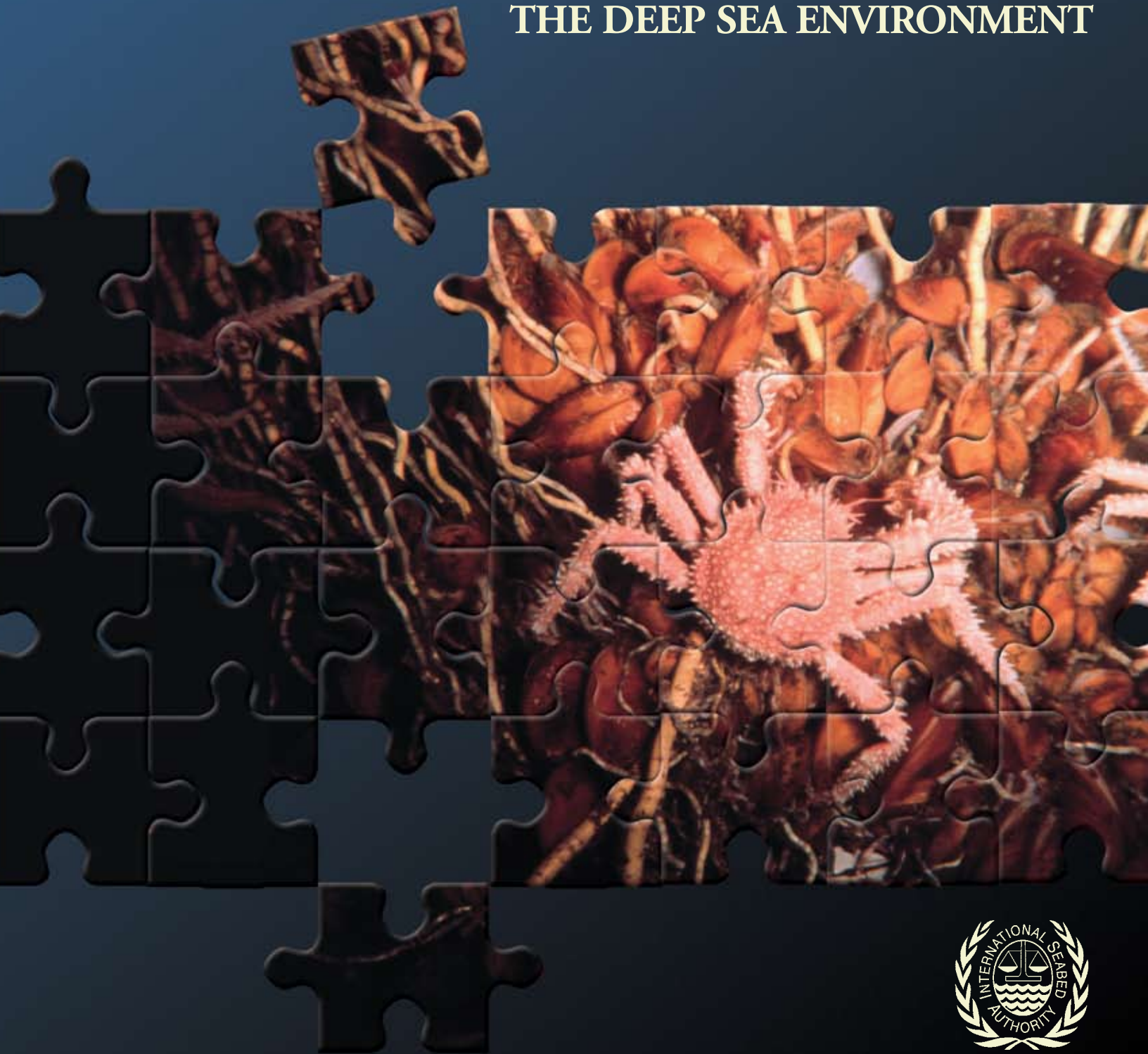


**PROSPECTS
FOR INTERNATIONAL COLLABORATION IN
MARINE ENVIRONMENTAL RESEARCH
TO ENHANCE UNDERSTANDING OF
THE DEEP SEA ENVIRONMENT**



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PROSPECTS FOR INTERNATIONAL COLLABORATION IN MARINE ENVIRONMENTAL RESEARCH TO ENHANCE UNDERSTANDING OF THE DEEP-SEA ENVIRONMENT

Proceedings of the International Seabed Authority's Workshop
held in Kingston, Jamaica, 29 July to 2 August 2002



Prepared by

Office of Resources and Environmental Monitoring
International Seabed Authority, Kingston, Jamaica

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FOREWORD

Satya N Nandan, International Seabed Authority Secretary-General

I hope that you will have a very fruitful meeting. It has almost become a practice for the Secretariat of the International Seabed Authority to precede the annual session of the Authority with a workshop. For these workshops, the Authority tries to select matters of practical interest to its work, and to increase the international community's knowledge base for the development of deep seabed mineral resources. The Authority's first workshop, to assist the Legal and Technical Commission to make recommendations to the Authority concerning the establishment of environmental baselines to evaluate the likely effects on the marine environment of activities in the Area, was held in Sanya, the People's Republic of China. This has been followed by several workshops held in Kingston, Jamaica. In all previous workshops we have focused on obtaining a better understanding of the mineral resources to be found in the international seabed area, the environment in which they are to be found, and to prepare to manage the impact of exploration and mining on the environment. This meeting is somewhat different in that we are trying to see how to proceed towards some practical cooperation in learning more about the international area and the marine environment. As you are aware, the Authority has a mandate, in broad terms, to administer the international seabed area and its resources. It also has a mandate to protect and preserve the marine environment and to promote marine scientific research. So the discussion in this workshop relates very much to these three areas; mining, environmental protection, and promotion of marine science.

Administering the Area requires knowledge of the Area. The only way one can learn about the Area is through scientific research and at the present time, mineral exploration. We have learnt something about the Area from contractors with the Authority. These contractors are engaged in exploration for polymetallic nodules and hope to start mining these mineral resources at the opportune time. They are not undertaking their activities in the broad pursuit of science. The contractors are working towards extracting mineral resources from the deep seabed. While there is a certain amount of science inherent in that effort, and under the Mining Code they are required to observe certain aspects of the oceans in relation to the activities that they undertake, theirs is not marine scientific research. In order to be able to manage mining the resources of the Area in such a way as to prevent serious harm to the marine environment, however, the Authority has to have broader knowledge of the Area.

Information gathered through marine scientific research has proven very useful for the Authority in relation to the knowledge that it has acquired on the resources that it administers and the environment in which they occur. Such information is also useful for contractors to obtain a better understanding of the marine environment in nodule bearing areas. With such

knowledge, the Authority can better regulate the activities of contractors in respect of environmental requirements. An example related to biodiversity and species extinctions from mining in the CCZ is that if it is established through scientific investigations that benthic species in potential nodule mining areas within the CCZ are distributed throughout the zone, and that genetic exchange takes place from one end of the CCZ to the other, then less regulation will be required to protect these species from mining since their recovery is assured. This is just one example of the way that marine science and broader knowledge of the Area would help the Authority in its role as Administrator of the Area, and at the same time reduce contractors development costs.

For scientists, these workshops are also very attractive because they provide a more focused rationale for undertaking marine scientific research that is relevant to the development of deep seabed mineral resources. I have met with many scientists at different meetings over the last few years. The issue of cooperation with other scientists and coordination of their efforts has been repeatedly raised. Most scientists welcome the opportunity to participate in some form of an international programme. In fact it was a meeting in Tokyo convened by Japanese scientists that preceded the ISA meeting in Sanya where I first recall having a discussion with a number of scientists who urged me to try to organize some programmes and projects where they could cooperate and participate.

Since then at each of the workshops this subject has arisen. The contractors have tried to see if they could organize something where they could cooperate. We have been discussing this over the last two years with them. And while we have made some progress, a specific project didn't quite fall into place. So I was very pleased to learn, in the course of the various discussions, about the Kaplan Project. I must thank Professor Smith and his colleagues for bringing it to the attention of the Authority and willingly agreeing to cooperate with the Authority on this project and other projects discussed during the workshop.

A small group of scientists was convened in March 2002 to help us prepare for this workshop. Concrete proposals for consideration at this workshop were sought from the scientists. At the March meeting specific research topics, whose results would facilitate the work of the Authority and of contractors in the protection of the marine environment from polymetallic nodule mining, were identified as the type of marine scientific research activities that the ISA should promote for cooperation.

Four research topics were identified for cooperative marine scientific research.

- Levels of biodiversities, species ranges and gene flow in nodule provinces,
- Sensitivity of the Clarion Clipperton Fracture Zone benthos towards elucidating the single dose response function,
- Mining plume impacts on water column ecosystems, and
- Natural spatial and temporal variability in deep ocean ecosystems.

