designed to enclose part of the surface of the bottom sediment. Decrease in oxygen or change in various solutes in the enclosed water are then monitored to estimate the rate of biological or geochemical processes in the sediment, pore water and benthic boundary layer water (Tengberg *et al.* 1995). To resolve the problem of variation in chamber design as part of the ALIPOR programme the University of Göteborg implemented intercalibration exercises at Tjarnö on the coast of Sweden where standard sediment samples were prepared and direct comparisons were possible between different chamber designs. This exercise involved 14 different chamber designs from Europe and North America.

Four workstations were set up:

1. Flow visualisation and mixing time experiments.

Chambers were filled with water containing suspended aluminium particles which were illuminated by a strong lamp through a 10mm slit. This allowed visualisation of vortexes, stagnant zones and circulation patterns with the chamber stirring system operating at the normal speed used in the deep sea.

2. Measurement of differential pressures.

The stirring action of an impeller in the chamber may result in differential pressures drawing pore water through the sediment which may alter flux rates observed. The test chambers were set up over a series of pressure measuring ports which enabled pressure profiles to be measured across the radius of the chamber.

3. Diffusive Layer Boundary Layer thickness.

The boundary layer thickness was measured from weight loss of alabaster plates set in an artificial bottom within the benthic chamber.

4. Comparative Flux-incubations.

An artificial substrate was made up from silty sediment obtained off the Swedish west coast (34m depth) mixed with sand and bottom water in a commercial concrete mixer. This produced a homogenous sediment which was spread to a depth of 17cm in large tanks at 8°C. The differing designs of chamber where then set up and run simultaneously measuring oxygen and silicate fluxes.

Statistical analysis showed that there were significant differences in flux measurements obtained from different chambers. This emphasises the need for standardisation and/or intercalibration of chamber designs. Certain features were defined as resulting in optimal results. A "Mississippi Wheel" type of impeller with a horizontal axis of rotation produced lower differential pressures than the more usual vertical axis rotor designs. The Tjarnö workshops are resulting in new standards for sediment interface chamber design.

8. SCIENTIFIC PAYLOADS AND MODULARITY.

A lander can be divided into two components.

1. The basic vehicle or delivery system comprising the chassis, buoyancy, recovery system and ballast release mechanism.

2. The scientific payload.

The basic vehicle poses generic problems which are discussed by Priede (1998) but the structure is to a large extent determined by the nature of the scientific payload. Some landers need to be work close to the sediment to insert microprobes or chambers whereas other landers are designed to stand high above the sediment so as to avoid disturbance to the water flow across the sediment. This means that it is not possible to entirely standardise lander design although there has been some development of modular approaches enabling landers to the readily adapted for different functions. Within ALIPOR the basic problems of lander design and operation are being investigated.

It is essential that the ballast release and buoyancy system of the lander is built to the highest standards of reliability. Otherwise the entire system can be lost. The University of Aberdeen ATTIS lander is designed as an ultra-compact system with all the electronics in a single housing known as the Acoustic Command Unit under the control of a single microprocessor. The ACU has two-way acoustic 10kHz communication between the lander and the surface ship. This allows transmission of ballast release commands from the surface and simple experiment control commands. Information on mission status can be coded and transmitted to the surface. The ATTIS has a release mechanism with twin electric motor drives to provide reliability through parallel redundancy. The ACU also contains data inputs, data storage and experiment control interfaces. Thus all the functions of a lander are combined. This system has been tested successfully.

The ATTIS and other landers often had a conditional or timed ballast release so that the lander would automatically rise to the surface after a certain time had elapsed or if the hard disc space was full. Trials during the first multi-lander cruise proved such apparently useful features to be quite detrimental in the weather conditions prevailing in the NE Atlantic. There was a high probability of a lander surfacing during rough weather when no recovery was possible or when the mother ship was too far away to take effective action.

Whilst the ATTIS ultra-compact concept was successful in the original application, extreme care is required when modifying software for new experiments to ensure that basic ballast release command integrity is maintained.

These experiences have resulted in a modular approach to lander design. It is recommended that all landers are equipped with two acoustic releases set up in parallel. These should be different models or designs from different manufactured batches to avoid common failure modes. Each ballast release should have its own power supply.

The power supplies and control of the scientific payload should be entirely separate from the ballast releases. A single lander may carry several scientific experiments and it is recommended these be functionally entirely separate from one another so that failure of one experiment does not endanger the whole mission. Experiments can then be innovative which often implies unreliability. Student experiments can be carried as "guest" packages on a modularised lander without any interfacing problems. Such an architecture has disadvantages in that data have to be down-loaded from separate ports for each experiment. Within ALIPOR a new modular architecture is being investigated using CAN network protocols which link all the scientific payload modules using a pair of conductors (Blandin *et al.* 1997). Each experiment is functionally autonomous and has its own power supply. However through the network a master controller can interrogate all the experiments and transfer data to a single archive in a coherent format. Such a system appears to combine the advantages of both unified and distributed architectures to produce a flexible yet reliable lander.

9. CONCLUSIONS.

Currently a very rapid rate of development of lander technology is taking place. Within months of this report being published it will be superseded by new results from the 1998 ALIPOR multi-lander cruise. The ALIPOR programme is based on close collaboration between design engineers and end-users so all hardware is designed for immediate use in scientific missions.

The most appropriate lander designs seem to relatively modest-sized vehicles typically with a total weight of less than one tonne. Over-investment in single sophisticated systems appears to be rejected in favour of use of batches of less sophisticated instruments adapted for different functions. The modular approach promises the possibility of *ad hoc* configuration of special experiments to answer specific scientific questions.

10. ACKNOWLEDGEMENTS.

I thank the ship's company of the *RRS Discovery* for making the multi-lander sea trials possible and over 80 colleagues throughout Europe who are contributing the programme.

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TITLE : Advanced Manipulation for Deep Underwater Sampling (project **AMADEUS** - Phase II)

CONTRACT: MAS3-CT95-0024

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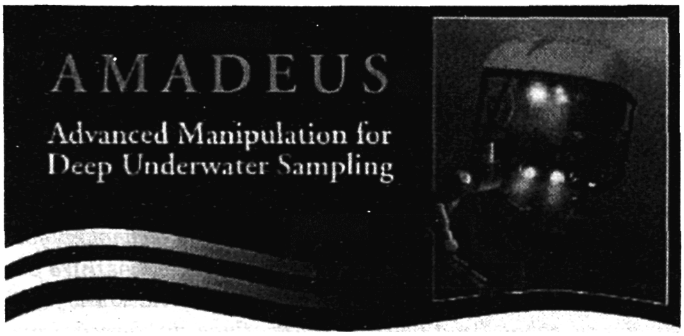

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Technological Developments for Improved Dextrous Grasping and Manipulation in the Ocean[1]

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Abstract

Progress in developing dextrous grasping and manipulation technology for use in the ocean is presented. As part of the EU MAST III programme, AMADEUS phase II, two testbed systems are being constructed for practical in water trials. The first involves a dextrous three finger robot hand, incorporating fingertip force/torque and slip sensors, mounted on a hydraulic robot arm as part of an ROV toolskid. The second involves a two arm testbed for grasping and manipulating large objects. For the latter, electric underwater arms have been specified and constructed. With the benefit of input from benthic science and marine geological partners, a set of realistic sampling and manipulation tasks have been defined to evaluate the testbeds' performance. The paper reviews the state of the project at the beginning of 1998, the half way point. This includes a description of the scientific tasks, the design and deployment of the robot hand testbed, the design of the two arms and the human computer interaction system, including an implementation of blind grasping for working in conditions of low/zero visibility.

1. INTRODUCTION

In marine geology and benthic science current practice for sampling rocks, sediment and fauna beyond diver depth is crude, often relying on grabs, corers and dredgers deployed from surface vessels. Such techniques are not selective, imprecise in sample location, disturb the surrounding environment during the sample and usually result in over or undersampling. The use of Unmanned Underwater Vehicles (UUVs) presents the possibility of a cost effective

[1] Presented at the 3rd European Marine Science & Technology Conference, Lisbon, Portugal, 23-27 May, 1998. European Commission, Brussels, Belgium, DGX11, MAST III.
[2] *http://www.cee.hw.ac.uk/oceans*

solution to this. However, the manipulative abilities of such vehicles are currently primitive, using manipulators with no dexterity or tactile feedback.
AMADEUS is a programme of work focused on improving the dexterity and sensory abilities of underwater manipulation systems, in support of marine geology and benthic science. Generic manipulation systems can be applicable across a range of applications, and can be more cost effective than development of numerous task specific devices.

Phase 1 of the programme was funded under EU DGXII MAST II, and developed a dextrous three fingered underwater gripper, incorporating force and slip sensors. It employed a control system to allow automatic grasping of objects up to 150mm diameter and 5Kg mass in conditions of zero visibility. At the end of phase 1, it operated as a prototype system in laboratory conditions, and demonstrated sampling operations on typical objects.

Phase II of the programme is enabled by the success of phase 1, and is progressing to grasping much larger and heavier objects, and to carrying out trials in the ocean with marine geologists and benthic scientists. Of particular interest in phase II is the co-ordinated use of a pair of UUV manipulators for grasping and manipulation, and the incorporation and use of additional sensory information such as optical and acoustic vision. Two testbeds are under development in Genoa and Edinburgh, suitable for wet trials. The first provides a platform for experimentation with a ruggedised gripper from phase 1, mounted on a single arm. The second provides a platform for deploying two collaborating arms with conventional grippers. Both testbeds are being suitably sensorized with tactile, video and acoustic sensors. Using the sensing and control techniques developed in phase 1, applied similarly to both testbeds, grasping and manipulation experiments are to be carried out on a wide variety of objects with benthic scientists and marine geologists at a trials site of interest to them. A scientific and engineering evaluation of the system and the technology will result.

Phase II forms a stepping stone to eventual use of such systems in conjunction with Unmanned Underwater Vehicle's (Figure 1) as a solution to practical manipulative needs.

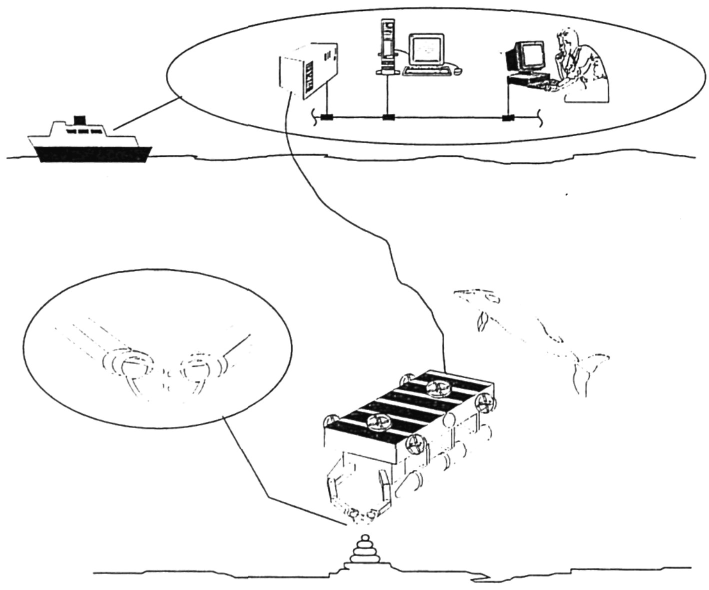


Fig 1: Dextrous Manipulation and Sampling

This paper reviews progress on the project up to the beginning of 1998. Section 2 briefly reviews the scientific requirements for the work, and section 3 presents details of the second prototype dextrous hand and associated testbed. Section 4 presents the dual arm testbed and section 5 some discussion on an automated operator interaction method termed 'blind grasping'. Section 6 presents the human-computer interface and finally section 7 concludes.

2. SCIENTIFIC REQUIREMENTS

Marine biologists and geologists need to undertake three kinds of underwater tasks: sampling, performing experiments and resolution of problems.

Traditional techniques such as dredging and coring from research vessels give rise to random sampling and have significant drawbacks; selective collection is not possible and samples are often destroyed by the mechanical action of the equipment, which has a high degree of impact on the environment (figure 2). In addition, there is no view of the sample surroundings or how the sampler is operating and there is no allowance for specific placement of samplers. Diver operations can overcome some of these problems but are strongly limited in depth and duration. In recent years remotely operated vehicles and manned submersibles have become increasingly accessible to the scientific community. These new technological advances have significantly improved the quality of scientific data, but they are still reliant on rough manipulative capabilities. This means that a wide range of substrates cannot be sampled, selective sampling of small objects is not possible, a significant amount of samples are destroyed, and sampling operations are very time consuming implying shorter diving transects and less efficient use of expensive ship time.

Figure 2: Samples are often destroyed by the mechanical action of the equipment

Performing experiments refers to taking measurements of biological or physio-chemical parameters of the sea-floor or the benthic environment. In most cases, to yield reliable and meaningful results, it is of paramount importance that experiments are performed in situ and with minimum disturbance of the seafloor. Present-day handling systems mounted on submersibles are not accurate enough for many tasks of this kind; most of the measuring devices cannot be grasped with reliability and precision and placement at specific locations, correct movement and / or recovery of sensors is difficult and not reliable. These often imply that the data collected do not reach the required standard of quality.

Several kinds of geophysical devices, landers and specialised benthic chambers are currently deployed on the sea-floor to perform long-term experiments. In situ access to these automatic mechanisms and the possibility of changing or fixing some components may allow an increase in the time span of the experiments. Performing longer experiments implies getting longer data series under homogeneous conditions (the experiment is performed exactly at the same location) which is very important in terms of statistically representative data. Sometimes, retrieval mechanisms may fail or benthic material may be stuck on the bottom by accident, and very expensive pieces of equipment, as well as valuable data, have to be abandoned. If highly dextrous underwater operations were possible most of these cases could be solved, which would significantly improve the quality and amount of data collected and the efficiency of scientific work at sea.

To obviate the above limitations, there is a clear need for the development of dextrous underwater handling techniques. Some scientific tasks need the use of a highly dextrous hands, while others would be better achieved by the use of two arms in co-ordination.

The role of the scientific partners within AMADEUS is to specify what are the environmental conditions to be found underwater and what tasks should the system be able to achieve, as well as to make a continuous assessment of the Dextrous Gripper, Dual Arms and the Human Computer Interface which are being developed.

A comprehensive list of 26 scientific tasks have been grouped into 13 different Task Categories. A first group correspond to handling unstructured objects (i.e. objects from which there is no previous knowledge about shape, size and position). These can be rigid and hard, rigid and fragile or soft and delicate. A second group correspond to handling structured objects (i.e. objects from which shape, size and position is known beforehand, such as tools or probes). Each of the 13 different Task Categories implies a different set of sensing requirements and a different set of actions to be performed. They can be broken down, through functional analysis, into a succession of sub-functions (or atomic actions) to reveal the details of what will have to be achieved by the future Dextrous Gripper and the Dual Arm systems. One of the main benefits of this approach is that it helps visualization of a particular task and reveals the functional relationship between the various pieces of equipment and other subtasks. The use of functional analysis also enables task methodology to be explored (e.g. the use of divers, two arm submersibles, ROVs, or task specific tooling could all be compared for a specific high level task by substituting different sets of functions).

At the outset, two sets of demonstrations to be performed at the end of the project were designed, to provide the engineering partners with specific benchmarks. They are simple tasks specifically designed to show the key characteristics of the AMADEUS system and will reveal if it reaches the minimum standards required by the marine scientists. They include tasks assimilated to real scientific work, and cover a wide range of abilities. There are four Dextrous Gripper demonstrations which are selective sampling (picking up specific objects among "uninteresting" ones), sampling rigid and hard objects (e.g. shells), sampling soft and delicate objects (e.g. sea cucumbers) and operation of a valve randomly oriented in space. The

Dextrous Gripper demonstrations are designed to highlight adaptability of the gripper to different sizes, shapes and textures, the gripper force and slip control, the ability of grasping in turbid conditions and the automation of movements. There are four Dual Arm demonstrations which are co-ordinated lift and recovery of a plate, break and recovery of a rigid and fragile glass cylinder, drive a probe into sediment along a single axis in space and connect and disconnect an underwater electric plug. The Dual Arm demonstrations are designed to show end effectors position control and precision, co-ordination between both arms, arms force control, and automation of movements. Both Dextrous Gripper and Dual Arm demonstrations sets are to be performed in water.

The scientific partners will also be responsible for the evaluation of the AMADEUS system at the end of the project. Evaluation will be made through the demonstrations specified above and will be both qualitative and quantitative. The qualitative evaluation will be based on factors such as energy consumption, weight, robustness, simplicity, miniaturization, simplicity of set-up and operation, ergonomic characteristics, as well as ease of learning and use of the Human Machine Interface. The quantitative evaluation will be based on a marking system which is designed to reflect some key factors such as speed, precision and reliability.

3. DEXTROUS GRIPPER TESTBED DESIGN AND DEPLOYMENT

This section describes key aspects of the dextrous gripper, and its associated testbed. The following section similarly considers the dual arm testbed.

A. Mechanical Design

The AMADEUS phase II gripper represents the latest in a series of developments in continuum robotics at Heriot-Watt University [1-6, 22-24]. The device generates tip motion by bending through smooth curves rather than at discrete joints. Operation relies on the elastic deformation of three parallel thin walled bellows due to internal fluid pressure and the structure contains no moving parts. The use of three parallel bellows enables both the direction and magnitude of the tip motion to be controlled (Figure 3).

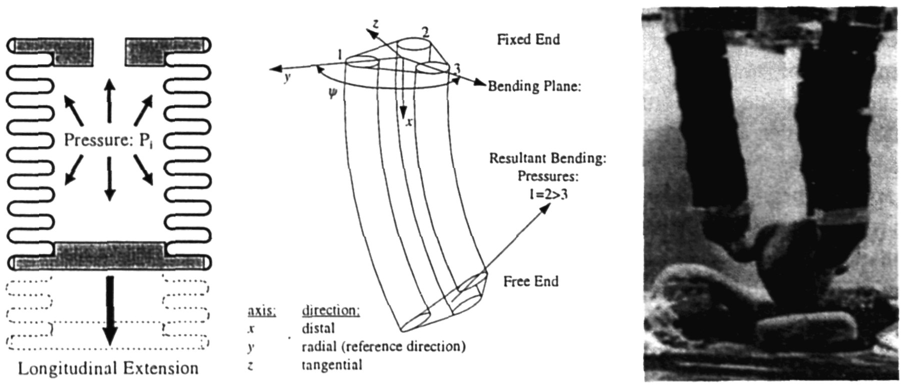


Figure 3: (a) Internal Pressure Causes Mainly Longitudinal Extension
(b) Bending of Flexible Actuator Caused By Internal Pressure Differential
(c) AMADEUS Phase I Prototype Dextrous Hand

Latterly work has focused on the development of dextrous robot grippers utilising continuum fingers. The continuum structures possess unique mechanical properties which can be exploited during grasping and manipulation activities.

- Omni-directional finger movement allows planar translational and rotational movements to be made on grasped objects solely using finger actuation.
- The continuum structure exhibits lateral passive compliance which enables the fingers to react to disturbances within the environment. This increases grasp stability by absorbing positional errors and other external effects which might otherwise lead to grasp failure. The mechanism also provides object compliance for assembly operations.

[2] describes the operation of a three fingered pneumatic gripper with continuum fingers. This device contained no sensor feedback and utilised intrinsic passive compliance to perform a variety of dextrous tasks via an open loop control scheme. Typical tasks included sequential manipulation and basic assembly operations.

The major objective of AMADEUS phase 1 was to transfer the benefits of continuum robot technology from pneumatic systems to a hydraulic gripper suitable for underwater operation. The phase 1 prototype was completed in 1996 and aspects of its mechanical, sensing, control and software design are described in [5-14, 16-21, 23-24]

This prototype was intended for laboratory investigations and has successfully performed underwater sampling and manipulation operations in its test environment. This provided a successful conclusion to AMADEUS phase 1 as the potential usefulness of applying continuum technology to underwater systems was demonstrated. An additional benefit of the continuum actuator is that it is naturally pressure compensated during normal operation.

Thus the goal of AMADEUS phase II mechanical design is to produce a second generation end-effector which will provide the functionality required by the project's scientific partners and be robust enough for trials in less structured marine environments.

The general layout of the phase II device is very similar to the first prototype and the principles of the continuum finger operation remain unchanged. However some significant modifications have been made in order to achieve the required performance goals.

The mechanical knuckle joint mechanism of the phase 1 gripper has been removed in favour of increasing the range of movement provided by each finger. The replacement palm unit will enable the finger configuration to be manually changed between deployments to optimally satisfy known mission requirements whilst providing a stable platform for finger operation during dextrous activities. The new unit houses a micro-controller to process data from the finger sensors and incorporates both a camera and sonar transducer.

The palm mounted camera provides the operator with a close up view of the grasp zone. This can be used to guide the gripper during the final stages of approach onto a target object and provides visual information regarding the current state of the grasp. Effectively the palm camera allows the operator to 'see through' the supporting manipulator arm. The utility of the arm as an inspection tool is also increased as the operator can look into and perform dextrous activities in areas which might otherwise be inaccessible. The camera housing includes an integral light ring to ensure operation during low light conditions.

The high frequency narrow beam sonar transducer provides range-to-object information to the computer control system again providing essential information during the final stages of approach onto a target object. This sonar has a range from 0.1 - 3 metres and a resolution of 0.7mm.

Obviously both these sensors become obscured once a target object is grasped between the gripper fingers and transportation of an object by arm movements must rely on toolskid mounted cameras. However the palm camera field of view is still sufficient to observe all dextrous manipulations performed by the gripper fingers.

B. Sensing and Sensor Processing

The AMADEUS phase II gripper is equipped with miscellaneous sensors for low level control. These sensors will allow the accurate measurement the force and torque at each fingertip, the position of the fingertips in cartesian space and the detection of slip.

Force/Torque Sensor
The special needs of AMADEUS gripper required that the force/torque sensor be designed and manufactured specifically for this application. Specifically, these are the fact the sensor should be completely sealed from the surrounding seawater, and yet pressure compensation must be achievable if the sensor is to operate within its full dynamic range at depth. The F/T sensor is a Maltese-cross arrangement instrumented with a total of 8 semiconductor strain gauges and capable of sensing the force and torque in all six degrees of freedom.

Slip Sensor
The function of the slip sensor is to detect the occurrence of slippage between the fingertip and the target object, and this information can be used in a variety of ways, for example providing a warning system to the force control loop or enhancing the gripper's exploratory capabilities in areas of poor visibility. It is an extremely small piezoelectric film based capable of being shaped to suit the curvature of almost any given fingertip shape, and operates by using fact that the relative motion between two surfaces causes mechanical vibrations in the direction normal to the plane of motion. The sensor acts as a vibration pickup device, and therefore can detect when slippage occurs between the gripper and the grasped object.

Position Sensor
The AMADEUS phase I gripper had no direct method of measuring the fingertip position relative to the palm. This facility has been incorporated in phase II of the project, and the sensing elements used are electrogoniometers. These are flexible devices whose resistance changes according to the angle subtended between the two ends, with each sensing element active in one direction only. There are a total of four sensors on each finger, arranged in orthogonal pairs. One pair is terminated at the distal end of the finger to measure the actual position in the finger reference frame, and the other pair is terminated half way down the length of the finger and is used in conjunction with the first pair to establish whether the grasp has caused the finger to bend into an S-shape.

Sensor Signal Processing

There are a total of 93 wires emerging from the force/torque, slip and position sensors. It is highly desirable that these raw sensor signals be processed as close to the sensor sites as possible, for two reasons:

- The VME computing platform resides in a pressure vessel on the trials toolskid (see below), and it would be greatly beneficial to route as few wires as possible to it, thus reducing bulk, the chances of failure and the outlay associated with expensive underwater connectors.
- The sensor signals are of a very low level and susceptible to transmission line noise pickup and voltage drops. Were they to travel through up to 10m of cable without amplification and processing the signals would be greatly degraded, if not unusable.

For the above reasons, all amplification, data acquisition and signal processing is carried out at the interface between the arm and the gripper itself. The physical constraints on the sensor processing hardware are severe considering the amount of work that needs to be carried out: the total volume that can be taken up by the sensor interface is no more than 100x100x60m^{m3}.

The sensor interface electronics consist of a custom designed surface mount PCB for signal amplification and some logic circuitry, which in turn interfaces to a single board 68HC11 microcontroller-based computer with analog I/O capabilities. The microcontroller manages data acquisition and transmission of the processed sensor data in real-time on a interrupt - driven basis to the main VME computer platform. An additional advantage of this distributed computing scheme is that it offloads some of the processing from the main computer, freeing up more resources for control loop implementation.

C. Finger Design for Vibration Damping and Fast Manipulation Tasks

Fast manipulative operations in the original design, due to the peculiar elastic structure of the finger, unavoidably lead to oscillations which could be very poorly counteracted for two main reasons: the uncertainty about the tip position and the low bandwidth of the actuators.

To further investigate this problem, a separate laboratory investigation is ongoing, researching different finger/actuator designs, and related control strategies. The solution results in a kinematically determined structure coupled with fast actuators. The solution adopted, evaluated in terms of the overall performances defined by the scientists, cover the medium-low contact force field at the maximum of velocity allowed by the system. An experimental set-up has been built in order to evaluate this performance in terms of constructive parameters like length and rigidity of the bellows, the size and force/displacement characteristic of the motors and to demonstrate the efficiency of the various control methodologies.

The adopted solution has the advantage to be able to be better modelled by lumped parameter equations then previous ones and makes sense to the experimental activity devoted to the sizing of the final prototype and to the investigation of advanced control strategies.

The new prototype, can be modelled by a simpler lumped parameter equation with respect to former solutions. One can write:

$$\begin{bmatrix} 0 \\ 0 \\ m_x \\ m_y \end{bmatrix} - \begin{bmatrix} k_{uu} & 0 & 0 & k_{u\varphi} \\ 0 & k_{uu} & -k_{u\varphi} & 0 \\ 0 & -k_{u\varphi} & k_{\varphi\varphi} & 0 \\ k_{u\varphi} & 0 & 0 & k_{\varphi\varphi} \end{bmatrix} \begin{bmatrix} u_x \\ u_y \\ \varphi_x \\ \varphi_y \end{bmatrix} = \begin{bmatrix} M & 0 & 0 & 0 \\ 0 & M & 0 & 0 \\ 0 & 0 & J_x & 0 \\ 0 & 0 & 0 & J_y \end{bmatrix} \begin{bmatrix} \ddot{u}_x \\ \ddot{u}_y \\ \ddot{\varphi}_x \\ \ddot{\varphi}_y \end{bmatrix}$$

where

$$m_x = \frac{\sqrt{3}}{2}(f_2 - f_3)d$$

$$m_y = \frac{1}{2}(f_2 + f_3 - 2f_1)d$$

and $u_x, u_y, \varphi_x, \varphi_y$ are the displacement of the centre of gravity of the tip and the rotation of the local co-ordinate frame with respect to the fixed frame, respectively. The rotation can be easily expressed in terms of the displacement of the control bellows. In this expression f_1, f_2, f_3 are the forces applied to the control bellows by the linear motors and the rigidity matrix components $k_{uu}, k_{u\varphi}, k_{\varphi\varphi}$, can be experimentally evaluated (with some possible imprecision) in any point in the workspace. The system can thus be considered uncertain and the control problem to be faced falls within the field of application of robust non-linear control techniques. So this system can be controlled by using a multi-input sliding mode approach of a particular type exploiting the three bellows structure of the finger.

This approach can be synthesized by the following steps:

1. choose a sliding manifold of the type
   $$\begin{cases} s_1 = \dot{\varphi}_y + c_2\varphi_y + c_1u_x = 0 \\ s_2 = \dot{\varphi}_x + c_2\varphi_x + c_1u_y = 0 \end{cases}$$
2. partition the (s_1, s_2) space by means of a simplex of vectors $f_1 f_2 f_3$; that is
   $s \in \Omega_i$ if $s = \sum_{j \neq i} \alpha_j f_j, \alpha_j > 0$
3. choose the following switching logic:
   if $s \in \Omega_i$ put $f_{j,j \neq i} = 0, f_i = F$ in the expression of the control vector $[m_x \quad m_y]$.

The application of this control law has proved to have some advantages with respect to classical P.I.D. controllers: fast tracking of unknown trajectories without oscillations, absence of phenomena like windup typical of dynamic regulators, efficiency and precision in generating contact forces within the allowed field.

D. Dextrous Gripper Deployment

To increase reach, the dextrous gripper presented in the previous section is mounted on a Slingsby TA9 hydraulic underwater manipulator, a typical robot used by the offshore oil and gas industries, (Fig. 4). The TA9 is approximately 1.5 m long, has a mass of 36 kg and is powered by a hydraulic supply operating at 175×10^5 N/m^2 giving a maximum payload of about 80 kg.

The hydraulic system consists of three parts, the hydraulic power supply, the electrohydraulic servovalve and the actuation mechanism, as shown in Fig. 5. The first part is used to provide the main power for the system. The second part, the electrohydraulic servovalve is used for the control of the opening of the central spool orifice which regulates the flow of hydraulic fluid. The third part, a linear actuator, is used to generate the movements of the joints.

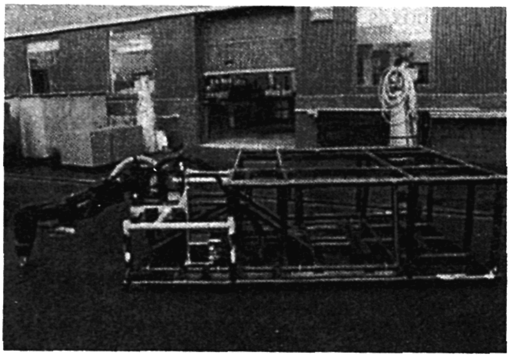


Figure 4: AMADEUS Phase II Gripper Deployment Using Slingsby TA-9 Hydraulic Manipulator. Toolskid in Preparation, December 1997

Because of the serious nonlinearity of the hydraulic system, a close-loop torque-compensation controller is designed to accurately position the robot arm. The signal from the seven potentiometers are compared with the desired inputs to produce an error signal which is further used to drive the servovalve [26-29].

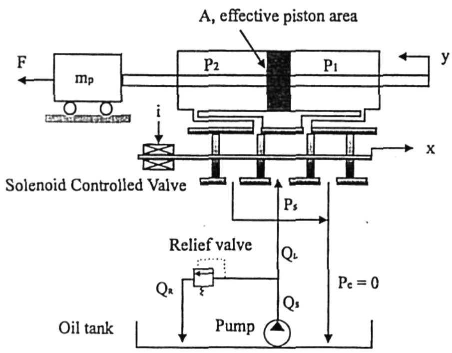


Fig 5: Hydraulic Servovalve Configuration

4. DUAL ARM TESTBED DESIGN

The characteristics of the dual-arm testbed (DA Testbed) for co-ordinated and co-operative manipulation are presented in the following. A key point pursued during the whole second phase of the AMADEUS program has been to design underwater robotic manipulating systems in close contact with potential end users coming from the scientific community (geologists and biologists in particular). This has led to the definition of a set of manipulation tasks to be considered as prototypes for a wide range of operations typically required during underwater sampling of specimen. At the end of the project the DA Testbed demonstrator is expected to show the feasibility of the proposed operations accordingly to the specific characteristics of the testbed. At this purpose, project partners IAN and DIST have defined a complete set of specifications for the design, and manufacturing of a two identical robotic arms for co-operative manipulation. These results have been based on:

- a detailed investigation concerning the most suitable, though commercially available components (motors, gear boxes, position, velocity and force-torque sensors, etc.)
- the basic requirements expressed by the scientific partners of the project
- the suggestions given by contacted manufacturers exhibiting a specific background on building underwater electromechanical devices.

The DA-Testbed consists of a deployable platform carrying two (7 degrees of freedom, 8 functions) robot manipulators. Each arm is about 1.3 m long, has a mass of 53 Kg (excluding the oil for pressure compensation), and is designed to operate to a maximum depth of 500 m. (fig 6)

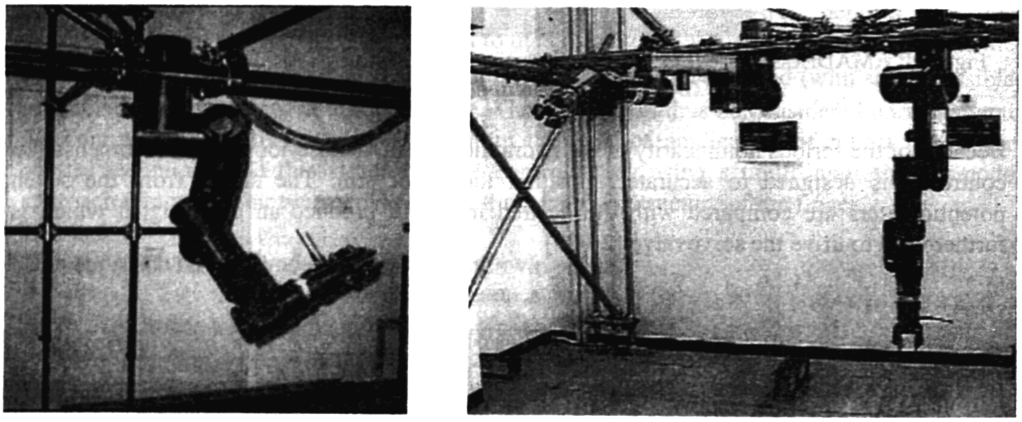


Fig 6: Dual Arm Testbed

The two arms have been designed to have redundant kinematics to overcome the problems due to singularities which could limit their manipulation capabilities, in particular during co-operative operations. The two robots are electrically actuated thus making control less critical and more accurate than using hydraulic power supply; furthermore a suitable mechanical design has made possible to achieve a load capacity (in air) of 50 N (with an accuracy of less than 1 mm), and with a capacity of sustain (in air) loads in excess of 100 N, finally the jaw grippers can apply forces up to 300 N. Technical specifications of both arms are summarized in Table 1.

Each arm is equipped with an underwater JR3 Inc. force/torque wrist sensor of adequate measurement range, located at the basis of the jaws. This sensor is connected by a high speed serial data channel to a relative digital signal processing (DSP) chip. Motors (DC), reduction gears (harmonic drives) and position/velocity sensors (resolvers) are all assembled inside the link vessel underwater coverings. Wirings (electric power, position/velocity signals and also wrist force/torque sensor signals) all run internally to the link vessel underwater coverings.

ARMs		
Number of DOFs		7
Maximum operational depth (not compensated)		3 m
Maximum operational depth (compensated)		500 m
Maximum load (in air)		50 N
Dimensions		
	arm	400 mm
	forearm	400 mm
Joint ranges		
	roll hand	± 180°
	pitch wrist	± 130°
	roll wrist	± 180°
	elbow	± 140°
	shoulder	−120° ÷ +90°
	base	± 175°
	pitch base	± 175°
Nominal velocities		
	roll hand	10 rad/min
	pitch wrist	5.89 rad/min
	roll wrist	4.2 rad/min
	elbow	2.88 rad/min
	shoulder	2.73 rad/min
	base	2.73 rad/min
	pitch base	3.2 rad/min
Jaws		
Dimensions		
	opening width	0 ÷ 96 mm
	jaw width	70 mm
	jaw length	80 mm
Force and velocity		
	closing force	0 ÷ 100 N
	velocity	30 mm/sec
Total weight		50 N

Table I: Electric Arm(s) Specification

The arms are coated with a particular anodization to resist marine corrosion; for deep waters, the applied treatment is specific: joints are filled with oil, with motors made oil-resistant, being appropriately pierced for oil penetration; appropriate O-rings are inserted between joints to keep oil from flushing away. Resolvers are assembled on slow axle in order to warrant calibration of the whole system.

The possibility to have further sensors mounted on the wrist is currently under investigation: additional cabling has been provided inside the arms for this purpose. On each gripper, detachable plaques will be mounted, to improve in the future the quality and quantity of sensors present (i.e. fingers' strain gauges). The project also allows the possibility to detach the gripper from the arm, for the purpose of trying different devices and tools operated through the arm itself. Currently, the DA-testbed has just been released from the manufacturer and is working at DIST laboratories for testing and developing of control algorithms.

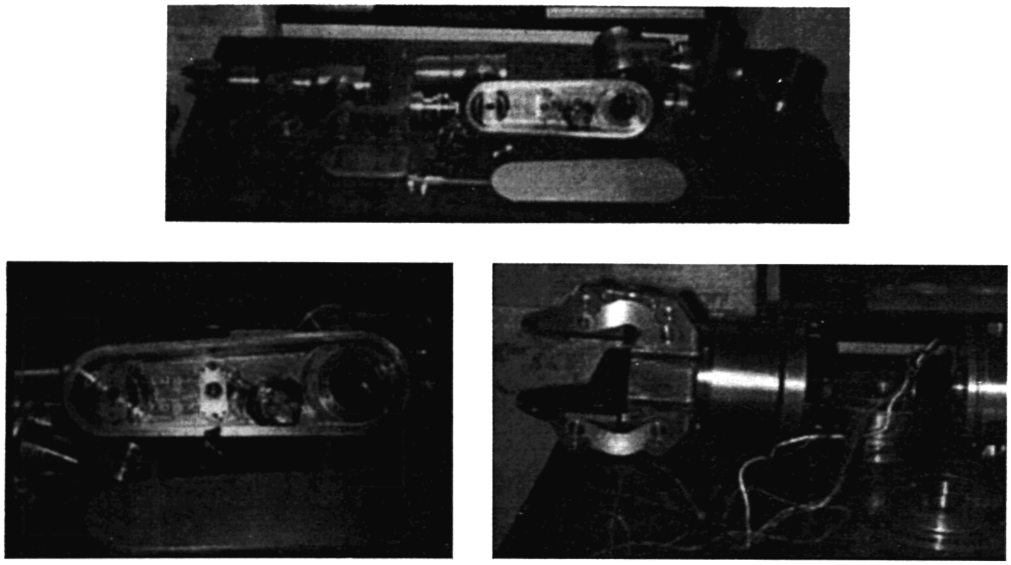


Fig 7: Detailed View of Arm Construction

The DA-Testbed will be teleoperated by a human operator providing commands through a special purpose human computer interface (HCI) feeding suitable commands to a task level control architecture.

The adoption of a task level control formulation, even though the system will not operate in autonomous modes, allows a simple control of the arms by the operator. Co-ordinated control of two co-operative arms performed at joint level may not be feasible since it would a large number of input signals to be provided by the operator. On the other hand suitable task level commands and automatic task switching allows to implement simple interface mechanisms.

The DA-Testbed, as one of the two testbeds of the second phase of the AMADEUS project, will demonstrate its capability in a set of pool experiments where 4 classes of tasks will be performed. These tasks consists of sampling and manipulating various types of objects corresponding to fragile/large/heavy candidates samples, furthermore other operations will consist in properly handling probes and other typical underwater scientific equipment.

5. BLIND GRASPING TELE-ASSISTANCE

The goal of the AMADEUS I high level control was to form a grasp planning strategy that would enable the system to execute a blind grasp, which is defined as "one where there is no a priori knowledge of the object that is being grasped, except that it exists and is in the vicinity of the gripper.". This would allow the user to perform grasping operations even in conditions of poor or no visibility. The second phase of the project presents wider goals for the high level control system, concerned with the automated control of the entire hand/arm system to assist the user (referred to as tele-assistance in contrast to tele-operation)[1]. These goals include the blind grasping operation of the first phase, but add various other operations with the system, such as classifying objects by their geometric and material properties, and simple co-ordinated finger motions for easy grasping and release of objects.

A reactive planning system has been implemented to carry out the high level control activities mentioned above [17]. Reactive planning is a subset of the more general planning techniques that have been developed in the artificial intelligence community over the last thirty years as problem solving tools. Such a planner was used because they are especially suited to controlling robotic systems in an unstructured environment (as that presented in the blind grasping problem is), by virtue of their behaviour based approach [15].

In general, three required properties of our reactive task planner are:
1. To be able to receive and process sensory data so that meaningful observations can be made, and to generate an internal model of the world based on the data.
2. To be able to make observations on and draw conclusions about the state of the world, based on the sensory data received and the internal model of the world.
3. To be able to form plans of action to alter the sensed world to achieve specified goals passed down from the user.

The high level control subsystem was developed in a modular fashion using object-oriented programming techniques, to ensure that future expansion of the system would be as easy as possible. This approach, when coupled with the desired properties listed above, resulted in the development of three separate program modules for the high level control (fig 8). These modules are called the geometric world model (GWM), the semantic world model (SWM) and the task sequence network (TSN).

The GWM takes data from the system sensors and builds a physical world model. This model contains geometric information about objects in the world, coupled with other physical properties that have been discovered.

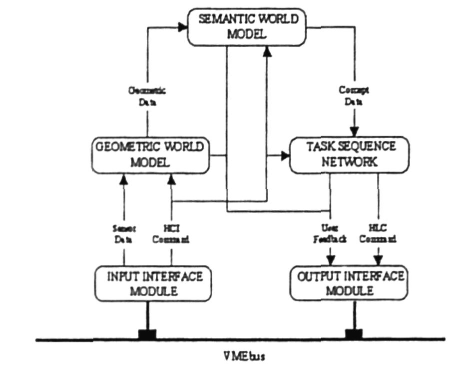
The SWM takes the physical data from the GWM and forms a semantic model of the world, that is the meaning of and relationship between the sensed physical properties.

The TSN holds lists (or sequences) of actions that must be performed in order to carry out the various tasks. These actions can be commands to the lower control levels (called primitive or atomic actions), or may themselves be lists of actions. These lists are called "task sequences", and each sequence and each action in a sequence is triggered by the non-existence of its goal in the world model. For example, if the goal was to pick up a rock, the sequence "pick up rock" would be triggered by the lack of a rock in the gripper. In this sense the planner can be said to be error driven.

The AMADEUS II high level control design has been expanded from the system implemented in the first phase to include provision for on-line entry of task sequences

[1] Tele-assistance using blind grasping is to be demonstrated using the dextrous hand/arm testbed. The dual arm testbed will use tele-operation.

(previously all sequences were entered at compile-time), a larger library of existing task sequences to carry out the range of operations required, and a wider set of commands to interface with the lower levels of the control system. The hardware platform has also been homogenised - the first prototype planning system ran on a UNIX-based platform, whereas the new version will run on the same VME bus based hardware as the rest of the control system.



Part of the planner design documentation

Fig 8: Schematic of Planner Architecture

Preliminary evaluation tasks have been specified for the second phase testbed, and will include both ordinary tele-operated and tele-assisted performance of the tasks where possible. One such task is to sample a desired type of object from a mixed group, once given an initial example of the object. Part of the task sequence for this "selective sampling" task is shown in the figure.

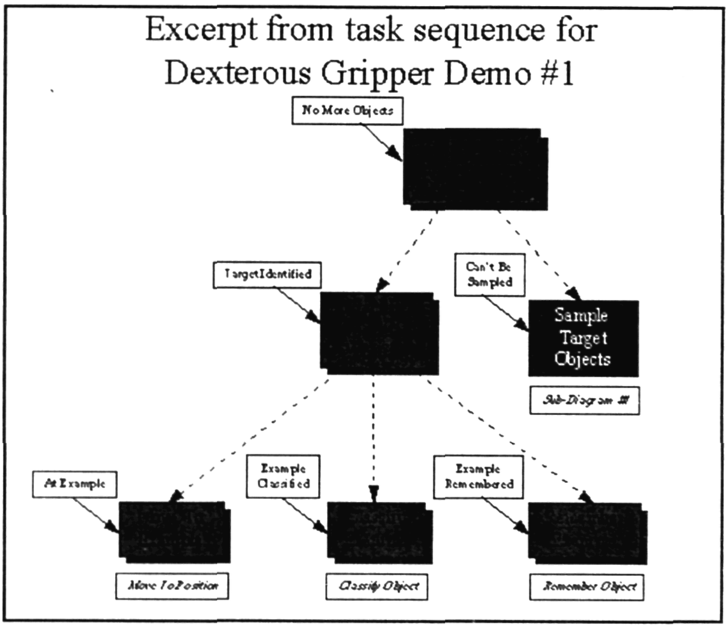


Fig 9: Task Sequence Network for A Scientific Task

6. HUMAN-COMPUTER INTERFACE

A. Tasks and Requirements

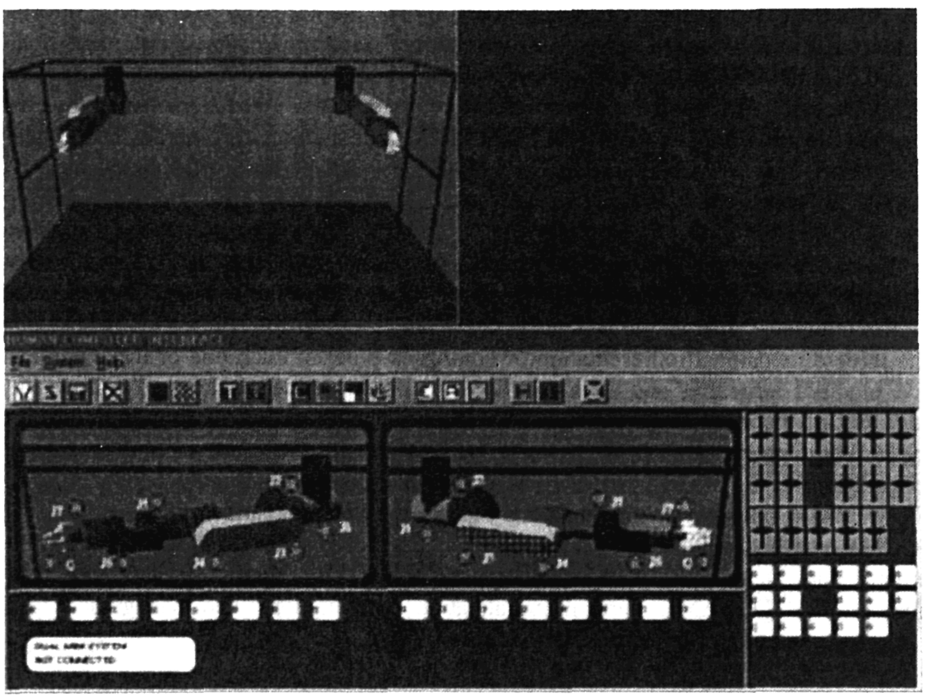
Different kind of tasks may be performed by the Human Computer Interface. They may be divided into two groups, called *data-visualization* (visualize position and attitude of the two arms, attitude of the gripper, telemetry, status data) and *commands-dispatching* (send a given command through appropriate complex windows, together with needed parameters, visualize the values of sent parameters).

B. Hardware Structure and Ergonomic Design

The Human Computer Interface system from an ergonomic point of view will be structured as follows: two differently configurable interfaces are the components of the HCI system : one for engineers and one for scientists: the first one with more emphasis on telemetry, to keep objects under control, the second on 3D visualization of objects, to manage objects with the minimum effort and as reliably as possible.

The two different AMADEUS robotics set-ups can be connected to the interface and operated through it: the following figures show examples of how the interface looks when connected to the two systems.

Commands can be sent through a Control Box, composed by different kinds of devices (joysticks, knobs, buttons), to be used in function of the different task which has to be carried on, and of the different operating mode. All devices are currently under study, and their practical use and configuration will be accomplished during the final integration trials.



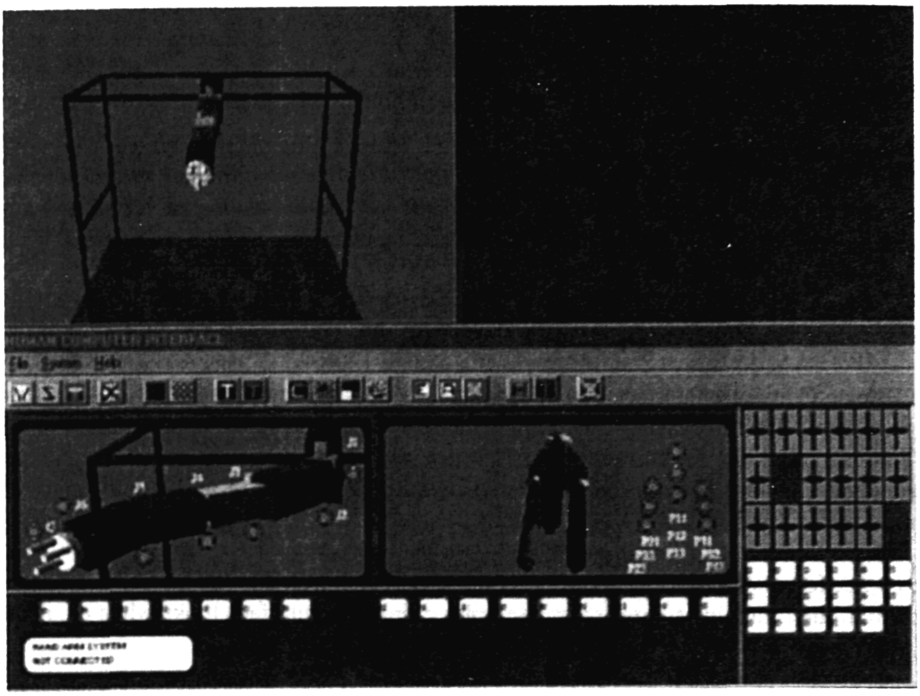


Fig 10: Human Computer Interface For Dual Arm and Dextrous Manipulator Testbeds

C. Software Structure

Some important HCI features can be listed as follows: the necessity to deal with information which is distributed over a series of different machines implies the implementation of a standard communication protocol; a correct definition of tasks and a good designing practice imply the use of a fully modular structure; the continuous data exchange between the HCI and controlled modules implies the use of a well-defined data structure. The devised structure is derived from the one used and successfully implemented during the first phase of the AMADEUS Project.

The Human Computer Interface is composed of two different applications: the *mmi* application, whose purpose is to run the kernel of the interface, manage communications, visualize telemetry, operate global configuration, and the *3dgraph* application, whose purpose is the three-dimensional representation of controlled objects. Each one of these applications has a modular structure, and each module has a well-defined task: here follows a brief description of the most important ones. The names and meanings of some modules are the same for both applications. These modules: perform all global initialization, management and configuration tasks (BOSS); take care of communications with Control Modules through a LAN (NMM); manage communications within applications and with the Control Box (INCOM); run an internal test and simulation procedure (SIMUL - *mmi* only); visualize telemetry data and graphical representations of objects on screen (VISM); log data and actions on disk (LODAM); perform initialization, computations and management tasks for the 3D representation of objects (GEN - *3dgraph* only).

The HCI has been designed in a way that allows complete reuse for different robotics applications, thanks to the total modularity and flexibility of its structure; the same way, it grants the possibility of adding new functionalities or hardware parts to the system, without any substantial change in the control flow or having to redesign the global control structure. The HCI system is currently used both as a development platform for technicians and as an interface to allow scientists to control a real machine: the devised structure is well fitted, and only configuration changes are needed to use the two HCI versions.

7. CONCLUSION

We have described progress to the beginning of 1998 developing improved dextrous grasping and manipulation systems for use in the ocean. Two testbeds are involved. The first comprises a dextrous three fingered robot hand with force, slip, vision and acoustic sensing, mounted on a hydraulic underwater manipulator and ROV toolskid. The second comprises two symmetrical electric manipulators with conventional grippers, similarly mounted. The first is designed for sampling and manipulating small, light objects. The second is similarly designed, but for much larger and heavier objects.

To date the project has progressed well, and the forward plan anticipates first dry and wet trials during 1998, with final wet trials in 1999. A crucial aspect has been the collaboration between engineers and marine scientists in technology specification and development. In particular, the project has benefited from the in built formal user evaluation, which is providing a sharp focus to guide planning and evolution.

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TITLE: Miniaturized and Reconfigurable Instrumentation for Multipurpose Survey with a Mini Autonomous Underwater VEhicle **(MAUVE)**

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MINIATURIZED AND RECONFIGURABLE INSTRUMENTATION FOR MULTIPURPOSE SURVEY WITH A MINI AUTONOMOUS UNDERWATER VEHICLE (MAUVE)

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1. ABSTRACT

The overall objective of the MAUVE project is to develop and validate at sea a miniaturized, reconfigurable, mobile and autonomous instrumented vehicle, dedicated to multipurpose survey, in particular in coastal waters. It is characterized by coherent integration of a compact automatic multiparameter measurement module with a small and low cost Autonomous Underwater Vehicle.

Such a demonstrative vehicle, derived from an existing "Torpedo-shaped" AUV (CALAS), has been developed and will be validated at sea in mediterranean coastal waters; the potentialities of the "MAUVE" vehicle open a much wider spectrum of potential applications, ranging from the monitoring of sub-ice conditions to the surveillance of various dumping sites, through the inspection of the quality of bathing waters.

Moreover, a technique has been developed within the frame of the project in order to accomplish benthic survey mission in coastal waters.

2. INTRODUCTION

Instrumented mobile unmanned platforms are a very promising alternative to current undersea measurement methods, being able to conduct fast 3-Dimensional survey in highly variable environment, such as coastal areas. Such measurements acquire a growing importance for the construction and validation of oceanographic models, but these instrumented vehicles will be economically viable only if they really offer affordable elementary data acquisition and simple exploitation and support means.

The overall aim of this program is to demonstrate both the operational interest and the technical feasibility of a small, affordable vehicle for multiple measurements in coastal waters. System operational specifications are the following:

- low cost baseline, with multi-instrument capability
- easy maintenability and fast new-mission reconfiguration
- low risk mission achievement and reliable recovery
- single or maximum two persons handling, from a small ship or from the shore
- multi-vehicle configuration capability.

The project has taken advantage of an existing vehicle recently developed by TMS S.A.S. and CNIM for the french navy (CALAS training target), and of the fundamental experience gained from developing such a small and low-cost AUV. The main functions related to the vehicle (body, propulsion, energy sources) has been maintained as much as possible. Improvement in vehicle autonomy (or intelligent control) has been achieved via:

1. A multifunction acoustical unit, composed of an on-board part and of an off-board part (two acoustical references), which has been designed as an aid for the navigation and for the recovery of the vehicle.
2. A mission management unit and a navigation system able to maintain an estimate of the vehicle position and offer the possibility to perform distinct navigation modes, using both internal sensors and acoustical external references.

A small and reconfigurable instrumented payload consisting of measurement units, with basic instrumentation and specific sensors has been developed.

3. BASIC SYSTEM

The basic vehicle is derived from an existing one, called CALAS, developed as a training vehicle for the french Navy ([1] and see photo 1 of the MAUVE vehicle). It is a "Torpedo-shaped"' AUV, designed as a modular structure with the "vector" function at the rear (including thruster, navigation fins, power pack and control electronics) and the front end being totally devoted to the payload and additional positioning and guidance systems.

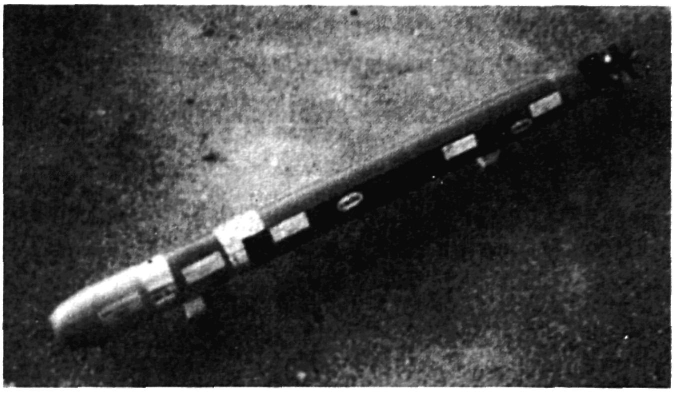

Photo 1 - MAUVE vehicle

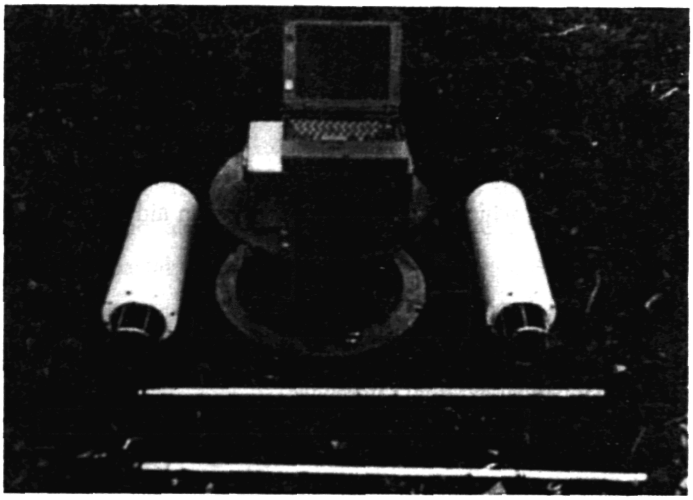

Photo 2 - PC + references

An electric DC motor drives a single propeller rotating around a fixed rear shaft. The rotating torque of the motor is compensated by a fixed rear stabilizer, acting hydrodynamically as a contra-rotating propeller.