The way it is emerging, there does not appear to be a need for all potential participants to work on the same project. Different groups could work in different areas according to their desire, specialty or sense of priority. It is hoped at this meeting that these projects are discussed by all participants to determine how best to proceed. The recommendations of the workshop will be submitted to the Authority's Legal and Technical Commission and eventually to the various bodies of the Authority, in particular, the Council.

I am very pleased by the cooperative spirit developed at the March 2002 meeting. I hope that concrete proposals are made at this workshop and that steps are taken to implement these research topics. I think the outcome of this meeting will be very important and I hope that you will work during this week in earnest and come up with the best advice you can give us so that the Authority can consider your proposals.

I don't want to prejudge or preempt the discussions and for that reason I want to conclude by thanking you all for coming, and by thanking the group that met in March 2002 for the preparatory work for this workshop. I would like to introduce the chairman for this workshop, Professor Craig Smith to all participants. Professor Smith from the University of Hawaii is one of the proponents of the Kaplan Project and he will describe the goals of the workshop.

WORKSHOP INTRODUCTION

Dr. Craig R. Smith, University of Hawaii, USA

The workshop is focused on prospects for international collaboration. In discussing the workshop goals, it is important to start with some assumptions or premises that underlie this workshop.

There are a number of premises or starting points for these discussions on collaborative research. The first assumption is that substantially more scientific information is needed to predict and manage the environmental impacts of deep seabed manganese nodule mining. We really don't know very much about deep-sea ecology and biogeography. In order to effectively manage nodule mining we need to know a lot more. Much of the information that is needed is generic or one could say general in nature. In other words, it could be collected in one or a few sites or perhaps by a single study; one coherent scientific study and then broadly applied to nodule mining impacts in the deep sea. There is a lot of general information that we are still lacking.

Another premise is that contractors and external scientists can most efficiently collect this generic information by pooling some of their resources, by combining their efforts and conducting collaborative research programmes; rather than working independently, and, in some cases, conducting redundant research.

Scientific collaborations ideally would provide a common pool of scientific knowledge available to all contractors, to ISA members, and to the scientific community for predicting nodule-mining impacts. The collaboration also has the potential to provide a research framework in which to train young scientists in critical research fields. Research fields such as taxonomy, molecular genetics, and quantitative ecology. Training in these areas is of relevance not only to deep-sea ecology or manganese nodule mining impacts, but also towards understanding human impacts in coastal ecosystems. As a result this training could benefit developing countries and scientists from developing countries.

A final assumption or premise is that because a number of contractors and independent scientists are conducting or planning environmental field programmes in nodule provinces, the time is ripe to organize scientific collaborations. There are a number of parallel projects going on. If we can pool our resources and work on generic problems, then all of us benefit.

At the preparatory meeting, several generic research topics of general interest towards understanding impacts of manganese nodule mining were suggested that could serve as a starting point for this workshop. The four topics that we identified having high priority for collaboration are:

- Levels of biodiversity, species ranges and gene flow in abyssal nodule provinces,
- Disturbance and re-colonization processes at the seafloor following mining track creation and plume re-sedimentation,
- Mining plume impact on the water column ecosystem; enhanced turbidity, heavy metal toxicity, and enhanced oxygen demand, and
- Natural variability in nodule province ecosystems.

How do the baseline conditions vary before and during nodule mining? This essential background information is necessary to understand and to monitor potential mining impacts.

The goals of this workshop are:

- To refine this list of generic topics that could serve as collaborative research efforts,
- To identify interested collaborators both from within the contractor community and independent scientific community who would like to work together on these various collaborative research programmes addressing key scientific questions,
- To design collaborative research projects to address the keys topics or key research questions,
- To tabulate the resources required in terms of personnel, monetary resources, and ship time in order to conduct these collaborative programmes, and
- To identify potential sources of additional support, and develop proposals to obtain this support.

The workshop plan is summarized below. Let me briefly explain the different stages that we hope to pass through in this workshop. First, there will be a number of presentations and discussions on the four key research topics that were proposed in the March preparatory meeting. Again these topics are biodiversity, disturbance and re-colonization, water column impacts and natural variability. We will next have presentations from the contractors regarding their planned field activities, their interest in collaboration, and which topics or research areas they might be interested in developing collaboration. We will then divide into working groups to deliberate on the research topics. For each key topic, a working group which includes scientific experts, some from the contractors and other interested parties will start to formulate the research, to look at some of the research or collaborative plans that have been prepared or develop new ones for those that don't have plans yet, identify the collaborators that would like to be involved in these projects, and the resources required. Each working group will then compile a list of the resources that are currently available and also identify potential sources of the additional support needed and outline a proposal to obtain those resources. We want to

start out with formulating projects and end up with proposals or at least outlines of proposals that are designed to obtain new resources to make this collaboration work.

After the working groups have deliberated for about a day, each working group will report to the whole group informing the workshop on where they are in terms of status on collaboration, what are the key topics, or what is the design of their study, who are involved with the potential collaborative study to address key topics, and where and how they anticipate getting resources. Finally, we will have a discussion at the end of the workshop to decide whether we really have achieved our goals.

Those are the premises and goals of the workshop. I believe we have the critical mass to make a very functional and productive working group to address each of these topics.

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EXECUTIVE SUMMARY

The International Seabed Authority held the fifth in its series of workshops to address issues of concern to its members. The Workshop entitled, "Workshop on Prospects for International Collaboration in Marine Environmental Research to Enhance Understanding of the Deep-Sea Environment," was held on 29 July to 2 August 2002 in Kingston, Jamaica.

Forty-five participants from 26 countries including members of the Legal and Technical Commission and representatives from the contractors were in attendance.

Prior to the workshop, a preparatory meeting of scientists was held in March 2002 to identify key scientific issues that needed to be addressed to evaluate the environmental impact of manganese nodule mining at the deep-sea floor. Additionally, the scientists formulated research proposals around identified critical topics.

Four key research topics with high priority for collaborative research were suggested at a preparatory meeting of scientists in March 2002 and were as follows:

- Levels of biodiversity, species ranges and gene flow in the deep-sea nodule province,
- Disturbance and biological recolonization processes at the seafloor following mining track creation and plume re-sedimentation,
- Mining plume impacts on the water column ecosystem including examination of nutrient enrichment, enhanced turbidity, heavy-metal toxicity, and enhanced oxygen demand, and
- Natural variability in the nodule province ecosystem.

The goals of the workshop were to refine the list of generic topics to address collaborative research efforts, identify interested collaborators from both contractor and independent scientific communities, design collaborative research projects to address key topics or research questions, tabulate the resources required for such research together with those presently available, identify potential sources of additional support and develop proposals to obtain their support.

The Workshop incorporated into its proposals some of the latest advances in genetic testing, in a project that would use DNA analysis to identify denizens of the deep ocean, much of them still undescribed by science.

The research would be conducted mainly in the Central Pacific Ocean, where most known deposits of polymetallic nodules occur. The selected topics deal entirely with the marine environment of the nodules and the potential environmental effects of their commercial extraction. The topics are:

- Biodiversity, species ranges and rates of gene flow in the nodule areas. This research, an extension of a project due to start early next year, is intended to establish a basic understanding of what animals live there, how they are distributed geographically and the extent to which they interbreed.
- Burial sensitivity of deep-sea animals and their response to the type of disturbance caused when nodules are scraped from the sea floor, as well as the recovery of animal communities in space and time. The aim is to learn what proportion of animals might be killed, and how long it might take for their populations to return to pre-disturbance levels under different mining scenarios.
- Impacts on the ocean layers above a mine site caused by unwanted materials from a mining operation. The bottom water, sediment and nodule wastes likely to be brought to the surface with the nodules will change the ocean environment in ways that this project would seek to clarify.
- Natural variability in deep-ocean ecosystems over space and time. These month-to-month, year-to-year and place-to-place changes are to be investigated as a further means of understanding the environment in which deep-sea mining will operate, especially so that the effects of mining can be distinguished from natural processes.

The biodiversity project would incorporate the techniques of molecular biology or genetic testing – widely known as a tool of criminal investigation – for the purpose of rapidly identifying specimens collected from the deep ocean and correlating them with identified specimens preserved at several institutions around the world. The Workshop was told that genetic testing could produce in 48 hours, through largely automated techniques, results that conventionally take highly trained specialists three months to achieve. The plan is to advance the development of these techniques and apply them to deep-sea research.

As the Workshop saw it, all of this research would be organized, managed and conducted by interested scientific institutions and the entities that have contracts with the Authority allowing them to explore specified seabed areas for polymetallic nodules. The organizers would report results to the Authority, which would disseminate them. The Authority would also facilitate this work by convening workshops, seeking financial support from other organizations and promoting training opportunities.