The principal characteristics are the following:

- Diameter: 150 mm
- Length: 1,8 m
- Dry weight: 30 kg
- Velocity: min: 3 knots - max: 6 knots
- Endurance: 5 to 6 hours, according to the actual speed
- Depth capability: min: 5 m - max: 100 m with payload / 300 m for the vehicle itself

- Auto-depth and auto-heading navigation capability
- Reliable recovering capability.

The PC station and the acoustical references (with the associated electronics) which are needed for the exploitation of the vehicle are shown in the photo 2.

4. MAUVE VEHICLE TECHNICAL DESCRIPTION

Multifunction acoustical unit

The acoustical components of the system consist of:

- 2 off-board acoustical references, with their associated electronics
- the on-board acoustical sensor with the front end and signal processing electronics.

The design of these components has been guided by basic considerations about the navigation and the recovery of the vehicle.

The acoustics which has been implemented has to improve the reliability of recovery of the vehicle, which induces the ability for the vehicle to transmit a sufficient power in all the directions. With respect to the place which is available in the vehicle, a good compromise was to implement a spherical transducer in the front of the vehicle; as there is no place for other acoustical sensors on the hull, this spherical sensor is used for several acoustical functions, by multiplexing in time.

The following acoustical modes have been defined:

1. *Passive localisation mode:*
   The two references transmit pulsed signals; the vehicle measures the difference of the arrival times for the two signals as well as the doppler of each signal.
2. *Active localisation mode (transponder mode):*
   The vehicle transmits a signal which is received by the acoustical references and immediately "reflected" towards the vehicle. The vehicle measures the range from each reference.
3. *Echo mode:*
   This mode is an active one which allows to carry out the echo sounder function as well as a simplified obstacle avoidance function.
4. *Communication modes:*
   These modes allow communications between the references and the vehicle.

5. *Pinger mode:*

   In case of emergency the vehicle will raise at the sea surface, and the vehicle will permanently transmit signals which are used by the references for the localisation of the vehicle.

6. *Trajectography mode:*

   In this mode, the position of the vehicle is measured by the references and displayed on the external PC-station. So the operator can control the evolution of the vehicle when it is in the range of the references.

7. *Test mode and wait mode:*

   These modes are basic modes during the test phase and the preparation phase; they can also be useful for the mission itself.

Before the mission, a sequence of acoustical modes is defined, like an agenda. The clocks of the references and of the vehicle are synchronized and the mode sequence will be loaded as input data into both systems.

Mission Manager and Mission preparation

The mission management of the MAUVE vehicle essentially deals with the problem of its unexpensiveness, with limited on board calculation capabilities.

Identification of all the possible foreseen "events" (external and internal), which may be met during the mission execution, is currently being done on the basis of realistic mission scenarios. This includes for instance remaining energy status, possible hardware failures, differences between navigation requirements and navigation parameters, etc. and specification of possible a priori reactive behaviours to be fired when these events occur.

A main guide-line of the project is to separate the mission preparation from the on-line (at sea) mission control. Most of the work related to mission planning must be performed during the mission preparation phase (before the vehicle launching). Only (simple) reactive (vs. reflective) behaviours will be implemented into the vehicle in order to guarantee mission execution and vehicle safety and homing/surfacing.

The Mission Management, embedded in the on-board computer, will thus be set much less risky by limiting the whole reconfiguration capability, while the most difficult task planning and global resources (pre)allocation will be provided by an external shore-based PC-station and thus under the operator's control. This architecture will thus minimize the risk of "loosing" the system, and will still offer a smart local autonomy.

Navigation (Positioning and Guidance) System

The navigation system is a key module of the vehicle: *(i)* it provides estimates of its position/velocity and of external perturbation currents; and *(ii)* it generates appropriate steering information for the low-level control unit.

The two major overall concerns in the design of the navigation system of MAUVE are:

(i) its stability and robustness, and
(ii) its security.

Map/Positioning

The vehicle has access to three distinct data sets for positioning:

(i) *on-board sensors* (compass, inclinometer, depth, rotating speed of the shaft);
(ii) *active acoustic measures* (distance with respect to two external acoustic references);
(iii) *passive acoustic measures* (differential delay of the signal received from two synchronized emitters, and the radial speed with respect to each of these emitters).

Data set **(i)** is (nominally) always available, while the data sets **(ii)** and **(iii)** are alternative.

The positioning module of MAUVE, illustrated in figure 1 comprises five distinct filters, which are activated depending on the data sets available: the angular filters are used whenever new observation of the corresponding on-board sensors are available, the active (passive) filter is activated when new active (passive) acoustic measures are received, along with the on-board depth measure, and the inertial filter when neither of these data sets are available.

Finally, current estimation is updated when passive measures -- where velocity is directly observed -- are received. Since velocity estimation is best when the vehicle follows a non-accelerating mode, a "probing" sub-mission is performed before the actual (user-defined) missions are executed.

During this initial sub-mission, the vehicle executes, in open loop, a simple geometric trajectory, identifying:

(i) the ocean current and
(ii) a mean compass bias.

The gain of the current filter during this (acquisition) submission is considerably higher that its value during the course of the rest of the mission, where only slight updates of the estimated current are expected.

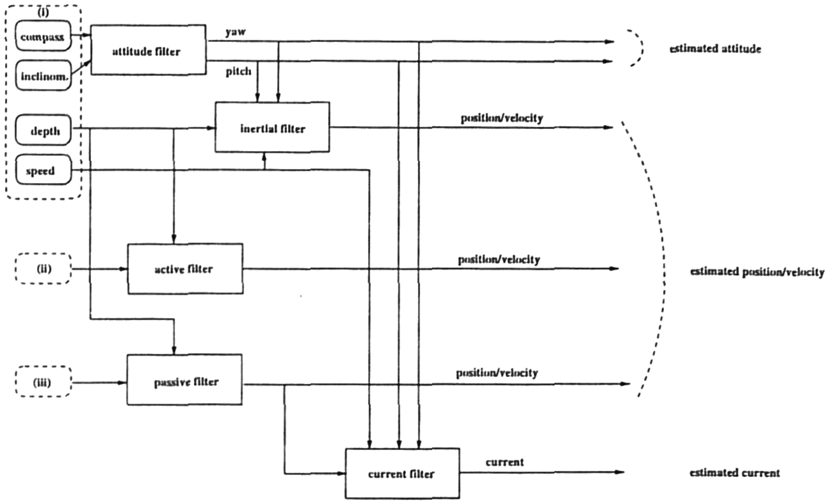

Figure 1- Positioning Module of MAUVE.

All filters are designed using a model-based approach, based on:

(i) locally constant attitude angles;
(ii) locally constant acceleration; and
(iii) locally constant ocean current. Moreover, outlier detection is performed, and signaled to the mission manager unit, providing an indication of possible sensors/subsystems failures. Details on the design of each filter can be found elsewhere [2].

Since the stability of the control loop depends strongly on the reliability of the confidence level associated to the estimate produced by the positioning block, an analysis of the innovation process of each filter is used to constantly tune the gains of the filters, providing a reliable estimation of the expected error level at each instant.

The potential availability of distinct data sets, with different observability characteristics, offers a supplementary degree of freedom, corresponding to the choice of the temporal sequence of acoustic measures. To minimize consumed energy, the use of active measures (which require emission of an interrogation signal) must be minimized, making the passive mode the preferred navigation mode.

However, the error of the passive filter can be shown to be a divergent process, and thus its prolonged use can lead to situations of vehicle lost. Moreover, the acoustic link must be periodically used for high-level communications with the base station, imposing a fixed periodic use of the inertial filter, which induces a rapid increase of the uncertainty concerning the vehicle position. To minimize the complexity of the acoustic sub-system, a finite number (two) of *acoustic cycles* have been defined. One, is a long cycle, with a minimal use of the active mode, and extended passive navigation, interleaved with the communication (inertial) periods.

The other one is a short "repairing" cycle, providing exclusive use of active measures, interleaved with the required inertial periods. The choice of the next cycle is performed on-line, based on the actual mission execution conditions and user defined required performance. The "repairing mode" can be activated in the following situations:

(i) the uncertainty concerning the accuracy of the sampled points grows above the user-defined minimal accuracy of the spatial sampling;
(ii) a way-point (in grid mode) is being approached, where the vehicle must enter a more active maneuvering trajectory;
(iii) the probability of successful homing falls below a fixed threshold.

The determination of this last probability, responding to the major reliability constraint of the MAUVE system, is constantly performed by an additional block of the positioning sub-system. It relies on analytical models of the evolution of the distinct error processes (for the different positioning filters) and is described in detail in [2].

Control of the vehicle

The global control of the MAUVE vehicle consists of two separate modules: the {\em high-level} control, and the {\em low-level} control. The first generates a set of reference values (desired yaw, depth and speed) that are used by the low-level classic control unit, that generates the control signals for the propeller and control surfaces.

Two distinct control modes are at present available on MAUVE:

(i) a *grid* mode, where a sequence of way-points is defined, along with the passage times at each way-point;
(ii) the *homing* mode, that must take the vehicle to the vicinity of the acoustic references;

as there are uncertainties about the dynamic model of the vehicle, the emphasis has been put on the stability of the vehicle, coping with the existing level of uncertainty (with respect to the controlled system, the vehicle position/velocity, and external perturbations. Stability and performance during mode **(i)** is ensured by using tolerance regions around each way-point dependent on the estimated state confidence level. During mode **(ii)**, if the confidence about nominal vehicle position is poor (which can be the case during an "emergency" homing), the passive acoustic measures are directly fed to the high-level control unit, and reference values for the low-level module are generated, based on the minimization of the differential delay and maximization of the radial speed of the vehicle with respect to the center of the base-line external array (middle point between references).

Miniaturized and reconfigurable instrumentation

A small size self-contained payload unit has been developed for the MAUVE vehicle, to carry out measurements of oceanographic parameters.

The basic design of this payload is a pump-driven flow chamber system with sensors for temperature, conductivity, turbidity, fluorescence and water ingression. Intake and outlet manifolds for the flow chamber will be in the payload flanges.

A micro-processor controlled datalogger with 512Kb non-volatile memory for data storage will log data during a mission and also manage communications with the Mission Manager. The datalogger samples data through a multiplexed 12 bit A/D converter. Conversion time for all 8 data channel is approximately 0.8 ms.

Temperature will be measured with a Pt-100 sensor. Response time is less than 0.1 second, resolution 0.01°C, accuracy 0.01°C.

Conductivity will be measured with a proprietary 3 electrode conductivity cell manufactured by GMI (resolution 0.01 ms/cm3, accuracy 0.01 ms/cm3).

It has been decided to use a combined Fluorometer-Turbidimeter sensor in the payload, with glasfibre optics to transmit light to and from a common sensor-head. The sensor head is inserted through a watertight connector into one end of a 90 mm long measuring chamber, with a diameter of 25 mm.

The sensor-head is mounted with 22 cm of fiberoptical cable which will be bent back along measuring chamber, and ending up connected to 2 Printed circuit boards with the electronic circuitry necessary for fluorescence and turbidity measurements.

This results in a payload configuration which will be validated at sea in order to demonstrate the flexibility of the system.

5. AT-SEA DEMONSTRATION WITH THE MAUVE VEHICLE

A demonstration sea trial will be done in the Rhône plume which corresponds to a "plume survey" mission, or investigation of mixing processes between waters of different origins. This type of mission will contribute to a better understanding of 3D phenomena involving both physical and suspended particulate processes, such as:

- dilution or urban waste effluents in coastal water
- mixing of river plumes in salt water.

These phenomena are presently difficult to investigate with conventional means.

A series of numerical simulations have been conducted for the Rhône plume using a three-dimensional hydrodynamical model. Test runs have been defined to analyse the role of different forcing mechanisms (river discharge, forcing at the open sea boundaries, wind direction and magnitude, time variability) on the circulation and the structure and evolution of the plume. The results are used to deduce ranges for the physical parameters which can be implemented in the Mission Preparation System of the MAUVE vehicle.

6. AT-SEA DEMONSTRATION FOR THE SURVEY OF COASTAL WATERS

In parallel with the development of the MAUVE system, a multisensor technique has been developed in order to accomplish benthic survey mission.

This mission is especially devoted to detect, and to characterize different sea bottom areas populated by aquatic vegetation. This subject is meaningfully related to a plenty of applications interesting both ecological, biological, and practical aspects.

The wide Posidonia Oceanica meadows were chosen for the sea trials. This plant is really important for the Mediterranean ecosystem both from the ecological (e.g., oxygen enriching, favourable environment for fish species and marine animals) and from the practical point of view (e.g., protection against coastal erosion).

The acoustic analysis of the seabed vegetation is performed by means of a high frequency (1.5 ÷ 2.0 MHz) monostatic sector-scan sonar. The high frequency of the device assures a suitable resolution capability, dense and accurate sampling of the investigated area and high sensitivity to low strength targets as represented by aquatic leaves.

An AUV - which at the moment cannot be the MAUVE vehicle for reason of dimensions - can be employed to carry the head slowly navigating at few meters from the sea bottom. While navigating, the head scans and insonifies a vertical sector towards the seafloor, hence acquiring backscattered signals from the seabed.

Experiments were initially organized in a tank of the Genoa Aquarium where Posidonia Oceanica plants live over a small pebbles substratum some decimeter high. Several tests were performed in order to acquire vegetation in different conditions for what concern its state of health, growth, density, and so on.

As an example, figure 2, created by placing side-by-side a sequence of acquired scanlines, show, two sequences of raw data acquired by different sonar parameters setting (TVG, head output filter bandwidth, and baud rates). The bottom of the tank and the plant arrangements are clearly visible.

Moreover, also the aquatic vegetation status was changed in the two test sessions: fig. 2a refers to young green leaves in a good state of health, fig. 2b. corresponds to sparse, old, and encrusted plants.

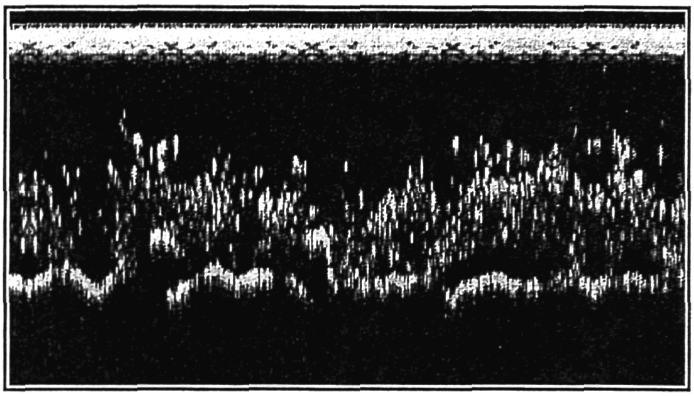

Figure 2a

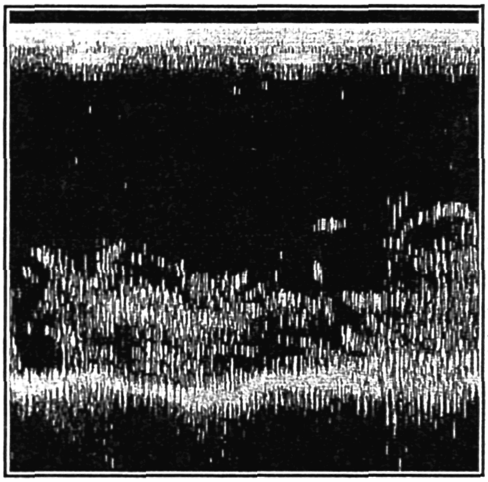

Figure 2b

7. CONCLUSION AND PERSPECTIVES

The MAST III - MAUVE project is intended to validate at sea the concept of using an autonomous mobile instrumented platform. Such a concept will be viable only if low-cost and ease of exploitation are achieved. This will be carefully assessed through a demonstration phase in Mediterranean coastal areas.

The basic parameters which shall be measured with qualified reliability and accuracy are: temperature, conductivity, turbidity and fluorescence. A small payload unit has been developed to carry out these measurements.

Parallely a technique has been developed in order to accomplish benthic survey mission.

However, the potentialities of the "MAUVE" project open a much wider spectrum of potential applications - with mono or multivehicle configurations - ranging from the monitoring of sub-ice conditions to the surveillance of various dumping sites, through the inspection of the quality of bathing waters.

These topics are of growing importance, in particular in most european coastal waters.

8. ACKNOWLEDGEMENT

The author would like to thank its partners from the consortium and the project officer from the commission of the European Union (DG XII) for their strong support.

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TITLE: A novel microsensor for measurement of flow and diffusivity (MicroFlow)

CONTRACT NO: MAS3-CT970078

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A NOVEL MICROSENSOR FOR MEASUREMENT OF FLOW AND DIFFUSIVITY

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SUMMARY:

A microsensor for flow and diffusivity based on an innovative principle has been invented under MAST project MAS3-CT-950029 "Micromare". It is based on the diffusive loss of tracer from a reservoir through a membrane filled pore. The tip of a transducer specific for the tracer is situated near the outer side of the membrane where it detects changes in concentration or partial pressure. As the concentration/partial pressure gradient through the membrane is dependent on both the diffusive properties of the tracer in the membrane and the transport coefficient in the external medium, the reading from the microsensor represents flow or, in stagnant media, diffusivity. The new microsensor is small (down to 10 micrometers in outside tip diameter), inexpensive, has a low energy requirement, and can quantify flow velocities over 4 orders of magnitude plus diffusivity in stagnant media. The aim of the MicroFlow project is to optimise this new sensor by choosing the best suited tracer and by optimising the detection of this tracer by the built-in transducer. As the signal from the new sensor is dependent on temperature, means of optical or thermo-couple temperature compensation will be investigated and implemented. The new sensor with built-in temperature compensation will be used on a microprofiling benthic lander in both shallow waters and in the deep sea. For deep sea use, a pressure stable (600 bar) sensor filled with liquid will be developed. The new sensor will be protected by patent and the feasibility of commercial production and marketing will be investigated. The potential use of the new sensor in fields like medicine, physiological research, and micromechanics will also be investigated. Calibration of the novel sensor is demonstrated and the first results are discussed.

INTRODUCTION:

For our understanding of processes in aquatic environments it is extremely important to have a detailed knowledge about the transport processes, i.e., advection and diffusion. Within a distance of millimetres or fraction of a mm, the transport at the sediment-water interface changes from being dominated by advection in the water to being mainly diffusive just above and within the sediment (Jørgensen and Revsbech, 1985). If we want to describe processes near the sediment-water interface we thus need analytical methods with a high spatial resolution.

One of the conclusions from the June 1996 international conference "The benthic boundary layer" (held in Bremen, Germany) was that a lack of suitable methods for the analysis of transport processes at the sediment-water interface constituted the perhaps most important limitation for our understanding of the processes in this environment. As an example, it is now evident that pore-water flow may constitute an important transport mechanism in coarse sediments, but methods to quantify flow between the sand grains have not been available. It seems that many of our determinations of solute exchange over the sediment-water interface based on incubations in closed chambers underestimate the in situ exchange rates due to reduced currents and absence of wave action which would otherwise cause pore water flow.

Four main approaches have been widely used to investigate flow in marine science: The Doppler method (based on acoustics or lasers), the induction method (Faradays principle), the hot film method, and methods based on direct mechanical action (propellers). For measurement near the sediment-water interface (at a mm scale) only Laser-Doppler and hot film anemometry are usable, since the acoustic Doppler and the induction method require more space and since the mechanical method requires flow velocities at least in the cm/s range. The Laser-Doppler method, however, has some disadvantages: It is expensive (ca. 150 kECU), it cannot resolve flow velocities below 1 mm/s in practical use, and it cannot be used in situ. The hot film technique requires relatively much energy, it cannot resolve flows below the mm/s range since it creates convection due to the heating of the film, and due to its relatively large size it disturbs the flow, especially when applied close to surfaces. Alternative techniques such as visual tracking of small particles and simple electrochemical registration by for example stirring sensitive oxygen sensors are either to inaccurate or can only be used in model systems.

There have been several approaches to obtain estimates of the diffusive properties of sediments and similar substrates. For sediments the most widely used approach has been electrical resistivity measurements (Andrews and Bennett, 1981) from which the diffusivity can be estimated. Resistivity probes have also been used on benthic landers (Reimers, 1987) where they give information about both the position of the sediment-water interface and about diffusivity. The resisitivity measurements have, however, the disadvantage that they cannot resolve the diffusivity in the uppermost few mm of the sediment, and this is particularly unfortunate as the uppermost layer has the highest biological activity and governs the diffusive exchange of solutes across the sediment-water interface.

A very direct approach to determine diffusivity is to add the chemical species in question to the sediment surface and then after some time determine the distribution of the chemical species in the sediment. In its classical approach the sediment is sectioned before analysis of the chemical species (Duursma and Bosch, 1970), but it is also possible to use microsensors for analysis of the chemical species, and it is then possible to analyse the surface layer (e.g., surface 1 mm) for diffusion coefficient (Revsbech 1989b). To obtain information about diffusivity (porosity multiplied with sediment diffusion coefficient) it is, however, necessary to transfer the sediment to a diffusion chamber (Revsbech 1989b) and it is difficult to do this without disturbing the zonation. Furthermore it is often necessary to poison the sediment to avoid consumption of the tracers, and this poisoning may change the diffusive properties (Glud et al. 1995).

OBJECTIVES:

The objectives of this project are to improve the microsensor so that it can be used routinely in aquatic sciences, to develop a deep sea version of the sensor, and to investigate the feasibility of commercial production and marketing.

The novel flow and diffusivity sensor is based on the diffusive loss of a tracer from a reservoir through a membrane-filled pore. The loss of tracer from the reservoir is so small that the source of tracer is constant for any practical considerations, and the principle is based on changes in concentration gradient near the tip due to changes in transport characteristics (flow, diffusivity) of the medium. A built-in microscale transducer specific for the tracer has its tip positioned near the outer side of the membrane where it detects concentration changes. As the concentration gradient through the membrane is dependent on the advective transport or diffusive properties of the tracer in the external medium, the reading from the microsensor represents flow or, in stagnant media, diffusivity. The new sensor is very simple, inexpensive, and low energy requiring, and it offers measurements with an ease, spatial resolution, and range of transport characteristics not offered by any other method.

The detailed objectives are:

1) Selection of tracer molecule and detection principle
The sensors made until now have been based on oxygen or hydrogen as tracer molecules, and electrochemical microsensors have been used for transducers. We will investigate the use of other tracer molecules including dyes and investigate the possibilities for optical detection.

2) Implementation of optical or thermocouple temperature compensation
The optical and electrochemical detection of a tracer molecule will be dependent on temperature as will the diffusion of the tracer molecule through the membrane in the new sensor. It is therefore essential to have an on-line temperature reading near the tip of the sensor, and we will construct and test both thermocouples and optodes (fibre-optical sensing devices) for temperature compensation.

3) Demonstration of sensor use in the aquatic sciences
The new sensor will be calibrated for use both as a flow sensor and a diffusivity sensor. Equipment and routines will be developed for the calibration. The new sensor will be used for both laboratory investigations of water flow and diffusivity and will also be implemented on a benthic lander for use both in shallow environments and in the deep sea. In the laboratory, results obtained with the new sensor will be compared with Laser-Doppler analysis of flow fields.

4) Patenting and market analysis
A Danish patent application on the sensor has been filed. Within the project a claim for international patent (PCT) will be filed. Furthermore the feasibility of commercial production and marketing will be investigated and in this context the use of the sensor in other fields will be investigated. The medical field constitutes a large field of future use, but the sensor may also be used to detect low flows in industrial applications including flow in miniature devices.

RESULTS:

In the following a description of the sensor and a presentation of the first results of the new flow/diffusivity sensor will be given. Initially flow will be discussed, then diffusivity.

Flow sensor: The flow sensor developed during the MAST project (CT-950029) is shown in Fig. 1. It consists of a glass capillary with a 10-40 μm thick silicone membrane at the tip. The inner lumen of the glass capillary is filled with a gas, which can only escape through the very narrow membrane-filled pore, and the concentration inside the sensor is therefore practically constant with time. The concentration of the tracer at the sensor tip can be measured with a built-in gas transducer protruding almost through the membrane at the tip. The transducer senses changes in concentration gradient caused by altered transport characteristics in the external medium.

The gas inside the capillary might be substituted with a liquid if the membrane is permeable to this liquid and a sensor specific for the liquid (or a substance dissolved in the liquid) can be devised. The tracer gas in question (in our work primarily hydrogen) will diffuse through the silicone membrane and out in the external liquid as illustrated in Fig. 1.

In a stagnant medium there will be a spherical diffusion field around the tip with decreasing concentrations with increasing distance to the tip. Spherical diffusion comes to a steady state (Crank, 1985), and after a short time the concentration field around the tip will not change, i.e., the gas sensor at the membrane surface reads a constant concentration. If a flow is imposed, the diffusion field will be disturbed as advection will now contribute to the transport of molecules away from the tip, i.e., the concentration at the membrane surface will decrease. The higher the flow, the lower the concentration will be at the membrane surface, where it is sensed by the built-in transducer. The average diffusion time of a gas molecule through a 20 μm thick membrane is about 0.05 s, so the response to changes in flow rate has about the same time constant with a 90% response below 1 s. The response time will increase with the diameter of the sensor and will be largest at low flows when the diffusion sphere around the sensor is wide.

The first gas to be tested inside the sensor was oxygen, as a transducer (microsensor) for oxygen (Revsbech 1989a) was readily available, and the sensor then worked as predicted, but only in environments with a homogeneous distribution of oxygen. The next gas to be tested was hydrogen, and as natural environments with appreciable concentrations of hydrogen are rare and as hydrogen does not react spontaneously at high rates in non-biological systems, this seems an almost ideal choice. Furthermore it is relatively easy to make an electrochemical hydrogen microsensor (Witty 1991) for use as a transducer. The calibration curves shown in Fig. 2 are obtained with an oxygen based sensor with 25 μm wide tip, but similar calibration could have been done with a hydrogen transducer. By use of a hydrogen transducer, the current for very high flow will be close to zero whereas a background of oxygen in air-saturated water gives a relatively high current at high flow when oxygen is used as a tracer. A single sensor can cover the range from 5 μm/s and up to more than 60 mm/s, a range of 4 orders of magnitude. No other method can cover a similar range (apart from image-analysis aided microscopic tracing of particle movement).

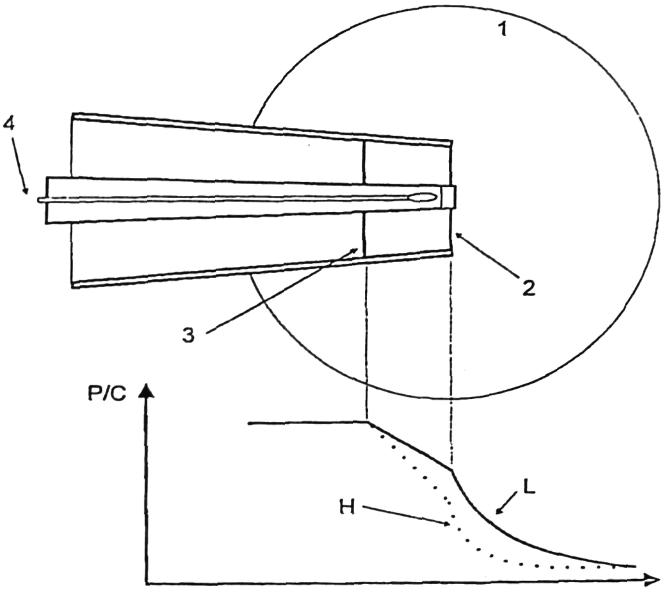

Fig. 1. Microsensor for liquid flow based on the diffusion field of a tracer molecule. 1) illustration of diffusion sphere, 2) surface of silicone membrane facing the exterior, 3) surface of silicone membrane facing the gas reservoir, 4) microscale gas transducer. On the partial pressure versus distance diagram below the sensor: L) partial pressure profile representing low flow velocity, H) partial pressure profile representing higher flow velocity. (From Gundersen et al., 1998)

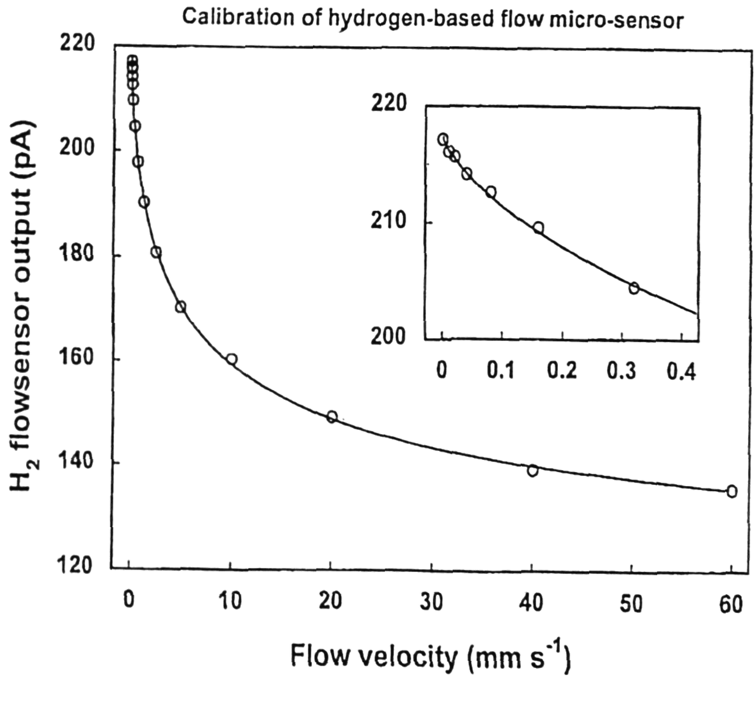

Fig. 2. Calibration curve of a flow microsensor tested at flows between 0.005 mm/s and up to 60 mm/s water flow. (From Gundersen et al., 1998).

..L&O flow MS\sand_med.fpw

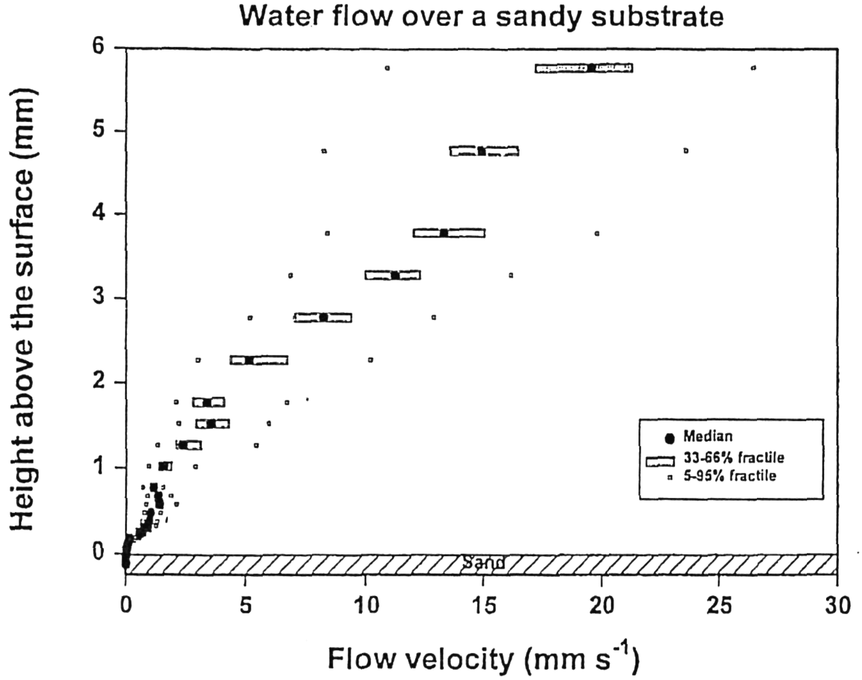

Fig. 3. Flow profile above a sand substratum as measured by a flow microsensor. (From Gundersen et al., 1998)

An example of a flow profile close to a solid surface as recorded with the new flow sensor is shown in Fig. 3. The flow profile is very much as previously observed by for example Laser-Doppler measurements, and the *U has been calculated to 0.48 cm s^{-1}. Also by this sensor it is possible to observe the rapid oscillations in flow giving rise to a rather wide range in flow rates at any given distance from the solid substratum (Fig. 3).

Diffusivity sensor: It is now possible to determine concentration profiles of various substances in sediments and other substrata with a wide range of techniques. To be able to calculate fluxes and reaction rates from such profiles we must, however, know the diffusivity of the substance in question in our sediment, and the techniques for this have been laborious and often inaccurate due to extensive laboratory manipulation of the samples to be analysed (Duursma and Bosch 1970; Revsbech 1989b). The novel sensor can, however, also be used as a diffusivity sensor, although the dimensions then should be somewhat different from those of the flow sensor. First of all, it is not necessary to know the diffusivity at extremely high spatial resolution. In a sandy sediment for example one would like to know the overall diffusivity, and not the diffusivity in between two sand grains or very close to a sand grain. The optimal diameter of a diffusivity sensor is therefore around 100 µm for analysis of most sediments, but in coarse sediments it should be even thicker. Also, to resolve relatively small changes in diffusivity, a significant part of the concentration gradient has to be found inside the sensor, i.e., the membrane should be long. It is relatively easy to calibrate a sensor to various flow rates, but to do the same with a range of different diffusivities is not a simple task. An analytical solution for the sensor response as a function of diffusivity is thus to prefer, and to be able to obtain such an analytical solution a simple geometry with a parallel sided capillary in the region containing the silicone membrane is advantageous (Fig.4).

A mathematical treatment of the diffusional problems associated with a diffusivity sensor results in the following expression for the signal (s) (Revsbech et al., 1998):

$$s = a/(b*D + 1)$$

where a and b are constants specific for the given sensor and D is the diffusivity (effective diffusion coefficient times porosity) of oxygen (or hydrogen with a hydrogen transducer) in the analysed medium. The expression above is a somewhat simplified version as all constants dependent on membrane dimensions etc. are contained in "a" and "b". To construct a calibration curve (i.e., to find a and b) for a given sensor one thus need the signal for two known values of D, for example stagnant water (for example 0.3 % agar) and an artificial sediment composed of equal-sized glass beads. The information obtained is limited to the diffusivity of the tracer gas, but with this knowledge it is usually possible to calculate good estimates of the diffusivity of other chemical species.

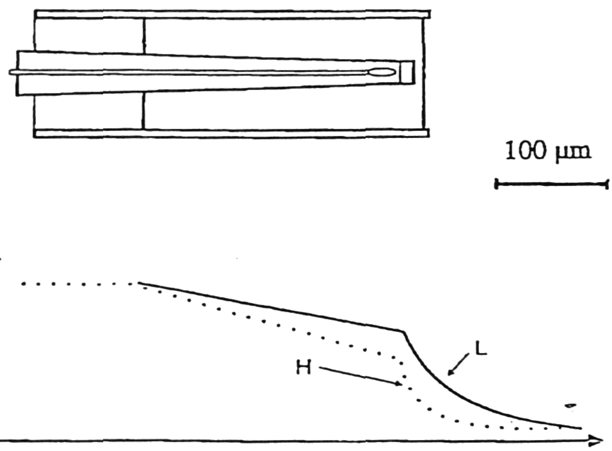

Fig.4. Microsensor for the determination of the diffusivity in various media such as sediments. See Fig. 1 for explanation about the individual parts. (From Revsbech et al., 1998)

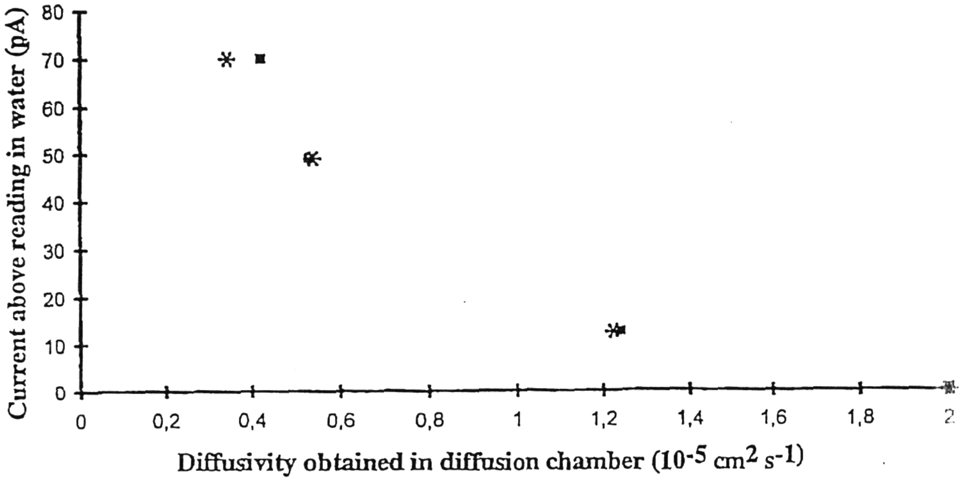

Fig.5. Calibration curve for diffusivity sensor. All determinations were carried out at 20 °C. (From Revsbech et al., 1998).

A calibration curve is shown in Fig.5 for a diffusivity sensor, where readings in water and in glass beads were used to obtain estimates of a and b. All readings obtained with the sensor were calibrated against determinations of diffusivity obtained by use of a diffusion chamber (Revsbech 1989b). In contrast to the very rapid response times obtained with thin sensors for flow, the about 100 µm thick sensors for diffusivity need about 5 min of equilibrium time at each depth before the signal stabilises. A sample of fine-grained calcium carbonate in water gave exactly the expected reading, whereas granules of potato starch gave much too high a reading (i.e., too low a diffusivity) with the sensor. Measurements with a wide range of other substrates indicated that relatively loose sediments gave close to correct readings, whereas some substances, here exemplified with starch, tend to pack to a dense mass in front of the sensor thereby resulting in unrealistically low estimates of diffusivity. These issues have to be investigated in more detail, but we do not expect such packing effects in front of the sensor to be a serious problem by insertion in sediments.

DISCUSSION:

With an optimised and temperature-compensated microsensor for flow and diffusivity it will:
-For the first time be possible to obtain precise data on the microdistribution of flow in diffusive boundary layers associated with all solid surfaces in the aquatic environment
-For the first time be possible to obtain on-line information about the microdistribution of diffusivity in sediments
- For the first time be possible to equip benthic landers with profiling equipment for water flow and diffusivity .
-For the first time be possible to perform in situ studies of liquid flow associated with meiofauna, etc.

Additionally the novel microsensor has multi-sectoral possibilities such as use in:
- Physiological research (flow in blood vessels, transport in tissue like liver and brain)
- Industry (flow patterns in micro pumps, leakage tests)
- Biotechnology (complex transport phenomena in biofilms, etc.)

ACKNOWLEDGEMENTS:

The European Commisssion is thanked for supporting this project through the MAST Programme.

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TITLE: Advanced ROV Package for Automatic Mobile Inspection of Sediments (Aramis)

CONTRACT NO: MAS3-CT97-0083

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ADAVANCED ROV PACKAGE for AUTOMATIC MOBILE INSPECTION OF SEDIMENTS (ARAMIS)

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SUMMARY

The RTD Project ARAMIS, an activity for developing the technology to carry out integrated multidisciplinary seabed research, started on December 1st, 1997 and runs for 36 months. The main aims of the project ARAMIS are decribed and the work plan is outlined. No results are yet available from the project.

1. INTRODUCTION

Up to the present day the majority of marine measurements (chemical, biological and physical) are taken by remote sampling techniques. These techniques have inherent problems:

- sampling cannot be precision related to an underwater feature, with the capability of repeatable sampling
- there is no view of the surroundings from which the samples/measurements are taken and therefore no knowledge of their representativity
- integrated *in situ* sampling is not well developed and single sampler deployment is inefficient
- near-bottom waters are very difficult to investigate (for example, CTD's are never lowered to the sea-bed)
- remote sampling equipment can have a high degree of impact on the environment
- the majority of *in situ* data logging instruments record 'off-line'
- towed systems take a long time to deploy and it is difficult to judge the correct amount of cable to pay out and also to maintain the correct speed. Video resolution is good at no more than 1.5 knot (for a focal distance of 1.5 m) which is the minimum steerage speed for most ships.

Manned submersibles are quite effective since they allow direct observation by the scientist and are not constrained by cumbersome umbilicals. However, they are characterised by poor maneuverability, limited diving time, limited number of observers whilst presenting substantial capital investment, operational costs and risks. There are only three operational submarines available to the scientific community within the European Union (Nautile, Cyanea, Jago).

ROV's potentially offer several advantages including increased endurance and depth capabilities, observation by multiple users, computerized control and the possibility of access hazardous sites. They have been used by the scientific community for the past 20 years or so, but this has almost always been with 'off-the-shelf' models. In 1974 there were approximately 20 ROV's in operation around the world mainly used in military and scientific applications. The number rose to 100 in 1978 and is estimated to be over 2000 in 1996 with over 150 different types available (Bell *et al.*, 1994). In scientific applications they have been used for either video or photographic recording (eg. Auster *et al.*, 1989), including density measurements of some species (Bergstroem *et al.*, 1987; Robinson *et al.*, 1991). In a few cases they have been used for single sample collection (Dando *et al.*, 1994), multiple sample collection (Sprunk *et al.*, 1993) and continuous parameter measurement (Horn, 1991). The only ROV's dedicated to support deep sea research (1000+ m) are based at the Japan Marine Science Technology Centre (Hattori, 1989; Takagawa, 1995), IFREMER (Alayse-Danet, 1991), Woods Hole Oceanographic Institution (Yoerger *et al.*, 1989) and Monterey Bay Aquarium Research Institute (Newman & Robison, 1993).

The state of the art reveals an increasing demand by the scientific community for a mobile scientific platform capable of automatically obtaining samples of, for example, water and sediment, of carrying out accurate quantitative photo and video transects, etc. In particular, the possibility of actually inferring accurate and real-time dimensional information from TV pictures with the capability for absolute size measurements is deemed extremely valuable.

The proposed ARAMIS system represents a large step forward beyond traditional sampling methodologies for single mission integrated multidisciplinary studies.. It provides scientists with a high precision, low impact sampling platform for well defined missions, incorporating samples from the water column, benthos, multidirectional instrument profiling and quantifiable imaging. It addresses the needs of state of the art scientific investigations to promote and support efficient and economic data collection.

This is achieved through a system capable of highly automating present generation of ROV's and benthic instrumentation packages, including quantitative video tools, auto visual recognition of interesting sea beds, sediment profiling sensors, multi-purpose multi-sampler of the sediment and of the water column.

2. WORKPLAN

(A) REFERENCE SCIENTIFIC MISSION

Scientific mission strategy

The targeted missions for the ARAMIS system is in tasks requiring a degree of mobility, such as surveys and sampling over specific areas, or activities in zones where other sampling methodologies are not able to operate, for example, where topographic features do not allow efficient sampling (e.g. steep slopes). The ROV will have a high degree of automation for mission definition and sampling procedure, but will allow the operator to override the system and chose when and where to take a particular sample.

Benthic capability missions for the ARAMIS system will include:

1. Near bottom instrument profiles and transects :

CTD (conductivity, temperature, pressure)

Dissolved oxygen

Chlorophyllous pigments

Particles - nephelometer

Prefiltered water samples: nutrients, metals, other elements

The ROV will have a minimum requirement to swim in a particular pattern in very close vicinity to the bottom to map distribution patterns along the benthic boundary layer.

2. Benthic samples

The benthic sampling package will consist of multiple cores, with a penetration depth of 15 cm (small size) and 50 cm (medium size) with 40 mm and 60 mm internal diameter respectively. Sampling can be done in replicate or singly on transect at known distances in relation to a known object. Cores will be recovered for physical and chemical analysis.

A probe system will allow precise sedimentary profiles (millimetre step to 20 cm penetration) to be made for pH, dissolved oxygen, sulphide and temperature.

3. Video Transects

The ROV will be able to locate the bottom and follow a particular track at very precise altitude. The stereo video camera system will allow:

- General observation
- Quantified Imaging: Density measurements along transects for population estimations of commercially important megafaunal species
- Size Measurements: Measurement of particular size parameters (length, diameter, etc.) for estimation of population characteristics, growth and production.

(B) SYSTEM DESCRIPTION

ARAMIS is composed of two parts to be interfaced with the ROV (see the scheme in fig. 1). The two parts are:

- Instrumented ROV Skid(s) including:
    - data acquisition and skid control system (including interface with ROV telemetry)
    - stereovision TV camera for the quantitative bottom imaging
    - object recognition TV camera for increasing the visual coverage of the travelled area

- obstacle avoidance sonar
- echosounder
- Doppler sonar or correlation log and dead-reckoning set
- CTD (conductivity, temperature, pressure) and oxygen sensors, nephelometer fluorometer
- water multiple sampler
- medium size and small size sediment multiple samplers
- sediment sensors - oxygen, pH, temperature and sulphide
- micro bathymetric sonar.

Surface controller to be interfaced with the ROV controller, including:

- the ARAMIS Man Machine Interface (MMI)
- the supervisory computer (including interface with ROV controller)
- the stereovision processing unit
- the video processing unit for automatic recognition of "interesting" objects
- the sonar processing unit
- a data base storage unit of acquired imagery and observational data

The resulting system will provide scientists with a high precision, low impact sampling platform for well defined missions, incorporating samples from the water column, benthos, multidirectional instrument profiling and quantifiable imaging.

Core skid
The core skid features all the basic technological devices needed for navigation / positioning and data acquisition/telemetry plus part of the scientific equipment. As such it can be considered as a stand alone package that can be installed under medium size ROVs, typically used for shallow waters (like ROMEO used in the project).

Larger frame skid
The larger frame skid provides additional capabilities: medium size sediment samplers, bathymetric sonar and additional motion sensors with relevant control bottle. The size fits large ROV's, typically those for deep waters (like VICTOR used in the project).

Auxiliary positioning sensors are also shown in the figure:

- acoustic positioning sensor at the bottom, such as Long Base Line (LBL)
- absolute positioning system of the support vessel, such as GPS.

They are not indispensable, even though important to enhance the ARAMIS capabilities in terms of automatic navigation and localization of the recorded data.

In the following an overview of the main items is provided.

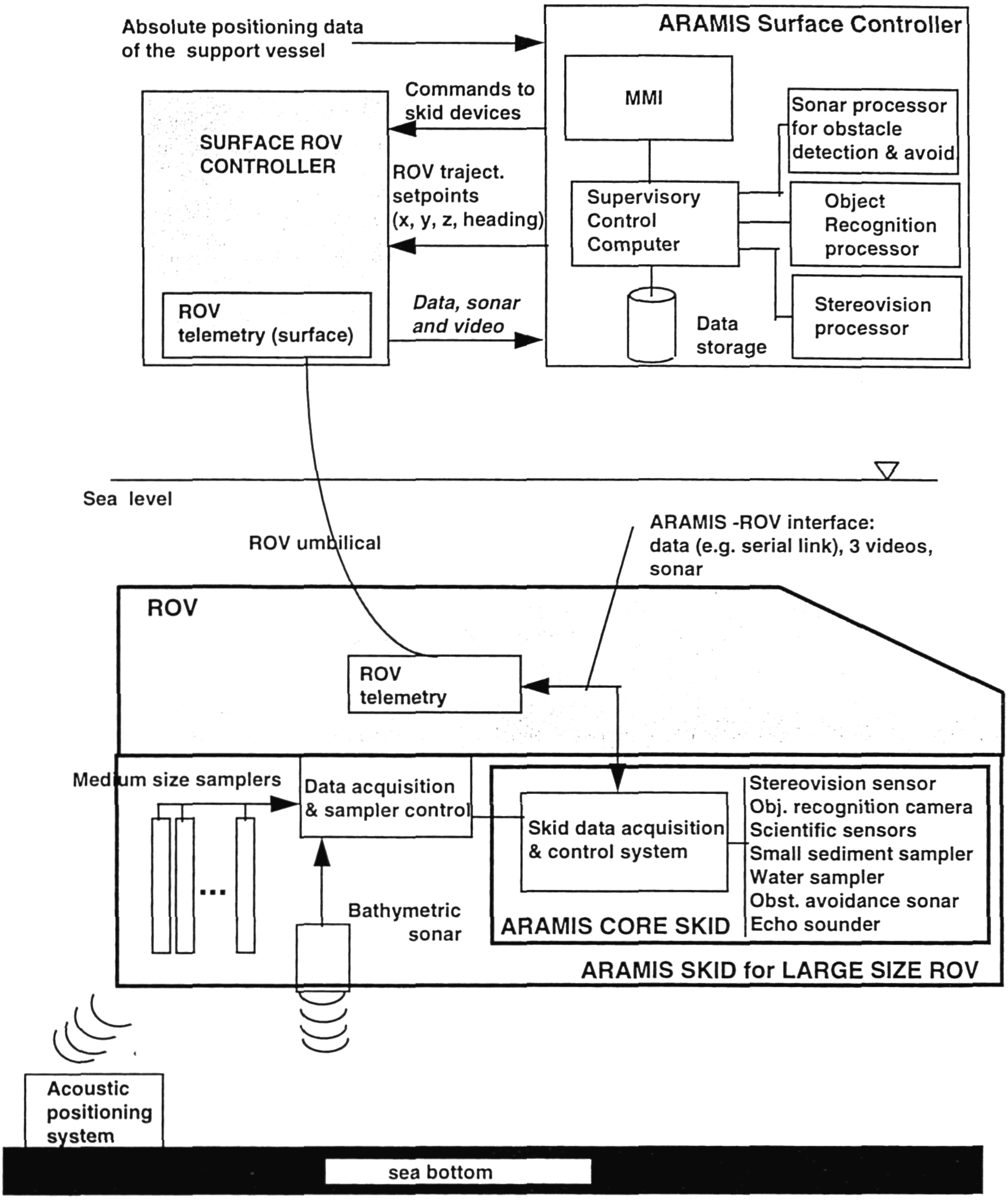


Fig. 1 Scheme of the integrated ROV + ARAMIS system

(B) DESCRIPTION OF SUBSYSTEMS

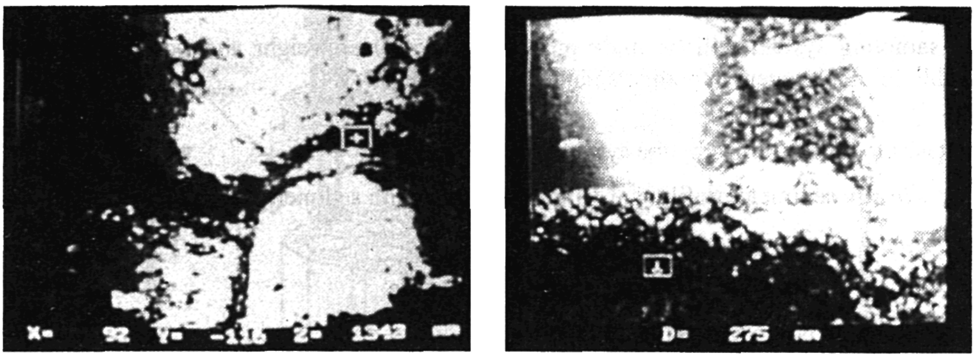
Stereovision system

The stereovision sensor implements two main functions in ARAMIS:

- extraction of real-time quantitative information from video transects, either "on the fly" or by post processing recorded stereo images: accurate measurement of objects/species on or in vicinity of the sea bottom and bottom elevation mapping;
- accurate measurement of ROV movements with respect to the bottom, e.g for hovering control or for estimation of the ROV speed.

This stereovision sensor is based on an existing system, the TV Trackmeter, developed for industrial application in the offshore industry, to be suitably modified and finalised for the use foreseen in ARAMIS, mainly scientific.

It is a computer stereovision system, with pre-calibrated subsea TV cameras unit and surface monoscopic monitor where the operator can select the points to be measured (see example in fig. 2).



(a) (b)

Fig. 2 By moving the cursor(s) on the monoscopic surface monitor, the operator can extract 3D information from the scene taken by the stereo camera:
(a) measurement of the x-y-z coordinates of the point marked by the cursor
(b) measurement of the 3D distance between two marked points.

Object Recognition Camera

The monoscopic TV camera and relevant processing unit are dedicated to support the scientist in discovering scientifically interesting bottom zones. It will feature the following functionalities:

- Automatic detection with real time processing of scientifically interesting objects or areas, from which scientific samples should be collected, with estimates of interest levels.
- Classification of objects of scientific interest using appropriate a-priori knowledge.

- Location of interesting objects relative to the vision system, with calibrated and uncalibrated cameras

Bathymetric sonar

A bathymetric sonar will be integrated in the larger frame skid in order to build a Micro-bathymetric map for geological and morphological analysis of the workplace. It appears very important to have maps at small scale (one meter isobath), not possible to obtain by multibeam bathymetry on board research vessel, before systematic video exploration and local work.

The ROV will hover the sea floor at rather high altitude (up to 100 meters). During this exploration, remarkable sites will be acoustically marked to facilitate a further return for a local work dive of the ROV.

Multipurpose water and sediment small samplers

The sampling system will be multi-purpose and of size, weight and power consumption suitable for deployment in both a 'small' and 'big' ROV.

The tasks to be performed by the system are:

In situ sediment profiling at 1 mm resolution with sediment depth for:

- dissolved porewater oxygen
- dissolved porewater hydrogen sulphide
- porewater pH
- sediment temperature

Providing cores and sediment-seawater interface samples by:

- taking short (10 cm) cores in a controlled fashion
- taking multicores in a controlled fashion
- taking cores in time series
- adding preservatives after sampling

Providing water samples (with a single multipurpose system) by:

- actuating water bottle multisamplers for dissolved species
- actuating a filtration multisampler for suspended particulates
- actuating an ion exchange multisampler for dissolved moieties
- taking and preserving microbiological samples

The sampling systems are based around two developed components
a) a small, low power, high torque linear actuator and

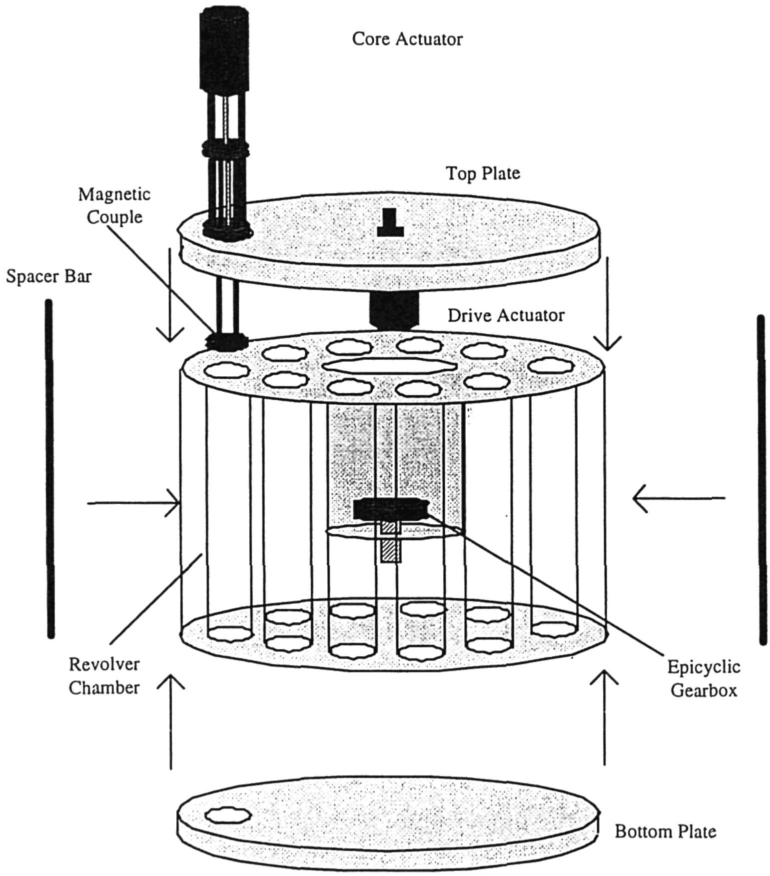
b) a small, low power, high pressure head gear pump.

Application to the implementation of the new innovative multi-purpose multi-sampler: the idea is to use one compact multisampler body to suit all types of sampling:

- multiple water samples at different depths for organic and inorganic analysis of both the water and the suspended particles
- multiple core samples to be taken in different locations on the same deployment.

The basic principle is to fit the samplers into a rotating chamber (similar to loading bullets into a revolver, as conceptually shown in fig. 3).

Application to the implementation of sediment profiling sensors: linear actuator is also used to drive into the sediment the profiling sensors.



Exploded View of Multi-sampler Set for Coreing

Fig. 3

Medium size sediment samplers

These samplers are corers located in the large frame skid. Sediment samplers design is for "medium" penetration, 500 to 600 mm.
The "standard" dimensions of the medium sediment sample are 500mm penetration and minimum diameter 60mm. It is foreseen to use a standard PVC tubing of 600mm long and 63 mm of internal diameter to undertake and store the sediment sample. All the corers will be assembled in a single package and each one sunk in the sediment under action of a linear jack. Similarly to the small size multiple sampler concept, only one jack will be used to sink all the corers, with a specially designed and actuated corer package for automatic tool changing.

Supervisory Control Computer

The Supervisory Control Computer allows the operator/scientist to enter high level commands via the ARAMIS MMI. The commands are directed to the ROV and to the scientific package.

The ROV commands are translated into vehicle position and/or speed setpoints to be fed to the ROV Controller which in turn takes care of the ROV servo loops.

Man Machine Interface (MMI) and Data Base

The MMI module provides the operator with the following capabilities:

1. enter/ edit a mission program
2. interaction during the mission execution
3. collected data presentation.

The MMI module will help the scientific programmer, based on knowledge of vehicle configuration, vehicle and sensors status, environment status.

According with overall mission programming, and asynchronous events occurring in the mission itself, a number of sets of data are collected and stored. Results of a mission are therefore collections of data of different types, all of which are related.
The data base will be managed and accessed through the ARAMIS MMI.

The medium size ROV

The medium size ROV, ROMEO, is an eight-thruster open-frame configuration. The four, symmetric horizontal thrusters permit heading and hovering control, as well as horizontal motion in any direction, while the four vertical thrusters allow depth and attitude control.

It is structurally stable in terms of pitch and roll and fully controllable in linear motion (x,y,z) and heading. The vehicle is equipped with a traditional autopilot for autoheading, autodepth and autospeed (referenced to the water).

The vehicle is structurally neutral and its vertical thrusters are located in the top part of the body. Thanks to this naval-mechanical architecture, it can perform benthic surveys with minimum impact on the operating environment, i.e. the sea bottom.

Physical specifications:

- Size: 130 x 90 x 90 cm (lwh)
- Weight: approx. 370 kg in air
- Max. Depth: 500 m.

The large size ROV

The large size ROV, VICTOR (fig. 4), is a scientific remote operated vehicle for very deep water. It is operated from the ship and the dives are controlled from a information processing system.
The vehicle is of a modular design.Physical specifications:

Depth capability	6000 m	
Vehicle dimensions	Height	: 1820 mm
	Width	: 2150 mm
	Length : 3150 mm	
	Depth	: +/- 10 cm

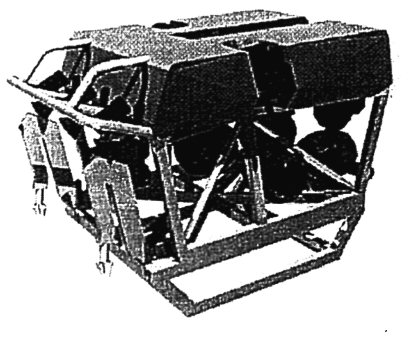


Fig. 4: The ROV VICTOR

(D) FINAL TESTS AND FUTURE DEVELOPMENTS

The developed system will be field tested at previously well studied sites in the Mediterranean, using the two ROVs: ROMEO and VICTOR.

The results/product from the project will be further used by benthic scientists (primarily those involved in the project) for their investigations.

The ARAMIS system will not be considered as a finished commercial product. More extensive field testing will be required to achieve suitable levels of reliability and a commercial version would require handbooks, maintenance procedures, definition of spare parts, etc and should incorporate all the safety features required by the operational regulations.

The project exploitation plan will take into account the necessary steps towards commercialisation.

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TITLE: EURODOCKER
A Universal Docking-Downloading-Recharging System for AUVs

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ADVANCED CONCEPTS FOR AUV DOCKING DOWNLOADING AND RECHARGING

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SUMMARY

The main objective of the RTD project EURODOCKER is to develop and demonstrate the technology necessary to accomplish the highly complex task of automatically docking an AUV to a docking station that is either lowered from a surface ship or mounted to an unmanned underwater platform. The project started on December 1st 1997 and runs for 36 months. This paper describes the current state-of-the-art in AUV docking and outlines the project's work plan. No results are yet available from the project.

1. INTRODUCTION

Autonomous Underwater Vehicles (AUVs) are widely accepted to become a major tool for oceanic science. Their performance and reliability have reached such a degree that a more widely use apart from trial runs and test missions is encouraged.
With an increasing degree of autonomous behaviour other limiting factors for oceanographic and commercial AUV missions have now come into view.

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The fundamental limitation for an untethered vehicle is the amount of energy that can be stored onboard. The range of an AUV mainly depends on the power consumption of the propulsion system, the hotel load, i. e. the power consumption of the computer system and basic instrumentation, and the payload, but a 50-60 km range and an 8 to 12 hours endurance seems to be an average at the present state.
Another important limitation on the endurance of AUVs stems from their limited capacity to store data. A typical survey mission generates data at a rate going from 10 to 40 Mbytes/hour, the latter figure applying to imaging sensors like medium resolution digital video or sidescan sonar. As full real-time data transmission to the support ship is not possible and onboard processing would lead to an increased hotel load, all data have to be kept in the AUV's mass storage system until the vehicle is recovered.
The necessity to repeatedly launch and recover the vehicle not only requires the costly presence of a support vessel on the surface. Adverse current, wave, wind and ship motions can greatly endanger unmanned vehicle operations, especially during the launch or recovery phase. For example the annual probability for a sea state >= 4 in the North Atlantic Ocean is almost 70 % and normally operations have to be cancelled under these conditions /7/.

A submerged docking station for AUVs, either as part of an unmanned underwater platform on the seabed or lowered from a support ship on the surface, is a suitable means to overcome these problems. The simultaneous operation of multiple AUVs or long-term missions of an AUV under a closed ice-sheet in the arctic or Antarctica region are further advantages.
The docking of an AUV to an underwater platform or another unmanned system is a complex task that requires combined research and engineering work in different areas including underwater robotics, intelligent control, underwater communication, navigation and positioning as well as wet mateable underwater connectors.
Within the EURODOCKER project the basic technology necessary to dock an AUV to a submerged platform will be developed and demonstrated. The Docking-Downloading-Recharging system shall be suited to various AUVs that are now operational or under successful development.

2. THE EURODOCKER DOCKING-DOWNLOADING-RECHARGING SYSTEM

During the definition phase of the project the following major functional units for a submerged docking station have been identified:

- A Mechatronic Docking Device (MDDP). The MDDP will contain mechanical, hydraulic or mechatronic subsystems which must be able to operate in the marine environment and which have to be actuated and controlled.
- An Advanced Operator Interface. In the present state of AUV use, missions are ship supported. Hence the MDDP is also ship supported and the docking operations are supervised by an operator. Thus an advanced operator interface has to be part of the near-term version of the DDLR system.
- A Mechanical Docking Device (MDDV). The counterpart of the MDDP onboard the vehicle. It is unlikely to be active, hence the name "Mechanical Docking Device".
- A Docking Connector (DC). A wet mateable connector for data and energy transfer.
- An Acoustic Communication System. Before the docking operation the AUV will communicate with the mission management system of the docking station to request the docking. Further communication is necessary during the docking operation. Thus both the manually operated and the autonomous version of the DDLR system will rely on an

advanced acoustic underwater communication system that solves problems such as propagation loss, limited bandwidth, frequency selectivity, multipath and fading.

- An Acoustic Location System (ALS). After the vehicle has returned from a longer mission its position data, which are at the current state based upon dead-reckoning, are not accurate enough to find the docking station. Thus external determination of the AUV position is necessary.
- USBL Homing System. An Ultra Short Baseline Positioning System that guides the AUV relative to the docking station when the distance between both is short.

At the present state, AUV missions require launch and recovery operations in relatively short succession either to recharge or exchange the vehicle's batteries aboard the support ship. With a docking station equipped with a device like the EURODOCKER DDLR system an AUV mission will start and end at the docking station and will in between typically follow a general pattern like this:

- As with normal AUV operations the docking station and the AUV fixed to it are launched from a surface ship, or in case of a station on the seabed, the vehicle is safely docked to the station (Fig. 1).
- The AUV is released from the docking station. The AUV Mission Management System takes control over the vehicle and starts its mission.
- During the mission the AUV keeps contact to the docking station by means of an acoustic communication system.
- At the end of the mission the AUV uses its onboard navigation system, usually a dead reckoning system based on compass, speed log, etc., to return to the docking station. An Acoustic Location System which makes the advantages of GPS and DGPS available to unmanned underwater vehicles computes the vehicle's exact position and transmits the data to the AUV (Fig. 2).
- Now the vehicle can calculate a path towards the docking station. It then assumes the correct position and orientation for docking, aided by the docking station's USBL homing system. This is done by the Docking Management System which computes the docking manoeuvres and forwards them to the AUV's control system for execution, for example like a ground-based aircraft assistance during landing (Fig 3).
- The Mechatronical Docking Device grabs and latches its counterpart onboard the vehicle. Once the vehicle is mechanically docked the connector is mated.
- The vehicle's energy storage is recharged. Data gathered during the mission are transmitted aboard the docking station. New mission parameter are fed into the mission management system of the AUV.
- Fully recharged and with full capacity to store new data, the AUV can start a new mission.

3. STATE-OF-THE-ART IN UUV DOCKING

At the time the EURODOCKER project was initially defined, the concept of docking an AUV was already suggested as a topic for AUV research /2/.

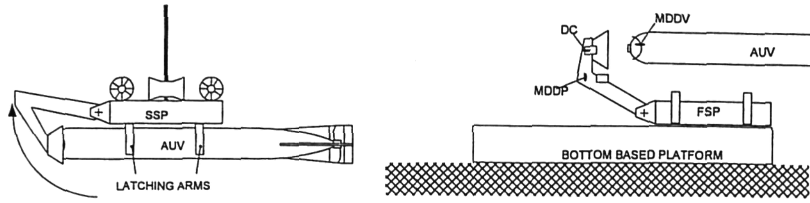

Fig.1: A possible EURODOCKER architecture for ship supported and bottom based applications

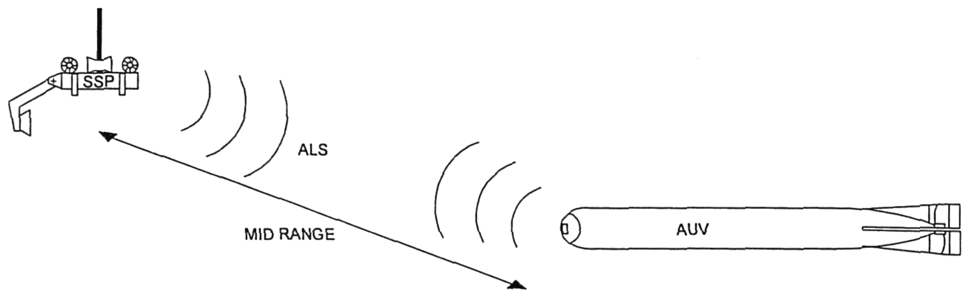

Fig.2: Approaching phase - Mid range - Use of ALS based on DGPS

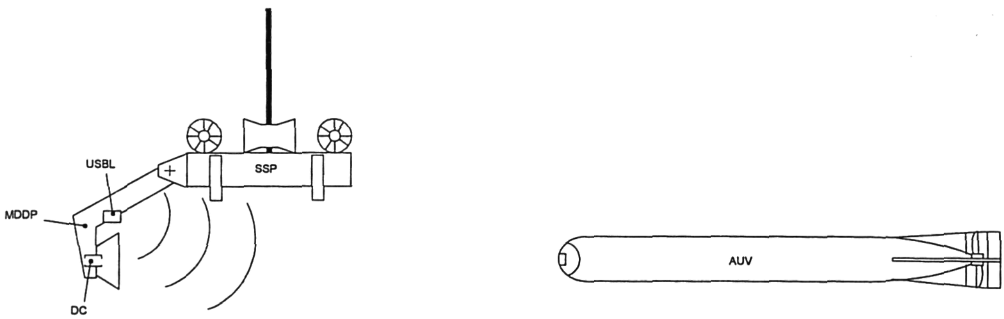

Fig.3: Approaching phase - Short-range - use of USBL homing system

Some of the required subsystems had already been developed for Remotely Operated Vehicles (ROVs). Among the different classes of ROVs some of the large work and support systems are equipped with docking clamps to fix the vehicle onto the workside. A less rigid connection can also be provided by using the vehicle‘s underwater manipulator. After it has established contact with the structure, sensor data from the manipulator joints are fed back into the control system of the ROV /6/.
Advanced concepts use ROV-mateable connectors for data transmission between the vehicle and an unmanned platform. Here simple mechanical guidance systems, for example two vertical cable ropes, are used to aid the operator /3/.
Meanwhile first trials for docking an Odyssey IIb AUV have been carried out within the

framework of the *Autonomous Ocean Sampling Networks (AOSN)* programme sponsored by the Office of Naval Research (ONR), USA. According to /1/ docking operations have been tested with a cone-like device the vehicle approaches and enters horizontally. A second technique employed a vertical pole onto which the vehicle latches. Whereas a cone allows for a relatively straight-forward establishment of the connection once the AUV is engaged a pole is insensitive to the approach direction and simplifies homing. For docking to the pole the AUV is equipped with a forked gripping structure mounted onto its nose. Two hoops, one above and one beneath the vehicle are centred on the hoop. After the AUV is attached, the bottom hoop rises to sandwich the vehicle against the top hoop. For the transfer of electrical power and Ethernet communications an inductive coupling technique is used.
This system is different to the EURODOCKER concept as the AUV is equipped with an active gripping device that demands higher efforts to integrate.
Also part of the AOSN is the concept of an expendable ROV that is linked to a support ship via an expendable fibre optic cable. This so called *Flying Plug* is guided into a socket onboard the submerged docking station to retrieve the data that stem from preceding AUV-missions. In addition to acoustical homing a light source is used to support the final docking phase.

4. EURODOCKER WORKPLAN AND STATE OF THE PROJECT

The core objectives of the project are to study the requirements of an underwater support to AUVs, relevant to re-energizing and data transfer, to identify and design both a ship supported and an autonomous Docking-Downloading-Recharging system, suited to the wide set of AUVs now under successful development, and to manufacture and test a prototype docking system. The project partner MARIDAN A/S will upgrade his AUV MARTIN 100[1] for the tests.
The final goal of the project is to provide the basic technologies and means to enter the world-wide market for commercial submodules for unmanned underwater vehicles with an advanced European product.
Thus the project has started with an extensive system engineering phase. During this initial phase the detailed requirements and the architecture of the EURODOCKER system will be identified based upon relevant data from AUV-related projects throughout the world.
This first task has highlighted the great variety of AUVs with regards to their primary tasks, dimensions, shape, propulsion system, etc.
For this reason, institutions and people involved in AUV development have been contacted and various meetings are planned to identify more specifically the needs of their projects for a standard AUV underwater support platform, either ship supported or sea-bottom based.
This survey encountered a prompt interest by the institutions contacted. These institutions will be kept informed along with the project with news on its advances in the mutual interest.

A detailed questionnaire has been prepared to identify basic requirements, such as:

- Operational scenarios (distance to shore, environment characteristics and water depth range)
- Typical AUV mission data (range, UUV/AUV mode shares, tasks)
- AUV main characteristics (size, shape, displacement and mass, attitude control characteristics, subsystem technology updates)

[1]. MARTIN will be exhibited at EXPO 98 in Lisbon

- Detailed DDLR system requirements of each project for near and long-term market prospects (energy type, energy storage capacity, data storage capacity, recharging power, charging process management requirements, etc.)
- Envisaged logistic support for the near and mid/long-term applications.

The information gathered will then be collected for a project database.

The best trade-off for near (3-5 years) and mid/long term (5-10 years) applications of the EURODOCKER system will be identified.
The requirements of the endusers will be monitored throughout the whole duration of the project.
The preliminary system architecture foresees a docking interface that consists of three major submodules: First a mechatronic docking device which will be combined later on with either a ship supported platform or a support platform that is part of a larger submerged station on the seabed. Second a mechanical docking device that is integrated onboard the autonomous underwater vehicle and third an advanced wet mateable docking connector for data and energy transfer between AUV and docking station.
An operator interface will be designed for the manual execution of the docking operation during the first tests and the monitoring of the automatic docking procedures during the final demonstration of the system's performance. A docking management system is responsible for the automatic docking operation. A computer simulation of the docking operation will be used to develop and test the management system software. System monitoring, failure detection, co-ordination of the AUV and the docking devices demand further high level control systems.
An acoustic communication system links the AUV and the docking station, so data can be transferred to and from the AUV in order to co-ordinate the docking manoeuvres. A precise high resolution positioning system allows the AUV to follow a suitable approach trajectory and to reach the correct hovering position for docking. The project ends with a series of sea trials to finally demonstrate the system performance.

5. CONCLUSIONS

The EURODOCKER project aims at developing and testing a submerged docking system for AUVs, relevant to re-energizing and data transfer. It will help to overcome some of the most prominent limitations of oceanological and commercial use of Autonomous Underwater Vehicles. The project aims to realise a practical standard Docking-Downloading-Recharging system, that shall be suited to various types of vehicles now operating or being under successful development.

ACKNOWLEDGEMENTS

The authors, on behalf of the whole EURODOCKER partnership, wish to express their gratitude to the Directorate General XII of the European Commission for the appreciation and final support of this project under the MAST III programme.

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TITLE: Advanced System Integration for Managing the Coordinated Operation of Robotic Ocean Vehicles (project ASIMOV)

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ADVANCED SYSTEM INTEGRATION FOR MANAGING THE COORDINATED OPERATION OF ROBOTIC OCEAN VEHICLES (ASIMOV)

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SUMMARY

The ASIMOV project is a three year research and development effort initiated in January 1998. The main objective of the project is twofold: i) enhancing and integrating proven technological systems to achieve the coordinated operation of an autonomous surface vehicle (ASV) and an autonomous underwater vehicle (AUV) while ensuring a fast communication link between the two platforms, and ii) using the systems developed to perform a series of scientific mission in the Azores, aimed at determining the extent of shallow water hydrothermalism and quantifying thermal fluxes and gas discharge rates at selected vent fields. The paper describes the scientific and technical objectives of the project and discusses some of the methodologies that will be used to accomplish them.

1. INTRODUCTION

Three major stumbling blocks have so far prevented demonstrating the potential applications of Autonomous Underwater Vehicle (AUVs) to demanding industrial and scientific missions. Namely, i) the lack of reliable navigation systems, ii) the difficulty of transmitting data at high rates between the AUV and a support ship at slant range, and iii) the unavailability of advanced mission control systems that can endow end-users with the ability to plan, program, and run scientific / industrial missions at sea while having access to oceanographic data in almost real-time so as to re-direct the AUV mission, if required.

As a contribution toward solving some of the abovementioned problems, this project puts forward the key concept of an Autonomous Surface Vehicle (ASV) operating in close cooperation with an AUV, thus acting as a mobile relay for fast communications; see the diagram in Figure 1. In the scenarios considered, the ASV will be

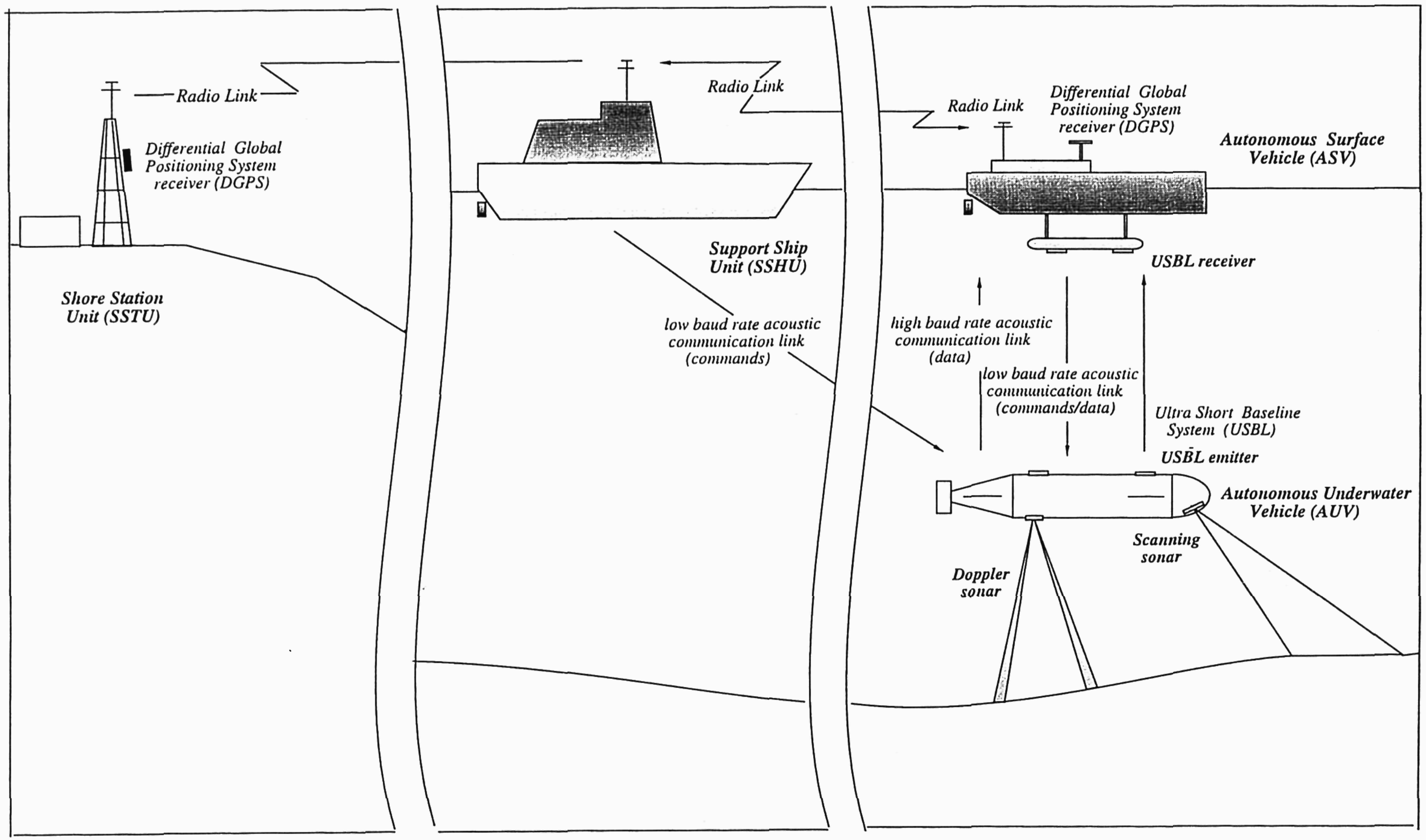

Fig. 1 Coordinated operation of robotic sea vehicles:
A step towards the development of Tele-Science Labs

equipped with a differential Global Positioning System (GPS) receiver, an ultra short baseline (USBL) unit, a radio link, and a high data rate communication link with the AUV that will be optimized for the vertical channel. Thus, by properly manoeuvring the ASV to always remain in the vicinity of a vertical line directed along the AUV, a fast communication link can be established to transmit navigational data from the DGPS and USBL unit to the AUV, and oceanographic data from the AUV to the ASV, and subsequently to an end-user located on board a support ship or on shore. Fast and reliable communications, as well as precise navigation, will thus be achieved by resorting to well established technologies.

The main thrust of the project is the enhancement and integration of proven technclogical systems to achieve the coordinated operation of the AUV and the ASV, while ensuring the integrity of the two platforms. To give the work greater focus, the final goal of the research and development effort is to perform a series of missions at sea - near the Azores islands, down to depths of 100 m - to determine the extent of shallow water hydrothermalism and quantifying thermal fluxes and gas discharge rates at selected vent fields. In the scenarios planned, the AUV will be required to maneuver close to the seabed and detect the occurrence of bubble emissions from the discharging vents. The detection of those phenomena will in turn trigger the acquisition and transmission - to the support unit - of time/space stamped sonar and video images through the vertical acoustic channel, via the ASV.

Obstacle avoidance and bubble detection will rely heavily on the development of a space-stabilized sonar head with vertical and horizontal transducer elements, and the associated signal processing algorithms. Programming, executing, and modifying on-line the plans for joint ASV/AUV operation will be made possible by developing dedicated systems for joint mission and vehicle control as well as appropriate Human-Machine interfaces. Special emphasis will be placed on demonstrating all the steps that are necessary to acquire, process, manage, and disseminate data on hydrothermal activity to a wide audience of scientists, over the Internet. The tests will be carried out with the *DELFIM* ASV and the *INFANTE* AUV, operated by the Instituto Superior Técnico.

2. SCIENTIFIC AND TECHNICAL OBJECTIVES. METHODOLOGY.

This section summarizes the scientific and technical contents of the project as well as the methodologies that will be used to achieve the objectives proposed. For the sake of clarity, the section includes a description of the general framework adopted for the coordinated operation of the AUV and the ASV. The reader is referred to [1] through [9] and the references therein for previous work carried out by members of the project team in this and related areas.

2.1 General System Organization for Coordinated ASV / AUV Operation. Mission Management and ASV / AUV Mission Control.

The general functional diagrams for advanced system integration and management of the coordinated operation of the ASV and AUV are depicted in Figures 2 and 3, which should be interpreted in light of the scenario summarized in Figure 1.

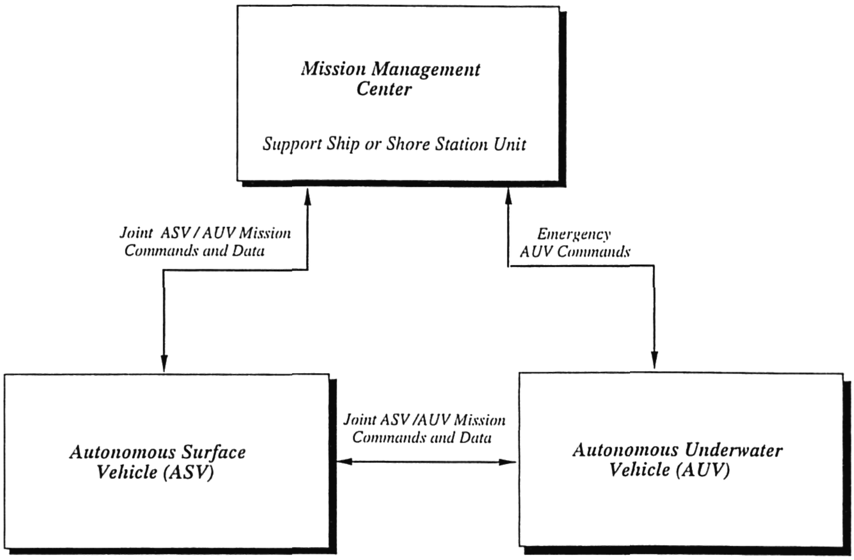

Figure 2. General System Organization

The Mission Management Center (MMC), installed on-board a support ship (or on shore), is the vital center for managing all the phases of coordinated vehicle operation. The MMC hosts the computers in charge of implementing a Mission Management System (MMS) that is responsible for the Mission Preparation phase. During this phase, a dedicated system allows an operator without detailed knowledge of the technical aspects of robotic ocean vehicles to program a desired mission in a high level language, have it checked for consistency, and translated into a mission program that can be compiled, downloaded to, and run in real time in the computers installed on-board the ASV and the AUV. During the mission execution, the MMS enables the operator to play a very active role in assessing the state of progress of the mission and modify the mission objectives, if required, based on mission-related and field data received from the AUV through the fast communications channel.

Each vehicle hosts a kernel of a Joint ASV / AUV Mission Control System that is in charge of accepting the joint mission program provided by the Mission Management System and scheduling and coordinating the concerted actions of the two vehicles, while enabling on-line intervention from the end-user. The corresponding flow of programs, commands, and data (in real time) is illustrated in Figure 3, that brings out the important role of the distributed Joint ASV / AUV Mission Control System as the main coordinator of the ASV / AUV ensemble behaviour. As seen in the figure, each vehicle hosts a local Mission Control System that is responsible for guaranteeing its own integrity, controlling its dynamic motion and the acquisition / transmission of scientific data, as well as interfacing itself with the respective Joint ASV / AUV Mission Control System kernel.

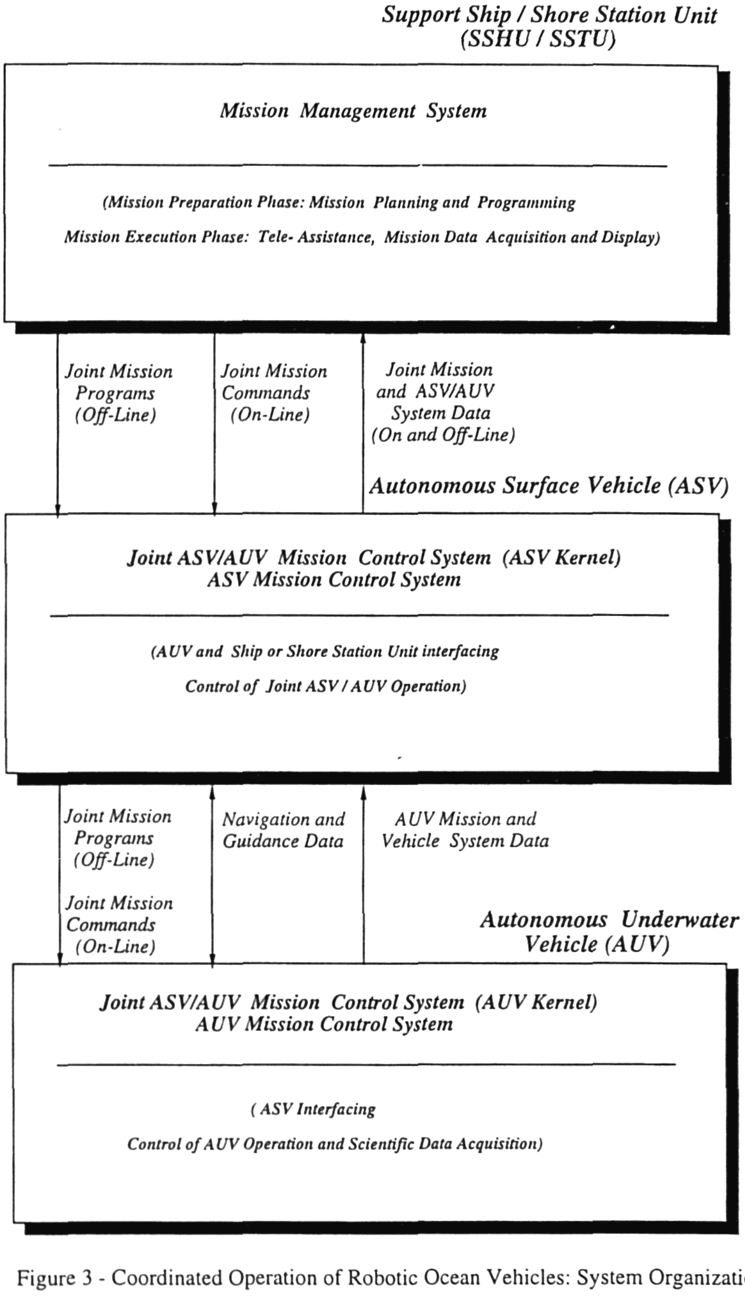

Figure 3 - Coordinated Operation of Robotic Ocean Vehicles: System Organization

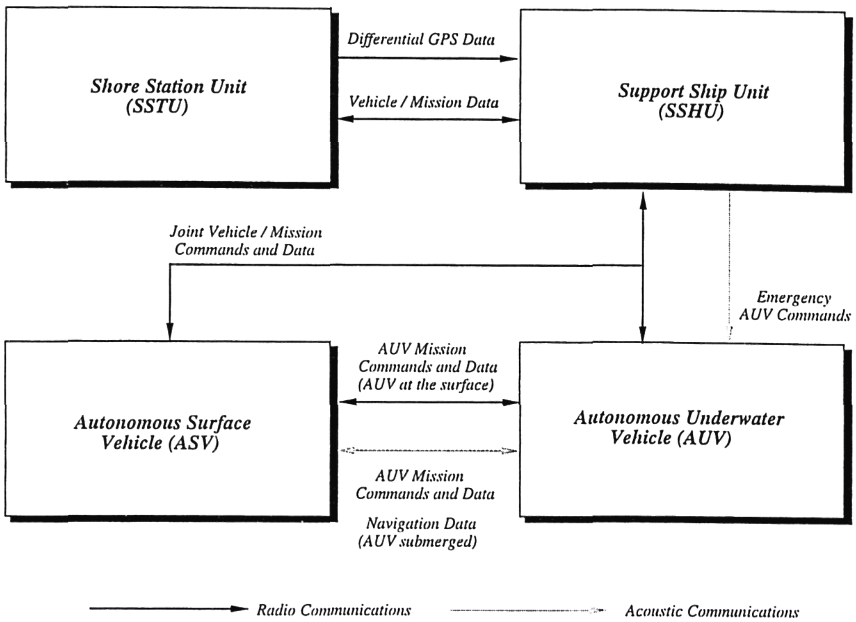

Figure 4. Radio and Acoustic Communications Link

2.2 The Communication and Navigation Systems.

Underlying the concerted operation of the two robotic vehicles are the radio / acoustic communication links depicted in Figure 4 and the DGPS / USBL navigation system that allows positioning the AUV with respect to an inertial reference frame without resorting to the cumbersome deployment of arrays of transponders; see Figure 5.

The radio link ensures communications between a support ship (or shore station) unit and the ASV, or with the AUV when it resurfaces. The communication link consists of a low data rate down link for emergency commands issued directly to the AUV, and a high data rate uplink to transfer oceanographic data from the AUV to the ASV. It is assumed that during operation the AUV and the ASV stay approximately along the same vertical line, so that the uplink may explore all the advantages inherent to vertical transmission. By operating at sufficiently high carrier frequencies and using video compression systems that are available off the shelf, it will be possible to even transmit video images at a sufficiently fast rhythm to allow the operator to intervene directly during mission execution based on the analysis of the data received.

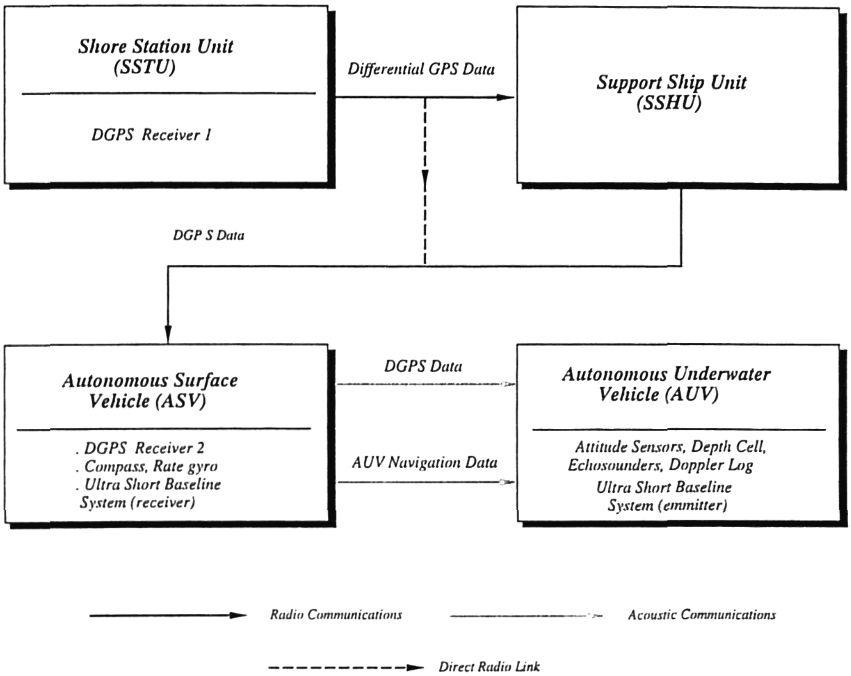

Figure 5. Navigation: Motion Sensors and Data Network
(AUV submerged)

2.3 The AUV System Organization. Advanced Sonar System.

The general system organization of the Autonomous Underwater Vehicle (AUV) that will work in close cooperation with the Autonomous Surface Vehicle (ASV) is depicted in figure 6. *The items marked A are enhancements of the systems that are available at the beginning of the project.* The following systems and respective interconnections can be identified [6, 7]:

Vehicle Support System (VSS) - The Vehicle Support System controls the distribution of energy to the electrical and electromechanical hardware installed on-board the vehicle and monitors its energy consumption. This system is also in charge of initializing all subsystems and, during operation, of detecting basic hardware failures and triggering emergency reflexive manoeuvres whenever required.

Actuator Control System (ACS) - The Actuator Control System is responsible for controlling the speed of rotation of the propellers and the deflections of the rudders, bow and stern planes. Actuator set points are provided by the vehicle's Guidance and Control System, in response to a mission related data file resident in the Mission Control System, or in response to the Autonomous Surface Vehicle (ASV) motion when this vehicle is in control of the operations. Actuator data (e.g., tracking errors of the propeller and surface control systems) are fed back to the Mission Control System for vehicle status assessment.

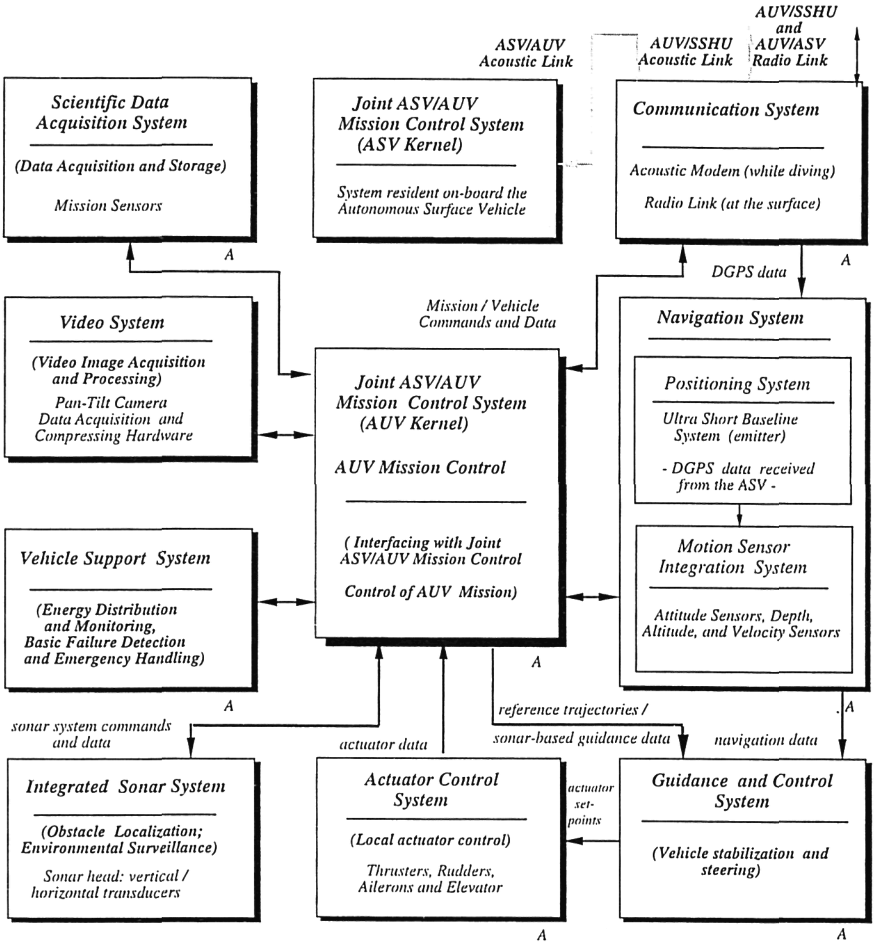

Figure 6. Autonomous Underwater Vehicle: System Organization
(The label A denotes that the systems are enhancements of existing ones)

Navigation System (NS) - The Navigation System provides estimates of the linear position and velocity of the vehicle, as well as of its orientation and angular velocity with respect to an inertial reference frame. This system merges information provided by the Positioning System and a Motion Sensor Integration System. The basic motion sensor package includes the following units:

1. *Attitude and Heading* reference unit consisting of rate gyros, pendulums, and a fluxgate compass.
2. *Depth Meter* (depth cell)
3 *Altitude and Bottom Profile Probes* (2 echosounders)
4. *Velocity Sensor* (Doppler Log)

In one envisioned scenario, the Positioning System will obtain updates on the inertial position of the vehicle, by relying on data available from an Ultra Short Baseline unit and a DGPS unit that will be transmitted over the acoustic channel from the ASV to the AUV. The outputs of the Navigation System are fed back to the Vehicle Guidance and Control System and sent to the Mission Control System for vehicle performance assessment.

Guidance and Dynamic Control System (GCS) - In a classical Vehicle Guidance and Control System, the inputs are reference paths issued by the Mission Control System and navigational data provided by the Navigation System. The outputs are commands to the Actuator Control System (set points for the speed of rotation of the propellers and deflection of the surfaces) so that the vehicle will achieve robust, precise trajectory following in the presence of shifting sea currents and vehicle parameter uncertainty. The proposed architecture goes on step further, and includes explicitly the situation where sonar data can be used directly for guidance. This extra functionality is included to perform the task of bottom following and / or obstacle avoidance, when the vehicle maneuvers close to the seabed. When required, this system is also in charge of ensuring that the AUV follows a trajectory traced by the ASV, in a slave / master configuration.

Acoustic Communications System (ACOMS) - The Acoustic Communications System is a bilateral digital acoustic link that is used by the operator to send new mission directives to the Mission Control System, and by the vehicle to relay back information regarding its status or mission related data [1,2,7]. Typically, short messages are sent across the acoustic channel, such as sensor readings, mission requested user commands and user requests for data. In the scenarios envisioned, however, this system will also guarantee high data rates in the uplink, in order to transmit sonar and video image data at rates that will allow the operator to re-direct the AUV mission, based on the analysis of the data received.

Scientific Data Acquisition System (SDAS) - The Scientific Data Acquisition System is designed to collect data from a suite of environmental sensors that may include units to measure conductivity, temperature, pressure, turbidity, fluorescence, oxygen and pH. Data acquisition is triggered by the Mission Control System. Selected data can be stored for post-mission analysis or sent to the operator via the fast link established through the ASV.

Integrated Sonar System (SS) - The purpose of the Sonar System is twofold: estimating the terrain features ahead of the vehicle, for safe manoeuvring above a possibly complex environment of canyons and rocks, and acquiring environmental data to assess the existence of hydrothermal activity, via bubble detection. The key element in the above system is a 3-axis stabilized sonar head with one narrow searchlight beam pointing downwards at slant range, and one steerable vertical fan beam, either one of which can be selected for use at any time. Interfaced with an obstacle avoidance module and an environmental data processing module, this system ultimately provides relevant data to the Guidance and Control System for vehicle steering, and to the Mission Control System to trigger the acquisition of video images.

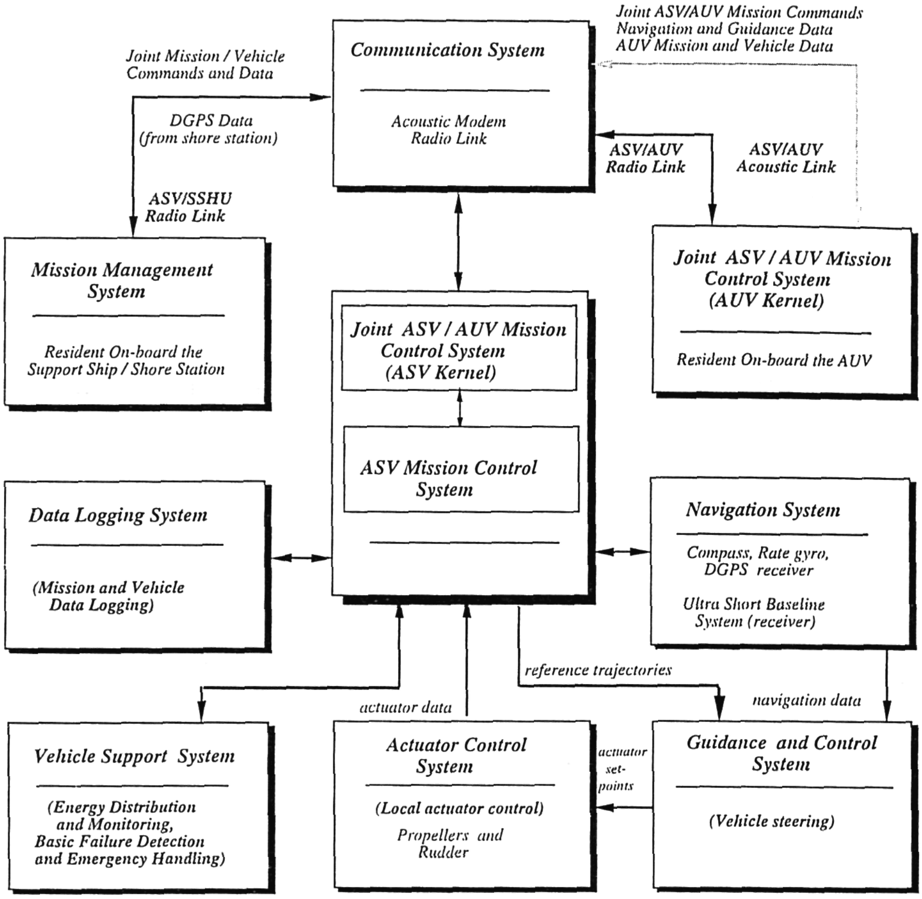

Figure 7 - Autonomous Surface Vehicle: System Organization

Video System (VS) - The Video System acquires, compresses, and stores data for further processing. When enabled by the Mission Control System (for example, upon detection of bubbles by the sonar system), compressed video data will be sent to the operator / scientific end-user through the acoustic channel, via the ASV.

Mission Control System (MCS) - In its simplest form, the role of the (AUV) Mission Control System can be described as follows:

> accept detailed AUV Mission Programs and AUV Mission Commands issued by the Joint ASV / AUV Mission Control System (see Figure 3), and schedule and coordinate the concerted operation of the vehicle systems, including those in charge of scientific data acquisition, while ensuring the integrity of the platform and its safe recovery from malfunctions.

In the mission scenario off the coast of the Azores, the AUV Mission Control System will direct the AUV to operate as a slave of the ASV, during the transit phase to an inspection site. However, once a survey phase gets started, the AUV will attribute the highest priority to the safety of the vehicle, which will then act as a master for the ASV.

2.4 The ASV System Organization.

The ASV system organization follows closely that adopted for the AUV; see Figure 7, which illustrates clearly the interactions of the ASV systems with the Mission Management Systems installed on-board the Mission Management Center, and with the AUV Mission Control System installed on-board the AUV. Except for the Joint ASV / AUV Mission Control System, all the blocks bear great affinity to those explained for the AUV.

2.5 Scientific Missions. Data Acquisition and Management.

The technical work proposed is firmly rooted in very specific missions that will be carried out with the AUV and ASV vehicles in the Azores, with the objective of determining the extent, distribution, and types of shallow water submarine hydrothermalism in selected areas around the islands.

In recent years, there have been an increasing number of reports on submarine hydrothermal systems on mid-ocean ridges and in back-arc basins. However, these systems are present in very deep water and are only accessible using manned submersibles or costly ROVs. Reports of shallow water submarine hydrothermal activity have been much less frequent, even though such occurrences can often be observed directly by scuba divers or with cheap ROVs. Many of the most important features of submarine hydrothermal systems, such as their composition, thermal and chemical flux rates, etc. can be examined equally well in shallow water at a much lesser cost, and therefore their study warrants further attention. Most of the work done on shallow water submarine hydrothermal systems has been carried out in the Mediterranean Sea. Here, submarine hydrothermal vents can be found along the Hellenic Volcanic Arc, mainly off Milos, Santorini and Kos/Nisseros, in water depths ranging from a few centimetres to more than 100 metres [3,4].

Identical systems occur in the Azores, but their study is still at a very early stage. This is a matter of great concern in view of the fact that new volcanic activity is a major potential hazard in the Azores and submarine hydrothermal venting may have a significant impact on the quality of fisheries and on the coastal zone [8]. Thus the importance of obtaining background data on the area and volume of submarine hydrothermal venting around the islands. Furthermore, the characterization and location of accessible sites close to the coast can be a major asset to scientists studying the bacteria and bacterial symbioses of possibly high biotechnological potential, which are endemic to such environments. The work proposed in the scope of this project is an advance in the state of the art in submarine hydrothermal research in that it will attempt to determine the extent of shallow water hydrothermalism and quantify thermal fluxes and gas discharge rates at selected vent fields using highly mobile stations.

Figure 8 describes the location of the Azores islands and the bathymetry of the D. João de Castro bank, where the final sea trials with the ASV and the AUV will take place. In the missions planned, the AUV will be instructed to conduct a survey of a given area, at a fixed depth, while avoiding unexpected terrain features. In this

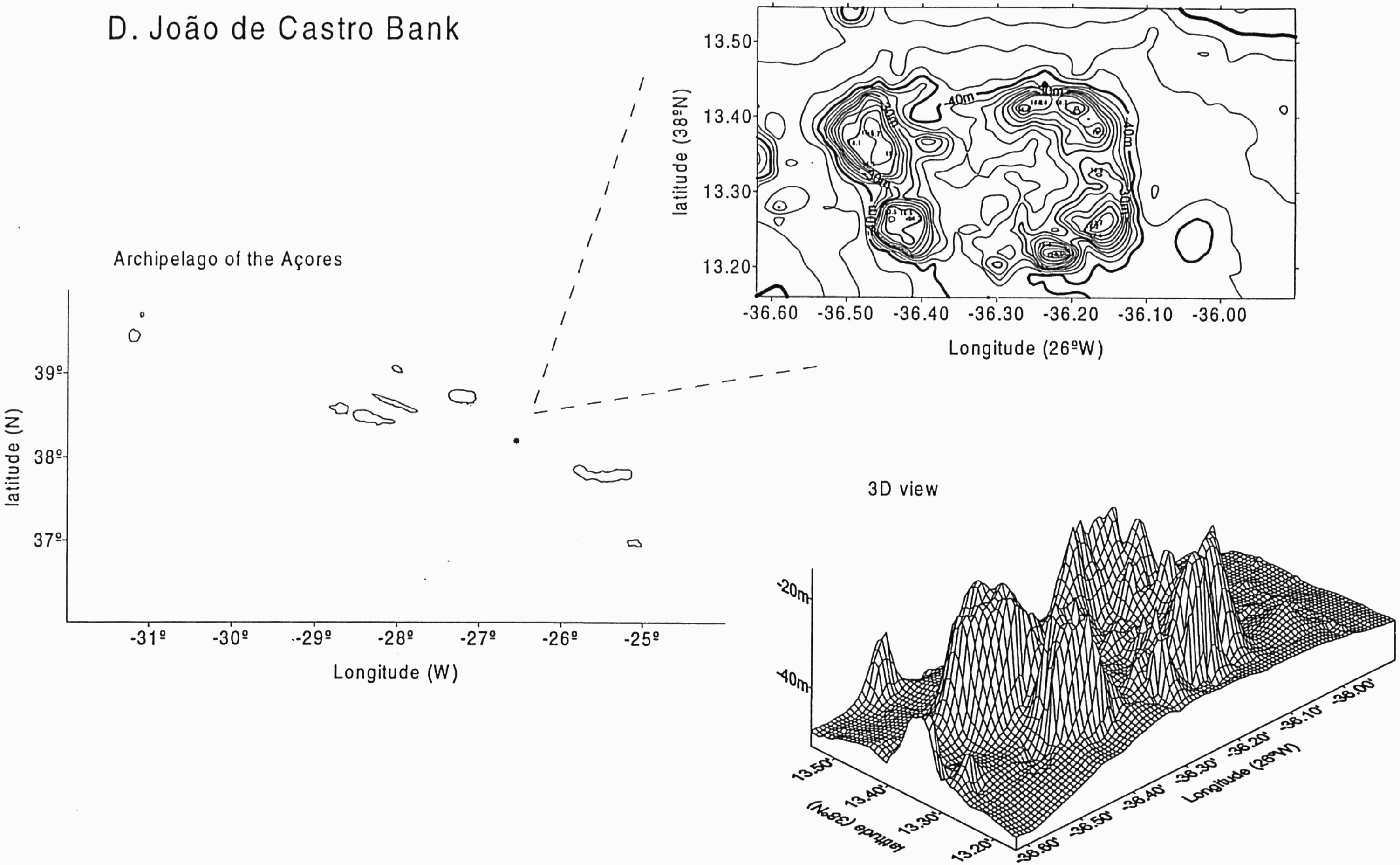

Fig. 8 The D. João de Castro Bank (Azores, PT)

scenario the AUV will act as a master of the ASV, probe the water column for the occurrence of bubbles and trigger the transmission of time / space-stamped sonar and video images to a scientific end-user who will be free to re-direct the joint ASV / AUV mission.

The project addresses also the very important problem of scientific data management, which aims at collecting the mission data acquired, processing it, and making it available to a wide audience via the World Wide Web. To that effect, emphasis will be placed on the problem of developing adequate algorithms for video and sonar image processing, with the objective of determining the size, velocity, and structural modification of the bubbles that emanate from active vents. Video image processing will also be used for remote detection of benthic communities.

2.6 *System Integration*

The complexity inherent to the development and integration of all the vehicle subsystems described above can hardly be overemphasized. As a consequence, considerable care will be taken to ensure that the process of system development and system integration will be guided by clear, efficient rules aimed at making their development modular and their integration simple. Software quality control plays a fundamental role in this process, since all vehicle systems are ultimately controlled and interact with each other by using software code that runs on target computers, in real-time. Therefore, strict rules have been issued for software development and integration.

The basic premise adopted is that no software from two different partners will run on the same target machine, in the same environment. The systems to be developed by the partners will reside on different machines, and will communicate through a set of well defined, fixed messages, using the existing vehicle's local area network (CAN Bus). This is possible, in view of the of : i) the hierarchical organization adopted for the vehicle's mission control / mission management system [6,7], and ii) the distribute computer architecture implemented on-board the AUV. By adopting this basic rule, software development becomes modular and the task of total software system integration is greatly simplified. Clear rules will also be followed for the development of each partner software package. Namely, using object oriented programming tools (e.g. C++) to make software development modular, efficient, and easily comprehensible. Procedures agreed upon by all partners at the beginning of the project will be adopted to evolve towards the implementation of automatic mechanisms for controlling different software versions, with the objective of simplifying the task of documenting and making the software available to a wide audience; e.g. using tools akin to the "Source Code Control System (SCCS)" of Unix.

In what concerns the development of the Mission Management and the Joint AUV / ASV Mission Control Systems, the following guidelines will be observed in order to make their development modular:

"Low level" layer (direct interfacing with physical systems - input of external sensor data and output of actuator / device commands) - based on a network of local processors, where each processor has a very limited and well defined functionality. The processors are nodes in a local area network. This modularizes and simplifies the task of developing hardware/software for interfacing with all physical systems installed on-board the vehicles.