During the Workshop, participants from several contractors and scientific bodies voiced a willingness to participate in one or more of the projects by providing funds, personnel and other support, including time aboard their research vessels for the scientists involved. Four working groups convened during the week, one on each topic, drew up preliminary outlines covering objectives, costs and duration. (Cost figures given during the Workshop are subject to revision in its final report.) The groups also identified persons from among the participants who will be responsible for developing plans, contacting potential contributors and seeking support for each project.

Each project has a training component, to give advanced students, especially from developing countries, a chance at hands-on training in laboratories and aboard ship.

In view of the high cost of research aboard specially equipped ships that spend months each year on the high seas, the Workshop urged collaboration among researchers to allocate ship time – the most expensive component of all the research schemes -- in the most cost-effective manner. It was argued that avoiding duplication of research by several vessels conducting similar studies in adjacent ocean areas could save millions of dollars, at a time when seabed contractors have reduced their exploration activities to await more attractive market conditions for the minerals lying on the seabed. Despite the likely delay in exploitation, many participants saw no less urgency in conducting environmental studies, some of which could take decades to complete.

The Secretary-General of the Authority, outlining the objectives of the Workshop at its start, observed that previous workshops organized by the Authority had been aimed at understanding what was happening in the seabed environment, since the Authority needed such information to administer the area. This Workshop, however, differed by its focus on practical co-operation in marine research. Contractors were not interested in doing science for the sake of science; their emphasis was on extraction of mineral resources. The Authority, by contrast, took a broader approach.

Speaking of potential benefits to contractors, he said that if it turned out, for example, that mining resulted in species extinctions in one area but population of those species existed elsewhere, environmental restrictions on mining could be less severe. Further, using such information in regulating mining could benefit scientists by giving their environmental research a more purposeful focus. Projects and recommendations developed by the Workshop would be passed on to the Legal and Technical Commission and other organs of the Authority.

Introducing the discussion, the moderator, Craig R. Smith, Professor in the Department of Oceanography of the University of Hawaii, pointed out that substantially more information was needed to predict and manage the environmental impact of deep-sea mining. Much of the needed information was generic, of a kind that could be collected at one site and applied broadly. For this, collaboration was preferable to redundant research. Such collaboration also afforded a means of training young scientists, including those from developing countries.

Details of Proposed Projects

Biodiversity

The Workshop has proposed additions to a project already being planned to study biodiversity, species ranges and gene flow in the deep ocean of the Central Pacific Ocean, in the area of polymetallic nodule deposits. This is known as the Kaplan project, from its main source of funds, the J.S. Kaplan Foundation, a private United States organization. The research will address the fact that evaluation of the environmental threat exposed by seabed mining is difficult without much better knowledge of the organisms living there and the ecosystems in which they function.

Knowledge of geographical ranges is important to judge the biological impact of mining. Inevitably, animals will be killed by the mining vehicle and by resettlement of the sediment cloud it raises. If the affected species has a broad range, there is a good chance that other individuals will recolonize the area; if not, the species might become extinct. However, recolonization will also depend on the mobility of individuals, which can be measured by gauging the rate of gene flow to assess how widely the individuals travel during their life span.

Identification of species is a major requirement of this research. Traditionally, this has been a time-consuming task depending on close study of a specimen's morphology, or anatomical characteristics. This requires the skills of specialists in taxonomy, who are often familiar with only a limited number of animal groups. The Workshop has proposed to speed this work by employing the techniques of molecular biology, a rapidly developing science that uses chemical analysis of genes to identify the relationship of one genetic specimen to another. In this way, once a species has been coded according to its genes, unknown samples – even from animals that have been crushed beyond visual recognition – can be compared with a known type and identified much more rapidly than before.

In proposing to apply this gene sampling technique for species identification in the Kaplan project, the Workshop suggests using it not only to study the new specimens that will be collected but also some of those preserved from past expeditions. It also proposes work to advance the microarray technique. A microarray, or "DNA chip" holds a collection of thousands of specimens – actually slices of genetic material arranged on glass slides -- serving as a key to which unknown specimens can be compared. In this way, for example, collections sampled from two locations can be compared to determine how many species from one place match those from another.

The Workshop also identified offers by some contractors to provide ship time for research as well as training opportunities for advanced students from various countries. The Kaplan project organizers offered at least nine traineeships for the molecular biology component.

The original Kaplan project envisaged a budget of \$3.4 million over three and a half years. During the Workshop a rough price tag of \$600,000 was placed on the additional molecular biology component, but a participant calculated that, once DNA chip templates had been produced, scientists anywhere could buy one for \$100-400. Another participant suggested that Germany might be willing to provide up to half of the \$400,000 cost of extending the research to megafauna – animals large enough to be seen in standard photographs.

The Workshop was informed that a group of scientists would meet towards the end of 2002 at Cambridge, England, to identify other bodies interested in the project, select a sampling site in the eastern area of the nodule zone and design a sampling programme. The project will be submitted to the Global Environment Fund for possible support.

Led by the University of Hawaii, the Kaplan project will also have the support of the Natural History Museum of London, the British Antarctic Survey, the Southampton Oceanography Centre (United Kingdom), the French Research Institute for Exploitation of the Sea (IFREMER) and the Japan Marine Science and Technology Centre (JAMSTEC), as well as the Authority. Representatives of several contractors and other institutions informed the Workshop of their interest in participating, by sending scientists along on the research cruises, offering time on their own vessels and providing training opportunities.

Mining effects

The second proposed project would investigate the burial sensitivity of seabed organisms – that is, the consequences of dumping a layer of sediment on top of them, as would happen when a mining vehicle digs into the ocean floor. Specifically, it would seek to elucidate the “single-dose response function” – the reaction to an artificially induced, one-time burial disturbance. This investigation would require two dives to the bottom by a remotely operated vehicle (ROV): one dive to deposit sediment in small areas at four different thicknesses, and another dive, one month later, to see what proportion of the animals had been killed. In later years, surveys would examine the rate at which organisms recolonized the disturbed area.

The cost of such an experiment was estimated at \$8.7 million. Most of this would be for the expense of operating an ROV and for ship time. Scientists were expected to meet in a workshop within the year to move the project forward.

The proponents of this project in the Workshop disagreed with objections raised by some that there was no urgency to such a study, given the fact that nobody currently planned to mine in the deep seas. They acknowledged that no contractor present had expressed interest in the experiment. However, the supporters argued that, if recovery of an ecosystem took 20 years or more, it would be risky to start such experiments only two to five years before mining. If that happened, the Authority might want to delay mining pending further environmental evaluation.

Water-column impacts:

Assessment of potential effects of mining on the overlying ocean is the topic of the third proposed study. Specifically, it would test the results when the unwanted materials that might be brought up in a mining operation -- sediment, trace metals from nodule waste, and cold, nutrient-rich bottom water -- were discharged into the upper ocean. The upper layer was chosen because it would be the most economical area for miners to discharge wastes, though some scientists believe that deep or mid-level discharges would be less harmful to the environment. The project would inject small quantities of experimental substances and sample the effects using bottles and bags. For cost reasons, it would not try to duplicate the mining process in a large-scale experiment.

The investigation would run for four years and be conducted in the Indian and Pacific oceans in alternate years. The rough cost estimate amounts to \$3 million over five years. Those interested would consult during the coming year, either by electronic mail or in a workshop, to draw up a proposal covering methods, budget and experimental design.

Natural variability:

Studying the possible effects of a mining disturbance is complicated by the fact that natural variations over time and from place to place must be taken into account, and many of these factors are unknown. Therefore, the fourth project proposed by the Workshop would send ships to measure a wide range of environmental parameters and to repeat the measurements in later years.

This three-year project would cover the \$7 million cost of one expedition per year, with a possible extension to five years. The ships would visit the western, central and eastern sectors of the Clarion-Clipperton Fracture Zone, the nodule bearing area south and southeast of Hawaii.

This project would be co-ordinated with similar research already being conducted by the China Ocean Mineral Resources Research and Development Association (COMRA), which has begun studies in the seabed exploration area south of Hawaii allocated to it by the Authority. During the Workshop, COMRA voiced particular interest in this topic and has offered to co-ordinate the plan in its initial stage.

Biodiversity Studies

The Workshop spent its first three days listening to presentations by participants centring on the topics identified by the preparatory meeting in March, before reworking those suggestions into its final recommendations.

Dr. Smith outlined the aims and scope of a project entitled "Biodiversity, species ranges and gene flow in the abyssal Pacific nodule province: Predicting and managing the impacts of

deep-seabed mining". Plans called for exploratory cruises to gather animal specimens from three locales in the Clarion-Clipperton Fracture Zone (CCZ), the area of greatest interest to potential miners of polymetallic nodules, where several entities have exploratory contracts with the Authority. The collections would focus on three abundant groups of invertebrate animals previously used for biodiversity studies: nematodes (roundworms), polychaetes (segmented worms) and foraminiferans (a type of microscopic one-celled animal). (The Workshop subsequently suggested adding the larger animals grouped as megafauna to this list.)