"Medium level" layer - in charge of executing: i) the algorithms that implement basic Vehicle System Tas (e.g., navigation, control, and obstacle data acquisition), ii) more advanced Vehicle Primitives that are obtain by coordinating the concerted operation of a number of System Tasks (e.g., following a straight line at giv depth and speed, with a certain heading), and iii) the discrete-event systems that control the calling of differe Vehicle System Tasks, as embodied into specific Petri net structures [6]. This hierarchical organization h proven efficient in the past [6,7], and is specially suited to keeping the software development modular. Tl medium layer level will be fully implemented on well-proven GESPAC machines, running the OS-9 operatir system, which allows for real-time multi-tasking operation, process and memory management, and interproce communication facilities that include shared memory and events.

"High level" layer - in charge of controlling the activation of the different Vehicle Primitives, as determined t Mission Programs, and in response to external events. Its design will be modular; its implementation will t done utilizing high level languages and object oriented programming. This will allow controlling ar accelerating the development of software, as well as reducing the occurrence of programming errors.

"High level graphical interfaces" (for mission programming and mission follow-up) will be developed usin environments that allow for the use of object oriented programming methodologies and ensure portability (e.; C++ and Java).

3. CONCLUSIONS

The paper described the main objectives of the ASIMOV project, a three year research and development effo that focuses on advanced system integration for the coordinated operation of robotic ocean vehicles. The fin goal of the project is to operate two prototype vehicles - the INFANTE Autonomous Underwater Vehicl (AUV) and the DELFIM Autonomous Surface Vehicle (ASV) - in the Azores, with the objective of determinin the extent of shallow water hydrothermalism and quantifying thermal fluxes and gas discharge rates at selecte vent fields.

4. ACKNOWLEDGEMENT

We gratefully acknowledge the contribution of the following colleagues, who were instrumental in putting th ASIMOV proposal together: P. Oliveira, C. Silvestre, V. Barroso, and L. Sebastião - ISR/IST; J. Coudeville ORCA Instrumentation; Joel Champeau and Valerie Lemarquand - ENSIETA; Eric Maillard - GESMA; J Goncalves - IMAR.

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TITLE: YOYO 2001: Ocean Odyssey

CONTRACT NO: MAS3-CT97-0130

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yoyo 2001 : Ocean Odyssey

ꞁBSTRACT

ıeral description of project yoyo-2001- ocean odyssey

An autonomous profiling instrument package will be developed which will provide a e range of oceanographical data over the top 1000m of the water column. Data will be smitted in near real time to a shore base by satellite link on fundamental physical, bio-optical, chemical and biological variables with a temporal resolution of 24hrs or less and a duration p to one year.

The scientific background to this proposal lies in the urgent requirement to provide a long ı monitoring capability of the complex physical and biogeochemical interactions of the ıns particularly those with relevance to carbon cycling and climate modelling. Such processes ɹr at significantly higher rates in the upper mixed layer and as such, increased data coverage be obtained in the top 100m of the water column.

The project presents some major technological challenges both in terms of development ovel sensors, data transmission and the profiling vehicle itself.

ides the necessary modifications to the existing profiling vehicle, a major effort is to be ɔted to the mooring itself: the surface buoy and the mooring line. A new surface buoy is to be ɜloped. The sensor package is a major innovation. The physical and bio-optical sensors need e interfaced to the profiler (mechanically, electronically). Other sensors have to see their ɹumption reduced before adaptation to a profiler (optical biomass). Others do not exist and ɔeing developed as prototype in academic laboratories, this is the case for the nutrient sensor, ɔCO2 sensor and the trace metal sensors.
system will be totally integrated. It means that the profiler will supply the energy for the ors and the central brain of the vehicle will command each sensor when it is time to make the surement, will receive back the data, will store it in memory and send it at the proper time for ɜmission. Data transmission from the vehicle to the buoy presents yet another technological lenge. On the other hand, data transmission from the buoy to land is more standard

Seven leading research laboratories and one industrial partner from 4 countries will ıborate to provide the required blend of skills. Furthermore the project will ensure effective ication and dissemination of the novel technologies for use elsewhere in the coming decades.

1 OBJECTIVES AND METHODOLOGY

Objectives:

An autonomous system able to profile from 1000 m up to the very surface at a fix location is to be developed and tested out. The instrument will be capable of gathering fundamental physical, bio-optical, geochemical and biological observations describing the oce state, over the whole water column with a particularly refined resolution in the upper 100 mete The system will be equipped so that it can transmit its measurements in real time to shore (af each profile). The instrument will be able to maintain a multidisciplinary time-series stati autonomously.

There are many advantages of such a profiling system over a traditional mooring: i) permits a fine vertical resolution (vertical changes occurring over short distances (e.g. 1 met cannot be examined by fixed sensors) ii) only one sensor covers the whole water column (sens cost and calibration work are greatly diminished).

The scientific objective behind this development is to monitor over the mixed layer a the main thermocline the complex physical and biogeochemical interactions governing carbon cycle and its associated elements in the ocean.

Methodology:

The propulsion system to be used is based on volume changes : it provides a profili vehicle decoupled from the cable and allows regulation of the profiling velocity (Provost and Chaffaut, 1996). Necessary modifications to the propulsion system are minor. On the other hai a major effort is to be devoted to the mooring itself: the surface buoy and the mooring line. new surface buoy is to be developed. This new buoy should allow the cable to be attached abc the sea surface, and should be constructed in order to experience small vertical motions.

The sensor package is a major innovation. The physical parameters to be measu include pressure, temperature, salinity. The bio-optical observations comprise the up and do irradiences. The geochemical observations include oxygen, nitrates, phosphates, silicates, pC and some trace metals (Cd, Fe). Biological observations are provided by an optical plankt counter.

The system is to be totally integrated. It means that the profiler will supply the energy the sensors and the central brain of the vehicle will command each sensor when it is time to ma the measurement, will receive back the data, will store it in memory and send it at the pro time for transmission. Ideal sensors should have the following properties: required accura short response time, low consumption, relatively small size, ability to sustain 100 bar pressu and numerical recording of processed data. Physical and bio-optical sensors exist which sati those requirements. They need to be interfaced to the profiler (mechanically, electronically). T optical plankton counter has to see its consumption reduced before adaptation to a profiler. T nutrient, pCO2 and trace metal sensors are being developed as prototype in acader laboratories.

2. TASK STRUCTURE OF THE PROJECT

The development will be undertaken through implementation of the following 4 work packages and their corresponding activities:

1 WORK PACKAGE 1. THE CARRIER SYSTEM.
1.1 Surface Buoy
1.2 Mooring line
1.3 Data transmission
1.4 Profiler

WORK PACKAGE 2. SENSORS
2.1 Physical sensors
2.2 Bio-optical sensors
2.3 Nutrient analysers
2.4 Trace metal sensors
2.5 pCO2 sensor
2.6 Optical plankton counter
2.7 sensor integration

WORK PACKAGE 3. FIELD TESTS
3.1 Coordination of field tests at the Casablanca oil rig.
3.2 Coordination of field tests at the Blanes Canyon
3.3 Coordination of field tests with the Minibat
3.4 Coordination of field test with the mooring line and surface buoy
3.5 Coordination of the final test
3.6 Tests at the Casablanca oil rig
3.7 Tests at the Blanes Canyon
3.8 Tests with the minibat
3.9 Tests of the mooring (mooring line + surface buoy - no profiler)
3.10 Open ocean test profiling to the surface with all sensors

WORK PACKAGE 4. DATA MANAGEMENT
4.1: data collection
4.2: data format and quality control
4.3: data banking
4.4: data bank update and data exchange

III.2.2. Oceanographic measurement and sampling equipment

TITLE: Operational Modelling for Regional Seas and Coastal Waters (OPMOD)

CONTRACT NO.: EUREKA EUROMAR EU 429

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Operational Modelling of Coastal Waters and Regional Seas: Application to the Tagus Estuary

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1 Introduction

The task of the EUREKA EUROMAR project OPMOD (**Op**erational **Mod**elling for Coastal Waters and Regional Seas) was to develop an operational system consisting of measurement devices, data links, data evaluation, and numerical models. Partners included organisations from Spain, Portugal, France, Finland, Sweden, Norway, and Germany.

The system was developed for estuaries, coastal waters and marginal seas individually by the participants but with close coordination as individual tasks, dynamic processes as well as fields of application, standards and interests vary locally.

This paper describes the OPMOD application to the Tagus estuary.

The Tagus Estuary is the largest in Europe, presenting complex hydrodynamic and ecological features. The population in the vicinity of the estuary exceeds 2 million people. Economic activities generate important ecological stress and often do have contrasting interests.

The coastal areas adjacent to the Tagus Estuary - Estoril Coast and Costa da Caparica respectively northward and southward - support an important recreational and tourism industry. The port of Lisboa is a major Portuguese commercial and fishing Port and the Tagus river basin is an important agriculture area. The estuary also boasts a very important natural area where a big number of migrating bird species breeds.

The model described in this text is a tool to support the understanding of the estuarine ecosystem and to predict its evolution according to different management scenarios. The hydrodynamical model is the basic part of this management tool. Some results are presented to illustrate the application.

2 The Estuary

The Tagus Estuary is represented in figure 1. Its surface is about 320 km^2, the length from mouth to the tidal propagation limit is about 80 km and the width can reach 15 km in the inner estuary. The mean depth is about 10 m, but maximum depths can reach 45 m at the mouth and in the 4-km wide channel entrance. The mean river flow is 300 m^3/s and the limit of the saline intrusion is 50 km (Vila Franca de Xira). Very large tidal flat and tidal marsh areas exist in the inner southern bank with a total area of about 130km^2. Most of these areas are integrated into the nature reserve of the estuary.

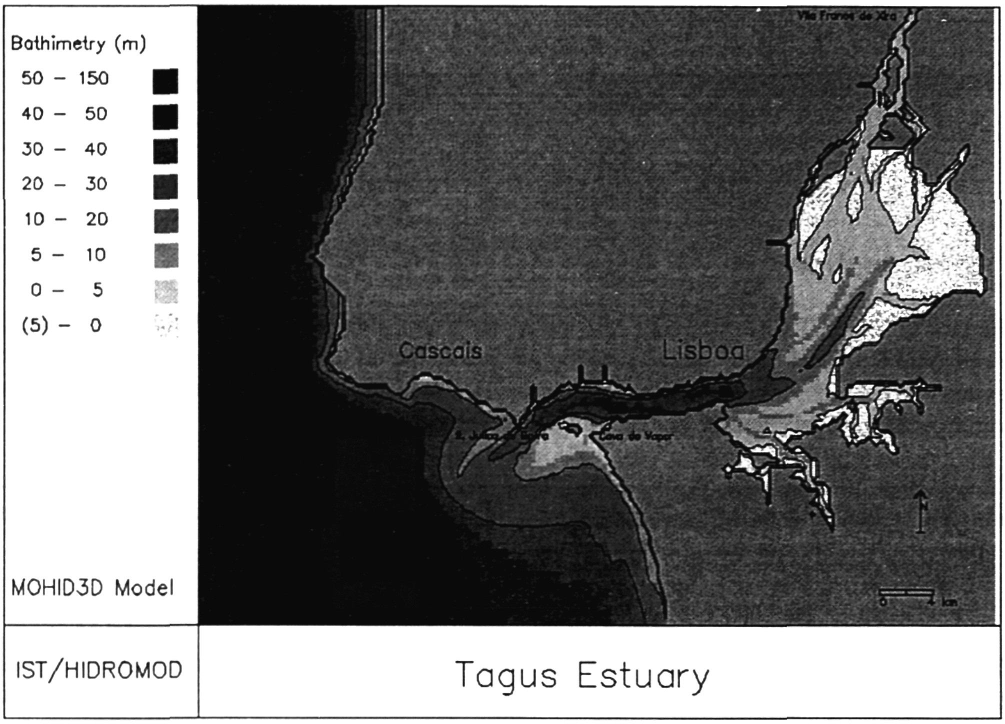

Figure 1, Tagus Estuary.

Currents are mainly tidally driven. Typical values are around 1 m/s, but 3 m/s can be reached at the mouth, during spring tides. The most important tide components are M2 (0.98 m), S2 (0.30 m) and N2 (0.22 m). Residual velocities can reach 0.15 m/s at the mouth and very large and well defined eddies are well correlated with topographical features.

Density effects are of secondary importance. They are confined to the upper estuary, except during the rainy seasons, when the river flow can reach 3000 m^3/s. The Tidal time lag between the mouth and the upper estuary is about 1 hour. Between Cascais and Lisbon the time lag is about 1/2 hour, with lower values at low slack water generating stronger currents during ebb.

3 The hydrodynamic model

A 3D hydrodynamic model, MOHID3D, is used to simulate both the hydrodynamics and ecological parameters. The model uses a non-regular rectangular mesh with 300 meters minimum step. A σ vertical grid is used. At the surface and bottom boundary layers the vertical resolution is of higher order to resolve more accurately the areas where larger velocity gradients are expected.

Five types of boundaries can be present in one application: free surface; bottom interface; lateral closed, lateral opened, and moving boundaries.

- In the free-surface boundary momentum, mass and heat fluxes are imposed.
- At the bottom fluxes are also imposed. Water and heat fluxes are assumed to be zero and momentum is calculated using a quadratic law.
- Closed boundaries are located at the land water-interface. In real domains the closed boundaries' surface is much smaller than the bottom surface and the horizontal resolution is not fine enough to resolve horizontal boundary layers. In these conditions impermeable and free slip conditions are used.
- Lateral open boundaries are artificial and confine the computation domain to the region of interest. This procedure saves computation resources but care must be taken to minimize the influence of the boundary condition errors on results. The condition to impose depends on the region where or the reason why the border has been set. For barotropic calculations two types have been implemented: imposed water flow in the river boundary; free surface elevation, in the tidal boundaries. Salinity and temperature are also imposed in baroclinic simulations when the flow enters into the modeling area. Values are modified during ebb and relaxed to typical values during flood.
- Moving boundaries are closed boundaries arising in domains with inter-tidal zones. They are closed boundaries whose position depends on the water level. A cell is considered as being dry whenever the water column is shallower then 5 cm and lower then the neighboring cells.

In this application to the Tagus Estuary we have two open boundaries (i), the Tagus river where 300 m^3/s was imposed and (ii), the Atlantic boundary where the tide level was imposed. The model was calibrated with data from tide gauges located inside the estuary.

4 Results

At the Atlantic border the sea level is imposed (Cascais' sea level). To calibrate and validate the model water levels from tide gauges inside the estuary were used. As an example figure 2 shows level generated by the model and taken from a tide gauge close to Lisbon. Another way to validate this model was by comparison of their results with these from MOHID2d (former bi-dimensional version of the MOHID system). With this 3D model results enhance where the vertical structure is more complex, for instance:

- In bends or over sharp bathymetry steps, where secondary flows are present;
- In areas with strong residual velocity (see below), where bottom and surface velocities eventually disagree;
- Upstream, where stratification may occur.

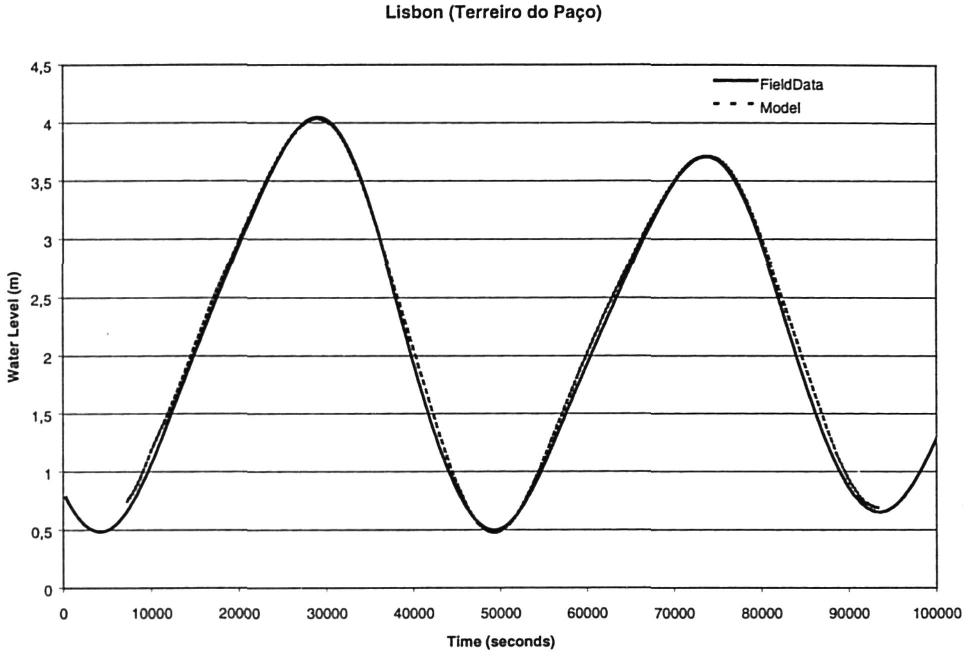

Figure 2, comparison between model and field data water level.

Residual velocities presented in Figure 3 (surface velocities) were obtained through time integration of transient velocities. Residual velocities do not usually provide much direct information but they can be helpful to understand long-term phenomena with time scales much larger than the tidal period. There is a jet outward the estuary associated with a strong anticyclone off Costa do Estoril; a cyclone and an anticyclone inside the channel reveals a very complex hydrodynamic system coupled with the topography.

Figure 4 shows instantaneous surface velocities during ebb (5h 44m after high water, tide amplitude 3m). The maximum velocity occurs in the channel. This figure shows the Cascais' bay periodic anticyclone (it appears during ebb time) and the outward jet. These features, also visible in the residual velocity (figure 3) have a strong influence in the bathing coastal area of Cascais; because of this gyre the estuarine ebb water weakly affects the area. Model results (and other field studies) strongly suggest that water quality in this area depends first of all on the proper control of local pollution sources.

5 Outlook

The model system described here is part of a system for monitoring the water quality in the vicinity of a subsurface effluent outfall. This comprises improvement of the coupling of measurement, data transfer and modelling. Statistical information about the dynamic variability and the variabvility of hydrographic parameters will be derived in future investigations to improve long-term operability and reliability of coastal operational models.

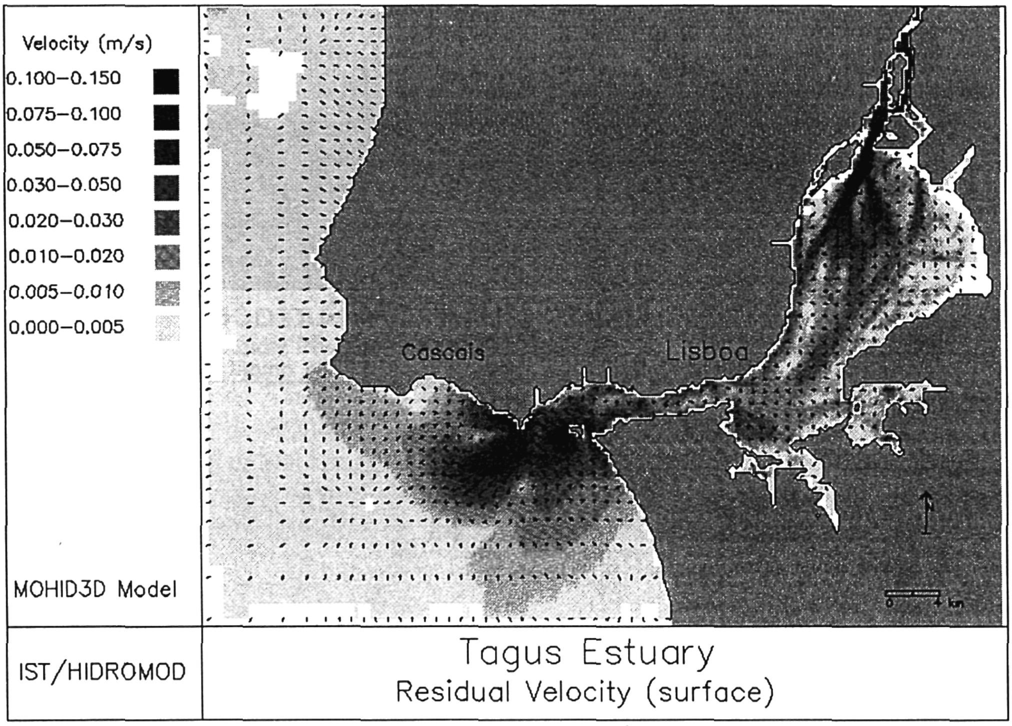

Figure 3, Tagus Estuary surface residual velocity field.

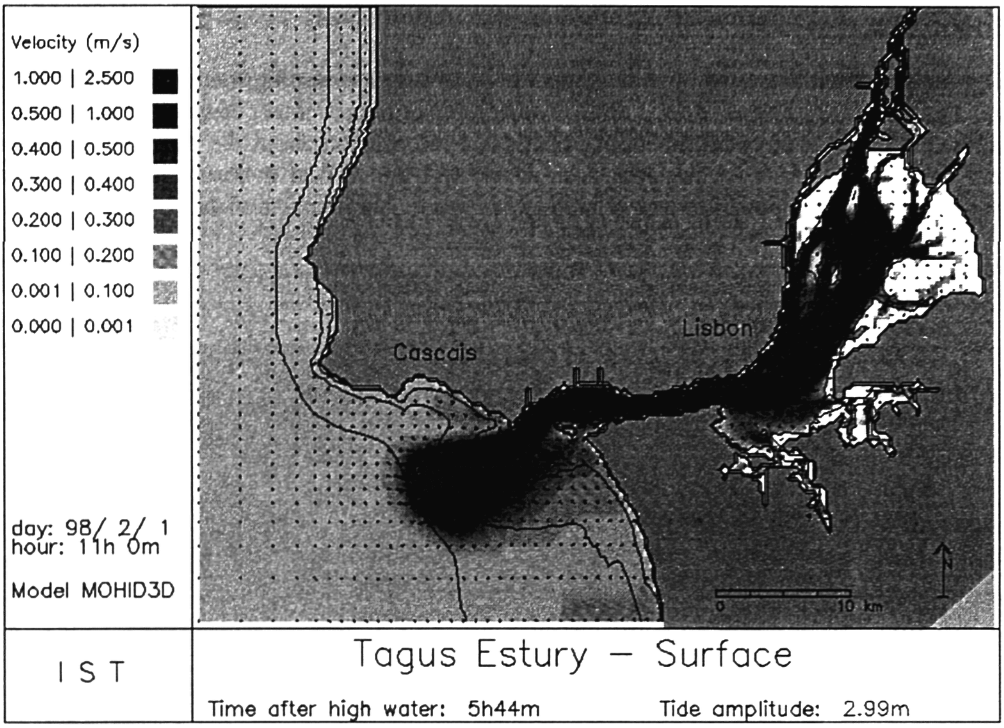

Figure 4, transient velocity field.

EUROMAR DAUGHTER PROJECT
EU-493 EUROMAR - ELANI

ELECTROANALYTICAL INSTRUMENTATION DEVELOPMENT FOR PHYSICO-CHEMICAL CHARACTERIZATIONS OF TRACE METALS IN THE MARINE ENVIRONMENT

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CONCENTRATION AND SPECIATION OF TRACE METALS IN THE MARINE ENVIRONMENT*

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SUMMARY

Physico-chemical characterization of species of trace metals at natural concentration level in the marine environment, using electrochemical methods, is presented. The fate and bioavailability of released toxic metals in aquatic systems strongly depend directly on chemical forms of dissolved metals.

Recent results of the ELANI EU-493 EUREKA (EUROMAR) Project: "Development of Electroanalytical Instrumentation for Physico-Chemical Characterization of Trace Metals in the Marine Environment" are presented. The aim of the Project is development of a highly sensitive, reproducible, selective and automated electrochemical system suitable to recognize and measure trace metals concentrations and species distribution in the marine environment. The system uses few different sensing techniques as well as innovative hardware and software for automatic determination of different physico-chemical states and reactivity of the dissolved trace metals.

The multi-national participants in the Project are experts in sampling and application of electrochemical instrumentation for measuring trace metals for years. The firm ECO CHEMIE B.V. is the manufacturer of the appropriate digital electroanalytical instrumentation. The joint working group suceeded in operationalizing the up-to-date knowledge by introducing the specific automation into the program resulting in the measuring system "ELANI" described here.

The measuring system has additionally been developed by combining special electrochemical cells, working electrodes, systems for efficient stirring of the solution all synchronized with the automatic burettes, pumps and valves, and supported by specifically developed software which controls the measuring procedure and enables required data treatment.

These requirements are fulfilled by developing three modes of electrochemical techniques: cathodic stripping voltammetry (CSV), anodic stripping voltammetry (ASV) and potentiometric stripping analysis (PSA), which are complementary with specific advantages.

* Partialy included in [1]

INTRODUCTION

During the last decades development of new procedures for the determination of total trace metals concentrations and chemical forms of dissolved trace metals has become necessary.

It is known that each metal reacts specifically in aquatic environment although many of them are mainly associated with a particular kind of the reaction mechanism. Trace metals in the marine environment are practically distributed between abiotic and biotic compartments (compartments A1-A3 and B1-B-4 in Fig. 1). Relative abundance of trace metals in each compartment depends on their physico-chemical reactivity characteristics, consequent to their chemical nature and levels of achieved equilibrium and/or steady state.

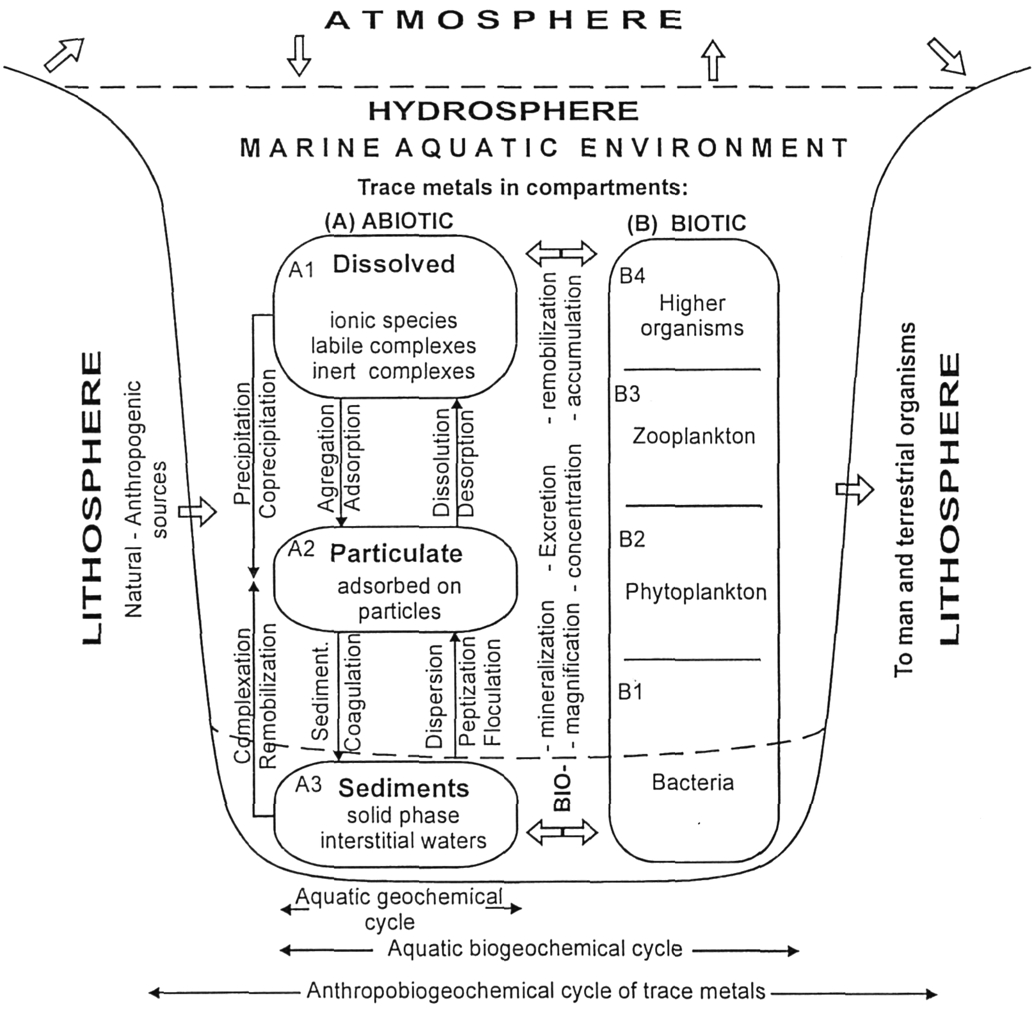

Fig. 1. Cycling of Trace Metals in the Environment

Various anthropogenic sources can exert an influence upon the content of trace metals in the aquatic compartments, as follows: (i) direct input of metal containing pollutants; (ii) release of other reactive compounds affecting the redistribution of trace metals within chemical species and environmental compartments (i.e. organic ligands, surfactants, redox reagent, etc.), and indirectly by (iii) thermal pollution.

Specifically, a direct input of pollutants, denoted as (i) affects the content of trace metals throughout the entire marine system, while the other two examples (ii) and (iii) can exert a considerable influence on fluxes and redistribution of metal species as well as their quantities between the marine compartments. Increasing the remobilization of the already inactivated toxic trace metals through their bioavailability, hazardous influence on mankind are thus enhanced [2].

Prior to measurements, modeling and/or simulation of the chemical reactions essential for the determination of reactive and total metal concentration and metal complexing capacity (MCC) complex network of chemical and electrochemical equilibria and reactions in the bulk

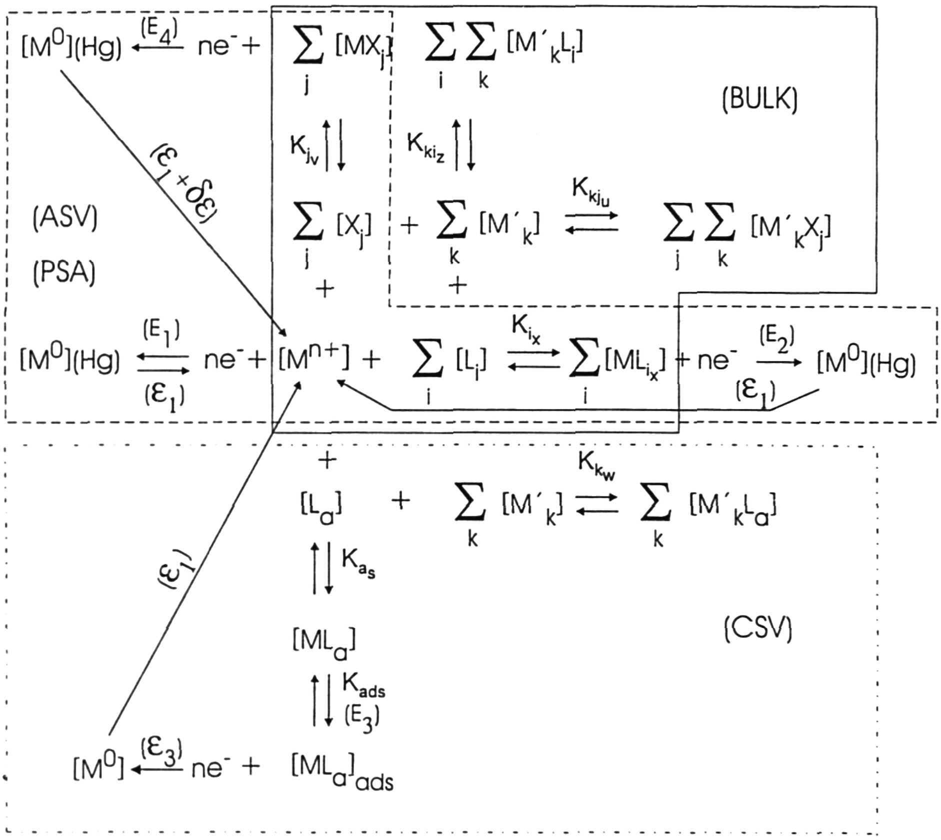

Figure 2. The network of chemical equilibria and electrochemical reactions in natural waater samples: M - metal which complexing capacity is determined; M'_k - k other present cations; L_i - i ligands that form inert complexes; X_j - j ligands that form labile metal; L_a - added ligand; ne^- - number of electrons in the reaction; K_{xy} - corresponding stability constants; E_1, E_2, E_4 - reduction potentials; E_3 - adsorption potential; ε_1, $\varepsilon_1+\delta\varepsilon$ -oxidation peak potentials; ε_3 - reduction peak potentials; Area surrounded by: solide line - represents reactions in the bulk of the sample solution, dash - takes part in ASV and PSA measurements, dash-dot - added because of CSV measurements.

of the sample solution as well as the possible influence of the measuring electrochemical procedure itself [3] must be taken into consideration. Figure 2 shows how the metal whose concentration and/or speciation is to be determined interacts and reacts with the other components in the solution. It also shows the distribution of the metal forms (i. e. speciation). In ASV measurements, portions of metal ΔM are subsequently added to the sample solution and left to equilibrate. "Titration" with electrons at the reduction potentials E_1 or E_2, results in the measurement of free and labile complexed, or total metal concentration with oxidation peak potential ε_1. For CSV, an additional ligand L_a has to be added to the solution. At the adsorption potential E_3, the intentionally formed metal ligand complex is accumulated at the surface of the working electrode. "Titration" with electrons results in a reduction process with a reduction peak potential ε_3. From the scheme it is evident that the stability constants of the metal complex formation are conditional ones and that they implicitly comprise the whole network of chemical reactions. The situation is still more complicated when, required by the measuring procedure, portions of metal $\Delta[M^{n+}]$ are added to the sample solution. The results of the consecutive measurements depend on the added metal distribution into the whole network, but in modeling , they are all implicitly included in the conditional stability constants.

"Metal ions in natural waters include essential elements (such as Cu, Zn, Mn, Fe) which may also be toxic at higher concentrations, and non-essential elements, which are mostly toxic such as Cd, Hg, Pb). Anthropogenic inputs have increased the concentrations of metal ions over the natural background in many rivers and lakes. Metal ions occur in natural waters in a variety of chemical forms, namely as free aqua ions, as complexes with inorganic and organic ligands, as particulate (or colloidal) phases or adsorbed on particulate (or colloidal) phases. These different chemical species have different reactivities and effects. Availability of metal ions to organisms, as well as toxic effects strongly depend on the chemical speciation. In many instances the effects have been shown to be related to the concentrations of free aqua ions (Sunda and Guillard, 1976; Anderson and Morel, 1982, Sunda and Ferguson, 1983)" [4].

"In addition to major nutrients (nitrogen, phosphorus, and silicon), certain trace metals, including iron, zinc, manganese, cobalt, copper, molybdenum, and nickel are essential for phytoplankton growth and metabolism. Historically, oceanographers have focused their attention on "classical nutrients" controlling growth and species composition of marine phytoplankton communities. Recent experiments have demonstrated that minute (1-5 nM) additions of iron can markedly stimulate phytoplankton growth in many remote oceanic regions (Martin et al., 1991). These and other recent findings indicate that trace metals (mainly their species) play a far more important role in regulating the growth and ecology of marine phytoplankton than previously recognized" [5].

The concern for the control of toxic metallic forms in the environment is reflected by the increasing number of legal regulations which insist upon the determination not only of total trace elements contents but also of chemical species. The European Commission has recognized the need to include such species in the list of dangerous substances to be monitored and some of them are already listed in EC Directives, e.g. on drinking water. Techniques used for the determination of metallic forms of elements generally involve many analytical steps such as extraction, separation and detection. These steps should be carried out in such a way that the speciation is unaltered during the analytical process [6,7].

The aim of the ELANI- EU 493 project is the development of a new procedure as a complex package of automated electrochemical instrumentation with corresponding software. In such a way, direct concentration determinations and the correlation of different species of trace metals (of natural and anthropogenic origin) and their physico-chemical properties in the aquatic environment, as well as metal complexation capacity measurements will be possible.

Recently obtained data show that not only in the open ocean, but also in unpolluted rivers as well as closed marine and coastal areas, the concentrations of dissolved trace metals are considerably lower than previously referred to in the literature. The intention of this Project is to contribute to elucidation of the appropriate treatment of water samples considering the entire process from sampling of natural waters to the analytical treatment, including determination and final evaluation of obtained data. Field observations and theoretical and experimental laboratory work are devoted to establishing the governing mechanisms and the influence of various parameters on the fluxes and transformation of species of different trace metals and pollutants in the European continental and marine aquatic environment.

In order to achieve this goal, correlated approaches to the aquatic system will be used:

- development of new and application of existing specific electroanalytical methods (voltammetry, potentiometry, pseudopolarography) with adequate automated sampling techniques for reliable qualitative and quantitative determination of trace metals with respect to their distribution, various forms, as well as and the kinetics of transformation reactions. Improved sensors, sampling and analytical procedures will be developed in order to achieve a high level of specificity, sensitivity, reproducibility and reliability of data;
- field observations will comprise the "model" aquatic system in the North Sea with the corresponding rivers and their estuaries, open ocean waters, the Mediterranean Sea open waters, the rivers Po and Krka with their estuaries;
- comparison of data obtained from the analyse carried out under "classical" laboratory conditions and from "in situ" measurements will be elaborated. Various electroanalytical methods will be compared and the most suitable one will be selected for the final combination of the instrumental assembly which will be commercially available.

RESULTS AND DISCUSSION

Digital multi-method polarograph μAUTOLAB (Eco-Chemie, Utrecht) is the instrumental basis of ELANI, so that it can be automated with a sample changer, a peristaltic pump, a hanging mercury drop electrode and an autoburette. Regarding software, a MS-Windows as a platform for objective-oriented programming is used, which enables necessary flexibility in automation.

The objectives of the Project are carried out in three different approaches: cathodic stripping voltammetry (CSV), anodic stripping voltammetry (ASV) and stripping potentiometry (PSA). They have been modified and specified according to the latest knowledge so that software packages could be prepared accordingly.

The Apparatus Assembly is illustrated in Fig. 3.

The possibility to determine metals which do not form an amalgam is a great advantage of CSV. Procedures for the determination of the chemical speciation and total concentration of some elements, as well as the concentrations of natural complexing ligands of several metals, have been developed. Each measurement is preceded by adsorptive preconcentration of the complex of the metal with an added specific chelating agent on the electrode surface.

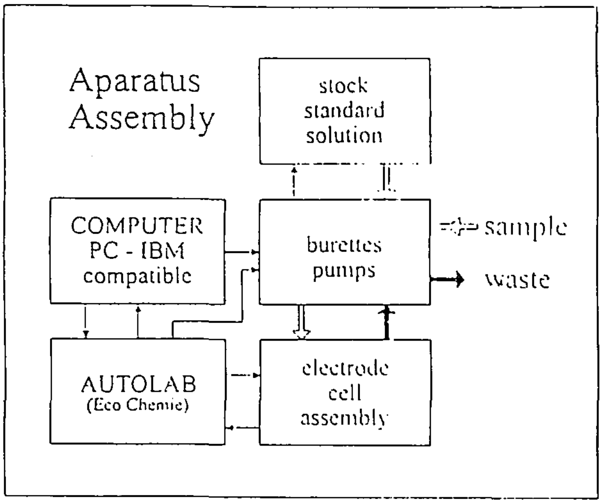

Fig. 3. Function tasks of the Apparatus Assembly:
1. Reactive metal concentration determination in pristine sea water
2. Total metal concentration determination by acidification of the natural sample
3. Metal complexing capacity determination by metal titration and/or pseudopolarography

Elements that can be determined by a CSV: A - those that can be determined by a CSV in unpolluted sea water without prior concentration of the sample; B - CSV procedures are available, but these elements can only be determined after some additional preconcentration step.

A	Al Ti V Cr Fe Co Ni Cu Zn Mo Cd Sn Sb I Pt U
B	Mn Ga Ge As Se Y Tc Pd La Ce Pr Th

The reduction current of the adsorbed complexes is directly related to the dissolved metal concentration. The sensitivity is very high as the entire adsorbed film is reduced during the potential scan. Competition of the ligand added with the naturally present ligands in the sample is the basis for the determination of chemical speciation. The stability of the natural complexes is evaluated relative to the stability of the complex with the known ligand added.

Bench top automated instrumentation for determining of dissolved concentrations of trace metals in seawater samples by cathodic stripping voltammetry has been developed. The instrument is connected to a sample changer, peristaltic pump, hanging mercury drop electrode and autoburettes. The software has been altered and advanced, so that the computer can automatically determine the quality of the measurements which are performed using the standard deviation of repeated scans. From the comparative magnitude of a standard addition and the original sample it evaluates whether the standard addition was sufficient, and then calculates the sample concentrations, or carries out another standard addition [8]. This instrumentation has worked well for the determination of trace metals (chromium [9], iron [10], vanadium [11] and copper) and also for complexing ligand concentrations. , The work has currently been done on the applications to other metals such as titanium, as well as on using a microelectrode instead of the standard mercury drop electrode. On-line UV digestion has been developed for the decomposition of organic substances interfering with the automated determination of metals in seawater [12]. The progress was made to enable a flow-analysis of metals with detection using cathodic stripping voltammetry. The existing batch cell of a commercial system (Metrohm electrode) was initially used, which was connected to an in-line UV-digestion system and pumps to obtain automated voltammetric analysis by comparatively conventional means. This system was recently used for a study of Ni, Cu and Zn in the Irish Sea [13], as well as of chromium and nickel speciation in the Mediterranean Sea [14]. The same system has even been used to determine uranium in kerosene after on-line extraction [15]. This system is therefore fully operational for field work on board and uses standard electricity supplies. A flow-cell for the attachment to a mercury drop electrode [16] was recently developed. It is fitted on the capillary of a conventional mercury drop electrode. The working volume of this flow cell is less than 10 microliter. This is interfaced with an on-line oxygen removal for an on-line voltammetric response. This system has also been taken to sea to determine cobalt and copper in the North Sea [17]. This system uses continuous flow analysis. A derivative method based on sequential-flow analysis has also recently been developed, which uses a low-power micro-pump and micro-valves and runs fully on 24 V with the aim of making it suitable for battery operation. An apparatus for an in-line oxygen removal has been developed, based on a tubing of semi-permeable composition which allows the oxygen to pass through but not the water. The residence time in this deoxygenator is about 25 seconds. The in-line system was initially used for continuous flow analysis, but recently was adapted for sequential flow analysis. This way a smaller sample volume can be used, and the analysis time optimized. A drawback is that the adsorption time is limited to 15 seconds [18].

AUTOLAB is also adequate for the derivative potentiometric stripping analysis (dPSA) for the determination of trace metals [19-22] and for solving problems concerning surface-active substances [23].

The effort has been made to develop electroanalytical cells, electrode and stirrer assembly. Extensive measurements were performed by anodic stripping voltammetry in comparison with the wall-jet electrode with a rotating disc electrode system . The wall-jet electrode exhibits more than twice higher accumulation efficiency than the rotating disk electrode. It allows shorter deposition time and faster analysis of trace metal samples, with the detection limit below 10^{-10} M [24]. The results obtained using glassy carbon and hanging mercury drop electrode (HMDE) were compared. Those two types of electrodes differ in the concentration sensitivity (favourable for glassy carbon), but also in a kinetic sense, glassy carbon being more capable of distinguishing between labile and inert complexes.

The newly developed mercury drop electrode assembly MDE-1 (Eco Chemie, Netherlands) has been tested and adjusted in order to diminish the adsorption of the reactants. The electrode adjusted in that manner showed variation of coefficients from 0.7% to 3.5% for the repetitive measurements of anodic peak currents for the concentration range between 10^{-7} and 2×10^{-10} M Cd^{2+}, respectively [25]. Software package which enables combination of automatic pseudopolarography with the automatic standard additions of the reagents by Cavro burettes is accomplished, which is an important step toward automation of the procedures for the metal complexing capacity determination and speciation.

The procedures for the determination of reactive as well as total concentration of Pb, Cd and Cu in seawater have been exactly defined for an ASV technique and thin mercury film glassy carbon disk electrode (TMFGCDE) [1] (Figure 4). In order to establish an automatic procedure for the Cu complexing capacity determination (Cu-CCD), pseudopolarograms of copper were constructed from a series of measurements and a great deal of model and actual sea water samples were [26] (Figure 5). The result is a software package which has been developed in order to automize the data treatment necessary for the metal CCD. The analysis

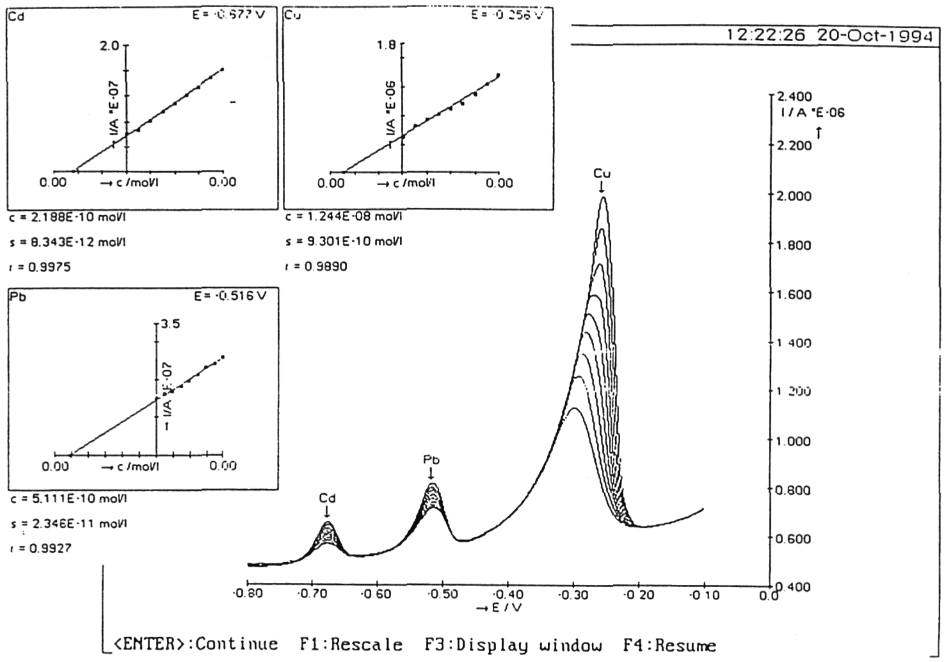

Figure 4. Direct and simultaneous reactive cadmium, lead and copper concentration determination in a pH natural seawater sample. Conditions: working electrode, thin mercury film glassy carbon disk electrode; method, differential pulse stripping voltammetry; deposition potential, -0.9 V; deposition time, 300 s.

of the data points for metal complexing capacity (MCC) determination is performed in order to be able, to fulfill the criteria necessary for the recognition of different ligand groups, i.e. their concentration and apparent stability constants from the experimental design viewpoint. The simulated data were selected taking into account the capabilities of the measuring systems available. They were fitted to the models which contain different numbers of inert ligands. The conclusion is that the product of the ligand concentration and the stability constant with the metal, should be larger than 1 in order to recognize its existence. If there are two ligands, their stability constants ratio should be at least 10, for the accurate distinction. Generally, it appears that the MCC can be determined with higher accuracy than the stability constants, that in the case when more ligands are present, the total MCC can be well determined, while partitioning among them as well as the values for the stability constants were less accurate, and practically not more than two different groups of ligands can be used in the evaluation of one set of experimental data points [3].

Problems regarding the metal complexing capacity determination (MCCD) procedure are elaborated by the measurements of the model solutions [27]. It has been observed that the pH of the sample solution influences the results of measurements and corresponding MCCD. pH of the solution is influenced by deaeration of the sample and can vary during the measurements. A need for monitoring and better control over pH of the sample solution is met.

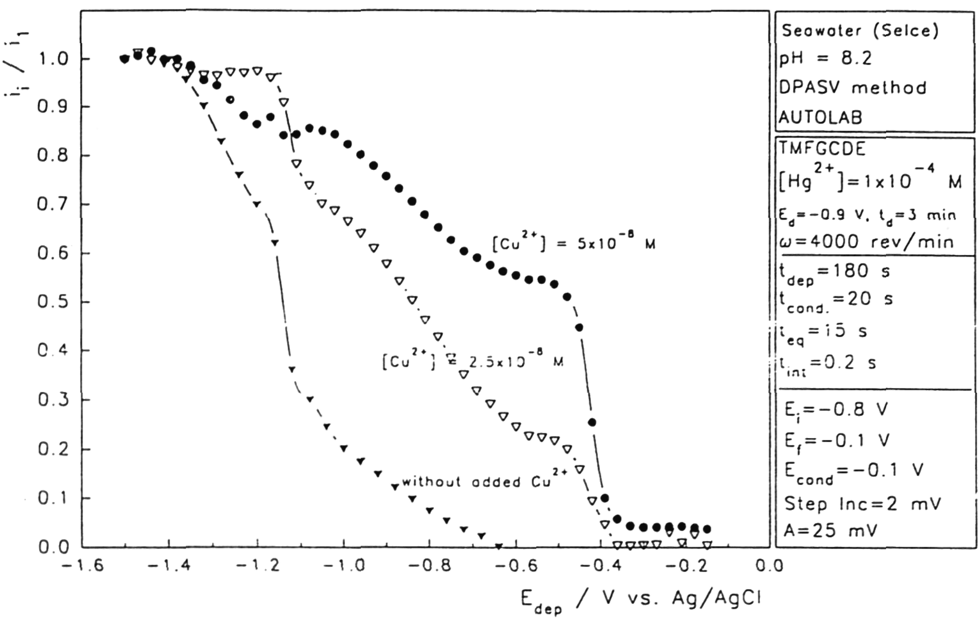

Figure 5. pseudopolarograms of copper of untreated, pH natural sea water sample (▼), and after addition of 2.5×10^{-8} mol^{-1} and 5×10^{-8} mol l^{-1} of copper (∇ and • respectively).

The work on establishing the procedure for automatic determination of labile, inert and particulate parts of the total dissolved metal is in progress. Software packages have been developed and the method worked out for data treatment in order to enhance the resolution of overlapped voltammetric peaks [28]. The method was tested and used on several specific electrochemical problems [29].

The applicability of thallium (I) as an internal standard redox process is elaborated [30]. It is shown that the electrode accumulation of thallium (I) could help in depressing and normalizing the influences of current fluctuations caused by unstable experimental parameters, which could help in automated routine field measurements envisaged in ELANI automatic system.

The adsorption of dissolved metal ionic species at the surface of sampler, electrochemical cell [31] as well as on sediments, dispersed inorganic particles and particulate organic matter is important subject of trace metal speciation measurements. On the basis of laboratory investigations of interactions between traces of lead, copper, zinc, cadmium and mercury ions and several minerals under different salinity conditions, the potentialities of the Krka River Estuary for the self-cleansing was already discussed [32]. The distribution of Pb, Cd, Cu and Zn in the selected grain fractions of carbonate sediments from the Krka River Estuary has been measured [33]. Metal ion adsorption can be increased if induced by inorganic anions [34]. Formation and properties of mixed ligand complexes have been studied as well as the problem of design of corresponding experiments and data treatment [35]. Analytical application of adsorptive accumulation is demonstrated for europium(III) [36]. The method of synergistic adsorption has been applied to determination of uranium and copper in natural water samples [37]. The sensitivity of this method can be enhanced by the application of a square-wave voltammetric stripping [38-40].

Adsorptive cathodic stripping voltammetry (CSV) has been applied for studying [41] and determination of traces of uranium [42] and its concentration has been measured in different sea water samples [43]. Specific measurements were oriented towards determination of predominant complex species of uranyl in the presence of hydrogen peroxide, chloride and carbonates [44]. Alkyllead behaviour and its stability constants with sodium salt of diethyldithiocarbamate (NaDDC) have been studied [45]. Electrochemical method developed for measurement of ionic alkyllead compounds in natural waters [46] has been applied to determination of their levels in surface waters [47,48] and wet atmospheric deposition [49].

Up-to-date achievements in the analysis of a water sample co4nsists of development procedures for:

- direct reactive and total dissolved trace metal concentration determination (approximately 10^{-11} mol l^{-1}),
- metal complexing ligand concentration determination which is of order of magnitude equivalent to reactive metal between 10^{-6} mol l^{-1} and 10^{-9} mol l^{-1} ,
- establishment of specific procedures for Cu and Fe complexing capacity determination, and,
- development of procedure for automated pseudopolarographical measurements enabling disolved metal speciation in aquatic systems.

Near future plans concern operationalization of the mentioned methods and procedures in the sense of automation by improved reproducibility and working simplicity for field observations.

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TITLE: Instrumentation for marine carbon dioxide measurements from Remote Platforms

(IMCORP)

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Instrumentation for marine carbon dioxide measurements from Remote Platforms

(IMCORP)

Towards a new generation of instruments for marine CO_2.

Andrew Watson, Liliane Merlivat, Ute Schuster, Richard Bellerby, Truls Johannessen.

SUMMARY

The IMCORP project seeks to develop instrumentation for the accurate and complete characterisation of the marine carbonate system, using a new generation of instruments which can operate without the frequent attention of trained personnel. Such instruments are needed since the marine carbon system is very variable both in space and time, so that a monitoring capacity to detect the consequences of global change will need many different observing systems. However, current instruments are normally only deployed on research vessels for short periods of time.

We are developing two instruments; one will measure the fugacity of CO_2, the total inorganic carbon concentration, and the pH from voluntary observing ships. It will typically be mounted in the engine room of such vessels, and will be able to run for 4-6 weeks between "services". It will be useful in obtaining wide spatial coverage of the CO_2 system over regularly repeated routes. The second system is mounted in a drifting buoy, and will measure pH and fCO_2. Because of the geometry of the sensors, many of the errors associated with the derivation of the other CO_2 parameters from these variables can be eliminated. The buoy will also sense the wind, temperature, conductivity and fluorescence of the water.

The project is now in its 3rd and last year; prototype instruments are under test in a fjord environment in Norway and at sea in the Greenland and Norwegian Seas. We have met most of our design criteria, and are now concentrating on obtaining the reliability needed from instruments that are designed for unattended operation.

THE REASONING BEHIND IMCORP

The importance of the oceans in the global carbon cycle is well recognised. Today, with atmospheric carbon dioxide rising rapidly, the oceans are acting as a buffer to global change by taking up about a third of the CO_2 that is released by human activities - some 2 Gt C yr^{-1} (Houghton, Jenkins et al. 1990). However, this situation is unlikely to continue indefinitely. Even if the oceans were completely passive in the climate system, no more than a large reservoir of salty water, their chemistry is such that their capacity to buffer CO_2 would decrease as the concentration rises (Liss and Crane, 1983).

However, in practice the oceans do not behave passively at all. Global change consequent on human activities is likely to impact the temperature, the circulation, the salinity and the biology of the seas, and these changes will all alter the rate of uptake of CO_2. The challenge is to understand the oceans well enough, and monitor them closely enough, to understand and predict how they will respond to these changes. The IMCORP project aims to develop "smart" instrumentation which can be used to monitor the state of the carbon system in the global oceans, using rugged automated equipment in a way that will greatly increase the amount of data available.

Our present understanding of the carbon cycle in the sea is based on many thousands of measurements made by oceanographers over the past several decades (see for instance, (Takahashi, Feely et al. 1997, for a compilation of fCO_2). The complexity and heterogeneity of the carbon system is so great, however, that the data set is by no means sufficient to describe the system in adequate detail to meet our needs.

The marine carbon cycle is very variable, both in space and time. The spatial scale of variation is sub-mesoscale -- a few kilometres only -- and the time scale is sometimes as short as a few days (Watson, Robinson et al. 1991). The most complete description of the oceanic carbon system to date has come from the WOCE and JGOFS programmes acting in concert together over the decade of the 1990s. However, even this enormous effort has only resulted in a "one-time" survey of the carbon system and related parameters at a series of widely spaced transects covering the oceans. But because the carbon system is changing, even as this survey finishes it is out of date. Measurements from research vessels are no answer to the problem of adequately characterising the carbon cycle, because there are simply not enough oceanographers and not enough research vessels in the world to do the task properly.

What is needed is a new generation of instruments which can collect data without the intervention of skilled personnel, and which can be mounted on platforms such as buoys or merchant "voluntary observing ships". Buoy-mounted systems could provide data in regions not frequented by merchant ships, at a fraction of the cost of research vessel based programmes. VOS-mounted systems can provide a low-cost method of covering large areas of the ocean with regular time series of data, but only for restricted regions of the world ocean. Both systems could be incorporated into the proposed Global Ocean Observing System to monitor the ocean and its role in the climate of the planet. The aim of the IMCORP project is to design and test such instrumentation.

THE STARTING POINTS: fCO_2 INSTRUMENTS

The carbon system in seawater can be characterised by measuring any two of four variables, TCO_2 (the total amount of carbon species present), fCO_2 (fugacity, nearly equivalent to the equilibrium partial pressure), pH and total alkalinity (TA). One variable of these, fCO_2, lends itself comparatively readily to being monitored by automated instrumentation. Prior to the start of IMCORP, the UEA group had developed an automated VOS system for this measurement (Cooper et al., in press), while the UPMC group had developed a buoy-mounted system for measurement of fCO_2, called CARIOCA. CARIOCA buoys are now being deployed both in the Equatorial Pacific, the Mediterranean and the Nordic seas under other projects funded by the EC. The VOS system has been tested over a 15 month period during 1994 and 1995, installed in the engine room of a commercial ship operating between Europe and the Caribbean.

However, useful as fCO_2 measurements are, they give only partial information about the processes which affect the carbon cycle in the ocean. Temperature and salinity both affect the carbon system, while biological activity affects it by two basic processes; organic photosynthate production/respiration, and calcium carbonate precipitation/dissolution. With measurement of only one of the four carbon system parameters, it is normally impossible to separate which of these processes is responsible for any measured change.

If the changes due to typical oceanic processes are plotted on a diagram of TCO_2 versus TA, each process has a characteristic signature, which enables them to be told apart, as shown in Figure 1. For example, carbonate precipitation/dissolution changes alkalinity by two equivalents for each mole of carbon, while air-sea exchange alters total carbon but does not change alkalinity (Broecker and Peng 1982). Our goal in IMCORP is to produce instrumentation which, while being as autonomous as the pre-existing fCO_2 instrumentation, is yet able to fully determine the marine carbonate system by making simultaneous measurements of at least two carbonate parameters. We are working towards the specifications of VOS and buoy mounted instruments shown in Table 1.

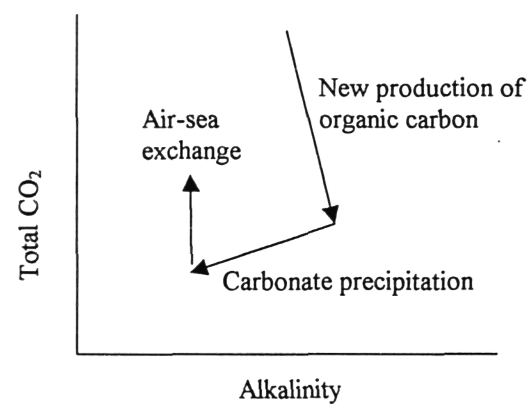

Figure 1: Changes in total carbon content and alkalinity for common processes

Table 1: Design specifications for IMCORP instruments

Instrument	Precision of fCO_2	Precision of TCO_2	Precision of pH	Precision of TA	Service interval
VOS	2 μatm	3 μmol kg^{-1}	0.005	---	4 weeks
Buoy	2 μatm	----	----	3 μEq kg^{-1}	6 months

The strategy adopted during IMCORP has been to design and assemble prototypes in the laboratory before sending them into the field for extensive testing. In the process, problems are identified with the design, which are then either worked on back at the home laboratory or, if possible, re-designed in the field. This process of iteration between laboratory and field has to be exhaustive; instruments designed to work for long periods unattendedly have to be tried extensively to "shake out" all the faults in their design. If they are not carefully field-tested, they may return from lengthy periods of use having failed at some early point in the measurement campaign, and have failed to collect vital data.

The rest of this article describes the designs we selected and the progress which has so far been made towards our goal, for each of these instruments.

THE IMCORP VOS SYSTEM

Total CO_2 concentration: The VOS system uses a LiCor 6262 infra-red analyser to measure TCO_2 concentration as well as fCO_2, a method first suggested by Goyet and Snover (1993). It differs from that usually used to quantify total carbon, the standard method being a "coulometric" titration technique which is highly accurate but which cannot be operated for long periods of time without substantial maintenance (Johnson, Wills et al. 1993). The LiCor is an accurate device, though not quite as precise as the standard method. However, an early decision was made to trade some accuracy for the robustness and potential for automation of the LiCor technique. The LiCor analyser has been proven to work at sea for the measurement of fCO_2, where it is now perhaps the most widely used analyser. More importantly, it is the analyser used in the pre-existing VOS instrument for fCO_2, so there is the potential to perform both fCO_2 and TCO_2 determinations with a single analyser.

Figure 2 shows a schematic diagram of the TCO_2 component within the IMCORP VOS instrument, which we are currently testing. A sample of 1 ml, accurately measured using a syringe pump, is acidified so that all the CO_2 is converted to the gaseous form, and the CO_2 gas is stripped into a nitrogen gas stream. The concentration of CO_2 in the gas is measured by the LiCor and integrated to obtain the total amount present. Our laboratory work during the first year of IMCORP has established a number of principles necessary for the measurement to achieve the desired precision:

1) The gas flow must be very precisely constant, and the gas must be dry prior to analysis in order to obtain the desired precision. This follows because the IR analyser responds to concentration in the gas flow rather than to the absolute amount of CO_2 present.
2) The IR analyser is optimised for concentrations of CO_2 near ambient levels in the atmosphere. Accurate calibration over the range 250 to 450 ppm is required for the fCO_2 analysis, but outside this region the response can be non-linear and difficult to calibrate. Introduction of a pulse of CO_2 from acidification of a sample of seawater would cause a concentration to be much larger than this, if it was done instantaneously. Therefore, the sample is not added all at once to the acid, but is metered in slowly over the course of several minutes. Instead of a sharp peak in the concentration of gas being analysed, a low and flat peak results which remains within the range in which the LiCor response is well characterised.
3) Though in principle it is possible to calculate absolutely the amount of CO_2 in the sample from the rates of flow and measured concentration, in practice it is better to calibrate using liquid standards. Such standards are now available to the oceanographic community from

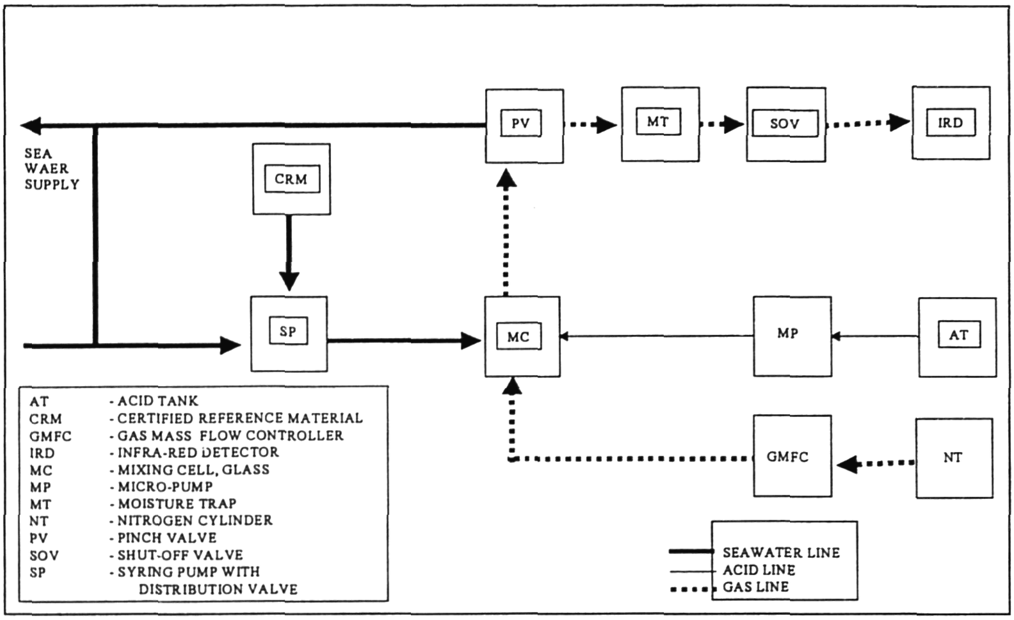

Figure 2: Block diagram of TCO_2 module in the VOS instrument

A. Dickson at Scripps Institution of Oceanography. In the design of an instrument for unattended use, the standard being used must be protected from contact with the atmosphere, and for this purpose we are using bags made with membranes designed to inhibit gas diffusion.

Figure 3 shows a sample of data from the new design, collected during our first sea trial in the Greenland Sea in March 1997. In a single measurement the precision is less than that from the coulometric method, but the measurement is much faster than the conventional technique. Therefore, in the time of one conventional measurement, three or four replicate analyses can be run with the new technique and the target specification can be achieved. The standard error on the measurements is generally of order 0.1% or 2 μmol kg^{-1}, which is well within the specification, but there are episodes of poorer precision. In winter 1997/1998, the instrument is field tested at the University of Bergen's station at Espeland, where the overall precision is improved. In March 1998 the second sea trial will be done, again in the Greenland Sea.

pH analysis: The method chosen for pH measurements in seawater is spectrophotometric flow injection analysis (Byrne 1996) with charge coupled device array detection. The pH-dependent absorption characteristics of a selected sulfonephthalein indicator are measured using dual miniature spectrometers. A schematic diagram of the manifold is shown in Figure 4. Light from a tungsten/halogen lamp is guided to spectrometers using fibre optic cables: light to the master spectrometer travels through a flowcell; light to the slave spectrometer solely through an attenuator. Seawater is pumped through the manifold at a constant rate and the absorption spectrum of the seawater is recorded by the master spectrometer. Thymol blue indicator is then injected into the seawater stream and after the seawater/indicator solution has been well mixed, the absorption spectra of the solution is measured.

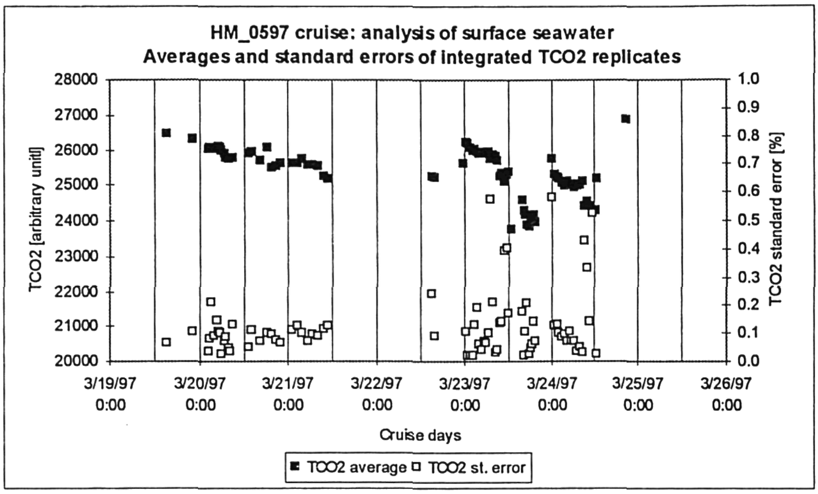

Figure 3: TCO_2 data and their standard errors measured with the VOS instrument in surface seawater in the Greenland Sea in March 1997

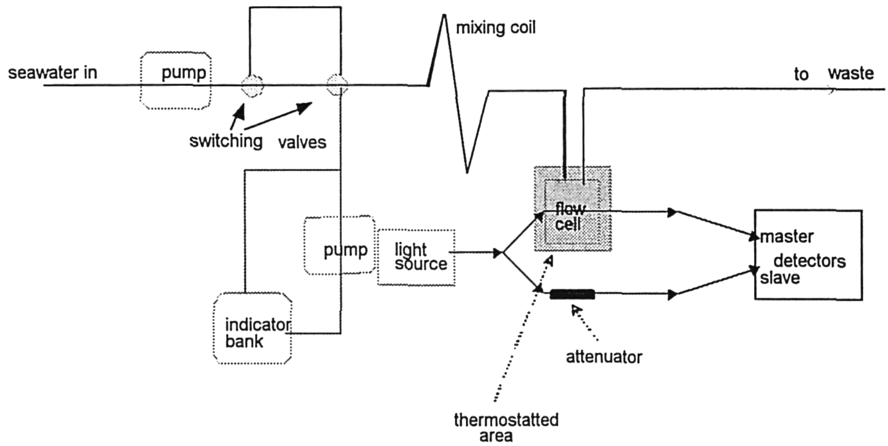

Figure 4: Block diagram of the VOS pH module

A comparison between the absorption spectra of pure seawater and of seawater with the addition of a small aliquot of indicator solution is used to calculate the pH on the total hydrogen ion pH scale. The module has been extensively tested both in the laboratory and during a 4 month field study at Espeland in Norway. It has a precision of ± 0.005 pH units and takes more than 30 measurements per hour over a 1 month period. The system is fully automated for instrument control and data acquisition and is miniaturised. The method is inherently calibrated as the physico-chemical properties of the indicator are constant. Therefore, no standards are required for routine measurement of seawater pH.

A sample of data from the system is shown in Figure 5. The instrument was measuring pH in a continuous stream of fjord water while at Espeland. As the incoming water warms, the pH changes in response. From the short-term variation it can be seen that the detection limit of the system is comfortably better than 0.005 pH units at this time.

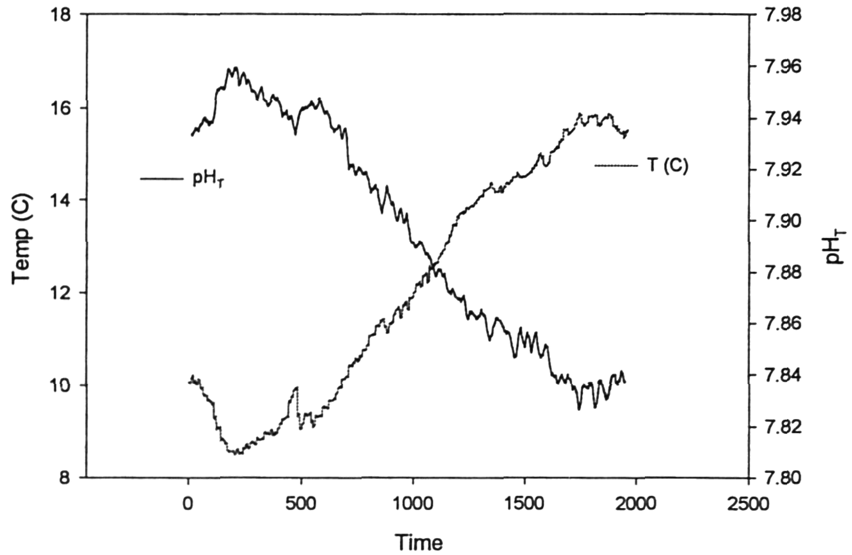

Figure 5: pH and temperature measurement done with the pH module of the VOS instrument

THE IMCORP BUOY

The IMCORP buoy is conceived as a free-drifting instrument which measures all the variables required to calculate the status of the marine carbon system and to interpret them. It builds on the design of the existing CARIOCA system developed by LODYC, which measures fCO_2 and chlorophyll fluorescence (Lefèvre, Ciabrini et al. 1993). In the IMCORP buoy, a new sensor for pH is added which operates in-line with the fCO_2 sensor. By using the two sensors on the same sample and in close proximity to one another, many of the errors normally associated with the determination of the second carbon system parameter can be made to cancel each other out. Therefore, a relatively accurate determination of the alkalinity from the other parameters of the carbonate system should be possible. In addition to the pH sensor, the new IMCORP buoy contains a conductivity cell and anemometer. Figure 6 shows a diagram of the buoy.

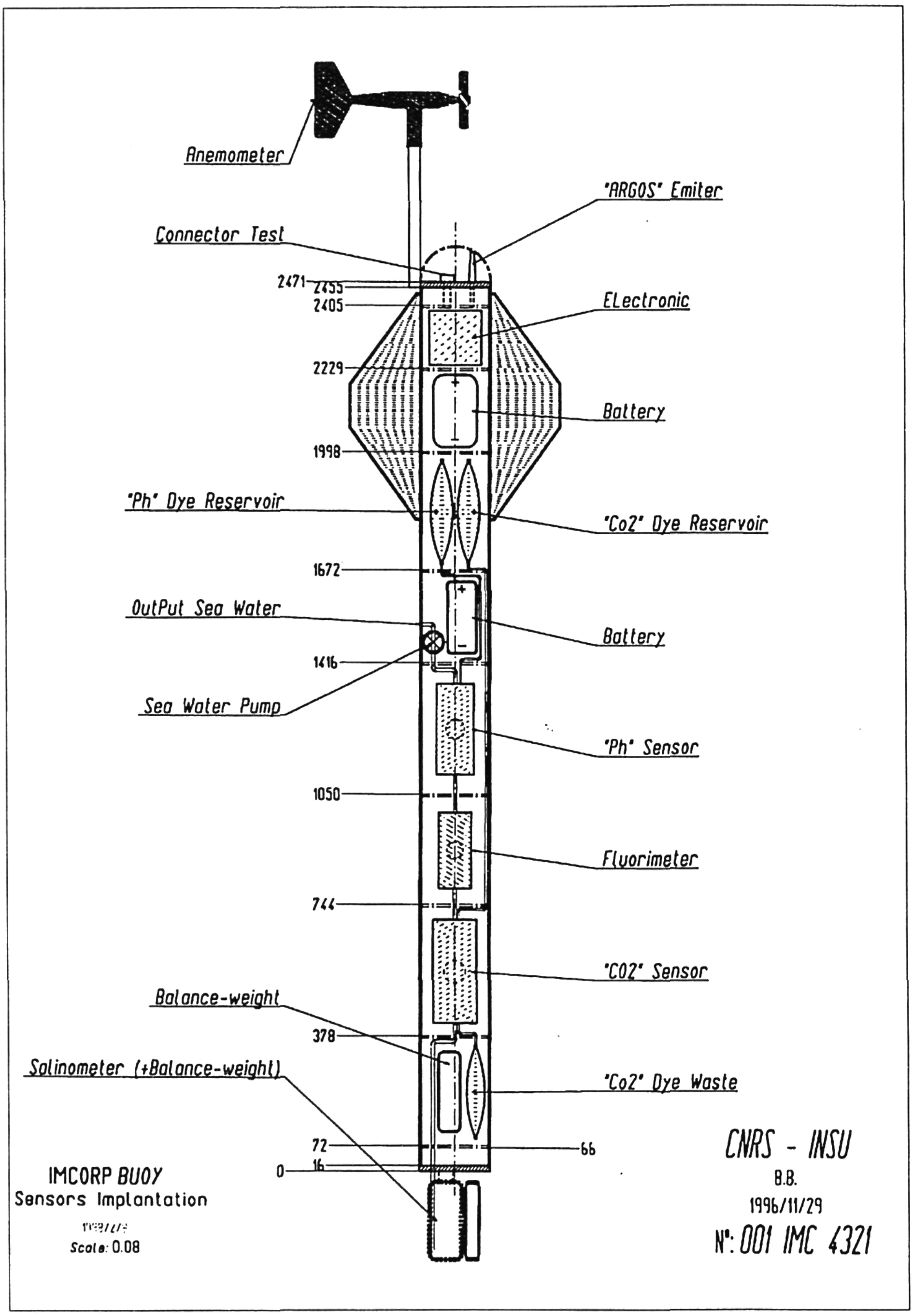

Figure 6: Diagram of the IMCORP buoy pointing out the location of the individual components

The buoy communicates to the laboratory via the ARGOS satellite network, and is designed for an unattended drifting time of at least 6 months. The attraction of such an instrument is that it will make possible the monitoring of the carbon cycle in the oceans far from any commercial shipping route. At present, our knowledge is most sketchy in regions such as the Southern Ocean, the Southeast Pacific and the Arctic seas where very few ships go, so that the development of ship-of-opportunity sensors will not help very much for these regions.

Extensive testing has been carried out of the new sensors on the IMCORP buoy, both in the laboratory and the field station of the University of Bergen at Espeland, Norway. Figure 7 shows the reproducability of pH carried out in the laboratory with more than 500 artificual seawater samples over a time period of 3 days. The standard deviation of each set of measurements was about 0.004 pH units.

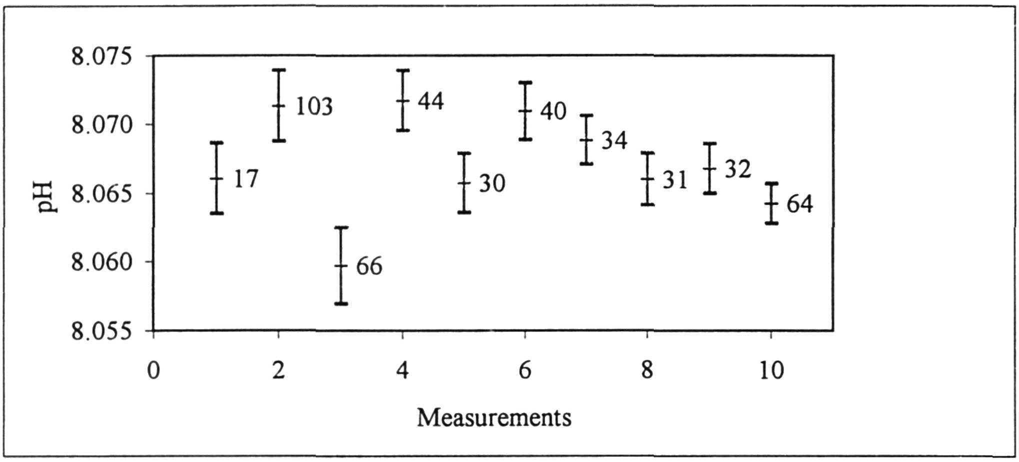

Figure 7: pH reproducibility (error bars: $\pm \sigma$) measured with the IMCORP buoy over 3 days

After further improvements have been completed, the buoy is field tested again at the Espeland station in spring 1998. It is going to be intercalibrated with the IMCORP VOS instrument, after the latter one has returned from the Greenland Sea. Open sea trials of the buoy are envisaged for later in 1998.

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TITLE: Biofouling Reduction on Optical Systems (project **BROS**)

CONTRACT NO: MAS3-CT95-0028

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BIOFOULING REDUCTION ON OPTICAL SYSTEMS
BROS

SUMMARY

This paper gives an overview of the objectives and work programme of the MAST-3 BROS project. The project is an interdisciplinary study of the methods by which biofouling on surfaces, and in particular optical surfaces, may be reduced or deterred in ways which would be suitable for implementation on underwater optical sensors. The project includes a number of tasks focussed on specific types of biofouling reduction technology, as well as a series of full-scale trials involving oceanographic instruments and underwater cameras. These technology-based tasks are supported by complementary fundamental studies of the development of biofilms and environmental risk assessment. The deliverables from the project will include guidelines for the use of specific biofouling reduction technologies for particular oceanographic campaigns. This is an important feature, since biofouling is a complex problem and any expectation of a single solution for deterring or reducing it is unrealistic.

1. INTRODUCTION

The project commenced in February 1996 and is due for completion in July 1998. The overall objective is to develop long life strategies to reduce marine fouling on optical underwater instruments to permit both measurements of a flux (for example, for fluorimeter, transmissometer and turbidostat applications) and to facilitate optical imaging (underwater cameras). Such developments will permit greater integrity of data collection from various platforms (i.e. fixed structures, floating buoys, moored buoys, unmanned underwater vehicles, benthic landers) used in extended deployments for oceanographic monitoring of the current state of the marine environment, and for the observation of environmental changes. The developments will also permit longer periods of deployment between maintenance, thus reducing the cost of monitoring. The protection of camera viewing systems against biofouling has applications in the inspection of structures and subsea installations, such as those used in the offshore oil and gas industries, as well as for oceanographic and marine environmental monitoring. Given the complexity and variability of the marine fouling problem, the objective is to develop a range of defensive strategies to improve the performance of optical sensors, since there is unlikely to be a universal solution to the problem

There are a large number of projects worldwide which involve the deployment of various combinations of sensors. Increasingly the deployment of sensors is occurring in lower latitudes where the fouling pressure is more intense and seasonal variations less noticeable. The long term deployment of such sensors will also increase when the use of autonomous underwater vehicles becomes more widespread. The effective gathering of reliable data from these sensors requires minimum maintenance over extended periods of time without deterioration of the accuracy of the measured parameters. All forms of instrumentation immersed in the ocean environment are subjected to marine fouling pressure which is often quite intense. Such fouling can significantly affect the measuring elements of various types of sensors. In most applications the sensor systems are deployed with limited power resources, which constrains the use of purely mechanical techniques.

Measures to control fouling on the structures/vehicles carrying sensor packs exist and have been the subject of recent research[1] aimed at improving the performance and limiting the environmental impact of the fouling resistant materials which are used. Similar techniques can be applied to the bodies of various sensors[2], although care is required to ensure that the antifouling treatments do not affect the measurements of the actual parameter being monitored nearby. Protection of the actual active elements of sensors against marine fouling is generally accepted as a very difficult[2] and largely unsolved problem (Table 1). It is this technical challenge which this project addresses, the results of which will provide increased resistance against marine fouling for specific types of sensors, i.e. those incorporating optical windows.

The usual range of optical marine sensors includes devices for the measurement of turbidity, radiance and fluorescence as well as cameras for imaging. Turbidity, radiance and fluorescence measurements involve optical windows for transmission and detection of a flux of radiation at a particular frequency. A summary of marine optical instruments is given in Table 2. Whilst typical sets of marine sensors employ a wide range of materials which must be treated in various ways as substrates for antifouling coatings[2], the materials involved in optical sensors form a much smaller set. In optical applications the 'windows' are usually polymethylmethacrylate (PMMA) for low pressure applications and quartz glass for higher pressure, deeper water applications.

There is no doubt that, in an untreated state, the normal development of micro and macrofouling[3], leads to deterioration in the measurements of the parameters. In optical applications the effect of this fouling will depend on the wavelength of the radiation.

There are broadly three major classes of methods for maintaining optical surfaces free of marine fouling. These classes of methods are:

(i) The use of biocide.
(ii) Mechanical cleaning.
(iii) Disrupting the mechanisms of bacterial and algal adhesion.

Within each class of method there are significant detailed approaches. These are listed in Table 1, together with a summary of the known effectiveness and the disadvantages of each method. The overall conclusion, from consideration of the background work which led to the construction of Table 1, is that there were no known successful methods for the maintenance of fouling free optical surfaces over reasonable periods of time. It is this gap in knowledge and ability which the BROS project addresses.

The formation of biofilms on solid surfaces immersed in the sea appears to be largely due to hydrophobic reactions[4,5] although there may be contributions from photo-and chemotaxis[6] and charge interactions[7]. The outermost parts of natural surface microlayers consist of hydrophobic substances, including various lipids, proteins and polysaaccharides[8]. The film formed by these substances strongly influences bacterial accumulation. Hydrophobic interactions are very important in the adhesion of micro-organisms to water/solid interfaces[5,9]. This natural surface hydrophobicity is generally non-selective in nature allowing the organisms to attach to a variety of substrates[10]. Very specific relationships

Table 1 Summary of Marine Fouling Reduction Methods with Potential for use with Optical Sensors

Methods	Performance	Disadvantages
No protection	Microfouling within a few days - transmission over range of wavelengths reduced	
Copper bezels or grids	Ineffective, performance as for unprotected surfaces	Ineffective
Clear TBT based coatings [B, C]	Acceptable performance for viewing applications	Effect on flux measurements unknown, environmental impact undesirable
Chlorine [C]	Unknown on optical surfaces but periodic treatment appears to maintain fouling free surfaces	Requires power. Total removal of biofilm sufficient for optical applications not known. Requires determination of dosage for specific applications. May affect other measurements.
Use of ultrasonics [B, C]	Deters biofilm formation, may be useful in biofilm removal.	Requires power.
Ultraviolet radiation [B, C]	Reduces biofilm formation on optical surfaces	Requires power. May affect other measurements of local environment. Difficult to provide adequate radiation levels where needed.
Magnetic fields [B]	Largely unknown	Requires power.
Mechanical cleaning [C]	Apparently successful, but well adhered components of biofilm difficult to remove completely.	Requires power. Long term performance of cleaning mechanisms subjected to fouling pressure is unknown.
Modified glasses [A, B]	Apparently successful in land based applications for resisting adherence of dust particles. Optical performance acceptable.	Performance in marine applications is unknown. Variety of formulations requires evaluation.
Low adherence coatings (siloxanes) [B]	Microfouling occurs but is more easily removed by mechanical means.	Effects on flux measurements over wavelength range not known. Durability under intermittent cleaning not known.
Hydrophillic coatings [A, B]	Microfouling deterred for up to 6 months. Optical performance suitable for flux measurements.	Life relatively short, soft materials, effects of temperature and pressure not understood. Field replacement not yet possible. Technology improvements required for imaging.
Hydrodynamics [B]	Has some effect on film formation but only very high velocities remove biofilms.	Effective treatment requires energy. The effect on film formation not understood, complicated by enhanced nutrient delivery.
Surface charge [A]	Can affect bacterial adhesion.	Requires energy, ability to deter biofilm formation unclear.
Electrode systems [A, B]	Appear to have some potential.	Require energy. Life and effectiveness of system unknown. Corrosion may be a problem.
Self-polishing (sloughing) surfaces [C]	Has potential if sloughing material does not affect optical properties or local environment.	May require energy source and may have environmental impact. Suitable systems for optical surfaces not yet identified.

Table 2 Optical Oceanographic Instruments

Measurement of Physical and Chemical Parameters

- CO_2 Analysers
- Colorimeters
- Fluorimeters
- Transmissometers
- Irradiance meters

Measurement of Solid Loading

- Turbidimeters
- Granulometers
- Underwater cameras

Measurement of Biological Processes

- Turbidostats
- Cytometers
- Underwater cameras

Inspection/Surveillance

- Underwater cameras

between micro-organisms and biotic surfaces exist and are attributed to hydrophobic interactions. Whilst natural cell surface hydrophobicity may be sufficient for initial adhesion to occur it does not ensure cell growth and proliferation. The stages of biofilm development following initial adhesion are determined by such factors as nutrient availability, metabolic pathways and various biological, physical and chemical stresses[(10)].

The impact of this complex microbiological picture on the prevention of marine fouling of optical surfaces is that there are three detailed alternative strategies to be investigated. The first is to prevent initial adhesion by disrupting the hydrophobic reaction[(11)]. The second is to deter the growth and development of adhered organisms so that a significant biofilm does not develop (and in addition to promote removal of the adhered organisms) and thirdly to remove mature biofilms which have been able to fully colonise an optical surface. In summary these strategies are referred to as:

[A] Prevention of adhesion of organisms
[B] Disruption of biofilm growth
[C] Periodic removal of mature biofilm

The methods described in Table 1 are annotated with the appropriate strategies referred to above.

Various methods are available to evaluate bacterial adhesion but because of the range of phenomena involved it is difficult to make meaningful measurements to distinguish between numbers of adhering cells, their immobilisation and the strength of attachment[(12)]. As well as charge interactions other important factors are surface roughness and shear rate across the surface. The dependence on these parameters is not straightforward due to cooperative effects between organisms. High flow rates past surfaces are known to inhibit settlement but once settlement has occurred the effectiveness of shear rate diminishes[(12)].

Much of the discussion above has referred to bacterial adhesion. However in relatively shallow water applications (<50m) there will be a simultaneous mechanism of micro-algae adhesion on, and colonisation of, surfaces. Many of the underlying mechanisms are similar and should be amenable to similar treatments with respect to avoidance and removal.

Any successful strategy to combat fouling on sensors should ideally not require any or much additional power. Hence the use of active self-cleaning sensors which incorporate ultrasonics[(13)] or periodic releases of biocidal[(14)] or inert gas[(15)] is not always appropriate. Whilst power requirements for some sensor applications must be minimised it is likely that some active strategies have a very useful role to play in the protection of optical surfaces against fouling. In particular, it is likely that a passive system, augmented by an active system requiring some power resource, could be successful. This is because of the need to remove 'dead' biofilm if some deposition occurs and the passive system prevents further development. Thus the active methods listed in Table 1 should not be dismissed simply because power resources are required. Marine biofouling, even at the microfouling level, is a complex phenomenon when considered over a wide range of locations. Hence it is unlikely that a single effective solution to the problem will ever be found. What is required is sufficient knowledge to permit optimisation, taking into account effectiveness of individual techniques, the additional synergy achieved by using techniques in combination, the relative priority of power consumption, environmental impact, etc.

Previous attempts at providing passive protection for optical windows have included the use of reservoirs of TBT[16] copper bezels and/or the use of copper mesh[17]. Other approaches have included the use of very thin low adherence coatings[18] based on polydimethyl siloxane. Previous work at GMTC showed that none of these treatments is sufficiently successful by itself in deterring microfouling, involving the settlement of bacterial and algal films, which have been observed[3] to affect the optical transmission of glass surfaces. The significant research effort elsewhere[19] on low-adherence, low-energy coatings for other applications is not particularly appropriate for such sensors. There have been some apparently successful attempts to produce modified glass surfaces which remain clean in a land-based environment[20] but this technology has not yet been proved in marine applications. Previous work[21] has shown that it is possible to adjust the surface characteristics of such glasses between the extremes of hydrophobic and hydrophilic behaviour. This ability to alter such surface characteristics is potentially useful in marine fouling applications since previous work at Glasgow showed that the physical characteristics of hydrophilic surfaces help to resist microfouling[22]. It is also possible to incorporate chemical species into glass surfaces to act as a local biocide. Other technology involves the use of ionic formulations so that the glass surface may be used as an electrode in an active system.

Previous work[22-25] produced some successes in the development of fouling resistant surfaces. The most successful materials employed hydrophilic coatings based on hydrogels dosed with an active agent. The agent may be incorporated directly into the gel or within a microcapsule although the use of microencapsulation is likely to be unsuitable for optical applications. Release studies on the active agents, using HPLC, when correlated with field trials, show that very low levels of such compounds are effective in preventing microfouling. Physical surface characteristics (hydrophilicity in particular) play a key role in the successful combinations. Some derivatives of the new hydrophillic materials have acceptable optical properties and have been used as a 'quick fix' in short term trials on marine optical sensors in a EUROMAR project[26]. However many aspects of this new materials technology required further fundamental research work, in particular extending the life of the coatings, improving mechanical performance, removing the need to keep the coatings wet at all times, optimising preparation techniques and application to the windows. Alternative solutions using active windows made from optical glass with surface modification also needed further study. It is these areas which the BROS project addressed with respect to optical sensors.

Given the above state of the art, the BROS research programme should lead to the following scientific and technical advances:

(i) Quantified understanding of the effects of biofouling on optical instruments and underwater cameras.

(ii) Performance envelopes for the effectiveness of various strategies for the reduction of biofouling on optical sensors.

(iii) New prototype methods for the protection of optical surfaces against marine fouling.

(iv) Recommendations for the protection of specific optical devices for particular missions.

(v) Preliminary understanding of the environmental and industrial risks and economics associated with the new technologies.

There is a growing industry within Europe for the manufacture of oceanographic instruments and underwater cameras. The world market is highly competitive with most competition coming from companies in the USA and Japan. The world market for oceanographic instruments has recently been estimated[27] to be approximately 400m ECU with a 5% growth rate per annum. The critical feature in the market is that of instrument performance. This is more important than price[27]. Consequently, to remain competitive the European manufacturers need to seek technological advances. The development of adequate biofouling resistance is potentially a major technological advance for such devices when deployed for extended periods.

2. EXPERIMENTAL PROGRAMME

The experimental components of the programme involved eight specific tasks. These included: two tasks dedicated to full-scale trials, one utilising oceanographic instruments and the other underwater cameras; one task concerned with polymer coatings; one task investigating the performance of possible alternative glass formulations; one task concerned with chemical strategies; one task investigating a limited range of mechanical methods for biofouling reduction/removal; one task concerned with electrical methods and a task to study the fundamental aspects of biofilm formation on a range of substrates over a geographic spread of locations.

The two tasks involving trials included matched pairs of oceanographic instruments (one pair of tranmissometers and one pair of fluorimeters) and two matched pairs of underwater colour zoom video cameras. The use of pairs of devices is a vital part of the study since the later stages of the trials involved comparative evaluations of the effectiveness of various biofouling reduction strategies and the earlier stages involved studies of the effects of the development of biofilms on the devices when compared to constantly clean devices.

The task involving polymer coatings had an experimental programme with many strands involving two of the partners. One of the major components of the programme was a study of hydrogel-based polymer coatings, originally developed in a previous MAST-2 project and further developed in the BROS project. A range of host hydrogel materials/methods of manufacture were included in the BROS study together with extensive evaluations of a range of active compounds included in the host materials. Large numbers of measurements of uptake and release of the range of active ingredients were carried out. The other major component of the coatings task concerned an extensive study of hybrid transparent coatings which can be formulated as either hydrophobic or hydrophilic coatings. This coatings technology is associated with organically substituted alkoxysilanes (hydrophobic) or organosiloxanes containing amine functions (hydrophilic). A key feature in the study of these coating compounds is their ability to coat glass as a substrate and the optical performance, as well as the biofouling resistance. The task involving glass (or glass-like) materials involved evaluation of the fouling resistance of a wide range of commercially available optical glasses as well as a selected number of synthetically produced inorganic glasses with different formulations. The study included PMMA and calcium fluoride both of which are variously used in optical instruments. As well as detailed fouling studies, to determine whether the substrate chemistry affected the developmental stages of biofilms, the task evaluated the changes in surface roughness as a function of time in the marine environment.

The aim of the task concerned with chemical strategies was to identify chemicals that may be suitable as biocides and to provide information on the characteristics of these chemicals and their activity against marine biofilms. A large number of potential biocides were identified and evaluated, in terms of general characteristics, for use in the target applications. A short list of such biocides was then subjected to laboratory trials involving microbial assays. In addition to evaluating the effectiveness of chemicals, this task also considered methods of deployment, in addition to those release mechanisms evaluated in the 'coatings' task.

In considering mechanical methods for deterring and/or removing marine biofilms, the BROS project team agreed, after a review of possible technologies, to limit further studies to the use of ultrasonics. The only other technology studies, involving a mechanical component, was the use of water jets in conjunction with some of the hybrid low adherence coatings.

The evaluation of electrical methods covered a wide range of technologies. These included the use of ultraviolet (U-V)light and copper electrodes, as well as the generation of chlorine directly from seawater and two techniques aimed at deterring bacteria from colonising a surface by the use of electrostatic methods. The use of U-V is widespread in land-based sterilisation applications and is applied, relatively crudely, for reducing physical maintenance in aquaria. The application of such techniques to sensitive instruments required fundamental studies of both its effectiveness and minimum useful dosage. Copper is known as an effective method of reducing marine macrofouling but much less is known about the use of copper to avoid/reduce microfouling. Chlorine generation is used to control biofouling in applications such as heat-exchangers. In the BROS project the feasibility of using a conductive optical surface to generate chlorine has been investigated. Clearly the power requirements and the durability of the conductive surfaces are important issues. The electrostatic techniques have been investigated since most bacteria are charged and hence might be repulsed from a charged surface. Two techniques, involving a.c. and d.c. potentials, respectively, have been evaluated.

Whilst much is known about the development of biofilms there were two particularly necessary areas of study of biofilm formation for the BROS project to undertake. These were (i) a study of variability of biofilm formation between geographically distant sites, each with its own environmental parameters, as a function of season, and (ii) a study of the effects of immersion depth on biofilm formation (about which little was previously known). The wide-ranging study of biofilm development with season and location involved very extensive experiments in three locations, the Clyde estuary, U.K., Den Helder, the Netherlands and Cascais on the Atlantic coast of Portugal, near Lisbon.

3. RESULTS AND DISCUSSION

The trials on unprotected oceanographic instruments and cameras showed that the performance of these devices deteriorated rapidly with time, even in the relatively cold waters of the Clyde estuary in summer. Figures 1-4 show the devices and the change in transmissometer and fluorimeter readings as a function of time compared to a clean instrument. The development of microfouling biofilm on the transmissometer windows reduced the transmission of light (660nm) from the source end of the device, through the two

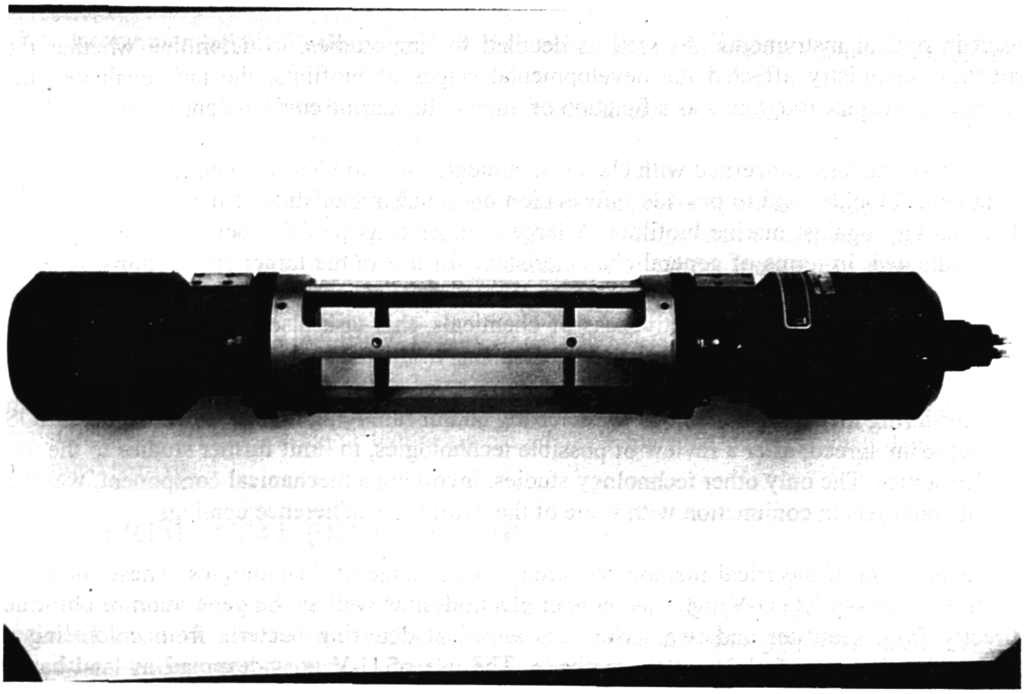

Figure 1 A transmissometer. The light source operates at 660nm wavelength and the light path is 250nm.

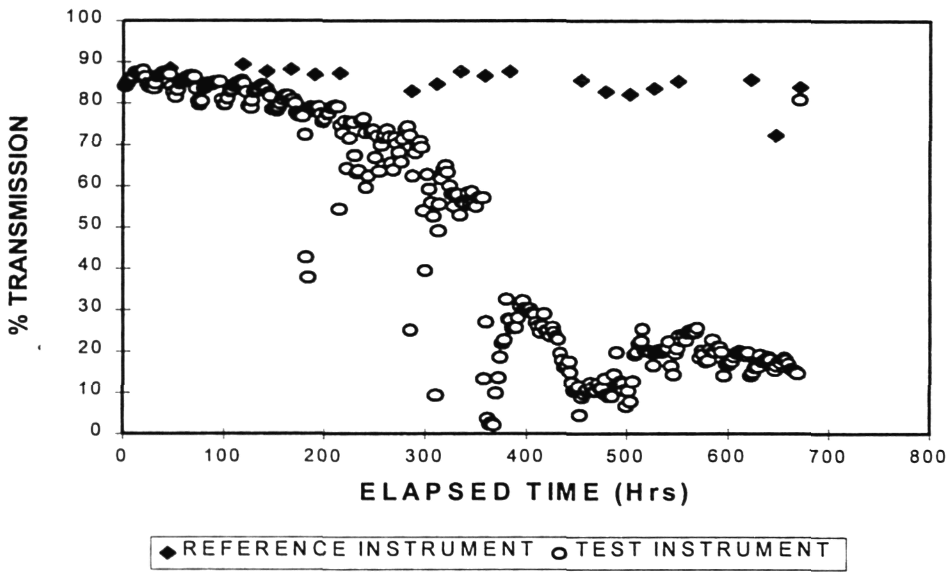

Figure 2 Transmissometer test. The reference was kept clean and used to record readings daily while the test piece was immersed in the tidal tank at UMBSM.

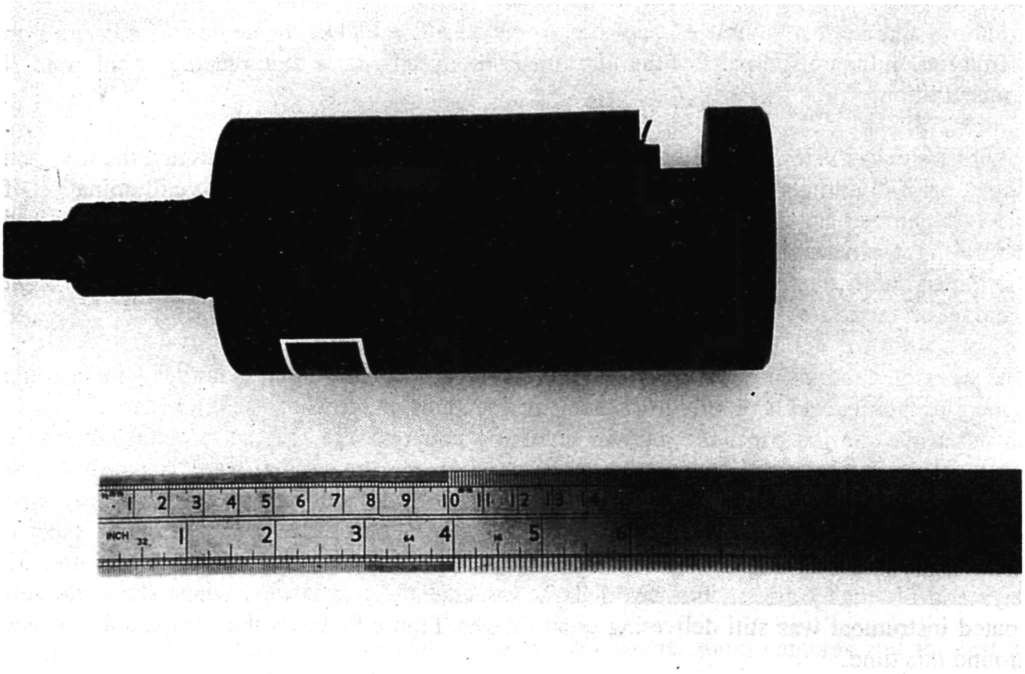

Figure 3 A fluorimeter. The light source operates at 430nm and the detector operates at 685nm. It detects the concentration of chlorophyll a.

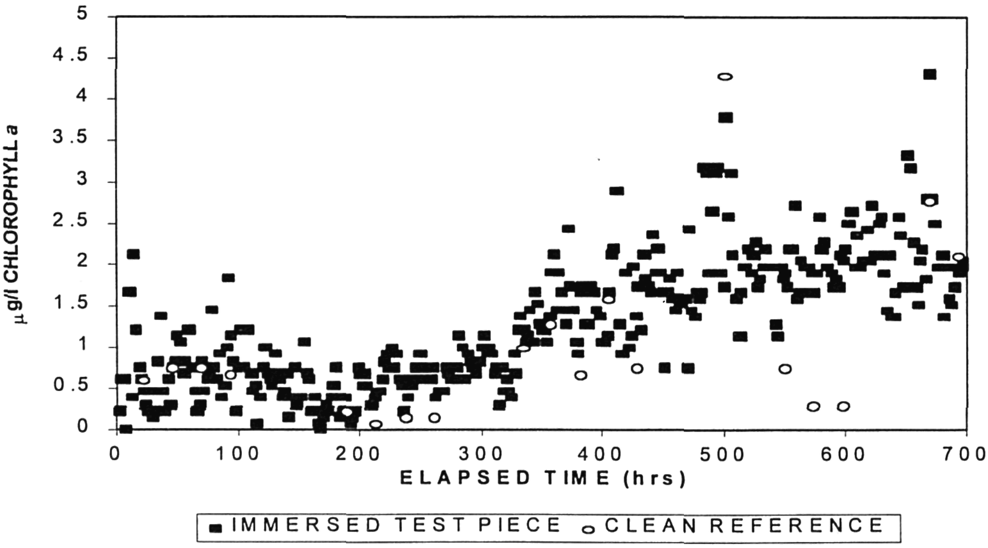

Figure 4 Fluorimeter test. The reference was kept clean and used to record readings daily while the test piece was immersed in the tidal tank at UMBSM.

windows and the test volume of seawater, to the detection end of the device. Similar biofilm formation on the optical parts of the fluorimeter produced a false high reading of chlorophyll concentration.

Figure 5 shows the test set-up for a pair of underwater colour zoom cameras and the test card target, prior to immersion for the first set of trials. Figure 6 shows the relative illumination of the two cameras as a function of time. These experiments (at a depth of 1-1.5m) utilised natural light penetrating from the water surface. As the biofilm developed the light entering the fouled camera appears to increase due to diffusion and scattering, by the biofilm, of light incident on the camera window.

The work on coatings has led to a range of successful hydrogel-based materials with suitable optical properties and relatively long lives (many month) in terms of fouling resistance. After an extensive marine trials programme involving coupons and the use of both laboratory assays and marine environmental exposure trials, the materials were deemed to be sufficiently promising to be used in full-scale trials on underwater cameras and oceanographic instruments. Figures 7 and 8 show the unprotected and hydrogel-protected cameras after a total exposure period of 67 days. The reference had been cleaned after 33 days and obviously after a further 34 days was essentially unusable, whilst the hydrogel-coated instrument was still delivering good images. Figure 9 shows the comparable images around this time.

Work on the hybrid coatings has produce some successes in that laboratory-based bioassays have demonstrated that a number of the formulations have reduced biofouling properties. Some of these coatings are low adherence and therefore are useful in combination with a cleaning strategy.

The extensive study of different commercial glass formulations and other glass-like materials has shown that, whilst none of these materials had very extensive biofouling resistance, there are differences between them. In addition a synthetically-produced glass formulation with significant biofouling resistance has been found. In certain oceanographic campaigns, lasting for a few weeks, the selection of an alternative glass formulation would realise significant benefits in terms of the quality of data collected. The results of the glass study also emphasised the importance of surface roughness in biofouling resistance and the changes in roughness which occur in some of these materials when exposed to the marine environment for significant periods.

The task which evaluated a range of biocides has produced a significant database of useful materials which have been evaluated against a number of criteria. The subsequent experimental study has ranked these according to effectiveness with respect to minimum concentration levels. The results of the study into the use of ultrasonic techniques to deter settlement of micro-organisms on surfaces have shown that the technique can be useful but that a major difficulty exists in determining the amount of energy being delivered at the surface to be protected. This is a problem which required to be resolved before practical guidance on the use of the technique can be established.

All of the given electrical methods investigated reduced biofouling under laboratory conditions. The experimental programme has been extended to evaluate these technologies in full-scale marine exposure trials and in instrument trials. These developmental aspects pose

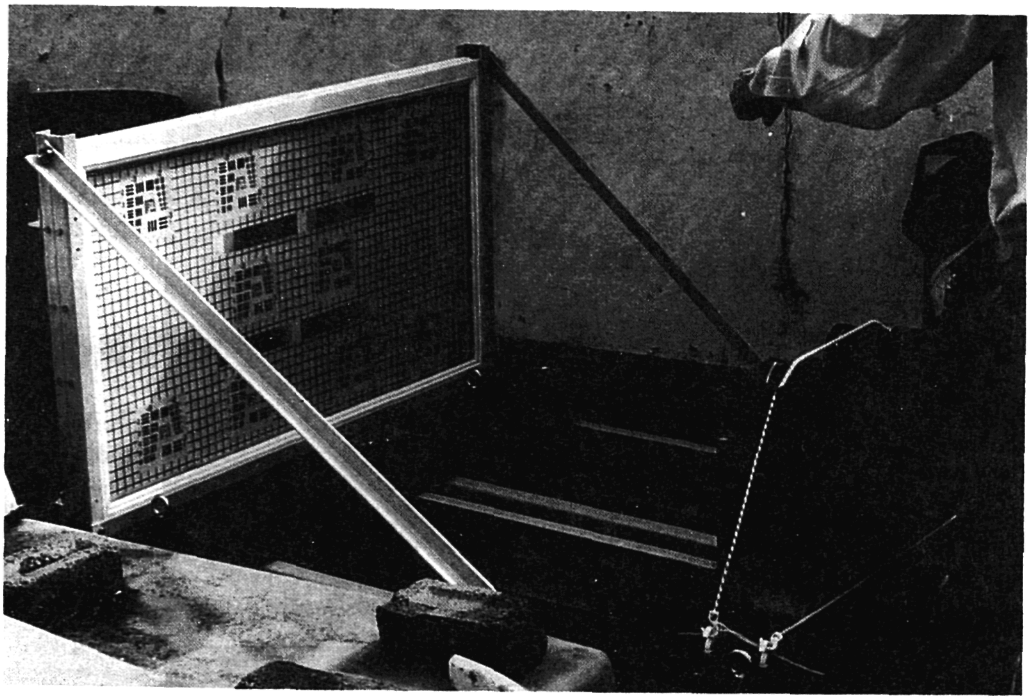

Figure 5 The test set-up for a pair of underwater colour zoom cameras and the test card target, prior to immersion for the first set of trials.

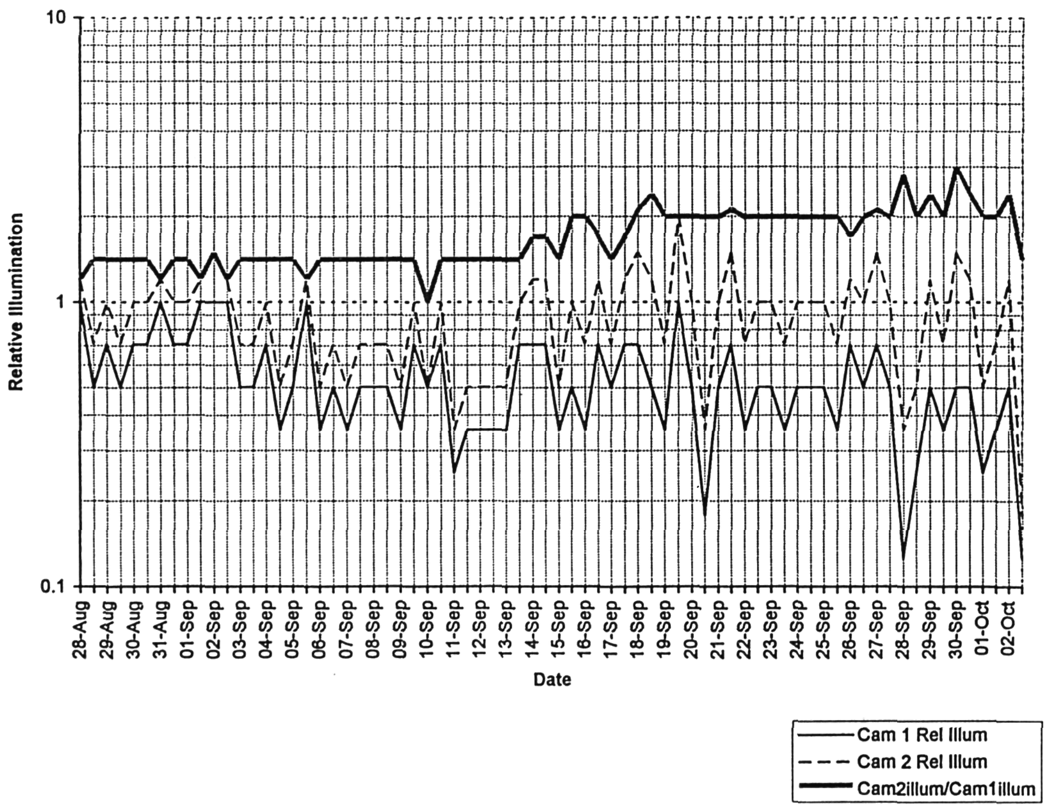

Figure 6 The relative illumination of the two cameras as a function of time.