The deep areas of the Indian and Pacific oceans were major reserves of biodiversity, Dr. Smith noted, yet the patterns and scales of diversity were poorly known, making it difficult to gauge mining effects. Relatively few samples had been collected; those gathered in the past had been preserved in such a way that their genetic information had been damaged, and species comparisons between animals collected at different sites were impossible due to the lack of standardized taxonomy (classification). Moreover, the number of taxonomists capable of identifying species was diminishing.

The first 18 months of the project, he explained, were to be financed largely by the J.F. Kaplan Fund, with input from the Authority. The project would measure diversity in terms of numbers of species, establish their geographic ranges, and study rates of gene flow, or the pattern of interbreeding through migration. Such knowledge would help the Authority to establish the likelihood of species extinctions as a result of mining. An initial cruise was planned for February/March 2003.

To a question about whether contractors would be consulted before research was done in areas allocated to them for polymetallic nodule exploration, Secretary-General Nandan observed that exploration contracts did not bar scientific research by others provided they do not affect the resource rights of the contractor. Dr. Smith added that contractors would be most welcome to collaborate.

Identifying Seabed Fauna

A "revolutionary" approach for identifying organisms by the molecular analysis of their genes was described by Alex D. Rogers, Principal Investigator for Biodiversity Research at the British Antarctic Survey, Cambridge, England. Contrasting this technique with the traditional method of describing and classifying animals morphologically, according to their structure, he said the new methods were much faster and could handle much greater numbers of specimens. They made it possible to distinguish species among animals that looked alike and they even brought out new evolutionary relationships among species. Moreover, the resulting identification was more objective, being based on molecular analysis rather than subjective criteria of appearance and form.

He described three different techniques of molecular analysis, in a field still under rapid development. All are based on an analysis of the structure of a selected segment of a

cell's chain of desoxyribonucleic acid (DNA), the stuff of an organism's genes, to identify its constituent nucleotides – the basic building blocks of life.

A recent advance, which he called a technique of enormous importance for future identification of species, is microarray technology, involving the production of "DNA chips". In this technique, tiny DNA samples are spotted in a lattice-like array onto a glass slide, each spot from a different species. Then, DNA from another set of specimens is squirted onto the slide. When the species match, the corresponding spot fluoresces. Thus, two sets of specimens – representing, for example, populations from different locations or taken at different times, could be compared to see how the species composition differs. Potentially, 30,000-40,000 species could be represented on a single slide. Moreover, copies of these "DNA chips" could be made robotically, in an industrial process. Theoretically, "spots" from every species of a large group of animals, such as nematodes, could be mounted on a single slide and used worldwide to compare and identify collected specimens.

Dr. Rogers cited two main drawbacks. One was cost – up to 150,000 pounds sterling for the necessary equipment, or \$20,000 for the commercial manufacture of a chip. More importantly, much effort would be required to assemble the original set of gene samples into a "clone library", serving as a key to species identification. Nevertheless, once the initial task was completed, the technique would revolutionize studies of community ecology and biomonitoring, removing the need to have "one person sitting at a microscope identifying species one by one".

Another proponent of these new techniques was P. John Lamshead, of the Natural History Museum, London. He cited the enormous savings in time, noting that species identification that once took three months could now be done in 48 hours, using automated techniques that did not require a skilled taxonomist. This was especially important for a group like the nematodes, of which perhaps only 1 per cent of species had been scientifically described. He explained that, to understand the geographic range of a species, a sure way must be found to know whether a specimen from one location was the same or a different species when compared to one found elsewhere. Molecular techniques could establish their identity with much greater assurance. He cited the need to collect new specimens, as preservation in formaldehyde – the medium used by biologists for decades – damaged the DNA. For genetic analysis, specimens were now being preserved in ethanol, a high-grade alcohol.

Measuring the Potential Effects of Mining

Gerd Schriever, head of the Biolab Research Institute, Germany, reported some results of a study in the southeast Pacific, in the German deep-sea mining-claim area off the Peruvian coast. The seven-year study (1988-1996), called DISCOL for Disturbance and Recolonization, had examined the effects on local animals caused by dragging a plough across the bottom to simulate the effects of nodule mining on the seabed, including the rates at which animals of the

same species returned to the disturbed area. The study had concentrated on megafauna – animals large enough to be seen in the 17,000 photographs taken of the ocean bottom.

The results showed that the population of many species had remained depressed up to at least seven years after the disturbance, when the last survey was undertaken. The effects were greatest on animals such as sponges that lived directly on the nodules, since their habitat had been destroyed. However, even many free-swimming species had suffered a reduction in numbers, presumably due to disruption of their food supply.

Dr. Schriever then described a three-year project, to be funded by Germany, aimed at making digitized copies of the photographs available on the Internet. He hoped this could be integrated with the data coming from the Kaplan project.

The proposed experiment to obtain more precise data on the sensitivity of seabed animals to burial by the sediment redistributed as a result of a mining operation was described by Dr. Smith. The aim would be to elucidate the “single-dose response function”, showing how mortality rates differed according to varying “doses” of resedimentation, from 5 to 40 millimetres thick. The resulting data could be used to calculate biological effects over the large areas in which a sediment plume generated by mining would bury animals living in the sediment.

To carry out this study, a remotely operated vehicle (ROV) on the seabed would inject sediment of specified thicknesses into fenced-in areas. One month later, before the animals could recolonize from the surrounding area, samples would be taken to measure the proportion of animals killed. He envisaged a three-and-a-half-year experiment costing \$3.4 million.

Paul Snelgrove, Assistant Professor at the Memorial University of Newfoundland, St. Johns, Canada, went beyond the initial mortality effect to discuss how recovery rates might be affected by different mining techniques. For example, mining a wide swath of seabed would have a greater effect on geochemistry, and thus on biology, than if the swath were narrow. Mining engineers could use such data when designing equipment and processes to minimize environmental impacts.

Water-Column Impacts

An ambitious, five-year programme to investigate the possible impact of nodule mining on the miles of ocean above a mine site was outlined by B. Nagender Nath, a scientist at the Geological Oceanography Division of the National Institute of Oceanography, Dona Paula, India. Introducing a paper prepared by several experts from three countries, he reviewed the many ways in which the recovery and processing of nodules could affect the overlying water column from its depths to the surface, at the same time that the seabed itself was impacted by habitat destruction and the resettlement of the sediment plume raised by the mineral collection device.

As the nodules were raised to the mining vessel at the surface, nodule particles, the surrounding sediment and even the cold water at the bottom would escape or be discharged. Some of the effects on the surrounding waters would include an increased flow of particles, the release of potentially poisonous heavy metals, changes in temperature and light transmission, and alterations in chemical components, nutrients, bacterial activity, oxygen consumption and photosynthesis. All of these changes would alter the numbers and composition of plant and animal communities, from plankton to fishes and whales, at all levels of the ocean.

Complicating the investigation of these myriad factors, he noted, was the fact that engineers had yet to decide on the kind of mining systems to be employed. That choice would depend on calculations of the composition and volume of discharge. Moreover, the depth of discharge, whether near the surface or the bottom, would make a great difference, with many conservation-minded scientists favouring the deepest feasible level, even if this meant pumping the discharge back to where it came from.

The study outlined by Dr. Nath calls for experiments in which scientists would introduce small quantities of nutrients and sediments into the ocean and then measure the resulting physical and chemical changes in the affected waters. Based on the data collected, they would draw up models to predict the likely effects of large-scale mining. The products of this experiment could then be used by the Authority to formulate policies. Dr. Nath estimated the cost at \$10.3 million over five years.

Natural Variability

Two participants pointed to the need to understand better how the oceans change naturally over time, seasonally and from year to year.

In the view of Huaiyang Zhou, Chief Scientist of COMRA, such research should take priority over impact studies, given the fact that no companies were currently contemplating commercial mining. Studies of natural variability could be used to establish standards against which to measure impacts, he observed. Long-term variations had to be understood, since mining operations would continue over several years.

David Billet, Head of the Deepseas Benthic Biology Group at the Southampton Oceanographic Centre, United Kingdom, also stressed the need for impact assessments to take account of natural variations. Citing data from the North Atlantic Ocean, he described enormous peaks and falls in population levels of a species of bottom-dwelling holothurian (an animal called a sea cucumber because of its shape), presumably in response to changes in the food particles descending from plant plankton near the surface. Species that were rare at one time became abundant at another.

Contractors' Plans

Participants from five seabed contractors informed the Workshop of their past marine research activities, their plans for future studies and their willingness to collaborate in joint projects to study the marine environment.