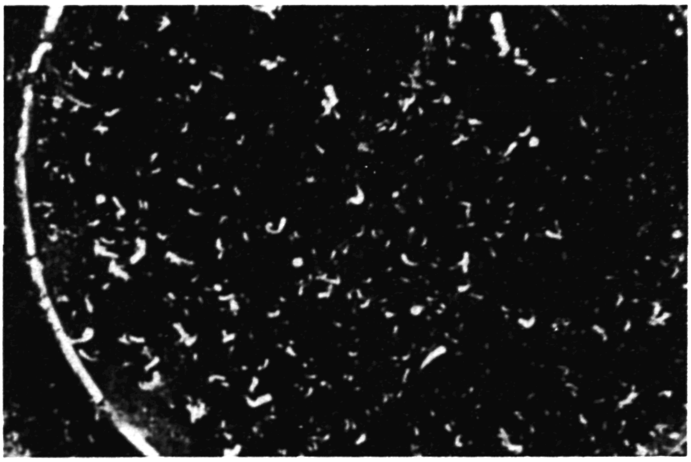

Figure 7 Unprotected camera (18 September 1997) – 10 weeks exposure.

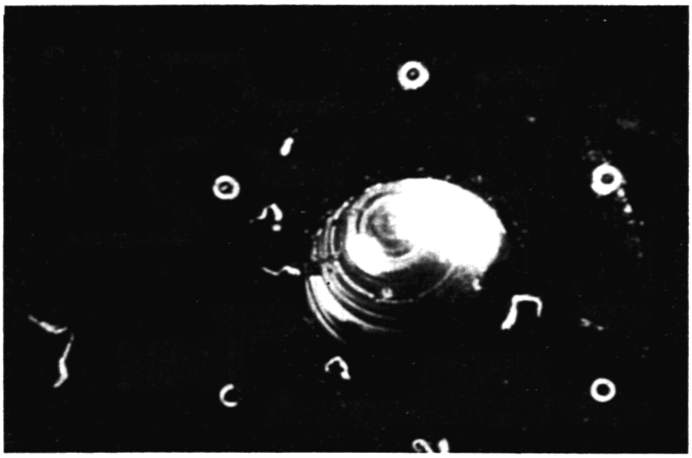

Figure 8 Protected camera (18 September 1997) – 10 weeks exposure.

Camera 1 Preset 3
12 September 1997 File mi030304.bmp

Camera 2 Preset 3
12 September 1997 File mi030305.bmp

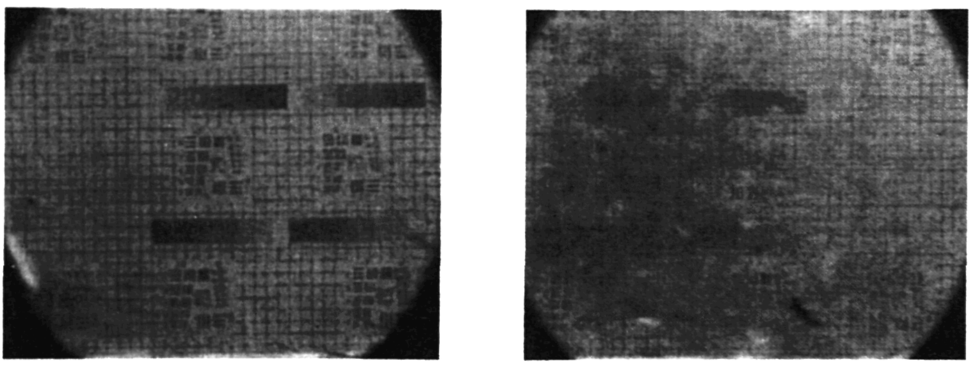

Figure 9 Third camera trial, day 67 (hydrogel coating – camera 1; unprotected – camera 2).

significant technological challenges. The power requirements for the different technologies vary significantly and hence each technique has a different sphere of application.

The study of fundamental aspects of biofilm development has yielded a vast amount of new and useful information including the detailed progression of microfouling and the seasonality of the communities, as well as the variation between the three sites. The depth studies have provided information on the depth range in which various organisms colonise surfaces.

4. CONCLUSIONS

1. Biofouling rapidity affects the performance of underwater instruments when the biofouling pressure is significant.

2. There are a range of options to deter or delay the onset of significant levels of biofouling on optical surfaces.

3. Some of the technologies developed in the BROS project are extremely successful in protecting optical surfaces.

4. The results of the BROS project are likely to lead to successful commercial products for use with underwater instruments.

5. ACKNOWLEDGEMENTS

This project would not have been possible without the efforts of a large number of researchers in the participating institutions. The lead scientists for each partners organisation are pleased to acknowledge these contributions.

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Title: Development of microsensors for use in the marine environment. (Micromare)

Contract no: MAS3-CT95- 0029

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DEVELOPMENT OF MICROSENSORS FOR USE IN THE MARINE ENVIRONMENT

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SUMMARY:

The overall objective of the project is to develop, improve and test microsensors and systems for their use in direct measurements of 1) oceanographic and environmental parameters, 2) sea floor characteristics, 3) biogeochemical processes and 4) fluxes across the sediment-water.

The sensors that will be developed or adapted can be classified in three major categories:

- biosensors for methane, nitrate, sulphate, and sulphide
- electrochemical sensors to characterise sediment diffusivity
- optical sensors (optodes) for oxygen, pH, carbon dioxide and a precise definition of the sediment surface.

Further, measuring devises, optical systems, opto-, signal- and system electronics will be developed and constructed in order to test the microsensors directly in marine environments.

Newly developed first-generation models of micro biosensors for nitrate and methane will be adapted for use in marine environments and their sensitivity, stability and functional duration (hours to days) improved. To this adds the development of micro biosensors for sulphate, sulphide and a micro sensor to characterise sediment/biofilm diffusivity. The functional duration of microscale biosensors will be enhanced by replacing the electrochemical sensing elements with optical sensors.

In order to design optodes for pH and carbon dioxide it is imperative to develop chemically sensitive materials that respond to pH and carbon dioxide by a change in their intrinsic fluorescence. These materials will be characterised in terms of their optical and mechanical properties, sensitivity to pH and carbon dioxide, long term (days to months) stability, compatibility with the fibre optics applied, and performance under marine conditions.

Opto-, signal-, and system electronics will be developed and adapted for use during development and tests of the optodes. Further the electronics will be adapted in terms of miniaturisation, decrease of power consumption and interfacing to existing electronics used in a free operating micro profiling, deep-sea, benthic lander.

The long term (months) stability of an existing micro optode for measurement of oxygen will be adapted and improved, and optodes for measurement of pH and carbon dioxide will be developed and miniaturised. Further, a microscale optical sensor for high resolution determination of the sediment-water interface relative to measured micro gradients will be developed. Finally, the sensors will be tested and adapted for use directly in the marine environment at full ocean depth.

A pressure stable (600 atm) optical system will be developed and applied on an existing profiling unit and on easy-to-deploy modular units which can be mounted on unmanned platforms such as buoys, ROV's, AUV's, benthic landers and stations. Shallow water and deep sea tests during long term (months) monitoring, water column profiling (full ocean depth) and fine scale (25 μm) profiling over the sediment-water interface will be performed.

INTRODUCTION:

In situ measurements in the marine environment are necessary for many applications. However, very few sensors are presently available for in situ use and the majority of these suffer from e.g. physical disturbance of the measuring environment, unpredictable pressure effects, poor long term stability, high power consumption, and long response times.

The planned intensification of deep-sea research in the coming years is thus likely to be limited not only by logistical problems, but by the availability of adequate sensors.

The introduction of the oxygen microsensor to the marine environment (Revsbech *et al.*, 1980), and the subsequent development of microsensors for measurement of sulphide and pH (Revsbech *et al.*, 1983) lead to availability of sensors without many of the above mentioned disadvantages. Microsensors have thus been used in the deep sea for high resolution profiling measurements over the sediment-water interface (Gundersen and Joergensen, 1990), registration of benthic fluxes in flux chambers of in deep-sea landers (Glud *et al.*, 1994) on modular units for deployments from submersibles and ROV´s, and for water column profiling and general environmental monitoring
(Fossing *et al.*, 1995).

RESULTS:

Presently, after 2 years of the project, a variety of microsensors and measuring systems have been developed. The highlights are briefly summarised. For more detailed information please consult the referred papers, the project homepage or the involved partners directly.

DEVELOPED MICRO-SENSORS

Nitrate bio-sensor (o.d. 25 µm)

Principle: Bacterial reduction of nitrate to nitrous oxide in the tip of a capillary, which acts like a micro-scale chemostat. Nitrous oxide is subsequently detected at a cathode.
Range 0.1 µM – 10 mM, Tolerance: salinity: 0-40 ‰, temperature: 3-37 °C.
Larsen, L.H. *et al.* (1998), Larsen *et al.* (1997), Larsen, H. L. (1998), Lorenzen, J. *et al.* (1998).

Methane biosensor (o.d. 25 µm)

Principle: Detection of oxygen consumption from a built-in reservoir due to oxidation of methane in the tip of the sensor. Range: 2 – 1000 µM, temperature: 10 – 30 °C; no influence by salinity.

Damgaard, L.R., *et al.* (1995), Damgaard, L.R. and Revsbech, N. P. (1997), Damgaard, L.R. and Revsbech, N.P. (1998), Damgaard, L. R., *et al.* (1998)

Flow sensor (o.d. 20 µm)

Principle: Detection of the diffusive out-flux of a tracer through a micro-pore.

Range: 10 µm – 60 mm/s.

Gundersen J.K., *et al.* (1998), Gundersen J.K. *et al.* (1998).

Diffusivity sensor (o.d. 100 µm)

Principle: Like flow sensor, measures gas diffusivity in aqueous matrices like sediments.

Range: 0.2 - 2.1 x 10^{-5} cm^2/s.

Revsbech, N. P. *et at.* (1998)

Oxygen optode (o.d. 30 µm)

Principle: Dynamic quenching of luminescence of immobilised on tapered silica fibres. Lifetime and intensity evaluation.

Ranges: 0-5%, 0-20%, 0-100% oxygen. No influence by salinity, stirring, or H_2S. Low effect of hydrostatic pressure. Temperature: 0-90°C.

Glud, R. N. *et al.* (1996), Glud, R.N., *et al.* (1998), Klimant I. *et al.* (1998), Klimant I. *et al.* (1995).

pH optode (o.d. 30 µm)

Principle:

1) Luminescent energy transfer from pH-independent luminophore to a coloured pH-indicator. Luminescence lifetime-based sensor.

Range pH 5-9. Response time 5-15 s. No influence by oxygen or stirring.

2) Colour change of absorption-based indicators in various matrices. Range: pH 7-9. No influence by stirring. Temperature: 0-50°C.
Kohls, O. *et al.* (1998), Kosch, U. *et al.* (1998)

Temperature optode (o.d. 40 μm)
Principle: Temperature dependence of luminescence lifetime of long-lived luminophores are immobilised into various matrices where oxygen does not interfere.
Range: 0-50°C with resolution of <0.1°C. No influence by salinity, stirring, or gases.
Holst, G. *et al.* (1997).

Carbon dioxide optode (o.d. 40 μm)
Principle: Severinghaus-principle with internal pH measurement by a dissolved pH-sensitive luminophore. Luminescence intensity.
Range: 2μM to saturation. No influence by salinity or stirring. Interference: gases penetrating the hydrophobic membrane and inducing a pH change of the buffer.
Temperature: 0-50°C.

Surface detection probe (o.d. 20-50 μm)
Principle: Measurement of back scattered NIR-light with a tapered silica fibre.
Range: Grain sizes from 0-100μm. No sensitivity to salinity or stirring. Temperature: 0-100°C.
Klimant, I. *et al.* (1998),

DEVELOPED MEASURING SYSTEMS

Single channel as well as 8-channel measuring system for use with oxygen and temperature micro-optodes in laboratory or field application

Principle: Phase modulation technique, fibre coupler set-up (either fibre coupler array or fibre optical switch), analogue and digital signal processing with serial interface to PC. Frequencies: 1.25, 5, 37.5 kHz, programmable, amplitude res.: 0.3mV, phase angle res.:0.01 degree, oxygen res.: 0.1% air saturation, accuracy < 1% air saturation.

Multi-channel modulated vector (i.e. magnitude and phase) measurement system
Applications: Fluorescence decay time, fluorescence intensity, optical absorption.
Frequency range: 0.1 to 50 kHz (expanded mode: 0.4 to 200 kHz), programmable
Resolution: 18 bits in magnitude, 18 bits in phase (i.e. 0.001 degree)
Gruber, W. (1997), Holst, G. *et al.* (1998).
DEVELOPED SYSTEMS FOR IN SITU USE

Pressure stable (600 bar) optical system for *in situ* optode measurements.
Modular, pressure stable (600 bar) unit and electronics allowing high resolution (25 μm) profiling measurements at the sediment-water interface with the new sensors Glud, R. N. *et al.* (1998).

Miniaturised single-channel measuring system for use of oxygen micro-optodes on benthic landers. Principle: Luminescence intensity, controlled excitation.
Frequency: 10 kHz, amplitude resolution: 12 bit.

Miniaturised dual-channel modulated vector (i.e. magnitude and phase) system.
Applications: Fluorescence decay time measurement, fluorescence intensity measurement, optical absorption measurement. Frequency range: 0.01 to 96 kHz, programmable.
Resolution: 16 bits in magnitude, 15 bits in phase (i.e. 0.01 degree)
Klimant, I. *et al.* (1998), Revsbech, N. P. *et al.* (1998), Kohls, O. *et al.* (1998), Kühl, M. and Revsbech, N. P. (1998), Kühl, M. *et al.* (1998), Lassen, C. *et al.* (1998).

DISCUSSION:

The first two years of the project has thus resulted in a dramatic increase in the amount of available microsensors and measuring systems for use in marine studies. All developed sensors and systems are functional for laboratory investigations of marine samples. (Foster, S. *et al.* (1998), Glud, R. N. *et al.* (1998), Glud, R. N., *et al.* (1997)).

Further oxygen micro-optodes are operational on profiling and chamber landers in shallow water and in the deep sea. The surface detection microsensor has been deployed at shallow water. The nitrate biosensor has been deployed for micro-profiling in shallow waters. (Glud, R. N. *et al.* (1998), Larsen, H. L. *et al.* (1998)).
During the coming last project year an additional series of the developed microsensors and measuring systems will be tested in situ.

ACKNOWLEDGEMENTS:

The European Commission is thanked for supporting this project through the MAST Programme.

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TITLE: CYTOBUOY
Upgrading Flow Cytometry for Buoy mounted operation: first experimental implementation.

CONTRACT NO: MAS3-CT95-0030

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CYTOBUOY: IN SITU OPTICAL SCANNING OF INDIVIDUAL PARTICLES WITH A BUOY MOUNTED FLOW CYTOMETER.

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SUMMARY

In situ and real time analysis of phytoplankton at sea will improve monitoring but also requires dedicated instrumentation. For this purpose, the CytoBuoy was developed, a flow cytometer which operates inside a 90 cm buoy to allow for moored operation. In flow cytometry (FCM), several correlated optical measurements are made simultaneously from individual particles in suspension. The CytoBuoy project aims at transferring this technology to in situ platforms. In the CytoBuoy, single particles are optically scanned with a laser and the pulse profiles of these individual particles are stored fur further analysis and classification. In situ flow cytometry will provide particle data for taxonomic and ecological analysis, as well as correlated distributions of optical properties for ocean optics and 'sea truth'. The development of the CytoBuoy prototype is described below.

1. INTRODUCTION

There is a need for technologies that allow rapid and adequate monitoring. The enormous diversity of the particle composition found in natural surface waters impairs the application of most particle analysis techniques, either owing to ambiguity in the relation between the measured entity and particle size, or by physical limitations to sensitivity, analysis resolution or particle size range. In addition, most techniques apply a single detection principle only, allowing hardly any discrimination beyond the elementary small-large assessment. In flow cytometry (FCM), several correlated measurements are made from individual particles (Melamed et al. 1990, Shapiro 1995). Light scatter and fluorescence of particles passing a focussed laser beam is measured as shown in Fig.1. The cells are pumped in single file through the analysis point at typically 1,000 cells or more per second, with a practical analysis speed of a few minutes per sample. The successive scattering and fluorescence signals generated by each passing particle are detected by photomultiplier tubes or photodiodes, with sufficient sensitivity to analyse particles down to a few µm in diameter or even smaller. The electronics convert these raw signals into correlated data, stored on disc for analysis and presentation as distributions or scatterplots or grey/colour maps. Instruments may have a sorting device, allowing the physical separation of selected cells from the main stream during analysis. The measured forward light scatter is governed by particle size and the sideward scatter is most sensitive for internal and external structures of the particle. The fluorescence intensity probed simultaneously in typically 2 to 4 excitation/ emission combinations is governed by the amount of cellular pigment and the composition which is different for major algal classes.

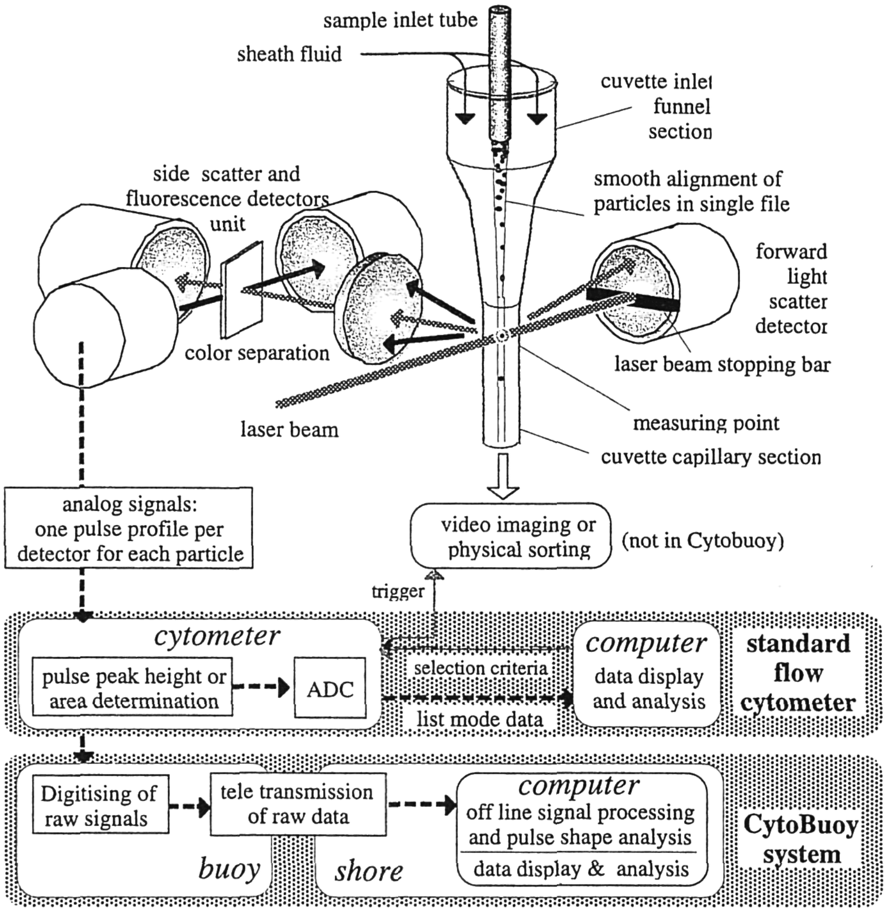

Figure 1: principle of flow cytometry

The small sizes of the phytoplankton studied by oceanographers allows the use of standard (biomedical) flow cytometers in the lab or even on shipboard. For the more diversely sized and shaped phytoplankton found in coastal zones and fresh waters, the Optical Plankton Analyser (OPA) was designed in the Netherlands (Balfoort et al: 1992, Dubelaar et al: 1989, Hofstraat et al: 1991, Hofstraat et al: 1994, Peeters et al: 1989). Its extended linearity allows analysis of field samples (Dubelaar and Van der Reijden: 1995). This technology was further developed in the MAST II project EurOPA, to achieve better routine applicability, also on shipboard, combined with a higher discriminating potential, and more automated data analysis (Jonker et al: 1995, Dubelaar et al: 1995). The CytoBuoy Project aims at transferring this flow cytometer technology to a real in-situ platform. The advantages will be the availability of continuous quantitative water column observations in time or space (tracks), yielding more insight in system dynamics and patchiness and more reliable overall results, important for intercalibration with other platforms and for input in ecological and biophysical models.

The direct project goal is to design, build and mount the smallest practical basic EurOPA type flow cytometer into a simple buoy, and use it as a test instrument. The quality of the generated data will be assessed with regard to applications of marine monitoring. Firstly this relates to the 'fingerprint' or analytic quality of the data. Next, sensitivity and dynamic range of the particle size measuring range, and the analysis of particle concentrations are important. Sampling should be aselective for particle types and the measuring process should not inflict morphological damage to the particles. Service cycle, data transmission robustness, corrosion and fouling resistance etc. will be evaluated. The longer term aim is successful development of autonomous real in situ cytometers with 'EurOPA' high definition quality, which may substantially stimulate the automation of environmental monitoring.

2. DESIGN AND CONSTRUCTION OF THE CYTOBUOY

The CytoBuoy is equipped with a small, single laser, high dynamic range flow cytometer without the options and accessory modules of the EurOPA instruments. Feed back from potential scientific users of the CytoBuoy concept was used in the design/engineering process. A comprehensive matrix was made of flow cytometer specifications and functions, governed by environmental aspects, basic physics, logistics and rules of thumb. The input of typical environmental values regarding particle concentration and preferred sampling rate allowed us to make basic choices with respect to signal processing and data transfer, and to assess interactions of changes in design of subunits. The buoy mounted flow cytometer had to meet the following overall restrictions:

- Limited power supply;
- Limited space and weight, 380 x 480 mm (diameter x height) cylinder, 25 kg;
- Platform movements and roll, the buoy is liable to shocks during hoisting;
- Stand-alone operation, up to half a year or more;
- Environment (water/salt, temperature).

OPTICS OF THE FLOW CYTOMETER UNIT

Excitation optics

Laser diodes provide an alternative to the more conventional types of laser system. Not only are they small and compact, they are far more efficient than their comparable alternatives. However, there are several complicating properties: susceptibility to damage from spiking currents; the so-called mode-hopping with output power changes of up to 10%. Their high, asymmetrical divergence angles cause laser focus deterioration by diffraction lobes. Helium Neon gas lasers, emitting at 633 nm have better beam quality, but sizes are large, and power use is 20 Watts or more. Similar power requirements are typical for the latest versions of the diode laser pumped Nd-Yag lasers, that emit at 532 nm. Diode and solid-state laser technologies have resulted in compact, highly efficient continuous wave laser sytems, providing exceptional lifetime, stability, and efficiency compared to air-cooled gas or lamp-pumped solid-state lasers. The size of the Coherent OEM device 'Compass' allowed mounting in the available space without problems. The implementation of this relatively expensive laser was decided to allow analysis of 2 fluorescence bands, whereas power demand is not too high. Excitation with a 635 laser diode remains preferable when only chlorophyll fluorescence (at 675 nm) suffices. Fibre delivery of the laser beam was not applied, because the main advantages, mechanical freedom and beam quality improvement, were not significant in the current design, whereas the disadvantage of light losses was substantial.

With the gaussian shaped intensity profile, the laser spot should be much larger then the sample core in order to obtain an acceptable low variation in light intensity over the sample core diameter. Two beams separated by a fraction of the beam waist size, yield a much larger region of constant illumination if their polarization states are chosen for minimal interference. With the small diode lasers, it is possible to couple an additional diode laser in orthogonally using a polarizing cube beam splitter. With a more expensive and larger laser such as a NdYag, a quarter wavelength retarder isolator set-up can be used to double the beam in the cuvette.

Colour separation

Normally the light emitted by the particles is collected by a microscope objective and 'imaged' to the detector system as a near parallel beam, passing subsequent dichroic beam splitters that reflect different portions of the colour spectrum. For a system requiring only side scatter and chlorophyll emission, the dichroic system is the most efficient. If many bands or nearby located bands are required, narrow band pass optical filters have to be used to prevent spectral overlap, reducing light collection efficiency. Spectrographs, both grating based (Fuller and Sweedler, 1996) and prism based (Gauci et. al. 1996) were demonstrated in flow cytometry for applications requiring higher resolution spectral analysis than achievable with dichroics. With an appropriate linear detector array, the spectral resolution is limited (roughly) by the size of the individual detector pixels. In stead of a grating, a dispersing prism may be used also. These have better throughput, but less dispersing capacity resulting in too large optical path lengths for the CytoBuoy. ISA inc. (Jobin Yvon) offers a rugged small focal length grating spectrograph (CP140) with a large input solid angle (f/2). The N.A. and slit width are sufficient to accommodate full throughput of the fluorescent light as collected by the CytoBuoy system.

Detectors

PMT's are most commonly used in flow cytometry to measure the fluorescence signals of the particles. However, photomultiplier tubes are voulnerable devices with respect to shock impact, and alternatives should are considered. Whereas solid state technology is capable of detecting the low light levels measured by the flow cytometers, the main restriction remains the short light pulses generated by our system and the requirement for linear (non-integrating) signal acquisition to allow pulse shape analysis. Flow cytometers without pulse shape requirements may have detector integration times of 10 to100 microseconds. The particles in CytoBuoy flow at 2 m/s through a 5 μm focus, resulting in a pulse rise time close to 1 μs. The detection system should be able to follow this, implying a sub-microsecond reponse of the detector system. The newly developed hybrid photomultiplier type (HPMT) detectors (Schomaker 1994) combine the performance of photomultipliers and solid state technology. Currently a 7 pixel device is mounted. These devices are expensive, but with a multipixel version, one device suffices for all parameters in principle, and only a single high voltage power supply is needed. New pixel configurations can be manufactured at will. This could mean that, using a particular grating with a spectrograph, the pixels can be placed on optimum positions for analysis of specific phytoplankton fluorescent emission bands.

FLUIDICS OF THE FLOW CYTOMETER UNIT

System

The limited space and weight require that the sheath fluid is recycled, allowing chemical treatment of the sheath fluid to prevent fouling and to adjust viscosity and density. The buoy will be a sealed hull, which demands the fluidics to be a rigid and closed system to be able to withstand pressure changes within the buoy hull. of 0.1 bar. The fluidics are in an open connection to the seawater for equalizing the pressure in the fluidic system and to discharge the surplus of taken in sampling water. The sample intake is liable to selectivity and fouling. For the buoy we will make a special design, after first test with a freely moving teflon tubing.

Sample dispensing.
The classic way of sample dispensing is pressure controlled or injection by a syringe system, which involves filling, dispensing and cleaning and requires 3-way valves. It would be more elegant to have a continuous flow system yielding 0.4 ml/min. or lower with:

- precise, viscosity and pressure independent flow to obtain reliable concentration data.
- reversibility, low dead volume, minimum valves, and low fluid shear to allow easy rinse, prevent clogging , and to minimize damage to vulnerable aggregates and colonies.
- fluctuation free flow to achieve minimal sample core diameter and burst free particle rate.

A valveless piston pump from Fluid Metering Inc. (FMI) was tested. The sinusoidal discharge was eliminated by programming the attached stepper motor in such a way that it gives a slow continuous discharge and a quick intake. We analyzed samples with colonies of Pediastrum. These colonies are vulnerable and easily break to single cells by shear forces within the fluid, but we could not measure significant de-aggregating of the colonies.
Alternatively, a peristaltic pump, was tested:

- Advantages: • No mechanical parts in contact with the fluid. Simple control.
- Disadvantages: • chance on tubing failure.

Tests were done with a home made pump. Pulsation was virtually eliminated by designing a slowly rotating, large diameter rolling section with only two rollers. Thick wall, narrow orifice tubing keeps fluid speed maximal to prevent settling, and reduces failure chance.

Sheath fluid
Injection of the sample and calibration beads suspension into the sheath fluid will be upwards to facilitate degassing. The sheath fluid should be denser then both sample fluid and calibration suspension. The density of the sample from the surrounding sea can be in-between 1.015 and 1.028 depending on water type. Because of re-use of the sheath fluid and slow dilution with sample water the density will approach the surrounding sea. The smallest particles we can detect are about 1 micron in size. This means that at least all parts greater than half the size, say 0.5 micron have to be filtered out of the sheath fluid. We choose 0.2 micron filters to trap air bubbles and to filter bacteria. The sheath flow should be pulsation free, stable and independent of pressure variations. Most positive displacement pumps pulsate. An air pressure system would react to pressure variations and to platform movements and requires a complex controlling system. A small gear pump, although expensive, seems the best choice. An I/R regulation may compensate for slow pressure build-up by clogging of the filters.

Calibration suspension
For reference, test beads have to be measured regularly. The concentration of the test suspension must be stable. These particles are liable to settle, aggregate and stick to walls. This problem can be reduced with surfactants and matching the density of the fluid and the particles, combined with stirring. We found polyvinyltoluene beads with a 1.027 density. These beads were tested and showed a good long term stable suspension. There was only some adhesion to the plastic container.

ELECTRONICS & DATA PROCESSING

General
The electronics and computer system need to be small and low in power consumption. The CytoBuoy will mainly use embedded software and the user interface of the operation of the instrument is limited. Data can only be analysed afterwards, when data is sent to the shore.
In commercially available cytometers, the signals from a particle are reduced to a single value per parameter. This implies that most of the morphological data is discarded. With one laser, only a limited amount of pigment-composition information can be obtained. This means that morphological data is even more important in order to discriminate individual species.

Therefore, the possibility to incorporate pulse shape analysis into the CytoBuoy electronics was pursued.

Alternatives

Normally, the electrical pulses from the photodetectors are amplified and fed to peak-sense circuits. Depending on the amplifier speed, the actual detector pulse maximum or the integrated pulse (fast vs. slow) is 'seen' by those peak sense circuits, that subsequently put these peaks into analog to digital converters (ADC's). The digital numbers are then send to a computer in so-called list mode data files. The large phytoplankton size spectrum impairs the use of conventional electronics. The (Eur)OPA therefore does not use peak-sense circuits, but fast ADC's directly digitize the output from fast amplifiers, so the actual pulses are fully available digitally (minimally 25 numbers per pulse). To reduce data load (design dating from 1986), the numbers from each pulse were summed directly in hard wired electronics to obtain the pulse area (integral) and only these resulting numbers (1 per pulse) were sent to the computer.
However, the (Eur)OPA electronics were not optimized for low power consumption, necessary for buoy operation, and the digital signal integration resulted in substantial printed circuit board sizes. Although more features can be extracted in real time using modern digital signal processor boards (Zilmer 1995), the use of DSP's yields similar disadvantages, and in addition, would require substantial development to implement flow cytometric data processing with high speed data collection (5 MHz, 3-4 channels). It also became clear that programming the DSP board does not allow for flexible analysis and display of the pulse waveforms. This led to the approach of acquiring and transmitting the complete raw pulse data to shore. There, pulse data analysis can be done with a PC, with greater flexibility. This option required development of a dedicated low power consuming, fast grabber card. Small processors can be used to control data acquisition and interfacing of the data to the central GENI processor. This processor takes care of initial data reduction and selection and storage and/ or transmittance of the data. Direct data reduction in the buoy CPU will become easier with future more powerful processors, and such developments can profit from the results obtained with the approach taken here.

Development

The pulse acquisition approach requires:

1. (semi)-logarithmic and fast amplifier;
2. data grabber for each parameter;
3. digital triggering circuitry;
4. interfacing of data grabbers to the GENI processor system of OCEANOR.

Basically the grabber card should consist of a logarithmic amplifier section, followed by digitising of the signals and subsequently some form of data buffering and a microprocessor.
It was necessary to develop a logarithmic amplifier having less than 0.5 microseconds risetime over 3 or 4 decades input range, tailored to the input levels of 8 bits (256 levels) linear, low power consuming fast ADC's. Available low power microprocessors can not handle the data stream, buffering is necessary therefore. As interfacing of DSP's is not simple, and FIFO's are not really low power, a RAM buffer was the best option, operating in batch processing (transfer all data of a measuring run to the processor) mode.

Datagrabber and triggering

A 16 bit (4x4) counter functions as address generator for the RAM. Two flipfop registers after the A/D convertor allow to store 2 data points measured before the trigger is generated. This allows to set the trigger level well above the noise level. Data acuisition requires no activity from the processor but is driven by a 4 MHz clock signal. The processor's I/O ports stay connected so that activity can be monitored. When the RAM is filled with data, the flipflops are put in a high impedance state to isolate the address generator and A/D from the RAM and have it available for the processor. Memory of the processor is sufficient for a small program.

Logarithmic Amplifier

The AD843 amplifiers of the prototype consume 130 mW each for a gain-bandwidth product of 43 MHz. Gain at 4 MHz is approximately 20, which is insufficient. Extra gain without extra consumption is realised by using transistors instead of the classical diodes to realise the log function. Gain requirenment at low inputs is limited by realising a LOG (1+x) function instead of a true logarithm. The circuit output was within 5% of the theoretically expected, over an input current range of 1 μA to 10000 μA. The gain of a logarithmic amplifier is proportional to the absolute temperature. Sea surface temperatures range from 270 Kelvin to 305 Kelvin. It is decided not to compensate for this 10% change by using temperature dependent resistors that are expensive and difficult to find. The temperature factor can be measuered by a test current and accounted for in software.

DATA ANALYSIS AND DATA STORAGE

Data analysis of the CytoBuoy data will take place both inside the CytoBuoy (future) and afterwards, when the data has been sent to the shore. The following tasks are necessary:

1. File handling and storage. Data will have to be stored as raw pulse data and as list mode data. Initial data storage will take place in the CytoBuoy and on shore.
2. Pulse shape analysis. The raw data from the CytoBuoy will be pulses from individual cells and from various detectors. These will need to be processed in order to compute particle characteristics from these.
3. Particle analysis. As soon as particle characteristics are available, particles can be classified. Phytoplankton can be discriminated from debris and noise. Within the phytoplankton, groups and species can de discriminated. Tools will be developed for this.
4. Graphical display, including integrated display of the raw pulse data and the list mode data (histograms, scatterplots).
5. Automated data processing. Based on using prototypes of the program and studying the most informative waveform features, the automated processing of CytoBuoy data will be developed. This will allow for automated sample analysis such that integrated water quality monitoring systems will be able to use the CytoBuoy data.

The Windows application **CytoWave** was developed in C++ so the resulting class libraries can be used for both data processing in the CytoBuoy (embedded software) and off line analysis afterwards on shore (Windows applications). The application allows display of both pulse waveforms and list mode data. Selections on subpopulations in the data can be made by setting graphical (mouse-driven) selections in the bivariate dotplot display of the data. Pulse waveforms of particles that meet certain criteria are displayed based on these selections. Features like signal length, integral, pulse height, ratio between parameters etc. can be derived. The data can be displayed, stored in ASCII and list mode format, and exported to commercial data analysis programs, including those for flow cytometry data. The file format for the application is called Cyto Pulse Format 1.0 (CPF1.0), and was derived from the FCS format; the internationally accepted file format for flow cytometry (spreadsheet). Each row contains data from one cell, and each column is one optical parameter, like light scatter and fluorescence. The FCS format consists of header data, followed by (normally) binary list mode data. The CytoBuoy data will be based on pulses from individual cells: the raw signal when a cell passes the laser beam. This is the data that is directly collected from the grabber cards and is one byte, semi-logarithmic data. CytoBuoy files consist of a header with information on the sample, date, time, position of the CytoBuoy, number of detectors etc. This header is followed by the data on individual events (particles); a set of pulses for each cell, such as from Forward Scatter, Side Scatter and fluorescence.

CONTROL OF THE FLOW CYTOMETER UNIT

The CPU executes or controls the:
- flow cytometer components
- data acquisition of parallel data from the data grabbers
- pre-processing of data (data selection, compression and storage)
- transmitting of pre-processed data to shore

The CPU (80c188EC MM) is a stand alone computer equipped with a powerful real-time operating system. Special hardware, together with the operating system ensures low power consumption. The system clock is frozen when the CPU is idle. If an interrupt occurs, or if data is written to the serial ports the clock restarts and the CPU continues to execute after less than a microsecond. The operating systems reads the battery clock to maintain time. Due to the limitation of IO lines at the CPU board, there is a need for an additional board for interfacing the data grabbers, the optics and the hydraulic units. Since there will be one data grabber for each parameter, chip select logic and control lines separate each data grabber. A Windows 32 bit test and configuring application for hardware and software communicates with the CytoBuoy via a serial RS232 line. Added OLE-2 server facilities allow transfer of CytoBuoy data to other applications like Microsoft Excel at runtime. This is particularly useful during the current experimental period for analysis of data from the CytoBuoy without having to make dedicated applications for this.

BUOY TECHNOLOGY

Power supply

The buoy power supply is a well developed technique, and was regarded a boundary condition in this project. Developments in e.g. solar technology will be considered for implementation if needed and standardly available. Standard Waveriders have Leclanche carbon-zinc batteries, but these are optimised for constant low currents and are not not suitable for the high current required by the laser when it starts up. The batteries to be used are specially developed lithium batteries, with a total of 22800 Wh for one package. Depending on the particle concentration of the sea water, one sampling requires about 3-5 Wh (with the green laser!). Radiotransmission may require a similar amount of power. This totals to over 2000 samples before new batteries have to be placed. This would be sufficient for most applications throughout a season, even for demanding applications, requiring for instance 10 samples per day.

Environmental

There are two major differences between a ship operated system and a buoy operated system in terms of shock and vibration elimination. The ship operated unit will not be seriously vulnerable to heavy shocks. The buoy mounted flow cytometer however may well experience shocks when hoisted. Also the danger of being hit by another ship while moored is realistic. At the other hand a ship operated unit suffers from heavy vibrations caused by the screw and the engine, not present in the buoy. The buoy mounted flow cytometer unit should survive shocks without damage or serious misalignment, without the need to keep on analysing during a shock. The main problem therefore is buoy movement. The strategy was to mount the flow cytometer unit fixed to the buoy, with a rather stiff damping and rigid optics construction to cope with shock load, and to design the fluidics section to minimize sensitivity for rolling and heave. Some typical significant values for the roll and heave as experienced by the Datawell buoy in the North Sea were 0.5 m to 3.65 m heights, and 5 degrees to 10 degrees angles. The frequency spectrum of the roll movement is approximately uniform from the significant wave period up to 0.6 Hz. In very rough seas roll angles up to 45 degrees must be expected occasionally. However, the need for accurate analysis during such circumstances is not considered important.

3. FIRST TESTS AND IMPROVEMENTS

The quality of the optics and the fluidics section determine the resulting data quality. The optical configuration with the green 532 nm laser provides a sharp primary laser focus of 5 μm [$1/e^2$]. Smaller would be possible, but the depth of focus then would be too small to accommodate the sample core width including trajectory fluctuations imposed by buoy movements. The laser beam is than reflected and recombined by a spherical mirror. This secondary, partly superimposed focus was of equal quality, allowing high resolution particle scanning. Figure 2 shows an example of detector output generated by the passage of a typical single algal cell. of sp. Ditylum brightwelli. The morphological information is overwhelming.

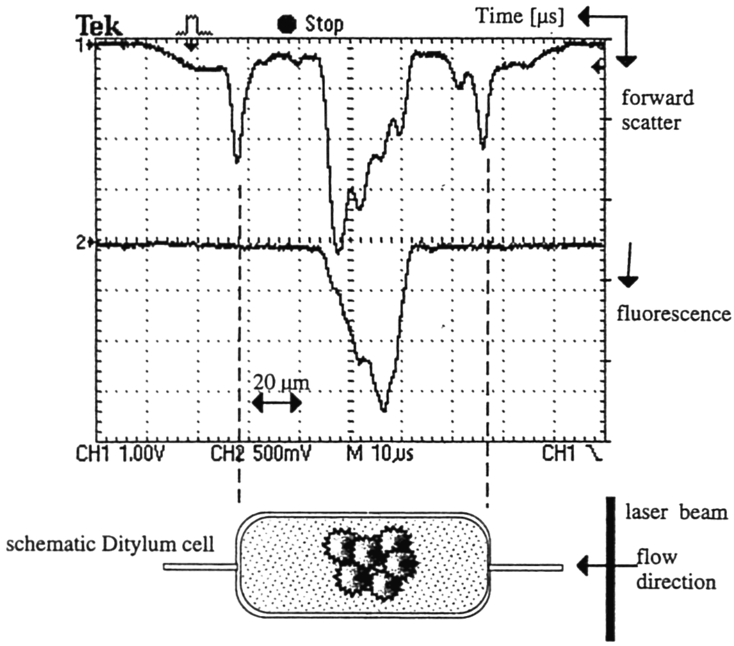

Figure 2: measured pulse of Ditylum Brightwelli

The spectrograph disperses light through an inlet slit into a series of coloured images on the detector pixels mounted on its other side. There is always some residual omnidirectional scattering from the grooves (lines) of the grating. The green light (scattered light from particles) was too intense relatively, so its residual scattering from the grating was equal or stronger than the other weaker fluorescence colours in the spectral image. This problem was solved by covering the fluorescence pixels from green light with a long pass colour filter. A prism spectrograph is would not have this problem, but its optical configuration would be too bulky. The approach was to use pixels of the multipixel detector for all signals. Forward scatter was coupled in through a fiber bundle. A problem was encountered with dark current pulses from the pixels of the hybrid photo multiplier detector. These appeared to be inherent to the type and production process of the detector. These pulses are narrower than real particle pulses, but too similar to eliminate by simple electronic filtering. Therefore we returned to measuring the forward light scatter by a separate photodiode, and using this signal to trigger the multipixel detector for fluorescence and side scatter. Triggering on light scatter also turned out to be necessary in view of the first series of particle scans examined by this system. Various species have fluorescing pigments concentrated at specific locations inside the cell (viz. Fig.2). With a system triggering on fluorescence, the non-fluorescing first part of the signal would be lost.

The influence of buoy motions on the measurements were investigated by placing the flow cytometer on a swing designed for the testing of buoys. A small video camera was placed on the optics, 'looking' at the cuvette and the sample core inside. The swing resembles a joy-wheel with a diameter of about 4 m, and rotates to simulate wave motions. Fixing the 'seat' in the swing provided translation without roll movements. The position of the laser focus and cuvette relative to the frame are not affected by the motions. It appeared that the linear accelerations had no effect whatsoever on system performance, as long as the density of sample and sheath fluids were well matched. The roll movement introduced fluctuations in the trajectory of the sample core in the cuvette. At typicall roll amplitudes in the test rig (10 to 15 degrees) this prohibited meaningful analysis because the sample core no longer intersected the laser focus and detection field of view in the middle. The effect is related to the inertia of the more or less round body of fluid in (and thus the size of) the funnel where the sample liquid is injected in the sheath fluid. The initial injector was copied from the EurOPA system which performed well (EurOPA had a mechanical solution to roll movements). Reducing overall size would impair the specifications regarding the particle size range and prevention of high fluid shear (damaging vulnerable colonies). Other solutions to minimize the fluid body size in the funnel are to place concentric funnels inside, or to put smaller funnels in series. The latter method is relatively easy to implement, and a first test model improved the situation to the extent that meaningful measurements are now possible at the roll amplitudes mentioned. Fine tuning of this design should largely eliminate trajectory fluctuations. A mechanical solution to the problem is to place the instrument in gimballs, such as the EurOPA shipboard suspension table. Although sufficient space is available, this option is not preferred if the above approach suffices.

Figure 3 shows an example of the graphical output of the data analysing software. Measured cells (Phaeodactylum sp.) and clumps of cells appear as single dots in a bivariate scatterplot. Some particles (dots) were selected in this graph to inspect their pulse shapes; these are shown to the right.

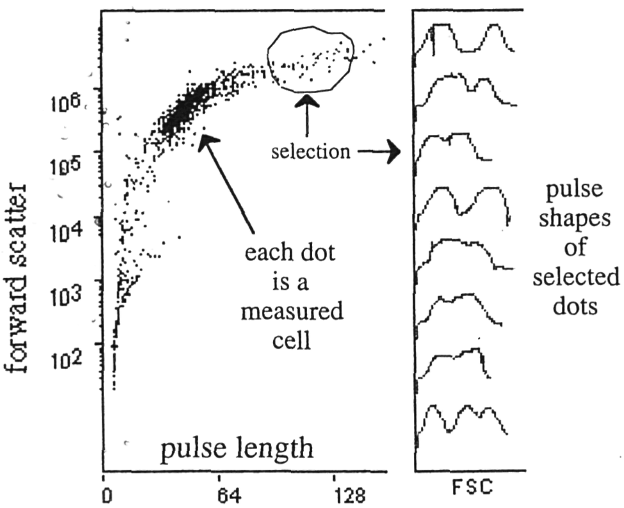

Figure 3: Example of output of data analysis software

4. DISCUSSION

The flow cytometer module is being mounted in the buoy now for a first in-situ testing in the water. After functionality tests, the instrument will be extensively tested in Norway, as part of the existing "Eureka" SEAWATCH buoy infrastructure for biological and physico-chemical monitoring (Hansen and Stel 1997, Tangen 1997).
The direct on-line estimation of phytoplankton biomass and the ability to discriminate between different phytoplankton groups allows analysis of undisturbed natural samples (growth, grazing, to follow the spatial or seasonal evolution of populations). Also, the application of specific fluorescent stains (bound to antibodies, or as DNA probes) may provide early detection of plankton groups of special interest, e.g. toxic species. An inherent drawback of flow cytometry is that it can only differentiate particles based on their optical characteristic as seen by the PMTs; this is better than the eye for the very small cells (picoplankton), and vice versa for the larger cells. The species-resolution is far below the Utermoehl-microscopy. The expectation that this situation can improve if the lack of specific fluorescent probes is reduced is widely supported. The laborious work to establish them remains a hurdle, and regarding the CytoBuoy: sample processing in-situ may prove a hurdle also, and would limit this technique to one or a few of the most important species. Particle scanning such as applied in The CytoBuoy may also improve the situation on shorter term, especially in subsequent versions with easy to implement higher spectral resolution and if necessary multicolor excitation.
In addition to the buoy, the flow cytometer module developed can be used for other in situ applications, like platforms or ship, including ships of opportunity. The development of autonomous underwater vehicles AUV's is ongoing, and testing flow cytometry on board is feasible.

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TITLE: **VAMP- Voltammetric Autonomous Measuring Probes for trace metals in the water column (500m, max. depth) and at water-sediment interfaces (6000m, max. depth).**

CONTRACT NO: **MAST3-CT95-0033**

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A NOVEL VOLTAMMETRIC *IN-SITU* PROFILING SYSTEM FOR CONTINUOUS, REAL-TIME MONITORING OF TRACE ELEMENTS IN NATURAL WATERS.

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SUMMARY.

The aim of the VAMP project is to develop, by means of several new technologies, voltammetric sensors and probes allowing *in-situ* automatic measurements of trace concentration profiles i) in the water column - sub-project I and ii) at the sediment-water interface - sub-project II. The work performed during the first two years of the project has allowed to successfully complete the sub-project I. The main technical developments and analytical tests carried out are summarised in this paper.

I. INTRODUCTION.

There is a growing need to monitor continuously chemical pollutants, and in particular trace elements, in natural aquatic systems both to get deeper insight into natural processes in general and to understand the relationship between anthropogenic releases and their long term inpact on man and the environment. Trace elements are not biodegradable but are involved in biogeochemical cycles and distributed under different physicochemical forms (i.e. simple inorganic species, organic complexes and metal ions adsorbed onto a variety of colloidal particles). The proportion of these different forms may vary continuously with space and time due to concurrently occurring physical, chemical and biological processes. Any variation in the speciation of an element will affect its bioavailability, its rate of transport to the sediment and its overall mobility in the aquatic system [1-4]. To understand and predict the role and the fate of these different metal species, new analytical instrumentation capable of performing *in-situ*, real time monitoring of specific forms of elements in continuous and reproducible manner, on a wide spatial network, is required [5]. The design of such a tool is still a challenge for analytical chemists since techniques that combine high sensitivity and reliability, speciation capability, integrity of the samples and unattended operation are prerequisite. This kind of development however is the only way : i) to minimise the large number of artefacts due to sampling and sample handling, ii) to allow rapid detection of pollutant inputs, iii) to accumulate detailed spatial and temporal data banks of complete ecosystems at low cost, and iv) to perform measurements in locations which are difficult to access. Amongst the analytical tools available, the potentiality of voltammetric techniques for trace compound analysis in natural waters has been demonstrated in the past [6-10]. However, most of the development done until now deal with on-line automatic voltammetric analyzers for laboratory or field measurements [9, 11-14]. Very little work has been reported concerning the development of submersible voltammetric probes for *in-situ* monitoring of trace elements [15-17] and none of these systems were usable for automatic, continuous measurements. This is mainly due to the fact that long term *in-situ* monitoring with such systems are limited by insufficient reliability of the commercially available voltammetric sensors and by the fouling of the sensor surface due to the adsorption of organic or inorganic matter.

In this paper the development of a novel **Voltammetric *In-situ* Profiling System** (VIP System), based on advanced microprocessor and telemetry technology, is reported. This system has been built by taking into account all the important criteria mentioned above as well as the problems related to field deployment, in particular: robustness, ease of handling and flexibility. The heart of the submersible voltammetric probe is a gel integrated either single or array microsensor. These microsensors have been specifically developed to enable continuous, reproducible and reliable measurements of analytes in complex media without physical and chemical interferences of the test solution [18-21]. The VIP system allows to perform direct *in-situ* measurement of the mobile fractions of Cu(II), Pb(II), Cd(II) and Zn(II) as well as Mn(II) and Fe(II) using either Square Wave Anodic Stripping Voltammetry (SWASV) or Square Wave Cathodic Sweep Voltammetry (SWCSV) down to 500 meters depth. Examples of environmental applications of the VIP system for *in-situ* monitoring of Cu(II), Pb(II) and Cd(II) in oxygen saturated sea water and Mn(II) profiling in lake water are given.

II. EXPERIMENTAL SECTION.

VIP System. A detailed technical description of the Voltammetric *In-situ* profiling System is given elsewhere [22]. Briefly, the VIP System consists of: a submersible voltammetric probe, an Idronaut Ocean Seven 301 multiparameter submersible probe (optional), a calibration deck unit, a surface deck unit and a IBM compatible PC (Fig. 1).

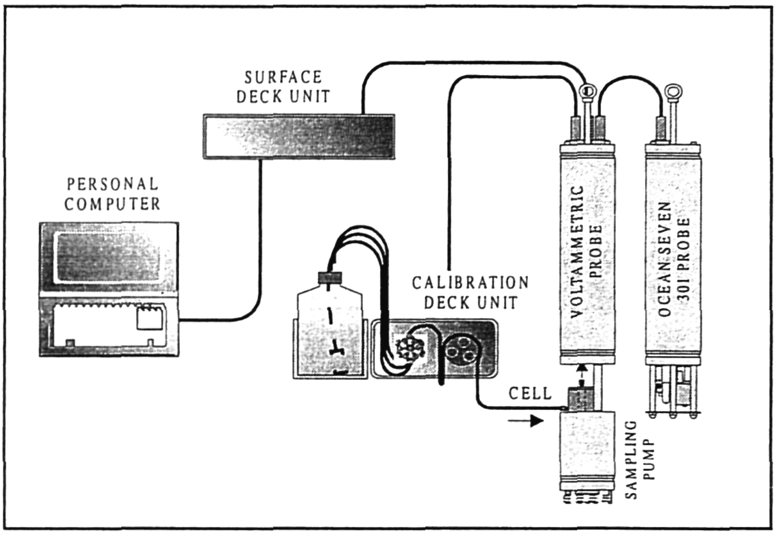

Fig. 1 : Schematic diagram of the whole Voltammetric In-situ Profiling System (VIP System)

The ***submersible voltammetric probe*** (dimensions : 86 cm length, 10 cm in diameter ; weight : 8 kg in air, 4 kg in water) is comprised of distinct specific modules : an electronic probe housing, a pressure compensated flow-through plexiglas voltammetric cell (internal volume = 1.5 ml) with a platinum ring auxiliary electrode and a home-made Ag/AgCl/KCl saturated gel reference electrode, a pressure case base incorporating the preamplifier for the voltàmmetric microsensor and a sampling submersible peristaltic pump. The electronic housing contains all the hardware and firmware necessary to manage : i) the voltammetric measurements, ii) the interfacing of the Ocean Seven 301 (via an RS232C interface), the calibration deck unit and the submersible peristaltic pump, and finally iii) the data transfer by telemetry. The interface between the Personal Computer and the voltammetric probe is carried out by using the Terminal Emulator under Windows. The VIP System software is divided in a management software and a firmware. The firmware, stored in a flash memory, allows the user to execute the processing operating functions and the data acquisition. The management software allows the user, through menus and pop-up data entry windows, to management software allows the user, through menus and pop-up data entry windows, to control and configure the voltammetric probe operating parameters and functions such as : electrochemical parameters, data acquisition, calibration and maintenance operations. Data files are stored in a non volatile memory having its own battery which guaranty high data retention and protection. The ***Ocean Seven 301*** probe allows to control the exact position of the voltammetric probe at depth and to measure simultaneously the following parameters : temperature, conductivity, salinity, dissolved oxygen, pH and Redox potential. The ***calibration deck unit*** enables to perform in laboratory, on shore or on boat i) the renewal of the microsensor Hg layer (see below), ii) the calibration of the probe, and iii) the measurements of standard and collected natural samples. The ***surface deck unit*** powers and interfaces, by telemetry, the measuring system with a Personal Computer. The telemetric signals superimposed to the system power supply flow all along the voltammetric probe holding cable. This unit allows an autonomy of about 35 hours and can be recharged either in continuous mode using solar captor or after use.

Gel integrated microsensors. The working sensor of the submersible voltammetric probe is an Agarose Membrane-covered Mercury-plated Ir-based either single or microelectrode arrays (μ-AMMIE and μ-AMMIA respectively) (Fig. 2).

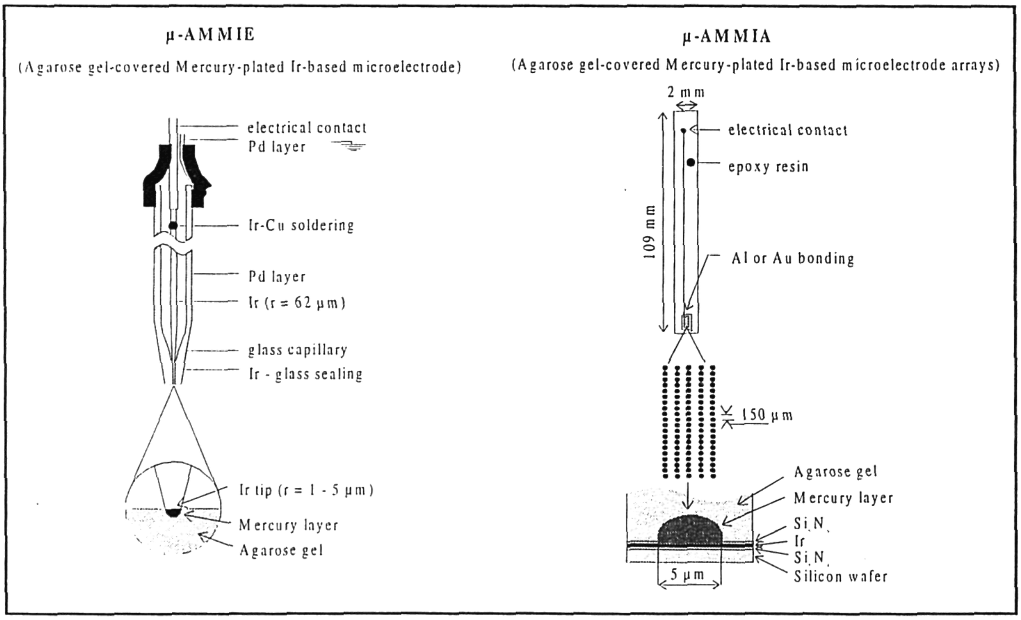

Fig. 2 : Schematic diagram of the single and array gel integrated microsensors

The voltammetric microsensors measure the test compounds within the gel after equilibration of the gel with the sample. Details of the fabrication and characteristics of these microsensors are reported elsewhere [18-21] and thus are only briefly summarised here. The single microelectrode was built by sealing an electroetched Ir wire with diameter of few micrometers in a shielded glass capillary followed by mechanical polishing [18]. The microelectrode arrays was produced by means of thin film technology on chips and photolitographic technique [20]. It consists of 5 x 20 interconnected iridium microdisc electrodes having a diameter of 5 µm and a centre to centre spacing of 150 µm surrounded of a 300 µm thick Epon SU8-8 containment ring. Both sensors are covered with a 1.5% LGL agarose protective gel membrane. Mercury semidrops were plated through the gel layer onto Ir substrates at -400 mV (vs Ag/AgCl/3M KCl/1M $NaNO_3$) in a deoxygenated 5 mM $Hg(CH_3COO)_2$ and 10^{-2}M $HClO_4$ solution. Reoxidation of the mercury was carried out by scanning the potential linearly from -300 mV to +300 mV , at 5 mV/s, in a degassed 1M KSCN solution. In both cases, the currents were recorded and, from the electric charge, the diameters of the mercury semidrops were determined by assuming they were parts of spheres. The same agarose antifouling gel membrane was used over an extended period of about one month. This kind of gel integrated microsensor has a number of advantages for in-situ measurements : i) the gel acts as a dialysis membrane, i.e. allows diffusion of small ions and molecules and retains colloidal and particulate material, and thus protects the electrode surface from fouling [19,21] allowing its operation for long periods of time ; ii) the gel membrane protects the electrode from ill-controlled hydrodynamic currents occuring inside the water column, i.e. analysis inside the gel is based on pure molecular diffusion ; iii) microelectrodes have low iR drop and reduced double-layer capacitance, thus direct voltammetric measurements without added electrolyte can be performed in freshwaters even if the ionic strength is as low as 10^{-4}M ; and iv) voltammetric currents, i, at micro-sized electrodes are controlled by spherical diffusion and reach a non zero steady state value at constant potential [23]. This last point is particularly important as it allows : i) to perform the SWASV deposition step without stirring, which is absolutly required to perform SWASV in the protective gel membrane, and ii) to define a maximum size cut-off limit of a few nanometer [5,24] for the so-called mobile species (i.e. free ions and small labile complexes) selectively measurable on the microelectrode. These considerations are important as they show that combination of VIPS *in-situ* measurements with complementary laboratory measurements of total concentration in raw and filtered samples, performed by laboratory measurements of total concentration in raw and filtered samples, performed by using classical techniques, allows to determine three key environmental fraction of trace element species : i) the mobile species (≤ of a few nm) by direct *in-situ* measurements in unperturbed sample which is a key feature to minimize analytical artefacts [10]; ii) the colloidal species (total concentration in filtered samples minus mobile concentration) ; and iii) the particulate species (> 1 μm) (difference in concentration between raw and filtered samples). Distinction between these three different fractions is important since the mobile species are the species the most easily bioavailable while the colloidal and particulate fractions play different roles in metal circulation and residence time. These three fractions are considered in the interpretation of the results reported below. Finally, it has been shown [21] that the current amplitude of the gel integrated microsensors is independent of the pressure, even up to pressure as high as 600 bars, and its temperature dependence can be taken into account using Arrhenius equation.

III. RESULTS AND DISCUSSION.

In-situ Mn(II) profiling in anoxic lake waters. The system was first tested for Mn(II) concentration profile measurements within the anoxic hypolimnion of lake Brêt (Switzerland). Lake Brêt is a shallow lake with a maximum depth of 20 meters and it is stratified roughly from May to the end of September. All through the field tests, μ-AMMIE or μ-AMMIA with the same mercury layers were used to calibrate the submersible voltammetric probe the day before and after each deployment as well as for *in-situ* measurements (i.e. no renewal of the mercury semidrops over three days). The calibrations were performed by standard additions in 0.2 μm filtered lake water samples degassed with a mixture N_2+CO_2 to maintain the pH at 7.5. Example of the results obtained (Fig. 3) illustrate the excellent stability of the gel integrated microsensor for at least three days and show no memory effect from in-situ measurements performed between both laboratory calibrations. In addition, all the different calibrations curves yielded an average slope of 17.5 ± 1.2 pA/μM (N = 6, 95% probability) and 30.6 ±1.6 pA/μM (N = 8 ; 95% probability) for SWASV (tdep = 5 s.) and SWCSV respectively using the μ-AMMIE (Hg layer radius :

8.8 to 9.3 μm) and 5.3 ± 0.29 nA/μM (N = 6 , 95% probability) for SWASV using the μ-AMMIA (Hg layer radius : 6.6 to 7 μm ; tdep = 10 s.). These results show the

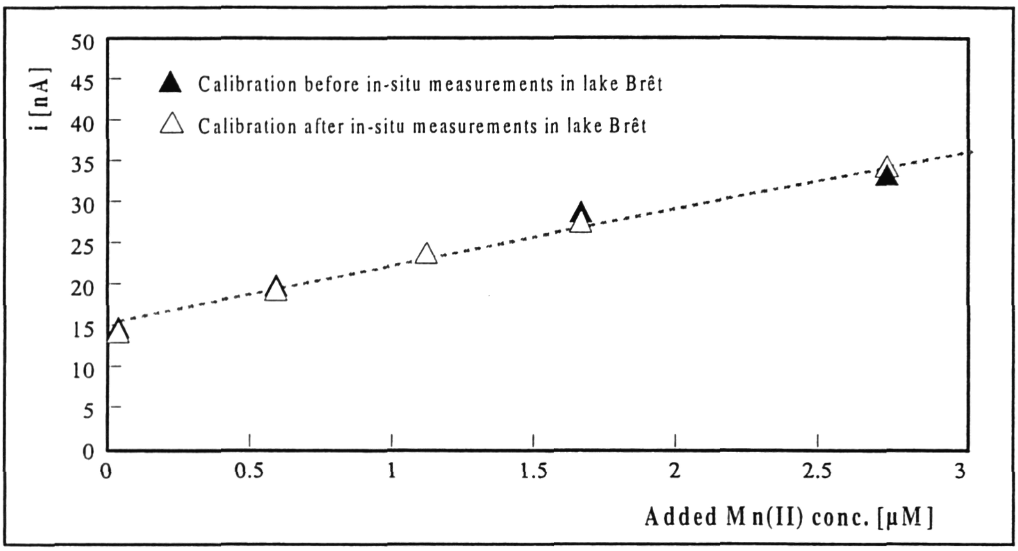

Fig. 3 : Typical SWASV results obtained for laboratory calibrations performed before and after in-situ measurements in lake Brêt using the same Hg layer over the three days. μ-AMMIA Hg radius = 6.7 μm. SWASV conditions : precleaning E= -800 mV ; precleaning t = 30 s. ; deposition E = -1600 mV ; deposition t = 10 s. ; final E = -1300 mV ; pulse amplitude = 25 mV ; step amplitude = 1 mV ; frequency = 5 Hz.

excellent reproducibility and reliability of both gel integrated microsensors developed. The main objective of field tests in lake Brêt were to check the validity and reliability of the measurements performed with the VIP System in real conditions. For this purpose, concentration profile obtained from *in-situ* voltammetric measurements were compared with on-field voltammetric measurements, performed at a constant temperature of 20°C, using microsensor arrays with and without protective gel layer. For on-field measurements, a Tygon sampling tubing was fixed to the VIP titanium protective cage at exactly the same level that the input of the pressure compensated flow-through cell and samples were pumped at each depth directly in a thermostated plexiglas cell using a peristaltic pump. For comparison purpose, samples were also withdrawn to allow laboratory Mn measurements in acidified (pH 2) raw samples, acidified samples filtered on 0.2 μm pore size membranes and acidified samples ultracentrifuged at 30000 rpm for 15 hours (which allowed to eliminate the species with a size ⊕ 5 nm assuming a density of 2) using either Atomic Absorption Spectroscopy (AAS) or Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).

Typical results obtained for Mn profiles in lake Brêt are shown in Fig. 4. Excellent agreement are observed in particular between:

- Mn(II) concentration profiles determined from *in-situ* voltammetric measurements after temperature effect correction, using the following equation ln(i) = 29.68-7091*1/T [21], and on-field voltammetric measurements performed at constant temperature of 20°C using microsensor arrays with gel.
- Mn(II) concentration profiles determined from voltammetric measurements with microsensor arrays with gel (mobile species with size of few nm) and ICP laboratory measurements of ultracentrifugated acidified samples (species ≤ 5 nm).

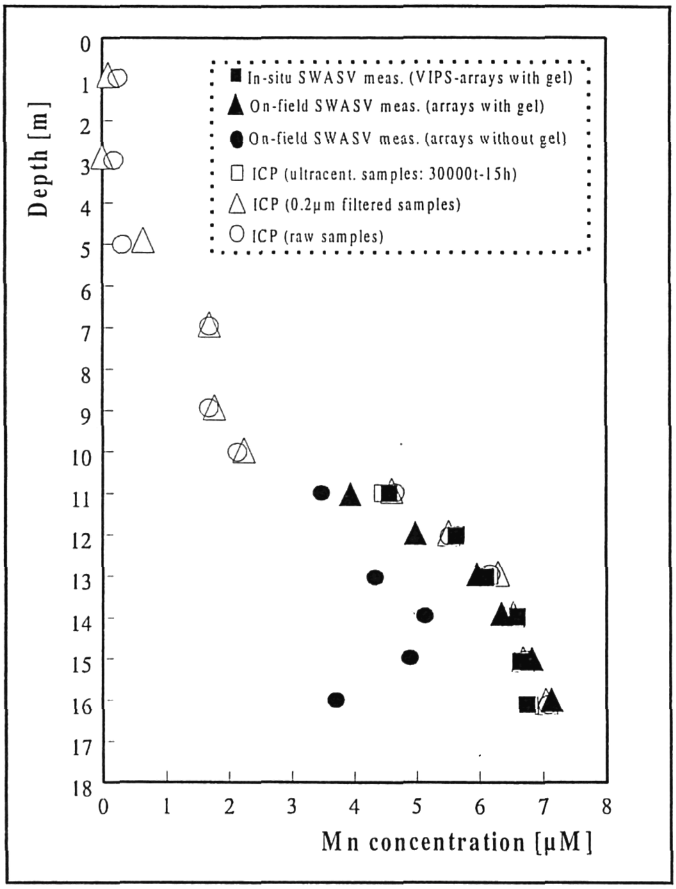

Fig. 4 : Typical profiles of Mn in the anoxic hypolimnion of lake Brêt, August 20 1997. (SWASV conditions as in Fig. 3).

These results demonstrate the reliability of the VIP System using gel integrated microsensors for *in-situ* monitoring. They also confirm that pressure has no effect on the current amplitude as well as the validity of the equation, determined in the laboratory, to take into account the temperature effect [21]. In addition, interesting information regarding the nature of Mn in lake Brêt is also obtained by comparing voltammetric measurements with the results obtained for ICP and AAS laboratory measurements of the raw, 0.2 μm filtered and ultracentrifuged acidified samples (Fig. 4). Since voltammetry measures only the mobile Mn species with sizes of a few nanometers, whereas AAS and ICP techniques measure the total metal concentration in the different samples, the results obtained indicate that Mn in lake Brêt is present predominantly in the mobile form, i.e. most likely Mn^{2+} and probably small inorganic complexes, at this period of the year. The protective role of the agarose gel membrane was also studied by comparing Mn(II) concentration profiles obtained using microsensor arrays with and without gel layer (Fig. 4). It can be seen that systematically too low concentrations are obtained for the unprotected microsensor. A detailed study showed that peak current attenuations observed for unprotected sensors are due to adsorption of lake born iron hydroxide particles, well-known for having strong adsorbing capabilities, onto the sensor Hg surface which then hinder the diffusion of Mn(II). These results clearly demonstrate the efficiency and necessity of the protective gel layer to eliminate fouling problems and thus allow reliable, direct measurements in natural media. It must be emphasised that no other perturbation in voltammetric peaks except a lowering in the current intensity was observed. Thus, in the absence of protective gel, the difference observed between *in-situ* voltammetry and classical techniques might be wrongly attributed to the presence of colloidal Mn species even though such species are not present in lake Brêt as demonstrated before. A more detailed study comparing the results obtained for Mn(II) profiling with the VIP System in different lakes can be found elsewhere [24].

In-situ trace metal monitoring in oxygen saturated sea water. Continuous trace metal monitoring for quality control is becoming of prime importance in many estuaries and coastal zones. The potentiality of the VIP System for such purpose was tested in Venice Lagoon (Venice-Italy) and in Gullmar Fjord (Sweden). In-situ measurements in Venice Lagoon were performed in three different stations: 1) Breda, which is located in the middle of the industrial area and represents one of the most polluted part of the Lagoon, 2) Fusina, which is at the limit of the southern part of the industrial area in a wide artificial

Fusina, which is at the limit of the southern part of the industrial area in a wide artificial navigation canal and finally 3) Bocca di Porto, which is located at the main entrance of the Lagoon. In Kullmar Fjord, two types of tests were caried out with the Vip System : i) *in-situ* measurements at different hydrographic stations aboard R/V Arne Tiselius, and ii) unattended, autonomous in-situ measurements at Kristineberg Marine Research Station located at the entrance of the Fjord. The probe was calibrated before and after deployment. Average slopes of 0.30 ± 0.01 nA/nM, 0.34 ± 0.01 nA/nM and 0.18 ± 0.02 nA/nM were found for Pb(II), Cd(II) and Cu(II) respectively (Hg layer radius = 7.9µm; deposition t = 15 min; N = 4; 95% probability).

Venice Lagoon VIP measurements (average of three replicates) together with total metal concentrations measured in UV irradiated, acidified samples in the laboratory are given in Table 1. As can be seen, the dissolved fraction of trace metals represent in general only 10

Table 1: VIP *in-situ* measurements (C_m = trace metal mobile fraction concentrations) and laboratory measurements (C_{tot} = total metal concentrations measured in UV irradiated, acidified samples) of Cd(II), Pb(II) and Cu(II) in Venice Lagoon (March 12-13, 1997).

Stations	Cd(II) [nM]		Pb(II) [nM]		Cu(II) [nM]	
	C_m (VIP)	C_{tot} (lab)	C_m (VIP)	C_{tot} (lab)	C_m (VIP)	C_{tot} (lab)
Breda	0.18 ± 0.04	1.33 ± 0.03	1.64 ± 0.11	3.03 ± 0.17	3.93 ± 0.86	10.57 ± 1.08
Fusina	0.25 ± 0.04	1.20 ± 0.01	0.42 ± 0.05	3.10 ± 0.10	1.56 ± 0.39	23.33 ± 2.60
Bocca di Porto	0.02 ± 0.005	0.23 ± 0.03	0.06 ± 0.01	1.0 ± 0.10	--	5.97 ± 0.81

to 20% of the total metal concentration and hence, as previously observed for trace metal measurements in freshwater [18], the colloidal/particulate fraction are predominant. Table 1 also shows that concentrations as well as variation in concentrations in the ppt level can be measured in-situ with a good reproducibility using the VIP System.

Stability tests of the VIP System for unattended autonomous in-situ monitoring performed at Kristineberg Marine Station are shown in Fig. 5. Measurements were automatically performed every hour for 52 hours with a renewal of the Hg layer after 22 hours. Interesting observation can be made from these preliminary results. Indeed, temperature, salinity and observation can be made from these preliminary results. Indeed, temperature, salinity and conductivity data (Fig. 5), measured simultaneously to in-situ VIP Pb(II) concentration using the *Ocean Seven 301* multiparameter probe, show that fresh, warmer water is advected into the measurement site during the experiment. At each of such event, significant variation of in-situ Pb(II) concentration were also observed. Even if more extensive studies are required for rigorous interpretation, these results show the potentiality of the VIP System as a tool to identify and study specific changes.

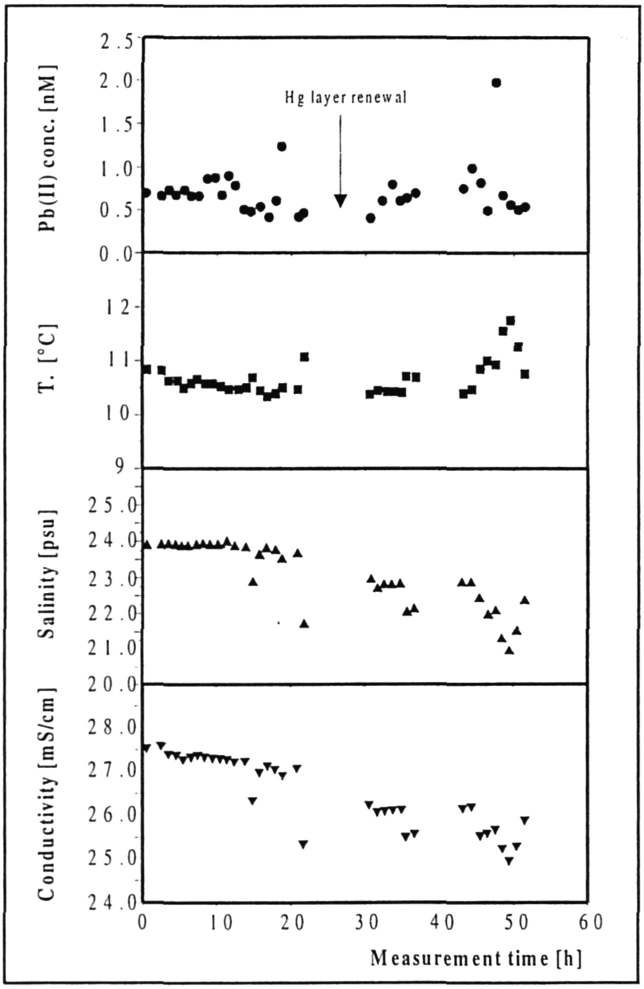

Fig. 5 : Unattended autonomous *in-situ* monitoring performed with the VIP System at Kristineberg Marine Station (Kullmar Fjord-Sweden May 16-18 1997).μ-AMMIE Hg radius = 8.3 μm. SWASV conditions : precleaning E = -80 mV ; precleaning t = 60 s. ; deposition E = -1300 mV ; deposition t = 15 min ; equilibration E = -1300 mV ; equilibration t = 10 s. ; final E = -80 mV ; pulse amplitude = 25 mV ; pulse step = 8 mV ; frequency = 200 Hz.

IV. CONCLUSION.

The combination of voltammetric principles with recent breakthrough in electonic and micromechanic has allowed the development of a sophisticated, compact, reliable, in-situ analytical device for trace element measurements in natural aquatic systems thanks to the use of reproducible and well characterised gel-integrated microsensors. In particular ppt level of the mobile fraction, which is the most difficult fraction to measure without analytical artefacts, can be determined by direct in-situ measurements, i.e. without perturbing the samples. In addition punctual changes can be identified and studied. Further development are under way to couple flow injection system to the flow-through voltammetric cell and to adapt the VIP System to a Radio Buoy Profiler. These will allow i) in-situ total metal concentration measurements in addition to the mobile fractions and ii) continuous, autonomous, automatic in-situ monitoring over extended period of time respectively. Both points are important for efficient envionmental monitoring and more rigourous interpretation of trace element cycles in natural aquatic systems.

ACKNOWLEDGEMENTS.

The authors thank : Fabio Confalonieri, Giuliano Riccardi and Antonio Sina (Idronaut team) who developed the sofware, the hardware and the submersible pump of the VIP System. Claude Bernard, François Bujard and Serge Rodak (CABE team) who built the flow-through voltammetric cell as well as all the instrumentation needed for constructing the voltammetric single microsensor. G.C. Fiaccabrino, M. Koudelka-Hep (Institute of Microtechnology, University of Neuchâtel) and Cécile Belmont-Hébert (CABE) who have developed the Ir-based microsensor arrays and performed systematic laboratory tests of this microsensor respectively. D. Turner (Department of Analytical and Marine Chemistry, Goteborg University) for providing all the logistic during field tests in Kullmar Fjord-Sweden.

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TITLE: GAS HYDRATE AUTOCLAVE CORING EQUIPMENT SYSTEM (HYACE)

CONTRACT NO MAS3-CT97-0102

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HYACE, AN AUTOCLAVE CORING EQUIPMENT FOR SYSTEMATIC OFFSHORE GASHYDRATE SAMPLING; MEASUREMENT AND GROUND TRUTHING

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1 Abstract: HYACE, an EU MAST III Project (EU MAS3 CT97-0102)

HYACE, standing for hydrate autoclave coring equipment system, is a research and development project sponsored by the European Union's Marine Science and Technology Program MAST, coming under the present Fourth Framework Program of Science and Technology, 1995-99. The project started in the fourth quarter of 1997 and it shall last for 30 months. There are eight partners involved from six countries, and it is coordinated by Technische Universität Berlin, Section Marine Technology.

Development and prototype testing of an innovative, down-hole controlled autoclave coring system constitute the central activities of the project. It will be designed to sample marine sediments at down-hole conditions and bring it on board while maintaining as many down-hole parameters as possible. Particular targets are sediments on the deepwater covered continental slopes containing gas hydrates. Sampling tools will include push, percussion and rotary corers. The rotary corer will be driven by a down-hole motor instead of the top drive on-board the research vessel/drillship. The main general goal of the project is to contribute to systematic ground truthing of a necessarily ephemeral phenomenon of growing global significance: sampling and analysing volatile gas hydrates in their natural environment. This must be achieved by obtaining quantitative and validated parameters of the in situ and down-hole conditions to the largest possible extent.

Complementary measurements while coring are therefore important. Temperature, pressure and acceleration monitoring had been selected upon request by the geoscientific community as the initial parameters. Further scientific-technical efforts to better use and analyse the autoclave cores after retrieval on board the research vessel are taken as well.

2 Introduction: The significance of marine gas hydrates

Large amounts of organic carbon occur mainly as methane in deepsea sediments in the form of clathrates. These solids are composed of penta/hexagonal host structures of water molecules with cavities which contain methane and other components of natural gas. Sediments with gas hydrates are found in high pressure and low temperature conditions that may stabilise the solid phase of the gas-water structure in permafrost regions and in the deepwater parts of continental margins, Fig.(1).

Locations and depths of gas hydrates can be inferred from the phase diagram, Fig.(2). Those occurrences, therefore, have been encountered in water depths of a few meters to a few hundred m in Arctic regions and in water depth to about 2000 m in the sediments on continental slopes of more equatorial latitudes. Gas hydrates have been found close to the sea floor and embedded to about 500 m depth. Gas hydrate sediments and free gas below them are often registered on seismic records, appearing as a bottom simulating reflector (BSR).

The amount of methane occurring in (offshore) gas hydrates is probably enormous. Estimates on quantities vary considerably due to still inadequate sampling equipment and evaluation methods. Estimates range widely between $10^{18} - 10^{21}$ g of organic carbon, Kvenvolden 1993,1996. If only partly and suddenly released, e.g. by sedimentary slope failure, the strong greenhouse gas methane from the gas hydrates may significantly influence global climate changes into further warming, Kenneth, 1996.

Gas hydrates also influence the stability of the sediments in which they occur. There is general agreement that large sediment slides on the continental slopes, such as sediment slides offshore South-West Africa, Summerhayes, 1979, the Blake Slide offshore the Carolinas, Paull, 1996, the Storegga Slide offshore Norway, Bugge, 1983, and the recently discovered slides in the Eastern Mediterranean, Woodside and Ivanov, 1996, are understood in the context of gas hydrates. They are thus important factors of offshore geohazards, especially if oil exploration, exploitation and transport take place in the vicinity of gas hydrate occurrences.

Marine gas hydrates may ultimately constitute an ecologically interesting energy resource if the large quantities assumed so far can be confirmed and if mobilisation and production technologies can be developed to produce gas from gas hydrates in an environmentally acceptable manner. The resource potential could be enormous. The gas hydrate field offshore the Carolinas, to give an example, where Leg 164 of ODP was performed in late 1995, contains estimated quantities of methane with an amount of carbon of 35 GT. If this could be mobilized and produced, the total US gas demand could be satisfied for about 100 years!, Dickens et al., 1996.

To validate existing theories as described above more specific and quantitative proof of the nature and extent of gas hydrates is required. This is only possible by collecting physical samples of the hydrates and surrounding sediments. Such samples need to be taken without affecting the hydrates and to be brought to the surface while maintaining the downhole pressure and controlling its temperature; in other words by the use of an autoclave sampler. Obviously special laboratory eqipment will be required to study samples in the autoclave sampler and outside the sampler while conserving downhole conditions. Beside samples, downhole measurements are reqired of the ambient pressure and temperature as they cannot be inferred from the samples. Acceleration monitoring, in addition, will give information on the location and attitude of the tool and on its operational performance.

3 Pressure coring and measurement, state of the art

Autoclave sediment sampling from deepsea areas of the continental rises has had limited success. Occasionally successful attempts were reported earlier, such as from ODP Leg 146. The first systematic attempt and partly successful operation for autoclave sampling of gas hydrates was on Leg 164 of the international Ocean Drilling Program (ODP) in November and December of 1995 on the Blake Rise offshore the US south-eastern Atlantic coast. A coring system with downhole sealing mechanisms, PCS, „pressure core sampler", Fig. (3),was used fairly successfully in push-in coring operations down to a depth below seafloor of about 400 m and into less consolidated sediment. Coring at greater drilling depths and/or in more consolidated sediments with an Auger cutting shoe gave only one satisfying result. Reasons for the failures were identified: probable destruction of the core by lateral-rotational movements of the bottom hole assembly (which is driven by the drillstring, top drive), rotating inner core barrel, possibly back pressure problems (water pressure against the core moving into the coring tube), malfunctioning of the core catchers, silt particles in essential seals and at the ball valve, kerf of the thick Auger cutting shoe and insufficiently controlled removal of cuttings, due to the thick cutting shoe and improper fluid circulation at the coring drill bit.

Onshore rotary pressure core barrels have been used in oil and gas exploration rather extensively in the former USSR and rarely in the rest of the world. Use of these for offshore hydrate exploration would require substantial further development to cope with a heaving vessel and to allow wireline operations of the corer.

Use a down-hole motor instead of the rotary mode of spinning the whole drill constitutes another outcome of an analysis of the state of the art. A down-hole motor, driven by the circulating fluid, would constitute a mechanically much more appropriate and down-hole controllable (radial position) drive for scientific coring. Although known in principle from commercial oil/gas drilling, its adaptation to scientific deepsea drilling is complicated. Wire-line coring methods, depending on top drive, are usually applied here. A notable exception is the motor driven core barrel (MDCB) of ODP. This tool has, however, a comparatively simple core barrel without an autoclave function. The same applies to the wireline operated down-hole rotary coring system (CCS) from Russia. Reviews show that incorporating the autoclave function requires a completely new design.

Push sampling techniques have been very successful in obtaining undisturbed samples and some attempts have been made in the past to keep the samples pressurized. Their application is limited to the non-indurated sediments.

Different methods developed by the geotechnical industry could be implemented for scientific drilling. Percussion techniques have been traditionally used in the geotechnical industry. Recently wireline operated downhole percussion hammers driven by the drilling fluid were introduced for offshore operations (Fugro,1997). Extending these to autoclave coring appears feasible.

Last but not least there are no facilities known to examine complete samples under pressure using standard methods of analysis.

Conclusions from the outlines of needs and of the state of the art are summarized as follows:

- available pushin corers for soft sediments, including the essential autoclave function, need to be adapted to fit into one standad bottom hole assembly to be more easily used world wide and in Europe in particular,
- a rotary autoclave corer with downhole drive and control, fitting into the standard bottom hole assembly, is needed for indurated sediments,
- friable, hard and large grain size sediments associated with gas hydrates and/or free gas from the hydrates-the most difficult area of coring- need a percussion autoclave sampler,
- facilities need to be developed to analyze hydrate samples under pressure or in such a way that downhole conditions are preserved,
- downhole measurements of those parameters which are neesed for the systematic and technically controlled research of gas hydrates, such as temperuture, pressure and acceleration,
- further.studies are required to see wether existing techniques of downhole or in-situ (below the borehole) measurements are adequate to map those ambient conditions which cannot be inferred from the core.

4. Technical scope of HYACE

On initiative of Marine Technology, a section of Technische Universität Berlin, who took part in ODP Leg 164, a consortium of European research institutes and public and private companies was formed in 1997 to improve available but insufficient technology with the financial aid of the EU Commission, which is acknowledged by all partners with gratitude.

The actual scope of work include the following tasks

- Available information on gas hydrates and experience from the group members are being scrutinized to define in more technical detail geological and performance requirements for the set of tools and methods to sample, measure in-situ and evaluate the gas hydrates, the emphasis being on the sampling and sample evaluation process,
- establish the measurements to be made on the recovered samples and the method of such monitoring under pressure to arrive at input data for computer modelling to predict what happens under a change of pressure and/or temperature and what hydrate dissociation processes could be expected.

It is foreseen to adapt available core logging systems to cope with an encapsulated sample. It is furthermore attempted to have equipment, with which discrete subsamples can be taken and analyzed, again under the in-situ,or, rather downhole conditions, either in the ship laboratory or in an onshore laboratory

In-situ parameters which cannot be inferred from data obtained from the sample need to be measured directly in-situ or downhole respectively. Those additional parameters beside temperature, pressure and acceleration (which are part of the HYACE Project) are being specified in further reseach and development efforts: density, shear strength, natural gamma, shape (of the hole and of the core) and acoustic/sonar properties.

The gist of the HYACE Project, however, is the development and prototype testing of the actual sampling tools, push coring, rotary coring and percussion coring, all aimed at providing undisturbed samples (as much as this is technically feasible) at ambient conditions. The outlines of those tools are given hereunder.

5. Rotary coring

The prototype of an autoclave system for gas hydrate sampling driven by a down-hole motor and instrumented for measurements of pressure, temperature and acceleration, Fig. (5), is expected to satisfy varying operating and scientific requirements in more indurated sediments, such as clay, consolidated clay and indurated into stone-clay. The motor will be a mud motor at low to medium rpm to provide for higher torque and less friction heat, which is obviously important for gas hydrate coring. Drilling fluid should be cold seawater from greater water depth (below 200 m), having well defined and constant properties.

Wireline action and the mud motor will be used to pull in and seal the autoclave corer, containing a core of about one m length. The corer should extend 1.50 m below the drill bit. The cutting shoe should have a slim kerf to facilitate cutting which should be furthermore assisted by a properly designed mud circulation system to remove cuttings more readily than in traditional systems.

This marriage of an autoclave corer with a downhole motor under those extremely narrow downhole conditions of scientific drilling (dia 120 to 140 mm) constitutes a major technical development task.

Downhole monitoring of temperature, pressure and acceleration (of the tool) will be incorporated as outlined above. Data should be preprocessed and stored in the tool for read-out and evaluation on board.

The downhole motor driven rotary corer will be tested in a large onshore test facility (ITE) to determine the basic design for optimum offshore performance. Final prototype tests could be performed in 2000 during scientific cruises of the RV "Joides Resolution" operating for ODP, the international Ocean Drilling Project and/or with a commercial European drilling/coring vessel such as the MV "Bucentaur".

6 Percussion and push sampling

Push and percussion sampling both aim at penetrating a tube into the sediment by force only. Both system capture 100 % of all sediments in the path of penetration and if an adequate core catcher is used 100% core recovery is achieved. This is also true for a rotary corer if the sediments are strongly cemented and/or have a high cohesion; the latter however is only the case in solid rock or in pure hard clays. Percussion/push samplers are therefore needed to capture all non-cohesive or friable, cemented sediments.

Push sampling is widely used in scientific and geotechnical drilling, either by the use of pressurised drilling fluid (ODP's Advanced Piston Corer, CCS' Rapid Piston Sampler or Fugro's XP-WIPsampler) or by downhole hydraulic or electric actuators (most geotechnical tools). The thrust developed in these tools is generally limited to about 100 kN and penetrable soils are, depending the form of the sampling tube, limited to clays with shear strengths less than 300 to 500 kPa and medium dense sands, silts or volcanic ash. One could increase the thrust on these tools, however this requires to further strengthen the sampling tubes (thickening the penetrating sampling shoe and tube) destroying the objective of achieving more penetration.

With the limitation in soil strength push sampling devices are good candidates to capture sediments with hydrates as proven by the success of their use during ODP Leg 164. During the latter operation the PCS rotary coring system was used with a stationary extended core barrel limiting the penetration to about one metre. HYACE will attempt to fit a pressure closing mechanism on real push sampling devices to enable long cores to be taken. Closing mechanisms are similar as for percussion samplers and are discussed below.

Percussion samplers were used extensively in the past in geotechnical investigations. They used downhole hammers operated by wireline from the surface. This method became inefficient in deeper waters. Nevertheless it was proven that by percussion a large range of semi-cemented sediments could be sampled.

In the USSR, and later in China extensive experience was gained with use of downhole hammers for rotary percussion drilling. With this method the hole is advanced by an impact percussion drill bit. The tool is driven by the drilling fluid and the method is very much like the use of air-driven hammers for blasthole drilling in solid rock in the "West". During the development of the "Nauka", the USSR's alternative (aborted in 1989) for ODP's "Joides Resolution" these percussion hammers became part of the intended set of tools for sampling. Through a joint venture between Fugro and Aquatic, the Russian heir of much of the former USSR scientific drilling technology this set of tools became the CCS drilling and testing system.

Percussion drilling requires very sharp impact blows on the bit and the hammers were tuned to this type of driving energy. For sampling where the penetration of the sampling tube is counteracted by resistance in rather elastic sediments a different form of energy is required. After various modifications downhole hammers were succesfully used in a program of investigations for gas hydrates for JAPEX, offshore Japan (Nankai Trough) in 1997. Samples, 1 to 3 metres long, were obtained in very hard silts and clays with shear strengths up to 700 kPa, tending to become siltstone, were recovered. .

It was decided then to use this tool as a basis for an alternative sampling tool for hydrates in sandy and silty host sediments and this tool will be specifically used to bridge the gap between the softer sediments which can be sampled by straightforward push sampling and the completely cemented or otherwise very cohesive sediments for which rotary coring is appropriate.

For hydrate sampling the percussion sampler has to be fitted with a pressure closing mechanism. As for the rotary coring tool it has been decided to close the sampling tube after it has been retracted from the sediment and returned inside the bottomhole assembly. Otherwise this mechanism has to penetrate the sediments as well, requiring considerable extra energy. No decision has yet been taken on the form of the mechanism and various alternatives are being researched.

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8. Figures

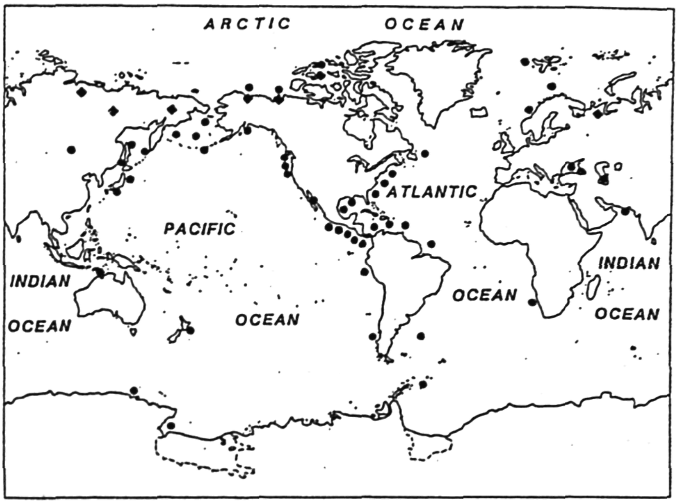

Fig. 1: Occurence of gas hydrates in oceanic sediments (●) and in continental regions (◆) (from Kvenvolden 1996)

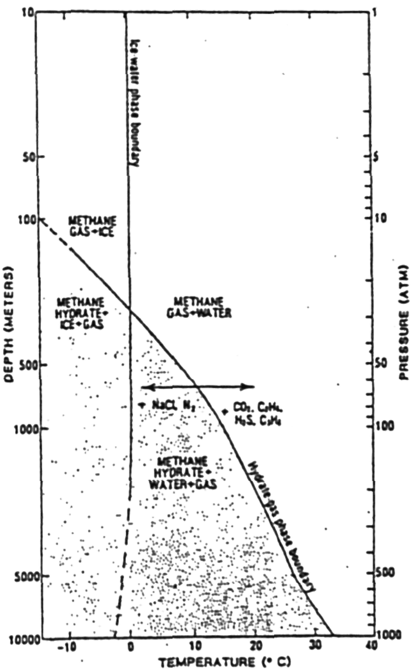

Fig. 2: Phase diagram of methane hydrate (from Kvenvolden 1996)

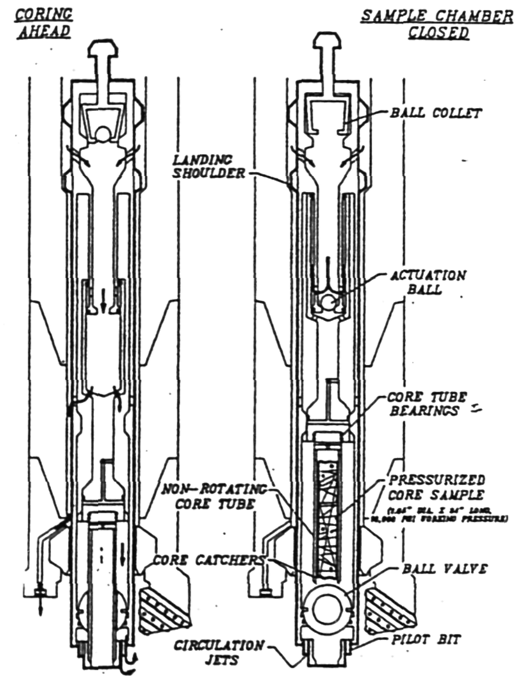

Fig. 3: PCS Operating Schematic (from Pettigrew 1992)

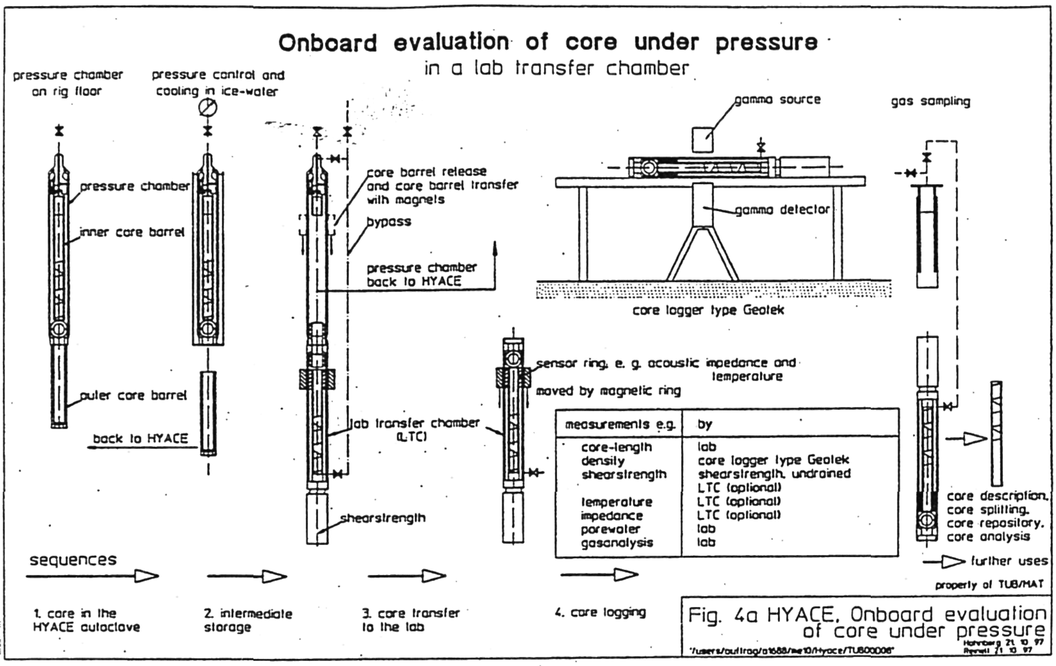

Fig. 4a HYACE, Onboard evaluation of core under pressure

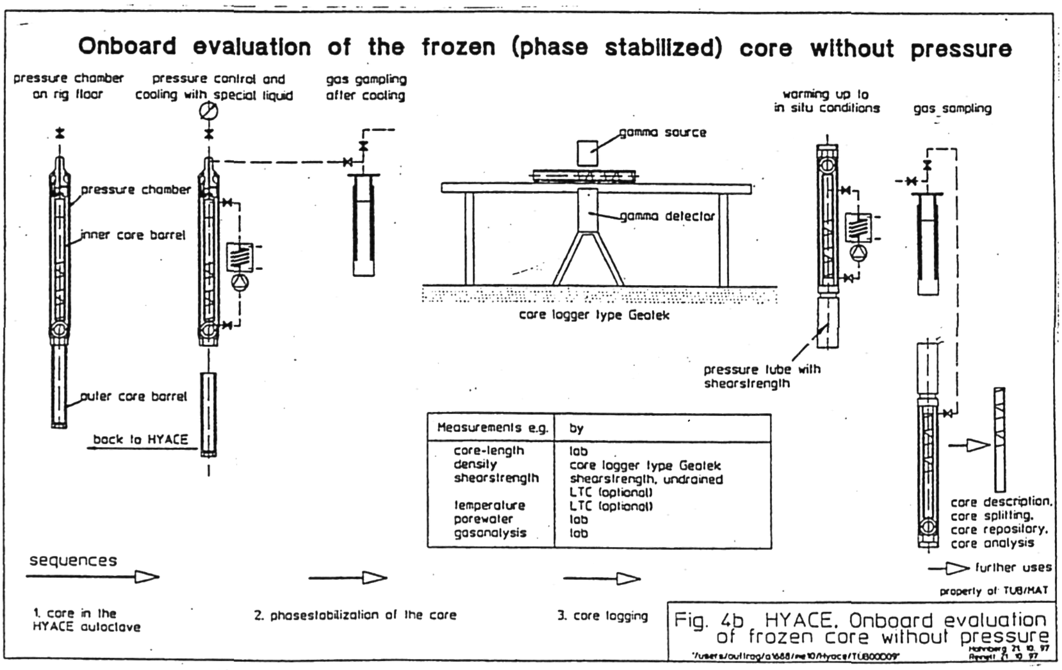

Fig. 4b HYACE, Onboard evaluation of frozen core without pressure

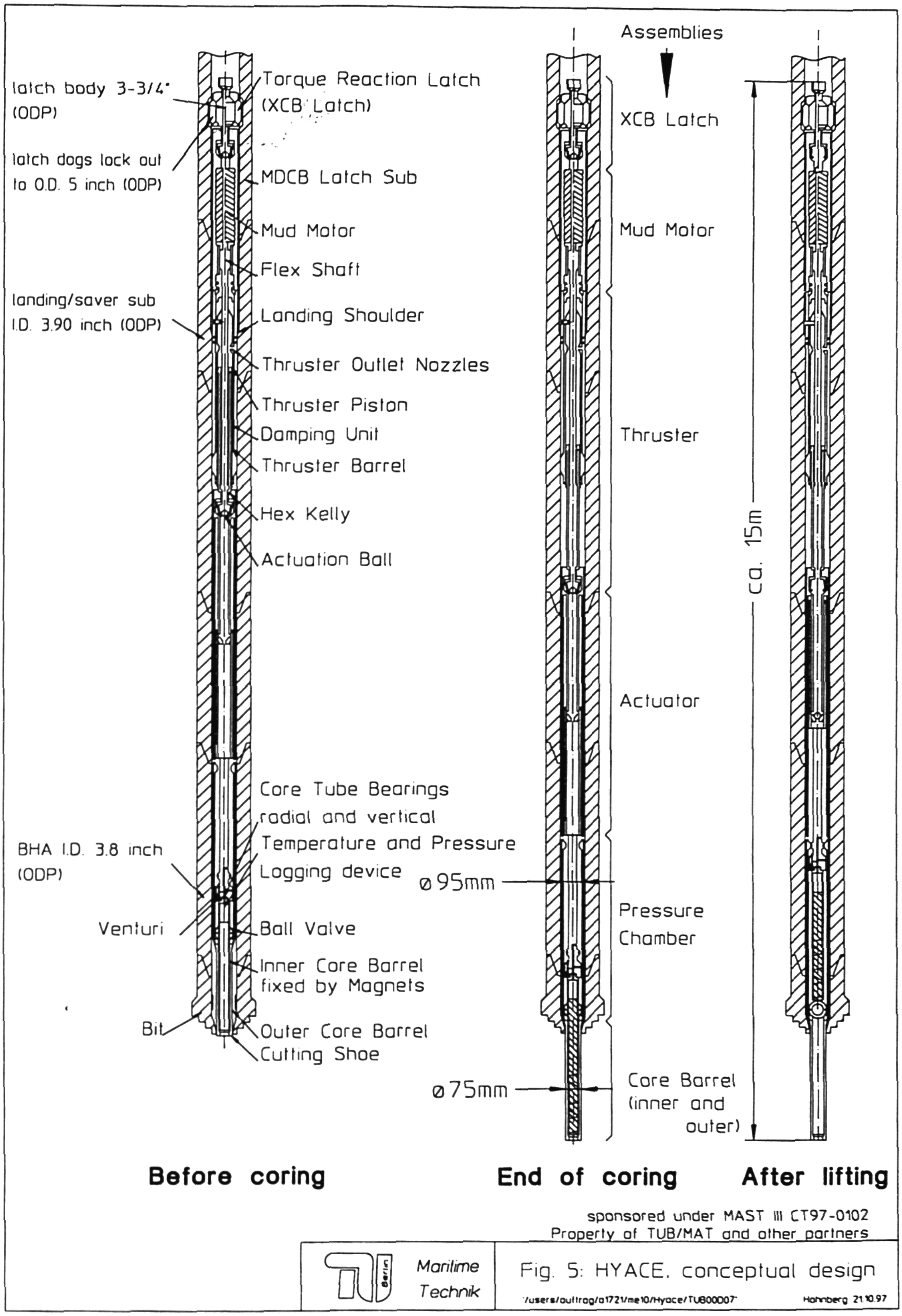

Fig. 5: HYACE, conceptual design

TITLE: Micro Analytical System For Total Organic Carbon In Sea Water
MASTOC

PROPOSAL NO: MAS3-CT97-0138

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MICRO ANALYTICAL SYSTEM FOR TOTAL ORGANIC CARBON IN SEA WATER MASTOC

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SUMMARY

The MAST III project MASTOC dealing with the determination of TOC (total organic carbon) in sea water started on 1 April 1998 and runs for 3 years. The main aim is the development and miniaturization of the instrumentation (filtration, photooxidation, carbon dioxide detection, fluidics) in order to realize an autonomous low power device demanding low reagent consumption. No results are yet available from the project, the workplan is outlined.

1. INTRODUCTION

Although organic matter in the oceans has recently been recognised as one of the earth's major reservoirs of carbon, its precise role in the global carbon cycle remains unclear. This reservoir is regarded as a potential sink for anthropogenic carbon deposited as atmospheric CO_2 and organic carbon compounds released into riverine and marine ecosystems. Therefore, the Total Organic Carbon parameter (TOC) is currently considered as critically important for modelling climate change and the development of sustainable ecosystems through cleaner technologies and life styles.

Currently, there are no instruments commercially available that can perform in-situ, real-time field analysis of TOC in submarine environments. As TOC contains a very significant labile fraction that can disappear within hours during sampling and storage, the accuracy of data from many studies is doubtful. These problems can arise from contaminants at the air-sea interface, particularly from research vessels. Another source of disagreement may be the TOC loss during storage and preservation, even during the first hours (i.e. loss of TOC volatiles by acidification preservation, microbial decomposition, losses during freezing). Moreover the TOC value can be altered during storage by the formation of colloids. Recently, sampling and storage conditions with negligible effects have been established. However, it is still unknown what alterations to TOC occur during sample retrieval from ocean depths to shipboard storage and measuring systems. Simultaneous and real-time sampling and analysis will therefore be a clear approvement for the correct determination of the TOC value.

As the key problem of the project is the catalytic photooxidation the aim is to achieve the complete mineralization of the organics to CO_2 and, eventually, inorganic anions by photocatalysis. Finally, the extensive testing and validation phase applying different marine locations will guarantee the highest quality standards and give an outlook for the requirements of a further development to an autonomous pressure stable underwater device.

2. AIMS

The main objective of the proposed research project is the development of a miniaturized demonstration prototype for the detection of TOC that can be further developed to an autonomous analysis system for measuring TOC directly at the location of sampling.

The miniaturized integrated on-site analytical demonstrator system will include a filtration unit, a low power high-efficiency photooxidation unit, a micromachined fluidic unit to achieve low reagent consumption, and a calibration-free coulometric carbon dioxide sensor as detection unit, exhibiting long duration and high stability. Furthermore, a well considered testing and validation phase will be implemented to guarantee the highest quality standards.

Silicon micromachining and miniaturization will be carried out for the fluidic system, the carbon dioxide sensor, the filtration and the photocatalysis unit. The efficacy of photocatalysis in the mineralisation of different organic species will lead to innovative concepts of less expensive and more versatile technology for the efficient photocatalytic degradation of organic compounds.
In detail the following objectives will be achieved within the project:

- Catalogue of requirements for TOC instrumentations for sea water applications.
- Appropriate filtration for separating predetermined TOC fractions (particulate > 0,7 µm; dissolved 0,7 µm; colloidal 0,01-0,7 µm); development of a miniaturized filtration unit.
- High oxidation rates of relevant organic pollutants within a short time of irradiation.
- Low energy consumption for the irradiation system component.
- Immobilization of a highly effective catalyst during the photodegradation.
- Development and validation of a miniaturized photooxidation unit.
- Micromachined fluidics in silicon technology in order to minimize the consumption of reagents and the size and weight of the analytical instrumentation.
- System integration of the filtration unit, photocatalytic oxidation unit and a coulometric carbon dioxide micro sensor into a micro fluidic system. Addition of conventional pumps and valves.
- Real-time simultaneous sampling and analysis to minimize storage and transportation effects.

Application oriented development of data acquisition, management and evaluation software, widely independent and not limited in application and transfer possibilities with respect to future information networks like GOOS, ELOISE or ECOPS Grand Challenges.

3. WORKPLAN

FILTRATION

During the first phase of the project a specification catalogue will prepared for a micro filtration unit compatible to microsystem technology to separate DOC from TOC. This will account for potential automated long-duration usage in remote environments without fouling and undesired alteration of the DOC value.
After extensive filtration experiments with different sized particles the optimized filtration unit will be integrated into the system concept.

PHOTOOXIDATION

As one of the key problems of TOC instrumentation is an incorrect and irreproducible oxidation of the organic compounds, the proposed project will focus on basic research in the promising photocatalytic oxidation of relevant organic species resulting in the development of a reliable low power photooxidation unit.

The main research will take place in the investigation of reaction kinetics during the photo degradation of the organic compounds, particularly in the presence of colloidal size organic components. Furthermore, the effect of different operational conditions, mainly the concentration of active species (eventual added oxidants, light intensity, catalyst's texture and form) on the amount of final products formed (CO_2 and anions) will be examined. The requirements for a highly efficient oxidation will consider the molecular mass, the biological liability and the chemical refractoriness of all TOC components. In particular, the maintenance service of the whole system depends on the lifetime of the employed catalyst and has to be taken into account.

Investigations concerning specificities of marine DOC towards photocatalytic mineralization will be carried out before analytical methodologies will be defined with regard to the detection of refractory marine constituents and refractory products of degradation and their photocatalytic mineralization.

For the final layout a miniaturization of the photooxidation unit is essential in order to minimize the consumption of reagent and energy. The scale down process and integration into the analytical system will be followed by extensive laboratory tests to get the final layout.

CARBON DIOXIDE DETECTION AND ANALYTICAL SYSTEM

Most techniques for TOC measurements consist of separating the organic carbon from the inorganic fraction, oxidation and measurement with a CO_2 detector. For the widely used NDIR detector the CO_2 has to be purged out of the solution and dried before a reliable detection can take place.

A new miniaturized and calibration-free coulometric carbon dioxide sensor can determine CO_2 in the sample solution. Therefore, it does not need any additional gases and fulfills the requirements for miniaturization and long-term service intervals.

Further research and development is focussed on the construction, miniaturization and interconnection of the different modules. The microfluidic system has to be manufactured and will integrate the photooxidation, the filtration unit and the coulometric carbon dioxide micro sensor.

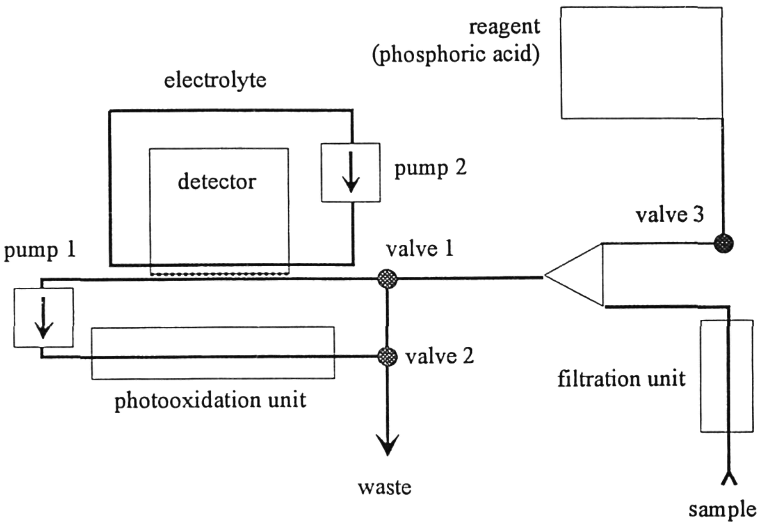

Fig. 1: Schematic arrangement of the analytical system

SOFTWARE

In close cooperation of the partners the requirements for software development, data acquisition, software adaption as well as the detailed specifications will be identified. The data management software package will be developed and adapted to the prototype instrumentation according to the system requirements. The developed software will guarantee a fully automated running of the whole system that means the control of pumps and values, the frequency of sampling, the flow rate, the sample cycling in the microfluidic channels, the check of the detection unit and the photocatalytic oxidation unit with time and intensity of irradiation, calibration cycles etc. Options for the modification of the analysis cycles and the software will be considered and documented for improvement of the instrumentation.

TESTING AND VALIDATION

The precise analytical procedures being employed in the project will be defined as well as accurate and stable measuring methods, validation and standardization. With regard to the testing phase of the TOC instrumentation a standard evaluation procedure will be developed regarding sampling methods for different depths of the sea and independent analytical methods to identify constituent compounds (HPLC, GC/MS).

A test protocol will be the base for comparative measurements on a ship during an oceanographic research in the coastal area in the mediteranean sea as well as measurements in the northern region. The found TOC values will be compared to classical analytical methods like GC/MS for quality control.

TITLE: TRACE METAL MONITORING IN SURFACE MARINE WATERS AND ESTUARIES
(project MEMOSEA)

CONTRACT NO: MAS3-CT97-0143

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TRACE METAL MONITORING IN SURFACE MARINE WATERS AND ESTUARIES (Cd, Zn, Hg, Pb, Cu, Fe, Mn and Co)

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SUMMARY

The aim of the MEMOSEA project is the monitoring of trace metals in sea waters *via* luminescence techniques. Cd and Zn will be determined by pulsed and steady fluorescence, Hg and Pb by room-temperature pulsed and steady phosphorescence and Cu, Mn, Fe and Co by chemiluminescence. An instrumentation suitable for the different approaches will be devised and an on-line preconcentrator system will be developed and will become an integral part of the optical trace metal monitoring instrument. The project has not yet started when writing this note.

1. INTRODUCTION

The need for monitoring of heavy-metals in marine environments is well established. Analytical techniques with the necessary sensitivity and accuracy are available, but often they are time consuming off-line methods. In a more cost-conscious world, new fast analytical tools devised in anticipation of automation are desirable and only the utilisation of these high-resolution methods will lead to a better understanding of ocean chemistry as a function of space and time.