Bin Mao, Secretary-General of COMRA, described past and planned activities under his organization's project called NaVaBa, for Natural Variability of the Baseline. Launched in 1996, the project had concentrated on four aspects: the effects of periodic weather cycles such as El Nino, the influence of Antarctic waters flowing towards the tropics, how the upper ocean interacted with the abyss, and how topographic, geological, chemical and biological factors varied from place to place. Between 1977 and 2001, four research cruises had gone to COMRA's seabed contract area in the Central Pacific. This work was to continue in 2003-07, with at least three more cruises by the recently renovated vessel Da Yang Yi Hao ("Big Ocean Number One"), specially designed for ocean research. In addition to its use by COMRA, this ship would be available to foreign researchers for collaborative work, for up to 45 days on each voyage, at a cost of \$30,000 a day for ship time.

COMRA believed that the collaborative projects under discussion should be integrated into a step-by step programme, as they could not all be carried out at once. COMRA was ready to take an active part in this collaborative effort, and felt that the Authority could play an important co-ordinating role.

Tomohiko Fukushima, Marine Biologist with the Deep Ocean Resources Development Company (DORD) and the Marine Biological Research Institute of Japan, said that DORD had no plans at this stage for marine environmental research in its contract area over the next five years, due to lack of funds. He described some of the results of work over the past 13 years, at a cost of some \$20 million. This work had centred on three projects: a cold-water dispersion study on the potential effects of discharging bottom water near the surface, JET (Japan Deep-Sea Impact Experiment) on the possible effects of seabed mining and DIETS (Direct Impact Experiment on a Seamount). The results were being used to prepare mathematical models of mining plume and water dispersion and of impact simulation.

He added that DORD was planning to make original environmental data from these studies available through the World Wide Web, covering such matters as the chemical properties of discharged material and photographs of sediment disturbance. In response to a question about the interest of Japanese scientists in participating in collaborative research, he said they were interested and ready to participate.

Woong-Seo Kim, Principal Scientist at the Korea Ocean Research and Development Institute (KORDI), said his institute's deep-ocean studies had begun in 1991, with research into the distribution patterns of nodules. Collection of biological specimens had begun in 1992. Between 1996 and 2000, additional environmental data had been gathered. In 2001, Dr Kim said that two research cruises, of 30 days each, had collected nodule data and limited

environmental information. Since 1995, the Korean Deep Ocean Study (KODOS) had studied environmental variability along north-south and east-west lines. This work had straddled the major climate changes of El Nino and La Nina, during which abrupt drops in water surface temperatures had been observed in 1998, followed by rapid rises in 1999.

Chemical, biological and meteorological studies were ongoing this year in the areas allocated to KORDI, he stated, and two cruises annually were contemplated over the next decade. Four days were available to foreign scientists on these cruises. They would concentrate on the development of environmentally friendly mining technology and studies of environmental variability.

Ryszard Kotlinski, Director-General of the Interoceanmetal Joint Organization (IOM), said his organization, as an intergovernmental body of mainly eastern European countries, was committed to international co-operation and was closely collaborating with other contractors, including an exchange of sea-going scientists. It was willing to contribute to such collaboration in whatever form it might take. It had gathered data on all four of the topics that the Workshop was examining for future research, but thought that natural variability was the most important topic for understanding the environment, since such variability could distort data generated by mining-impact studies. IOM would welcome the Authority's support in curating and digitizing the animal specimens it had collected on previous expeditions. He particularly urged efforts to intercalibrate taxonomy among various researchers.

The next IOM cruise to its allocated area in the Central Pacific would take place in 2004 or 2005, he stated. It would focus on nodule studies, mining conditions and natural variability. Days could be added to the planned schedule of five to six weeks, at a cost of about \$25,000-30,000 per day, to accommodate research by outside scientists.

Mikhail F. Pilipchuk, a marine geochemist from the State Scientific Centre Yuzhmorgeologiya (Russian Federation), reported that a research cruise to the Central Pacific had taken place last year, seven years after an experimental disturbance of the seafloor, to assess geological and ecological conditions. One conclusion drawn from the years of research was that, while organisms would be destroyed in mined areas, undisturbed areas would remain because of the complex terrain, serving as a biological reservoir for recolonizing the destroyed sections. Warning of the dangers arising from the release of underlying heavy metals when the upper sediment layer was removed, he said the change could disrupt geochemical systems that had been stabilized over geological time, possibly creating zones of reduced oxygen where life would become impossible.

In the interest of disseminating information, he spoke of plans to make the results of the Russian studies available in English. Regarding future work, he explained that the Ministry of Natural Resources, which now had authority over Yuzhmorgeologiya in place of the Academy of Sciences, was more interested in money-earning projects. However, he would like to receive proposals from foreign scientists and would pass them on to this Ministry including any ideas they would have on joint programmes.

Following Contractor presentations, Umit Unluata, Head of Ocean Sciences at the Intergovernmental Oceanographic Commission (IOC), spoke of support that could be given by that body, a part of the United Nations Educational, Scientific and Cultural Organization (UNESCO). The IOC was not a funding organization, he explained; rather, it facilitated, coordinated and promoted research, with special attention to training and education, or what it called capacity building – an important element of its budget. This activity was especially directed towards people from the least developed countries and island States. It had recently established a programme on ocean resources and environmental protection, and could aid the proposed research on natural variability – a topic that went well beyond the concerns of mining. It was also interested in the effects of human activities on the deep sea.

Continuing Machinery

The Workshop discussed a suggestion by two of its participants to create a forum for marine scientific research related to the seabed. The forum, to which contractors, scientists and others would be invited, would have three functions: (1) to promote and initiate international co-operation to achieve cost-effective, co-operative, integrated projects; (2) to evaluate and disseminate the results of research through the Authority, and (3) to identify research needs and suggest them to the international community. Supporters of this idea felt that millions of dollars could be saved by such collaborative efforts as the joint use of ship time.

Some other participants, however, questioned how proposals from such a body would be received by governments or contractors if they came without the endorsement of the Authority which is the body with the legal responsibility for regulating seabed activities. Some also voiced concern about the consequences of formalizing a consultation process through a forum.

Secretary-General Nandan observed that the suggested functions were already being carried out by the workshops organized by the Authority. Each project discussed by this year's Workshop would have its own group that would decide how the project would proceed. He preferred the present arrangement of a structure that was not rigid and was open to individual scientists, institutions and contractors. In any case, the secretariat would convey workshop proposals to governments and would also try to encourage them to identify personnel for training.

As an example of how this system would work, he envisaged that the organizers of the Kaplan project would report annually to the Authority and eventually submit the final results, which the Authority would then disseminate to the world.

Possible Role of the ISA

It was suggested that the ISA play a role in facilitating meetings of scientists in the working groups to further develop project proposals. Additionally, the ISA could assist in

coordinating participants from developing nations in particular research cruises and traineeships. The ISA in turn offered to disseminate the results of the research to interested parties.

An underlying theme of this workshop and previous meetings of scientists was the need for standardization of methods to insure comparability of research results. Standardization in marine research can best be achieved through collaborative efforts. The ISA has held and will hold future workshops on developing standards for oceanographic sampling, monitoring of environmental parameters and standardizing procedures for use of oceanographic equipment.

The workshop was successful in developing practical cooperation in marine research with an emphasis on scientific experiments critical to understanding the impact of extraction of the mineral resource in the international seabed area.

Part I

SCIENTIFIC INFORMATION NEEDED TO
PREDICT AND TO MANAGE THE
ENVIRONMENTAL IMPACTS OF DEEP SEABED
POLYMETALLIC NODULE MINING

Chapter 1

The Kaplan Fund Project: International Collaboration in Marine Scientific Research to Enhance Understanding of the Deep-Sea Environment

Dr. Craig R. Smith, University of Hawaii, USA

The first key topic that was identified in the preparatory meeting in March is what we generally are calling biodiversity. This topic will specifically address the levels of biodiversity, species ranges and rates of gene flow in the abyssal nodule provinces. The rationale or justification for why the scientists and variety of other people think that this is an important topic is outlined below.

Rationale

The abyssal Pacific and Indian Ocean floors are thought to be major reservoirs of biodiversity. Although it is controversial, it appears that, based on some extrapolations, levels of biodiversity are very high in the abyssal Pacific and Indian Oceans. Yet, the patterns and scales of this biodiversity are very poorly known. And thus it is very difficult to evaluate the threat of nodule mining to biodiversity (in particular, the likelihood of species extinctions), without knowledge of the following:

- The number of species residing within areas potentially perturbed by single mining operations, and
- The typical geographic ranges of species (and rates of gene flow) within the general nodule province.

How many species are there at a particular site potentially perturbed by a single mining operation? And an even more critical bit of information that is lacking for most of the organisms living in the nodule provinces is their typical geographic ranges within the nodule province. How broadly are they distributed? Are most species so narrowly distributed that they occur only in one area and might be caused to go extinct by a mining operation? Or are they so broadly distributed in the abyssal Pacific or Indian Ocean that we can mine one area and know that they will exist in some other part of the abyssal Pacific or Indian Ocean and have

no chance of going extinct. We know so little about typical species ranges that we don't know which extreme, or whether something in the middle, is true for species ranges of deep-sea fauna.

Why do we know so little about the levels of biodiversity of species ranges in the abyssal Pacific, for example? One reason for poor knowledge of levels of biodiversity and species ranges is inadequate sampling. Relatively few benthic samples have been collected in the nodule province and identified to the species level. Considering the size of this region, we have very few data.

The Clipperton Clarion Fracture Zone is the area of greatest interest in the abyssal Pacific for manganese nodule mining. Stations or sites for which we have species level identifications for a major portion of the fauna, such as nematodes, polychaetes, and crustaceans, are identified in Figure 1. Considering the extent of the potential mining area, we do not have sufficient data. There are more data for the megafauna or larger animals, but in general, the amount of sampling that has been performed in this region and identified to the species level is very small. We just do not have a very large data set.

A second problem in the sampling is the way in which samples have been collected. To date, the sampling is not suitable for modern molecular analysis. Virtually all the samples that have been collected in the abyssal Pacific and Indian Ocean have been preserved in formaldehyde. This makes it easy to study them, to look at their morphology and undertake standard taxonomic identifications. But it prevents use of, or makes it difficult to use, modern molecular techniques for studying species diversity, in particular DNA based techniques. Dr. Alex Rogers will address this issue later. Therefore, we cannot use some of the most modern techniques to study patterns of diversity, species ranges and gene flow in the deep Pacific or in the Indian Ocean.

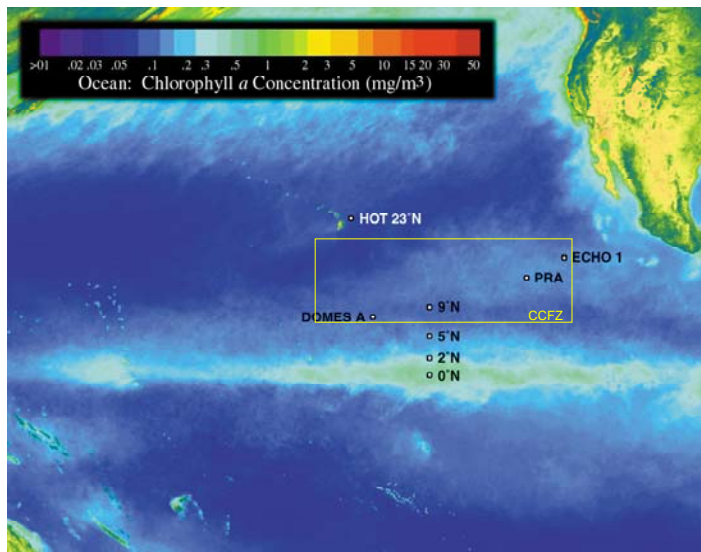


Figure 1. Sites in the CCZ that been previously investigated.

Another reason for this poor knowledge of the level of biodiversity and species ranges in the nodule provinces is the lack of taxonomic standardization. Samples collected in different parts of the Clipperton Clarion fracture zone, for example, have been identified by different groups of taxonomists. These taxonomic data sets have not been merged due to difficulty in comparing species identifications.

As an example, polychaetes are a group of worm-like animals that are very abundant in deep-sea communities. Polychaetes have been sampled and identified for a number of different areas in the abyssal Pacific nodule province. Polychaetes sampled from the HOTS station and along a transect from 0 – 9° N have been worked on by taxonomist from the Natural History Museum in London, whereas polychaetes identified from the three other sites (ECHO-1, PRA, and DOMES-1) have been worked on by another group of taxonomists at the Smithsonian Institution in Washington DC. The reason this is problematic is that most of the deep-sea polychaetes, in fact most of the species that are collected in this area, are new to science. They have not been described in detail by taxonomists. So, when scientists collect polychaete samples from one site and categorize diversity, most of the species end up being labeled species A, Species B, Species C, etc. To understand species ranges, we need to know whether what's called species A at one site by one group of taxonomist is the same as species A collected from another site or by other taxonomists. We need to merge these taxonomic databases in order to understand the real ranges of species in the nodule province.

A third reason that we have a poor understanding of the biodiversity in places like the Clarion Clipperton fracture zone is that the approaches that have been used to identify animals are based on morphology or classical taxonomy. Scientist collects the animals and examines them under a microscope to identify species based on their shapes. It is becoming increasingly evident that using just morphology may underestimate biodiversity. Recent work with molecular techniques in shallow water ecosystems and in the deep sea has shown repeatedly now that there are many cryptic or hidden species; species that look very much the same under the microscope but are actually genetically distinct. So if you want to understand and evaluate levels of biodiversity in deep-sea ecosystems like the Clarion Clipperton Fracture Zone, it is very important to use a modern molecular genetic technique, for example, looking at DNA sequences.

A final obstacle to lack of our knowledge of biodiversity in the deep ocean, and in the Clarion Clipperton fracture zone in particular is the vanishing number of taxonomists. Taxonomy has not been a very popular profession of late. A lot of taxonomists are retiring or have retired. Newly trained scientists, especially scientists capable of combining classical morphological based approaches with the new molecular taxonomy, have not replaced them. This has served as an impediment to undertaking needed ecological research in the deep sea. The lack of taxonomists with morphological and classical expertise has also been an impediment to understanding biodiversity in the abyssal ocean.

The Kaplan Project

The Kaplan Project is an example of an international collaborative research effort designed to help to fill some of the gaps in our knowledge of biodiversity, species ranges and gene flow in the Pacific nodule province. It is called the Kaplan Project because much of the support for this project comes from the Kaplan Foundation. The goal of this project is to study biodiversity in the nodule province to help understand, predict and manage the impacts of deep-sea mining. This is a collaborative programme that involves a number of investigators from the University of Hawaii, John Lamshead and Gordon Patterson from the Natural History Museum in London, Alex Rogers from the British Antarctic Survey, Dr. Andy Gooday from Southampton Oceanography Centre, Dr. Hiroshi Katazato from JAMSTEC in Japan, Myriam Sibuet from IFREMER in France and Karsten Zengler from Diversa Corporation in the USA.

The project was conceived as a two-part project. The Kaplan Foundation and International Seabed Authority have funded the first phase of this project. As a collaborative project, we are very interested in getting additional investigators or collaborations for this project. In fact, additional collaborations are necessary to really make the project productive, to achieve the goals that we've set for ourselves.

Kaplan Project Goals

There are three goals of the Kaplan Project. The first goal is to evaluate biodiversity levels, geographic ranges and rates of gene flow for three faunal components in the Clarion-Clipperton fracture zone. This involves estimating, using molecular techniques as well standard taxonomy, and the number of species in particularly important taxonomic groups. Those groups are polychaete worms, nematode worms and foraminiferans. Foraminiferans or forams are protozoan that are related to amoebas and are very important to deep-sea sediment communities. Our goals are to estimate the number of species in each of these groups at three stations spaced at 1500 kilometer intervals across the Clarion-Clipperton fracture zone.

A second goal of the Kaplan Project is to evaluate, using state-of-the-art techniques, the amount of species overlap and rates of gene flow among our stations. The stations range in distance from one another by 1500 to 3000 kilometers. In other words, to critically understand species ranges, we need to know how many of the species found at one station are also found at another. How much genetic continuity or gene flow is there between the populations at these different locations? And how broadly distributed are these species in the deep ocean?

A third goal is to broadly communicate our findings to the scientific and mining-management communities. Ultimately, we seek to make specific recommendations to the ISA on how to minimize the risks to biodiversity that might result from manganese nodule mining.

Field Programme

Our ultimate field programme is to study biodiversity by collecting samples at three stations across the Clarion-Clipperton fracture zone. The stations will be selected in the eastern, central and western portions of the fracture zone. Each station will be spaced at intervals of about 1500 kilometers. At each station, we plan to collect roughly 14 – 20 box core samples. Box cores are big boxy instruments that collect a cube of sediment about 50 centimeters on a side. These samples will be used to look at the sediment dwelling macrofauna. Macrofauna are animals that are retained when washed on a 300-micron sieve and are generally large enough to be seen by the naked eye. Macrofauna include organisms such as polychaete worms.

We also plan to collect 6 – 10 multiple core samples for meiofauna and microbes. A multiple core is an instrument that is lowered into the seafloor and collects relatively undisturbed cores of sediment that are very useful for looking at meiofauna and smaller organisms, and also sediment geochemistry. We also plan to collect one or two epibenthic sled samples. An epibenthic sled is a sled-like device that is dragged over the sea floor and collects large numbers of animals. Sled samples are particularly useful because they collect a large numbers of organisms, which can be used for molecular genetic techniques for looking at gene flow and species ranges.

One of the new aspects of this study is that samples collected will be treated with DNA-friendly techniques. Samples are treated in such a manner that the DNA is not destroyed or rendered unusable for molecular analysis. For the macrofaunal samples, the samples will be kept cold (2 - 4 °C) and will be washed with chilled seawater. We have designed and fabricated a special chilled seawater system that delivers large volumes for washing the samples. The macrofaunal samples are then fixed in 95% ethanol so that the DNA is not disrupted. This has not been done before in the Clarion-Clipperton fracture zone.