In open ocean environments, the response of the surface waters to anthropogenic perturbations is a long term process (time scale: years) and the main origin of the fluctuations of metal concentration is linked to biological activity (time scale: days). The metal concentrations are low (1 nM or less). On the other hand, in coastal areas and estuaries, variations of metal concentrations from riverine run-off are essentially linked to human activities. Temporal and spatial variations can occur in time periods of less than one hour and at distances of less than one kilometre. The concentrations of metals can be high in such environments and analysis can often be performed without preconcentration, whereas in open ocean, the analytical requirements must be met with the help of appropriate preconcentration techniques.

The major drawback of present analytical tools is that they are often fragile and their application in stand-alone systems is difficult. This is due to their size and weight (ICP-MS/GFAAS), operational difficulties and their sensitivity to matrix components present in sea water. In addition, the manual sample handling procedures involved in metal determination using present tools do not always preserve sample integrity.
Optical techniques based on molecular spectroscopy offer distinct advantages for reason of

simplicity and *absence of physical contact between the detection system and the analytes*. In particular, luminescence methods (fluorescence, phosphorescence, chemiluminescence) offer potential advantages in ion determination: high sensitivity, ease of automation and straightforward application of fibre optics based remote sensing. Furthermore, the treatment of optical signals are in a stage of fast development and one can foresee that their importance will continue to grow in the future.

The challenge of optical metal detection is that, at present, established analytical procedures for analyte monitoring in the useful concentration range are scarce.

The project is aiming at the development of a marine water optical sensor for metals detection and at the development of dedicated analytical procedures.

Depending on their nature and/or concentration, the metals considered here are either micronutrients or pollutants. In any case, their presence or quasi absence in sea water is of vital importance for the sustainability of key communities and for their exploitation. Their determination and speciation (organic/inorganic) by high resolution methods is one of the conditions to fulfill for ecosystem modelling in view of supporting management decision either to exploit or protect the sea.

Cu, Fe, Mn and Co will be addressed by chemiluminescence methods; Hg and Pb by steady and time dependent phosphorescence (ms time-range); Cd and Zn by steady and time-dependent fluorescence (ns time-range).

All these methodologies will be developed on home-built instrumentations with a view to establish and specify the technological specifications of an equipment that will be usable for the three distinct approaches.

A steady and time-dependent luminometer based on commercially available excitation, detection and optical parts will be designed. This equipment will be adapted to the requirements of all the partners in the project. It will thus be a further step towards a multi-element, multi-technique monitor.

In order to increase the useful concentration range of the mentioned analytical techniques, heavy metals preconcentration systems will be designed and construct. This preconcentration equipment will become an integral part of the shared luminescence detector.

Specific objectives are thus as follows :

a/ the participants in the project will develop optical sensing techniques for monitoring the metals by fluorescence (Cd, Zn), phosphorescence (Hg, Pb) or chemiluminescence (Cu, Fe, Mn, Co).

b/ an instrument for steady and time-dependent emission based on an optical transient recorder will be constructed and adapted to the specific requirements of the three luminescence techniques. Time-dependent intensities will provide a signature for the cations when needed.
c/ analytical methodologies based on optical sensing will be developed and assessed for the eight metals.

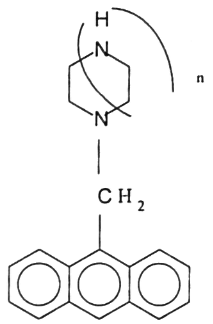
d/ speciation of the metals will be addressed; therefore analysis will be performed on untreated marine samples and U.V. digested samples.

e/ an automated operational on-line preconcentrator will be designed and constructed to meet the analytical requirements when appropriate.

2. WORK CONTENT

Cd and Zn

The monitoring of the two metals will be based on the enhanced luminescence intensities and modifications of the emission spectrum of chemosensors induced by the cations. The selected chemosensors are anthrylazamacrocycles [Figure 1] first studied by Czarnik (1992). They will be synthesized following known procedures.



Example of chemosensor

Figure 1

Two complementary approaches will be used; steady fluorescence and time-resolved spectrophotometry. Although to date, few applications of time-resolved fluorimetry to strictly analytical problems have been reported, some significant advantages of this technique have been put forward by Lytle *et al.* Among them, the possibility of eliminating interferences by luminophores having longer or shorter lifetimes than that of the useful signal is here particularly relevant. The sensitivity, precision, reproducibility and detection limit will be determined; the influence of various parameters will be tested: pH, temperature, salinity and concentration of the indicators. The effect of expected interferents will be addressed. The accuracy will be estimated and the method validated using certified reference materials. Sea water will be U.V. digested for total cations determination and compared to non-treated samples for labile metals analysis. In parallel, and depending on the time available, the benefits brought about by encapsulating the indicators in a solid state matrix (probably a hydrophilic porous glass sol-gel matrix) will be tested.

The light excitation and detection instrumentation based on a commercially available flash lamp system and streakscope or multiplexed luminescence decay detection system will be adapted in our laboratory, bearing in mind that it should be used not only for continuous or time-resolved fluorimetry but also for chemiluminescence measurements and RTP measurements. At this stage, no automation of this equipment is considered yet; this could be the subject of a further development.

Pb and Hg

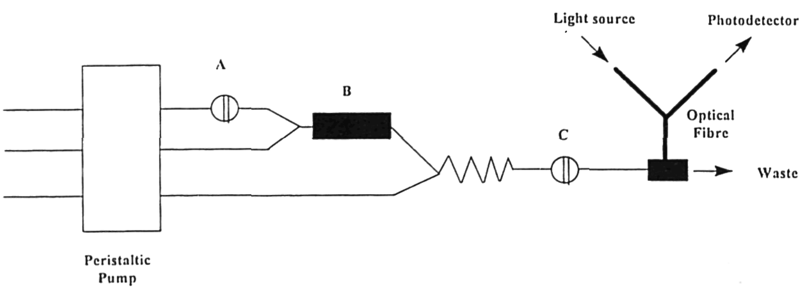
The determination of Pb and Hg will be based on Room-Temperature Phosphorescence (RTP) measurements of immobilized indicators.

Hydroxyquinoline derivatives will be preferentially chosen as possible indicators. Preliminary experiments have shown that the chelates formed between 5-sulphonic hydroxyquinoline or 7-sulphonic hydroxyquinoline and the heavy metals Hg^{2+} or Pb^{2+} exhibit strong RTP in aqueous media when immobilized on anion exchange resins.
Different types of supports (e.g. ion-exchange resins, non-ionic polymers, sol-gels, etc) and different immobilization procedures (covalent bond, entrapping, electrostatic interactions, adsorption, etc) on the solid support will be addressed.
Moreover, the use of organized media (e.g. micelles in flow systems) will also be evaluated as an alternative for the RTP sensing of lead and mercury.

The basic configuration of the optical sensors designed for RTP measurements will be of flow-through cell type. The configuration of the measurement cell will be one of the two following: a) the cell is a conventional flow cell, adequately packed, introduced in the light path of the detector; b) the light to, and from, the flow through cell is carried out via optical fibers and the design of the sensor is home-made. Both types of manifold will be evaluated. Conventional flow systems will be assayed with "organised media".

Fundamental parameters (e.g. absorption and emission wavelengths, lifetime, quantum yields, etc) of the "active phases" and also of the flow systems using organized media, required to evaluate the analytical potential of the sensing phases, and operation conditions of the systems (i.e. pH, flow rate) will be optimized to assess analytical performance of the methods developed.

The use of flow systems allows high versatility in terms of interference removal with pre-minicolumns incorporated on-line in the manifold (see Figure 2). This would be assayed to remove potential interferences such as Na^+, Cl^- or heavy atoms of anionic nature (such as iodide) present in sea-water.



Example of flow diagram for the "on-line" removal of interferences: A. Sample introduction. B. Minicolumn for retention of interfering ions. C. Releasing agent.

Figure 2

Cu, Fe, Mn and Co

A flow-injection (FI) chemiluminescence (CL) based monitor with a solid-state detection system for the determination of the four metals in sea water, incorporating a selective solid phase cartridge for preconcentration will be designed. This will also remove matrix effects and allow the instrumentation to be deployed in fresh, estuarine and coastal systems.

The attraction of the FI approach with CL detection for monitoring of marine systems is the simplicity, robustness and portability of instrumentation coupled with the excellent detection limits (typically nanomolar and below) and rapid response (seconds) that makes it ideally suited to shipboard and even submersible operation. This approach would therefore generate on-board ship validated trace metal data at a high topographical and temporal resolution and would eliminate the need for expensive sampling procedures and minimise the risk of sample contamination and change of sample integrity.

The FI-CL monitor will be computer controlled and have computerised data-acquisition. The monitor will allow the determination of inorganic/organic speciation : with the application of on-line UV digestion total dissolved metals will be determined, whereas without on-line UV digestion the labile (bioavailable) fraction will be determined. The main advantages of the CL based method over, for example, voltammetric methods include increased sensitivity (due to on-line preconcentration), reduced operator dependency, simplicity and robustness of the instrumentation. The CL monitor will form a fully integrated part of the spectroscopic ship-board metal monitoring system

As an example, for the CL detection of Cu: 1,10 phenanthroline is the CL reagent which is oxidised by hydrogen peroxide in alkaline media in the presence of Cu(II) as shown by Coale *et al*. The reaction proceeds *via* the formation of a Cu-phenanthroline complex $[\mathrm{Cu\ phen}_n]^{2+}$ (n=1,2) and the emitting molecule is an excited state diformyl species. This reaction is highly selective for Cu(II), with Pb(II), Zn(II) and Fe(II) giving only minor CL emissions at 0.1 mM levels. In addition, none of the major ions in the sea water interfere positively with the reaction although Mg(II) quenches the CL emission. Therefore, a preconcentration/matrix removal approach is required for the determination of Cu in seawater, and for this project suitable solid state chelation materials will be investigated including MetPac (iminodiacetate based) and cysteine immobilised on silica gel. The quality and validity of analytical work will be supported by analysis of certified reference sample material and by intra-laboratory comparisons with ICP-MS.

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TITLE: Ocean Tomography Operational Package and Utilization Support **(OCTOPUS)**

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Ocean Tomography Operational Package and Utilization Support (OCTOPUS)

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1. INTRODUCTION

Ocean acoustic tomography has recently evolved to become a recognized tool in oceanographic research. This is largely a consequence of the implementation and successful completion of several experiments, which used established tomography techniques to address scientific issues of interest. It is now foreseeable that tomography could become an element in many future oceanographic experiments, as is already the case in multi-national investigations in the Labrador Sea and the Canary-Azores region. Since acoustic tomography yields time series of horizontally integrated quantities, it is a useful complement to other physical observing techniques which usually obtain data at single locations in the ocean (hydrography, moorings) or which can only sense the sea surface (satellite remote sensing). In a sense, tomography is the only remote sensing method for the ocean interior, and therefore it is expected to play an increasingly important role both in future oceanographic research and monitoring/forecasting applications (e.g. GOOS).

Tomography, as currently used, still has some drawbacks which make it complicated and expensive to apply. These cause a lack of acceptance (and usage) in the wider oceanographic community. On the hardware side, various efforts are already under way, to make the instruments and the batteries they require more affordable (i.e. new sound sources which are simpler and have higher efficiency), some of them being carried out with MAST funding. However, the modelling, signal processing, and analysis necessary to successful perform tomography, is also rather demanding, time-consuming and non-standardized. It requires a cumulative knowledge of oceanography and geophysical data analysis, acoustics, wave propagation theory, signal processing, electronics, navigation techniques, and finally inverse problem theory, that is more than a small single group can rapidly gain access to. This is the problem that OCTOPUS plans to target. The project is centered around the development of a user-friendly computer package/environment, that allows oceanographers to use acoustic tomography without in-depth knowledge of all the specialties involved. The hope is that in the long run, tomography can be compared to the usage and processing of ship-board ADCP data: In principle this is also a very time-consuming and complicated task (which took years to analyze in the early days), but a standard processing package and format (developed by the University of Hawaii) is now widely used and allows to process a ship-ADCP data set within a few days.

The development of this software package will be augmented with other measures to bring tomography to a more wide-spread and operational usage, like standardized data format/banking, consideration of the needs for data assimilation, transfer of expertise to an SME, and establishment of an instrument service center. This is important for operational observing systems of the ocean which are now being designed and for which tomography is a candidate. The maintenance of such systems is beyond the role and capabilities of research institutions, and thus operational agencies or European private companies must be prepared for this type of demand.

2. OBJECTIVES:

As recently demonstrated, acoustic tomography is a non-intrusive technology ideally suited for remotely observing, studying and eventually monitoring the large-scale state and environmental changes in the sea. The proposed project intends to develop an integrated and easy-to-use acoustic tomography capability for the community of ocean scientists, service providers, and commercial enterprises. The long-term goals are

- to turn tomography into a routine, affordable, and operational tool for ocean research, monitoring and forecasting which also the non-specialist user has access to

- to increase the acceptance and usage of tomography results as "routine" data

- to enable small/medium enterprises (SME's) to carry out operational applications and provide instrument services in tomography.

3. WORKPLAN:

The above objectives will be achieved through the following measures:

A: Software Package

A suite of user-friendly (menu-oriented "point-and-click") machine-independent software routines will be developed for easy and rapid preparation, processing, and analysis of tomography experiments. The expectation is that this will make tomography more accessible and affordable for the non-specialist user. The package will include

- Ocean data routines to generate sound speed profiles, sections, EOF modes, etc. to be used as input for acoustic modelling

- Acoustic propagation modelling routines to calculate transmission losses, arrival patterns, influence coefficients for inversions

- Data pre-processing routines to perform the signal processing (cross-correlation, Doppler analysis, etc.), mooring motion compensation, clock drift correction

- Data analysis routines to perform arrival time/angle estimation, peak identification, and slice inversions.

B: Data Management and Exploitation

A standard data format will be established for archiving of tomography data, upstream compatible with the software package, downstream compatible with the end user (exploitation) requirements. Similar in a sense to satellite data, a whole set of companion corrections and standard levels of processing will be defined, which would need to be included in a tomography data archive. The outputs of tomography are of interest for both the community of oceanographers, acousticians, and numerical modellers interested in data assimilation. The needs corresponding to these possible uses of the data will be analyzed and taken account of.

Further, a data bank will be established for the European tomography data from this and previous MAST projects. These measures are important for dissemination and to make tomography more accepted and widely used as "standard" data for research and for future monitoring/forecasting applications like GOOS.

C: Routine Products and Instrument Service

The SME partner in OCTOPUS will be responsible for integration of the software package and will thus become familiar with the routine processing of tomography data. In addition, this SME will establish a calibration facility for tomography instruments and initiate an instrument service center. Since this company is already performing other sea-going marine services, it will thus be prepared to provide complete services for routine/operational applications of tomography.

TITLE: Spectroscopy using optical fibres in the marine environment
Project **SOFIE**

CONTRACT NO: MAS3-CT97-0157

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SPECTROSCOPY USING OPTICAL FIBRES IN THE MARINE ENVIRONMENT SOFIE

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SUMMARY

The SOFIE project aims to demonstrate the feasibility of using fibre optical probes in the marine environment for *in-situ* measuring of pollutants and salinity. The project started on 1st March 1998 and runs for 30 months. Objectives, key features and the planned work content are described. Results are not yet available from the project.

1. INTRODUCTION

The maintained health of our surrounding oceans requires frequent monitoring of their numerous physical, chemical, and biological parameters. With this aim the European Union has initiated several programmes and groups, for example HDP and MAST. These initiatives, and the international GOOS programme, aim not only to safe-guard our seas but also to develop a deeper understanding of the processes governing them. This also implies researching and developing advanced systems for ocean monitoring.

On considering the diversity of the constituents, the often harsh measurement conditions and the sheer size of this water mass, one immediately realises the enormity of the task. The most widespread method of generating these data involves sampling. Water samples are either collected in containers from different depths at different points, or, the water is pumped from different depths into a ship or floating platform. These samples are then either brought to a laboratory to be tested or analysed directly on-board.

There are however several inherent flaws with this sampling approach. The constituent concentration may change in the interval between sampling and measuring. In some cases the physical extraction of the sample also varies the concentration. In coastal zones, estuaries or effluents concentrations and conditions are changing rapidly, both spatially and temporally. Pollutants brought into the sea by rivers and effluent streams are subjected to rapid changes by dilution, adsorption to suspended matter, incorporation by organisms and metabolism etc. Sampling techniques are often just not fast enough to track these changes. The random point nature of sampling may also provide misleading information in these cases. As a consequence, an increasing number of samples is required making this process a very labour and hence cost intensive one. Obviously what is required are *in-situ* devices with fast response times, with a wide range of analyte applications and the possibility of long-term unmanned operation in harsh conditions.

This has initiated the SOFIE project. The overall objective is the development of an all fibre-optic measuring prototype device for the *in-situ* monitoring of pollutants in the marine environment. In particular the SOFIE project has three major goals:

1. to develop fibre-optic chemical sensors (FOCS) suitable for sea-water analysis
2. to design and construct a prototype device for this sensor system and
3. to demonstrate the feasibility of this concept in a series of tests including two final field tests.

The *in-situ* measuring process has as a prerequisite that it should be fast and non-intrusive, i.e. it should not alter the matrix. Optical methods in principle fulfil these requirements. However, they need to be very specific towards the target analyte. In laboratory based classical analysis this is achieved by careful pre-treatment or conditioning of the sample. In the *in-situ* application this is realised by the concept of an optode. The optode measures a specific reaction with the target analyte like its electrical counterpart, the electrode, but it utilises radiation instead of electricity.

The optode is a fibre-optic chemical sensor (FOCS). It is an innovative and very active branch of optical spectroscopy. As well as delivering and collecting radiation from the sample, a section of the fibre forms the actual sensor. This approach has several advantages. It is small and may be designed modular. The physical interaction of radiation with the analyte is restricted to a sensing area which is specially prepared. This introduces a certain selectivity and minimises the interference with other constituents. Optical methods are advantageous since they do not consume reagents for the analysis itself.

The proposed sensing device addresses in principle dissolved substances. In the demonstration stage we focus on a selection of the following analytes: heavy metals (copper, lead, mercury), chlorinated hydrocarbons (tri- and tetrachloro ethylene) and aromatic hydrocarbons (BTEX). However, as it is a modular system, adaptation of existing techniques or the incorporation of new modules allows the detection of further analytes if required.

No *in-situ* methods for any of these substances are currently available. The existing methods of analysis involve sampling, subsequent pre-treatment and laboratory analysis.

The envisaged deployment in estuaries or effluents affords measuring of salinity. Salinity will be also determined optically *via* the refractive index.

The overall system consists of four different optodes linked to a core instrument for spectroscopic analysis (Fig. 1). Light sources and detectors will be underwater with only the control unit and data processing on board. The four optodes employ the optical methods absorption, fluorescence, Raman scattering and refractivity. These methods cover a spectral region ranging from visible blue to the mid-infrared. Hence, the feasibility of the different wavelength regions and methods can be determined. Two detection modules are foreseen: a CCD-based multiple-fibre spectrometer with specific gratings for Raman, absorption and fluorescence measurements in the visible and near infrared region and an FT infrared/ tunable diode laser spectrometer for the mid infrared region.

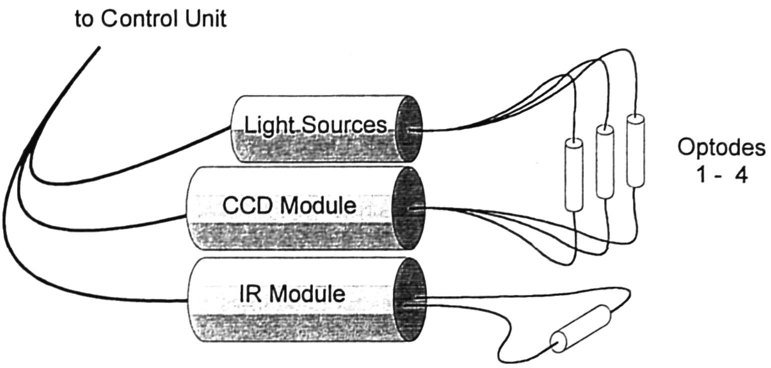

Figure 1. Schematic of the Underwater System

Following is a brief description of the four sensors.

Sensor 1 is based on surface-enhanced Raman scattering (SERS). A metal colloid is embedded in a thin layer of a silica sol-gel. The target analyte diffuses into the gel and adsorbs to the colloid. The sol-gel provides a specific response by discriminating and enriching the target analyte *via* chemical constitution, polarity and size. The Raman scattered signal collected from this surface is a fingerprinting spectra of the analyte. The SERS-active coating is applied to the fibre or to an exchangeable substrate. Target molecules are aromatic compounds (BTEX, naphthalene etc.) and chlorinated hydrocarbons.

Sensor 2 utilises fluorescence quenching and absorption for the detection of heavy metals. A metal-ion sensitive reagent dye is immobilised in a silica sol-gel. The target ions diffuse into the gel forming a complex with the dye. This changes the absorption of the complex or quenches the fluorescence of the dye. The formation of the complex is an equilibrium reaction, i.e. the process is reversible. The heavy metals: lead, copper, mercury are probed. The sol-gel is coated on the fibre or a sensing monolith is used.

Sensor 3 uses evanescent wave absorption of infrared radiation by the analyte. The fibre acts as an elongated Attenuated Total Reflection (ATR) element. The evanescent field of the propagating IR radiation is absorbed by the analyte. The optical fibre is coated with a polymer to enrich the analyte in this layer and to protect the fibre from the surrounding water. The chlorinated hydrocarbons trichloro- and tetrachloro-ethylene (TCE, PCE) are the target molecules. An FT IR-set up for multiple component detection or alternatively a tuneable-diode laser system for selective single component detection is foreseen.

Sensor 4 makes use of the excitation of surface plasmons by evanescent waves of the fibre. A section of the sensing fibre is etched, polished and then coated with a noble metal (e.g. gold, platinum). The evanescent waves of the radiation propagating through the fibre can, under certain conditions, resonantly couple to the surface plasmons of the metal layer. The sensor can detect small changes of the optical properties of the surrounding medium, i.e. the refractive index and hence salinity by comparison of measuring and reference arms.

2. WORK PLAN

The following sections are describing key activities of the project. The development of the different components of the system is done to a large extent in parallel. Planning and construction phases are accompanied by testing phases.

The project starts with an up-date of the specification according to recent progress in the accompanying fields of research and development.

The first step is the development of the sensors in the laboratory. Each of the four sensing principles is established and tested with a laboratory scale set-up. Two approaches for each sensor are tested with the best method proceeding to the next step.

This phase is accompanied by the characterisation of sensors in the laboratory: Dynamic range assessment, check for possible interfering components such as natural constituents of sea-water and chemical compounds which might interfere due to similar properties. Performance and design are optimised according to the results of the check procedures.

In parallel the underwater core instrumentation is designed and manufactured based on the specifications of the laboratory sensors under development. Existing spectrometers are modified and adapted to the needs of the marinised system.

At this point the laboratory sensor heads are marinised. Optodes are designed and constructed for use in sea-water. Based on the results of the laboratory tests prototype optodes are manufactured taking into account flow dynamic and practical aspects of links and handling.

Each of the prototype optodes is subjected to a series of tests in the laboratory to determine the limits of detection, working ranges and calibration functions and to check for possible interfering substances from sea-water constituents or other analytes. The programme is adapted to the demands of each optode based on the results of the characterisation of the laboratory sensors.

The core instrumentation is assembled and the optodes are integrated into the core instrument. The operation of the assembled unit is checked.

Flume tank experiments are performed under controlled field conditions to test the behaviour of the instrument under flow conditions. This is also the first operation of the assembled measuring system. Performance and operability will be determined.

The integration of the instrument into the tow vehicle and the buoy has two main parts: Planning and constructing the tow vehicle to house the instrument and the incorporation of the measuring device into the frame. Power supply, control unit and telemetry are built in.

The first field test is envisaged with the instrument on a mooring station. A buoy is positioned at a site near an effluent stream. The system is tested with the optodes and the spectrometers underwater while the control unit is onboard the buoy. The instrument will be tested for a realistic period to show operational behaviour under real life conditions.

The end-objective of this thirty month project is the testing of the various sensors *in-situ* using a tow-vehicle. The experimental objective is therefore a field trial in an estuary or an effluent. The concentration gradients observed in these plumes range from the normal (sub ppb) levels to several orders of magnitude higher. This demonstration will be performed at a referenced site along the EU coast. Data will then be compared to those collected from local data bases. Emphasis will be put on the dynamic range assessments. Profiles from high concentrations to noise levels will give realistic potentials for each of the sensors.

III.2.3. Biosensors

TITLE: **BIOMARKERS IN MARINE SPONGES: Molecular approaches to assess pollutional risks and ecosystem health in the ocean to support management for its sustainable use**
(project **BIOMARK**)

CONTRACT NO: **MAS3-CT97-0118**

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BIOMARKERS IN MARINE SPONGES:

Molecular approaches to assess pollutional risks and ecosystem health in the ocean to support management for its sustainable use

TOPIC HERE:

Novel multixenobiotic resistance mechanisms in marine sponges:
Mechanism for protection against hazardous xenobiotics in the marine environment

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SUMMARY

The aim of the project "BIOMARK" is to establish reliable biomarkers allowing *(i)* an assessment of pollutional risks and *(ii)* an estimation of the health status of animals in their natural marine milieu. The scientific prerequisites will be prepared to set up guidelines guaranteing a sustainable use of the sea. *As such, the present project is concentrated on the development of novel molecular biological techniques to monitor the quality of the marine environment.* By this approach, the scientific basis for an monitoring of the environmental risk by hazardous substances and chemicals will be strengthened by an improved, fast, efficient and standardized protocol incurring only low costs. Thus, the changes in the levels of different pollutans in the sea water will be assessed before they can cause irreversible damage to the environment.

Marker organisms: Sponges [Porifera], ubiquitously occurring aquatic organisms, are used as bioindicators to establish novel biomarkers for an estimatation of different types of pollution in the sea.

Biomarkers: The effect of environmental stress on sponges is studied by two types of biomarkers: *(i) Biomarkers of exposure*, which are indicative for an exposure to an environmental stress but not its possibly adverse, toxic effects on these organisms, and *(ii) Biomarkers of effect*, which are indicative of exposure and occurrence of adverse effects.

Focus on first results: The multixenobiotic resistance mechanism in marine sponges for the detection of a novel, hitherto neglected class of environmental pollutants was applied. In the present study we report firstly on the presence of • two novel multixenobiotic resistance mechanisms and • hitherto unknown compounds impairing these protection systems.

1 INTRODUCTION

Sponges [Porifera] are one of the major phyla, both with respect to the number of species and biomass, present in most aquatic environments from the surface level to the highest depths. As sessile filter-feeders sponges are exposed to the aqueous environment much closer than any other metazoan phylum; as an example a sponge specimen of 1 kg may filter ≈ 24000 liters d^{-1}. Hence, it is only logical to assume that sponges must have developed efficient strategies to resist against unfavorable environmental loads (Müller and Müller 1998).

Like many marine organisms, also sponges have been shown to possess multixenobiotic resistance [MXR] mechanism(s). By application of molecular techniques it became overt that two types of MXR exist; *(i)* the non-inducibel type of MXR and *(ii)* the inducible, or acquired type of MXR.

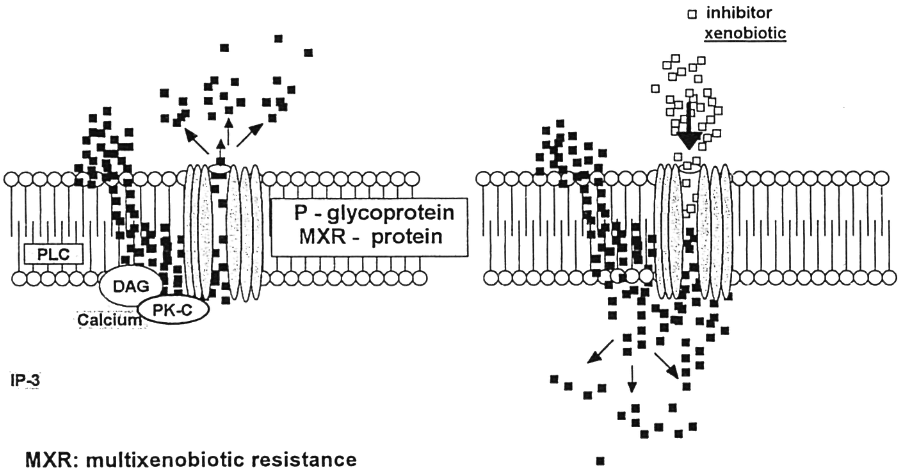

Fig. 1. The MXR, P-glycoprotein [Pgp], pump in the plasma is pressumably regulated by phosphorylation/dephosphorylation reactions mediated by protein kinase C [PK-C]. This scheme outlines that xenobiotics [■] activate plasma membrane-associated phospholipase C [PL-C] resulting in a local formation of diacylglycerol [DAG] and inositol-trisphosphate [IP-3] and an ultimate translocation of PKC [only shown on the left]. The PKC may activate the MXR, Pgp, pump which then extrudes the compound (**left**). Inhibitors of the pump [□] - some novel classes of xenobiotics - compete for the transport system and block extrusion of the compound [■] (**right**). We identified a Pgp-like pump in marine sponges and termed it multixenobiotic resistance protein [MXR protein].

1.1 Non-inducibel type of MXR

This type of MXR acts protective by extruding toxic xenobiotics taken up by bioindicator organisms thus preventing the potentially deleterious effects of environmental xenobiotics (Kurelec et al. 1992; Müller et al. 1996). We could demonstrate that sponges are provided with the MXR transporter, that might function as a protection system for sponges in polluted environments. This MXR transporter (also termed P-glycoprotein [Pgp]) functions as a plasma membrane extrusion pump (Kurelec 1992); *Fig. 1.*

The MXR is related to the multidrug resistance [MDR] that results in the acquisition of resistance e.g. of tumor cells to a variety of structurally unrelated cytotoxic drugs (Gottesman and Pastan 1993). The 170-kDa Pgp, a plasma membrane phospho-glycoprotein which is encoded by the MDR gene in mammals catalyzes an energy-dependent extrusion of many anticancer drugs and other cytotoxic agents. Proteins of 125 -135 kDa that immunologically cross-react with antibodies raised against mammalian 170-kDa Pgp have been identified in the marine sponges *Geodia cydonium* (Kurelec et al. 1992) and *Suberites domuncula* (Müller et al. 1996), and in the clam *Corbicula fluminea* (Waldmann et al. 1995).

***1.1.1 Application in the field; I. Toxins from the alga* Caulerpa taxifolia**: In the present study it is shown that the toxic effects of substances excreted from the rapidly expanding tropical alga *Caulerpa taxifolia* on the flora and fauna in the Mediterranean Sea may be due to an inhibition of the MXR pump. The extensive growth of this green alga that was accidentally introduced into the Mediterranean in 1984 (Meinesz et al. 1996) is altering the appearance of Mediterranean algal communities. Here it is reported that hydrophobic extracts from *C. taxifolia* contain MXR-reversing agents, that inhibit Pgp-ATPase activity at non-toxic levels. One main secondary metabolite isolated from the alga *Caulerpa racemosa*, caulerpin [a cyclooctatetraene ring containing pigment] (*Fig. 2*), was also investigated for its effect on MXR.

Previously we determined that the water pollutant, tributyltin, induces apoptosis in tissue of *G. cydonium* (Batel et al. 1993). Tributyltin, a stable pollutant, has become a permanent addition to the aquatic environment. This compound accumulates in animals organ- and organelle-specific and causes acute toxicity in animals. Here we show that MXR-reversing compounds excreted by *Caulerpa* potentiate the toxicity of tributyltin, resulting in apoptosis of sponge tissue.

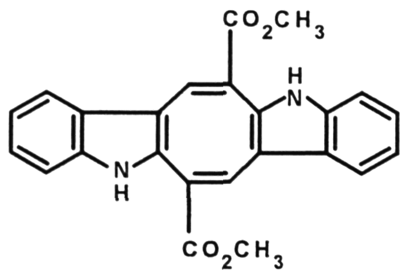

Fig. 2. Structure of caulerpin.

1.1.2 Application in the field; II. Marine snow particles: The formation of marine snow particles is considered to be one of the major mechanisms for the transfer of particulate organic carbon from the euphotic zone to deeper waters (Alldredge 1988). Such aggregates with dimensions of up to several meters have been reported for more than one century to occur occasionally in the Northern Adriatic Sea. The matrix of aggregates consists primarily of mucopolysaccharides. This sticky mucoid material traps organisms, particles, dissolved molecules and trace metals from the bulk water.

In the present study we present first evidence that metabolites of the microorganisms are associated with the gel-like aggregates which act as chemosensitizer(s) [inhibitor(s)] of the Pgp extrusion pump and/or are toxic to benthic metazoans and jointly responsible for the large scale mortality of macrofaunal organisms.

1.2 Inducibel or acquired type of MXR

Only recently it was shown that besides the MXR/Pgp resistance mechanism a further - and different - molecular mechanism is present which provides the cells with resistance against a broad variety of toxic compounds (Spataro et al. 1997). This finding that a system is present which confers resistance in a Pgp-independent manner gains importance especially with respect to those animals which live in a variety of habitats and are there affected by a load of different stressors. The Pgp-independent resistance mechanisms of the class Poh1 provides the cells with a protection against a series of chemicals but also against ultraviolet light. Originally this MDR-like, Pgp-independent resistance protein was identified in the fungus *Schizosaccharomyces pombe* (Shimanuki et al. 1995) and recently in three metazoan species. Here we describe that the MDR-like protein is present also in the sponge *G. cydonium*. It is reported for the first time that the expression of the gene depends on the load with environmental xenobiotics.

2 BIOINDICATOR ORGANISMS

2.1 *Geodia cydonium*

Specimens of the marine sponge *Geodia cydonium* Jameson [Demospongiae: Tetractinomorpha: Geodiidae] were collected in the Northern Adriatic near Rovinj (Croatia) and either frozen immediately to -80 °C or kept in aquaria for up to 1 week at a temperature of 16°C prior to use in the experiments.

2.1.1 Incubation of sponge cubes: Sponge specimens were cut into cubes (0.16 - 0.25 cm^3). The sponge cubes (5 g each; about 120 to 130 cubes) were put into flasks containing 50 ml of filtered, oxygenated seawater without compound or spiked with compound, and agitated by 15-s pulses from an air compressor at 16°C. The seawater was continuously oxygenized during these pulses. Under such conditions the sponge cubes may survive for at least five weeks (Müller and Müller 1998). Incubation of the sponge cubes in seawater containing 0.1 or 3 µM tributyltin without or together with 10 or 50 µg/ml of *C. taxifolia* extract, or 10 µg/ml caulerpin was performed for 24 h. Thereafter the sponge cubes were immediately frozen in liquid nitrogen and stored at -80°C. Control experiments revealed that exposure of sponge cubes for this time period to 0.05% DMSO (present in *Caulerpa* extract) or 0.05% ethanol (present in tributyltin stock) or both solvents had no influence on the results.

2.1.2 Apoptotic DNA fragmentation: Apoptotic DNA fragmentation was determined quantitatively by measuring the cytoplasmic histone-associated DNA fragments ("Cell Death Detection ELISA" [Boehringer, Mannheim, Germany]). Details were given recently (Schröder et al. 1998).

2.2 *Suberites domuncula*

Specimens of the marine sponge *Suberites domuncula* Olivi [Demospongiae: Tetractinomorpha: Suberitidae] were collected near Rovinj, and then kept in aquaria in Mainz (Germany) for up to 9 months at a temperature of 16 °C.

2.2.1 Single cell suspension: Single cells from the sponge *S. domuncula* were obtained by mechanical dissociation as described earlier (Müller et al. 1996). Briefly, the sponge was cut into cubes and incubated for 30 min in seawater containing penicillin, streptomycin, and nystatin. Then the tissue was pressed through a nylon net (pore size 20 µm). The single cells obtained were suspended in seawater containing penicillin and streptomycin, and centrifuged (10 min, 1000 *xg*). Finally they were re-suspended in seawater. The cells were cultured at 16 °C in a temperature-constant incubator and used for the experiments 1 or 2 d later.

2.2.2 Uptake studies: The method described originally by Hollo et al. (1994) was applied with modifications; details were given earlier (Müller et al. 1996). A suspension of 1×10^6 cells ml^{-1} of seawater was incubated in the presence of 2.5 μM calcein-AM in 96 microtiter plates and subjected to analysis during a 20 min incubation period, using a fluorescence microtiter reader (Fluoroskan II). Calibration of the dye concentration was based on the measurement of calcein fluorescein (Hollo et al. 1994), trapped by the cells. Where indicated, the cell suspension was incubated with the model Pgp inhibitor cyclosporin A (5 - 20 µM) as described (Kurelec et al. 1995; Schröder et al. 1998). The extracts were added to the cells at the incubation time zero.

The inhibitory potential of the extracts is expressed as cyclosporin A-equivalents (expressed in µM) per extract (Smital and Kurelec 1997). The concentration of extracts was normalized to 5 ml of original aqueous marine snow sample.

3 RESULTS

3.1 Non-inducibel type of MXR

3.1.1 Poisoning of the MXR pump by* Caulerpa *toxins: To determine the effects of different doses of tributyltin and *Caulerpa* extracts or combinations of them on DNA fragmentation, sponge cubes from *G. cydonium* were treated for 24 h. Subsequently, DNA fragmentation was determined by agarose gel electrophoresis. Control experiments revealed that sponge cubes incubated for 24 h in the absence of tributyltin and *Caulerpa* toxin showed no significant fragmentation of DNA. Application of tributyltin (1 µM) or *C. taxifolia* extracts (10 µg/ml) - applied as single components - did not result in accountable fragmentation of DNA. However, simultaneous treatment of sponge cubes with 1 µM tributyltin with 10 µg/ml of *C. taxifolia* extracts caused a strong increase in apoptotic fragmentation of DNA (data not shown).

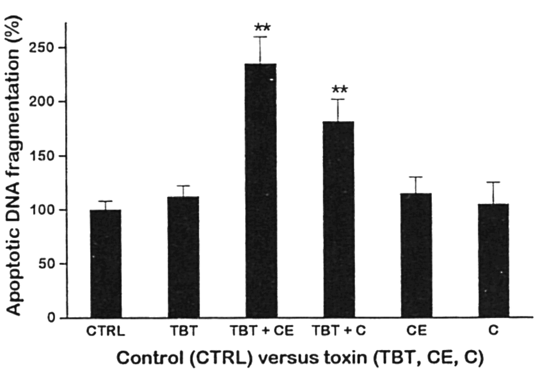

Fig. 3. Enhancement of apoptotic DNA fragmentation in *G. cydonium* tissue by combined treatment with tributyltin and *Caulerpa* extract or caulerpin. Sponge cubes were incubated in the absence of any compounds [CTRL] or presence of 1 µM tributyltin [TBT], or 1 µM tributyltin together with 10 µg/ml of *C. taxifolia* extract [TBT + CE], or 10 µg/ml of *C. taxifolia* extract alone [CE]. In one series of experiments, 10 µg/ml caulerpin was either added to the sponge tissue alone [C] or together with tributyltin [TBT + C]. The calculation of the percentage of DNA fragmentation compared to control is based on the sum of the amounts of oligonucleosomal fragments determined in lysate supernatants (Schröder et al. 1998). The mean values (± S.D.) from triplicate determinations are shown; ** level of significance, $p < 0.001$ compared to untreated control.

A quantitative analysis of the potency of *Caulerpa* extract to enhance tributyltin-induced apoptosis in *Geodia* tissue was achieved by applying the ELISA procedure. Incubation of sponge cubes for 24 h with 1 µM tributyltin or 10 µg/ml of *Caulerpa* extract alone did not cause a significant change in DNA fragmentation (*Fig. 3*). However, a combined treatment of 1 µM tributyltin and 10 µg/ml of *Caulerpa* extract resulted in a significant increase (by 2.2-fold; $p < 0.001$) of the release of mono- and oligonucleosomal fragments into the cytoplasm (*Fig. 3*). Higher concentrations of tributyltin (3 µM) were found to increase DNA fragmentation by about 50%, confirming previous results which indicated a dose-dependent increase in tributyltin-caused apoptosis (Batel et al. 1993). This result suggests that the enhancement of the apoptotic effect seen at 1 µM tributyltin and 10 µg/ml of *Caulerpa* extract is the consequence of an increase in cellular concentration of tributyltin (and very likely not of *Caulerpa* extract).

The same result was obtained with the pure compound from *Caulerpa,* caulerpin. If this toxin is added (10 µg/ml) alone no apoptotic DNA fragmentation is seen (*Fig. 3*). However, if the compound is added together with otherwise non-toxic concentrations of TBT a pronounced and signficant increase of the extent of apoptosis by 1.8-fold is measured (*Fig. 3*).

3.1.2 Marine snow: An example of a gel-like aggregate floating in the open water is given in *Fig. 4B; Fig. 4A* and *C* show a sedimented aggregate carpeting benthic organimsms.

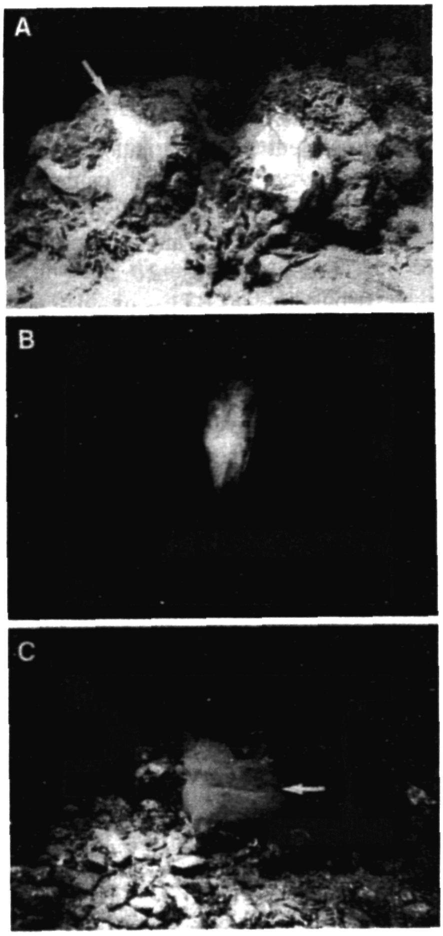

Fig. 4. Snow samples covering a surface area in a depth of 20 to 28 m (*A* and *C*) or floating in a depth of 12 m in the Limski Kanal [near Rovinj; Croatia] (*B*); the marine snow is marked by arrows. Magnifications x 30.

Study sites and sampling: The study was conducted in the Northern Adriatic Sea (45°05' N, 13°30' E) with sampling stations off Rovinj (Croatia) and in the central Northern Adriatic during the period July - September 1997. Aggregates were collected by SCUBA divers with 600 ml disposable syringes (pre-rinsed with 0.1 N HCl and distilled water) at depths between 10 and 28 m. Within 30 min the samples were brought to the laboratory of the Institute Ruder Boskovic (Rovinj) for analysis or processed immediately on board of the research vessel. Samples were taken from aggregates with a minimum diameter of one meter by taking subsamples from the central region and the outer sphere of each aggregate (subsequently referred to as inside and outside) without inclusion of ambient water (Müller and Müller-Niklas; submitted).

Poisoning of the MXR pump by extracts from marine snow: Extraction of marine snow samples was performed as described (Müller et al. 1985). A constant volume of 50 ml from each marine snow sample was extracted with 50 ml of ethyl acetate. After shaking overnight the organic phase was collected; the aqueous phase was re-extracted again with ethyl acetate; the organic phase was collected. After drying over Na_2SO_4 the two combined extracts were evaporated to dryness. The tar-like residues obtained were dissolved in 200 µl of dimethyl sulfoxide (DMSO).

	Effect on MXR pump (CYC-equivalents)		**Cytotoxicity** (reduction of growth ml^{-1})	
Samples	**Outer zone**	**Inner zone**	**Outer zone**	**Inner zone**
n = 15	5.3 ± 0.9	1.1 ± 0.2	6.6 ± 0.8	23.2 ± 2.7

Table 1. Inhibitory activity of the extracts obtained from 15 different samples of marine snow. The same samples were used for determination of both MXR inhibitory potency and cell toxicity. The effect of the extracts was assayed firstly, in uptake studies to determine the effect on MXR pump with cells from *S. domuncula* using Calcein-AM; the inhibitory activity of the extracts is expressed as cyclosporin A-equivalents (CYC-equivalents) and correlated to an extract volume, corresponding to 5 ml of original aqueous snow sample. Secondly, the cell toxicity was determined using the L5178y mouse lymphoma cell system; the reduction of cell number caused by an equivalent of 5 ml of the respective original aquaeous snow sample is given in percent.

For the accumulation experiments, single cell preparations from S. domuncula were used; they were obtained by mechanical dissociation. The suspensions comprised of > 50% choanocytes and approximately 20% of spherulous cells (*Fig. 5*). Using those cells the extracts have been tested for activity to alter the AM-dye trapping. The data summarized in *Table 1* indicate that most of the extracts obtained from the inner zones of the marine snow samples display only a low inhibitory activity on the Pgp extrusion pump. Only seven samples out of 15 samples show a detectable activity; the others are below the detection limit of 1 µM of cyclosporin A-equivalents. In contrast most of the extracts obtained from the outer zone display a significant increase of the uptake of the AM-dye, reflecting an inhibition of the Pgp mechanism. On the average, the inhibitory activity is 5.3 ± 0.9 cyclosporin A-equivalents.

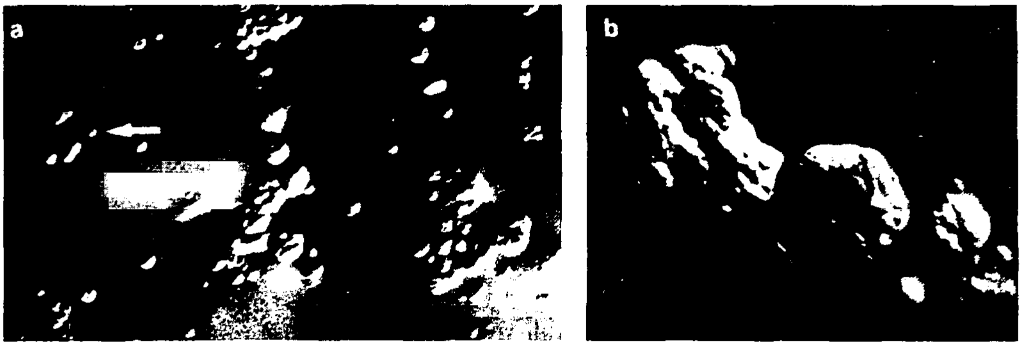

Fig. 5. Suberites domuncula. Dissociated cells. a Cell suspension, containing choanocytes (*long arrow*), and spherulous cells (*short arrow*); b spherulous cells. Magnifications: **a** x 400; **b** x 1200.

In parallel the extracts were tested for their activity to reduce growth of L5178y mouse lymphoma cells. The results revealed that the toxicity of the extracts, isolated from samples of the outer zones is lower than that measured from the inner parts of the marine snow samples. In the outer zone an average of 6.6 ± 0.8% reduction is measured while in the inner zone the inhibition is significantly higher ($p<0.001$) 23.2 ± 2.7% (*Table 1*).

Fig. 6. Pgp-independent, MDR-like, resistance protein from the class of Pohl proteins found in Metazoa as well as in yeast and in fungi. Alignment of the multidrug resistance protein homolog from the sponge *Geodia cydonium* [MDR1_GEOCY] with the related sequences from *I. Metazoa*: human [MDR1_HUMAN; assession number U86782], mouse [MDR1_MOUSE; Y13071], trematode *Schistosoma mansoni* [PAD1_SCHMA; AF014465], as well as from *II. yeasts: Saccharomyces cerevisiae* [MPR1_YEAST; P43588], slime mold (*Dictyostelium discoideum*) [SKS1_DICDI; U96916] as well as from *III. one fungus:* the two sequences from *Schizosaccharomyces pombe* [SKS1_SCHPO; D45047] and [PAD1_SCHPO; P41878] were used. Residues conserved in all sequences are shown in inverted type; those present in at least four sequences are shaded.

3.2 Inducibel or acquired type of MXR

3.2.1 Cloning of the gene from G. cydonium

The cDNA encoding the MDR-like protein from the sponge *G. cydonium*, termed MDRl_GEOCY, was isolated from a cDNA library. The deduced aa sequence, only the C-terminal region is shown here (*Fig. 6*), comprises all the characteristic aa found in related sequences from human, mouse and the trematode.

3.2.2 Expression of MDR-like protein in G. cydonium: Field study

Field studies were performed for the first time with the sponge *G. cydonium*. Specimens were collected from the area around Rovinj. Positions were selected which are known to be differentially loaded with genotoxic xenobiotics (Kurelec et al. 1977): *S-2* [heavily loaded, based on measurements of benzo(a)pyrene monooxygenase (BaPMO) activity in the livers of the fish *Blennius pavo*] ⇒ *S-1* ⇒ *S-3* ⇒ *S-4* ⇒ *S-5* [close to unstressed]; see *Fig. 7 A*. The expression of the MDR-like protein was measured by Northern blot experiments (*Fig. 7 B*). The results revealed that the MDR-like protein is expressed in areas of heavy load [animals collected at station *S-1*] 11.3-times higher than in specimen from the reference station S-5. Intermediate expression levels are found at stations *S-2* to *S-4* (*Fig. 7 A and B*). These data show for the first time that the MDR-like protein is present in marine animals and that its expression is correlated with the environmental load.

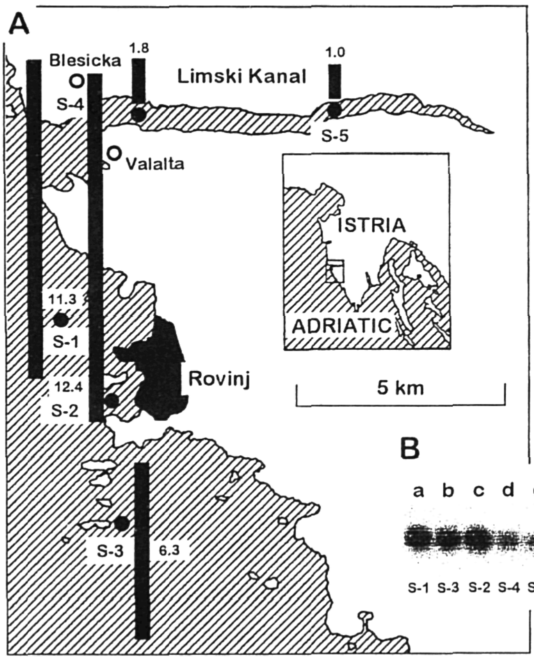

Fig. 7. **A.** Sampling sites for *G. cydonium* in the Rovinj area [Northern Adriatic]. The sites are termed *S-1* Rovinj [fish cannery], *S-2* Rovinj [harbor area], *S-3* S. Giovanni in Pelago, *S-4* entrance to the Limski Kanal [with the tourist camps Blesicka and Valalta] and *S-5* end of the Limski Kanal. The bars [and the added numbers] mark the level of MDR-like protein expression in the sponge tissue. **B.** Northern blot analysis to determine the size [1.6 kb] as well as the amount of transcripts of the mRNA encoding the sponge MDR-like protein. RNA from sponge tissue samples was extracted, size-separated and after blot transfer hybridized with the *GCMDRl* probe. 5 μg of total RNA, each, were analyzed.

4 DISCUSSION

4.1 Non-inducibel type of MXR

4.1.1 Poisoning of the MXR pump by* Caulerpa *toxins: Increasing evidence is available that the MXR mechanism represents an important biological defense system in aquatic organisms which are exposed to high levels of toxic compounds dissolved in the surrounding milieu. Previously we proved that the MXR pump is present in marine sponges (Kurelec et al. 1992). The MXR pump also extrudes non-toxic lipophilic compounds, that might saturate this system at higher concentrations, thereby reversing multixenobiotic resistance. It can be expected that many compounds that inactivate the MXR pump, are present in the marine environment. These toxic or non-toxic compounds enhance the toxicity of such compounds which are otherwise eliminated by the MXR mechanism (Kurelec et al. 1995). Such compounds, which can abolish the protection by the MXR defense mechanism, may be of high ecotoxicological importance.

In this paper we show that the toxic effect of the water pollutant tributyltin in the *G. cydonium* cube system (Batel et al. 1993) is strongly enhanced by agent(s) present in hydrophobic extracts from the green alga *C. taxifolia*. It is feared that the rapid spread of this alga in the Mediterranean Sea may influence the biodiversity of the marine biota. The concentrations of tributyltin used in the described experiments are relevant for the *in vivo* situation.

The effects observed for hydrophobic extracts of *C. taxifolia* are also relevant for the *in vivo* situation, since substances (or the potential) contained in them are excreted into the surrounding water by the weed. Seawater enriched with *C. taxifolia*-metabolites, obtained by bathing 2 kg of living seaweed in 10 L of seawater during 18 h, doubles the level of accumulated rhodamine 123 in gills of the mussel *Mytilus galloprovincialis*, i.e. inhibits MXR to a higher level than that caused by exposure to 5 µg/ml of *Caulerpa* extract (T. Smital, personal communication).

4.1.2 Marine snow: Cytotoxicity as well as chemosensitizing activity was measured in extracts obtained from samples of marine snow. The experiments showed that the cytotoxic potential is considerably high; an inhibition of cell growth of up to 54 % is reached if extracts from 5 ml were tested in this assay cell system. The presence of toxic compounds in samples of marine snow came not unexpected considering the large number of secondary metabolites produced by marine animals (Sarma et al. 1993).

Likewise, the inhibition potency towards the Pgp extrusion pump caused by the extracts was strong. Maximal activity was identified in the outer zone of the marine snow samples reaching values of 10 cyclosporin A-equivalents from 5 ml samples, that is extracts from 5 ml of marine snow samples display an inhibition of the Pgp extrusion pump which is as strong as the effect of 10 µM of the very potent MXR inhibitor cyclosporin A. From this finding it can be deduced that animals coming into contact to those regions of the samples have a low capacity to extrude environmental stressors and are hence very prone to their toxic effect. Consequently, it can be expected that secondary metabolites, produced by monocellular organisms in the surroundings of those sessile animals which are covered by marine snow, cause their toxic effects, under these conditions, even at low concentrations.

It can be postulated that the animals coming into contact with marine snow are in the first step poisoned by chemosensitizers and in turn are affected by low concentrations of xenobiotics present the aquatic milieu; in the absence of chemosensitizers they are not harmful to these animals. The organisms, already showing a reduced viability due to the combined effect of chemosensitizers and low toxins are subsequently, killed by the toxins, produced by the marine snow, especially in the inner zones of the samples.

4.2 Inducibel or acquired type of MXR

The MDR-like protein (cDNA) was isolated from the sponge *G. cydonium*. The size of the nt sequence as well as the deduced aa molecule correspond to those known from the few other sequences cloned, so far.

Of great impact for further studies is the finding that the expression of the MDR-like protein is dependent on the load with environmental xenobiotics. Hence this novel MDR-like protein has the potential to be a powerful bioindicator in the aquatic field.

5 CONCLUSION

Sponges have been shown to be suitable bioindicators for assessment of environmental pollution,. because they are highly exposed to pollutants present in the water filtered through the sponge body (Verdenal et al. 1990). The finding that the inhibition of the MXR mechanism in marine sponges by natural products may result in the enhancement of the toxic effects of environmental pollutants underlines the potential usefulness of these animals in environmental monitoring.

Taken together, this report demonstrates that algal toxins or toxins present in marine snow are chemosensitizer of the Pgp extrusion pump, which diminish the protection of metazoan animals towards dissolved compounds in their surrounding milieu which are otherwise not harmful for the animals and are extruded from their cells.

In addition first experimental evidence is presented which indicates that the second MDR-like system with the MDR-like protein of the class Pohl is present in the bioindicator organisms, the sponges. This finding is of importance for an understanding of the ecology of these animals. In addition, it can be expected that the MDR-like protein will become a suitable biomarker for the assessment of environment load, both of organic and physical origin.

Acknowledgements

This work was supported by grants from the Commission of the European Communities [MAST program] and the International Human Frontier Science Program [RG-333/96-M].

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EUR 18220 — Third European Marine Science and Technology Conference — Project Synopses — Volume IV: Advanced Systems

Luxembourg: Office for Official Publications of the European Communities

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