Kaplan Project Laboratory Programme

The laboratory programme has six major tasks, which will be conducted in a variety of different laboratories. The first task is macrofaunal sample processing and preliminary identification of the animals collected from box cores. This effort will be undertaken at the University of Hawaii with the possibility of some portion being performed at IFREMER in France.

A second task is identification and morphological taxonomic analysis of the polychaetes, including standardization of the taxonomy. Gordon Patterson of the Natural History Museum will lead this aspect.

The third task is nematode sample processing. John Lamshead of the Natural History Museum will direct sampling of the meiofaunal nematodes that are collected.

A fourth task is sample processing and identification of the foraminifera. Andy Gooday and Hiroshi Katazato from Southampton Oceanography Centre in England and JAMSTEC in Japan, respectively, will supervise the study of these amoeba-like animals.

A fifth major component of the laboratory programme is the molecular genetic analysis of the metazoans, both nematodes and polychaetes. Alex Rogers at the British Antarctic Survey will lead this effort. Microbial genetic analysis will also be conducted at Diversa Corporation.

Finally, all the principal investigators will undertake the integration of the results from the various taxa and scientific approaches, both molecular and morphological. A synthesis of our results and recommendations to the International Seabed Authority will be done by all of the principal investigators from their respective institutions.

Kaplan Project Anticipated Products

What will be the output created by the collaborative Kaplan Project? The primary output will be a detailed report to the ISA explaining the significance of our findings to the environmental impacts of nodule mining. We will then make explicit recommendations concerning the management of mining to minimize risks to biodiversity.

A second major output we envision is the creation of a DNA-friendly archive of biota; a sort of library of samples of different kinds of animals that can be studied with DNA or molecular-friendly techniques. This would be available to the general scientific community as a reference collection, so other groups can compare their findings from molecular techniques with those from the Kaplan Project. This is very important for generating a standardized taxonomy.

A third major output is the creation of Internet Web pages and a CD-ROM illustrating the common polychaete species from this region including their distinguishing features. This effort will be spearheaded by the Natural History Museum in London.

And finally, one of the anticipated consequences from this project is laboratory exchanges and collaborations with young scientists. We foresee training opportunities in molecular, taxonomic, and quantitative ecological techniques that can be used for not only studying nodule province biodiversity, but also biodiversity in other kinds of areas. Many of the techniques that are used in the Kaplan Project are generic and can be applied to biodiversity studies in the coastal zone as well as in the deep sea.

Kaplan Project Field Programme

Our currently funded field programme involves a 31-day cruise on the RV *New Horizon* in 2003. The research cruise is expected to fully sample the eastern station and possibly to begin collecting samples at the central station. To complete the project, we actually need an additional 14 days of ship time; 7 days at the central station and at least 7 days at the

western station, so we still need additional ship time to complete this project. Anticipated additional support for the Kaplan Project includes a second increment from the Kaplan Foundation. We also hope to have the opportunity to use ship time from IFREMER. IFREMER is planning to have a field programme in the nodule province area in the Pacific in 2004. We may be able to sample the central and western sites during that cruise. There may also be ship time or other resources from other groups that potentially might be contributing to the Kaplan Project. Finally, we may generate a proposal for additional funds from the Global Environmental Facility. This is one of the points of discussion we plan to have at this workshop.

As mentioned earlier, additional collaborations are extremely welcome to participate in the project. For example, we could set up a programme and perhaps get some resources to facilitate traineeships in taxonomy, molecular genetic, and ecology. The Kaplan Project can serve a function or play an important role in providing traineeship to scientists from other countries, from contractors, and from developing countries.

Another area of collaboration, which is very important, is taxonomic inter-calibration and training workshops. It would be very beneficial to the Kaplan Project and to the contractors if we could merge the standardized taxonomy that is being used in studying deep-sea biology in the deep Pacific Ocean. The faunal groups that are being addressed by the Kaplan Project are important, but they are limited. They do not include all the major biological components in the nodule ecosystems. For example, we are not able to include the megafauna and are hoping that through additional collaborations we can include megafaunal components. Dr. Gerd Schriever will be talking about this a little bit later in the workshop.

Benefits of the Kaplan Project

To paraphrase John F. Kennedy, instead of asking what you can do for the Kaplan Project, perhaps we should ask what the Kaplan Project could do for you. What benefits does the Kaplan Project provide to the contractors? The Kaplan Project is conceived to provide benefit to the contractors and to the general scientific community from a number of perspectives. First, the Kaplan Project will provide insights into species ranges and biodiversity levels in the Clarion-Clipperton Fracture Zone.

Why is this of benefit to the contractors? It provides insights into how we need to structure preservation reference areas or reserves that are designed to protect and preserve biodiversity in the nodule province. I believe that what we are likely to find is that most of the species in the nodule province are very broadly distributed. They are distributed throughout the zone. If that is the case, that might mean that we only need to have perhaps two preservation reference areas, one at each end of the nodule province, rather than having each contractor set up and monitor a preservation reference area in association with their mining activities. Potentially, this could be a big savings in terms of sampling and ship time for the contractor. By better understanding the biodiversity, we can more effectively design programmes to protect it. If we know nothing about biodiversity, then we need to take a very

conservative approach with very redundant preservational reference areas, for instance. If we understand it reasonably well, then we don't have to be as conservative; we may be able to require a more limited set of preservation areas and monitoring programme, for example. Some scientists may not agree with my statement, but I think it is true that by understanding the biodiversity we can design regulations more efficiently rather than having to be very conservative and to protect ourselves from our ignorance.

A second benefit to the contractors and to the scientific community in general is a standard taxonomy for key faunal groups from the Clarion-Clipperton fracture zone. For example, the polychaete information will be available on the World Wide Web so that scientists or contractors working on a research programme can pull up these web pages and use the scientific keys to identify their animals. This will be a great benefit to understanding the biology and potential impacts in each of the contractor's areas.

A third major benefit to the contractor and also to scientists from developing countries is the opportunity for training young scientists in modern molecular and morphological taxonomy and also in quantitative seafloor ecology. These methods, in general, are applicable to studies of biodiversity in any benthic ecosystem that is impacted by human activity, such as in the coastal zone. My work ranges from mangroves to the deep-sea abyss. Many of the same techniques are used in my laboratory to study biodiversity in each of these ecosystems: mangroves, continental shelves, and the deep sea. The Kaplan Project provides a scientific framework in which to train young scientists in these very useful scientific techniques.

In conclusion, full realization of these benefits depends on fostering additional collaboration and obtaining additional resources.

SUMMARY OF THE DISCUSSIONS

Several contractors noticed that the selected sampling areas were in regions allocated to various contractors and questioned whether sampling could occur in exploration areas where contractors had exclusive rights to the mineral resources without scientists from the contractors participating.

Professor Smith stated that the actual sampling sites were not yet determined. Specific locations would depend on topographic areas that were relatively flat and potentially representative of the broad region. He added that he would consult with the ISA prior to selecting sampling stations. The selected sampling stations would also depend on collaborations and known sediment characteristics. For scientific investigations, Professor Smith stated that none of the areas were restricted. He further stated that allocation of the areas was for the purpose of prospecting and exploring mineral resources and that marine scientific research was not restricted.

On a question about the likelihood of species overlap between the Pacific and Indian Oceans, Craig Smith provided an example of scavenging amphipods, which were fairly big

scavengers and were highly mobile and that appeared to occupy all ocean basins. He further noted that for species that live in the sediment, it was quite possible that some species were the same between the Indian and Pacific Ocean, but it was a very open research question as there were no data available to give an answer. The bright side of this issue was that the development of new molecular techniques, which were quite fast, allowed for objective methods to address this question.

Dr. Snelgrove added that the important part of the exercise was to develop techniques to address this issue that could be applied in the Indian Ocean to reduce species loss.

Colleagues studying the Indian Ocean Basin stated that the major groups were the same, but genetic structure was not known at present. The techniques being proposed, if adapted, could yield information on the diversity changes within the Pacific nodule province and the genetic structure in the Indian Ocean nodule areas as well.

Alex Rogers said that one particular species of amphipods, *Eurythenes Gryllus*, had been examined using new genetic sequencing techniques which indicated that the wide-ranging species was a species complex. He added that similar methods could start to give real information on species ranges and distribution in the abyssal zone.

Craig Smith emphasized that as Alex Roger had pointed out, molecular genetics were essential tools to understanding species ranges. As the example showed, animals studied under the microscope may look the same, but a number of hidden species could be recognized with molecular genetics.

An observer questioned the emphasis on only three groups of deep-sea fauna, namely, polychaetes, nematodes and foraminifera to which Craig Smith responded that the limitations were related to limited amount of resources for the study, and not limited interest. He would have liked to include megafauna, for example, but that it would require additional funding and additional collaborations by megafaunal experts. The molecular biological techniques however were not limited to the three selected groups. These groups provided the starting point for exploring deep-sea species ranges.

One participant asked if there were economic or medical uses for the deep-sea species.

Craig Smith said that while there was a potential within the highly diverse biota in the deep ocean that could be of use commercially or have biomedical applications, the Kaplan Project was not however focused on this aspect.

Alex Rogers mentioned that he would be discussing some new methods in molecular biology that would enable workshop participants to look at gene expression at the community level. The technology was so far advanced that samples from a community could be taken and gene activity studied in the same samples. In the present context, for example, one could find microorganisms associated with polymetallic nodules that could have genes, which potentially

could be useful in terms of bioremediation of mining spoil. This was only a possibility, but it was something to be looked at in the future.

A participant asked if the samples would be sorted and archived. Professor Smith responded that everything would be kept and archived. He added that in the future, more could be done with the samples which was why DNA friendly storage techniques were chosen. Non-targeted groups would also be preserved for later study by interested investigators.

John Lamshead mentioned that one of the functions of the Museum of Natural History was to formally archive specimens. One of its policies was to hold all specimen including non-target species from the Kaplan Project. Smith further commented that he planned to archive the macrofaunal samples at the University of Hawaii. The collections would be available for other groups to study.

A participant wanted further elaboration on a specific output of the Kaplan Project, namely management recommendations based on the impacts of the mining on biodiversity. Professor Smith responded that one of the objectives of the Kaplan Project was to examine species ranges for key faunal components of each of the major biological groups. The goal was to see if there was overlap. For example, were the same polychaete species found at each of the sites across the Clarion-Clipperton fracture zone? He suspected that many of these species had very broad ranges that if they were found all the way across the zone from east to west, then one could make a recommendation that this species was very broadly distributed, and mining in one area was unlikely to cause extinctions if it was in a very small percentage of this species geographic range. This could mean that having a preservational reference zone at every mining point across the province may not be needed.

He said that species range information was absolutely critical and extremely useful in designing and managing mining programmes. The biodiversity levels, such as the number of species were of secondary importance and that species ranges were a fundamental issue. It was fundamental not only to managing mining, but also to understanding the levels of biodiversity in the deep ocean and could be useful in other biotechnological spin offs.

A scientist inquired about how the three different stations within the Clarion-Clipperton Fracture Zone would be selected and whether the bottom topography, currents and plume direction would be considered in future mining operations as the site could be covered with resuspended sediment and of little use as a preservational site and suggested looking at a north-south transect.

Dr. Smith replied that one of the things under consideration in the final selection of sites was what information existed on topography and current information. He said that there was some information on near bottom current flow patterns from contractor programmes and from environmental studies that have been conducted in the past. In terms of understanding the species ranges, he said it did not really matter whether 20, 30, or 40 years later these areas became sedimented over by mining. The important point was to get the basic distribution

patterns regardless of whether or not the species were broadly distributed. The sampling sites were not being set up as baseline sites but rather as end members with a site in the middle to provide information on how broadly distributed the species were. He added that an idea worth considering was to select some sites that would never be impacted in mining for use as baseline.

A member of the Legal and Technical Commission asked about integration with other disciplines as the Kaplan Project as presented was a very narrow biologist's programme, and whether the scope could be expanded to look at sediment distribution and bottom currents.

Dr. Smith acknowledged that in an ideal world, scientists could map the geology, deploy current meter moorings and measure seafloor characteristics. One of the obstacles to that course of action was cost as there was barely enough resources to accomplish the biological studies at the three sites. At this point in time a multidisciplinary study at each of the sites could not be conducted, although this was where collaborations and interactions with the contractors could be very useful. He said that one reason to have sites within areas that contractors were conducting studies would be because they collected a lot of environmental information that could be useful in the biological studies. For example, sites that had descriptions of the sedimentology, mapped manganese nodules, and characterized flow regimes would be ideal candidates for the Kaplan Project biological studies.

One of the biologists questioned the sampling design of the Kaplan Project and asked what proportion of the community could be expected to be ascertained considering they were widely distributed. He added that deep-sea communities were often characterized by a few species that were quite common and widely distributed and a large number of species that were quite rare.

Dr. Smith responded that he could not be certain what proportion of the species range information would be available because every deep-sea community had a very long list of species that could not be adequately sampled to examine species ranges using molecular techniques. He added that no site had been sampled well enough to answer that question although the question could be answered with a subset of biota and finding out whether the results from that subset was generalized for the whole community. He continued that this was where value judgments and scientific perspective played a role, and that this was the challenge in undertaking the synthesis and making recommendations to the International Seabed Authority.

One scientist said that his concern was whether, as a result of these studies, there would be a reasonably good chance of a conclusion for which there was going to be a consensus about conservation applications.

Dr. Smith suggested that the picture was not quite as dismal as made out. He said that all species were not equally abundant and that there was a continuum. One way to recognize whether abundance affected species ranges was to perform statistical analyses. Making interpretations and recommendations would require careful scientific interpretation, however,

if every species studied was widely distributed, that could be a suggestion that broad distributions are the rule rather than the exception. He added that this was a very useful bit of information for making recommendations about environmental impacts.

One of the scientists was concerned about drawing conclusions on the variability of the different species and their abundances with limited horizontal and vertical sampling.

Dr. Smith responded that there were a number of studies examining smaller spatial scale distributional patterns of the biota in these communities. The Kaplan Project would be investigating patterns on a large spatial scale. With the limited number of samples that could feasibly be collected, Dr Smith said that the Kaplan Project would try to identify relatively similar sites across the nodule province. The Project could not look at all scales or all environmental parameters, but would look primarily at large scales within areas where nodules occur.

A participant asked about the variability of different species horizontally and vertically within the sediment in the deep sea based on to the present knowledge base.

Craig Smith advised that the horizontal distributions of species could not be defined very well because there was inadequate information. He cited molecular information from the deep seabed as an example, saying it was basically non-existent. In terms of vertical distributions, Dr Smith said that approximately 99% of the larger animals occurred in the top 5 centimeters of the sediment and their distributions were restricted vertically. He also added that these animals could be sampled easily by concentrating on the top ten centimeters using specially designed sampling tools to collect the upper, biologically rich zone for analysis.

Another scientist added that for megafauna, there was variability over large spatial scales as had been demonstrated in the Clarion-Clipperton fracture zone. Based on analysis of 200,000 photographs, the scientist said that the variability was correlated with the different habitats that were in existence using multivariate analysis.

A participant asked if there were any time constraints on the Kaplan Project, especially if the leaders needed time to compile and synthesize the available ISA data. He asked for a holistic view of site selection before embarking on intense biological sampling. He mentioned that the International Seabed Authority had a workshop planned to develop a geological model of the area, which would be an excellent resource for selecting the sites. The model probably would not be available until the middle of 2005-2006.

Dr. Smith confirmed that, unfortunately, there was a time constraint. The first increment of Kaplan funding had an 18-month time limit. There was also some constraint on when they could get ship time. They needed to collect samples early enough in the Kaplan Project to have sufficient time to complete the analyses. He thought it was very important to take the broader view of what was being done so that non-biologists could give a synopsis of what kinds of data were available and how biologists could obtain them to help in site selection.

Alex Rogers confirmed that with only three sites across the entire zone, the Project would only pick up the gross patterns which essentially would be able to tell whether or not populations were genetically similar between those sites. An important point with respect to this project was that it was a first step in reaching an understanding of the ranges that these species have in the abyssal zone; ranges over which these animals could actually disperse and migrate. He suggested that participants look at the Kaplan Project as a first step and that studies done thereafter could be designed to look at things on a much finer scale. He added that a lot would depend on what was found in the first steps of the investigation.

Chapter 2

Biodiversity and Taxonomic Issues: Particularly Standardization

Dr. John Lambshead, The Natural History Museum, United Kingdom

Introduction

The fundamental job of the Natural History Museum is the identification, taxonomy and evolutionary relationships of living things. This pursuit was considered old fashioned until about twenty years when various molecular techniques were developed and the field became 'sexy' again. Taxonomy is the fundamental building block of all biology. What you feed into most biological experiments at the base level is taxonomy.

When I first joined the Natural History Museum, I was a trained biochemist. My manager was one of the "old museum boys". He went to a lecture given by a biochemist. He relayed this story; the biochemist had carried out an experiment on these particular parasites to test their physiological reaction to a drug. He got two peaks on his curve so he spent considerable time in his lecture explaining why he had two peaks. And eventually my boss got up and from the back of the room said "young man, young man, are you aware you have two different species in your culture". This illustrates the importance of taxonomy.

Taxonomic Issues Related To Deep Seabed Mining

Accurate Taxonomy

Before we can undertake any ecological analysis, we need accurate taxonomy. Accurate taxonomy is essential to evaluate and monitor biodiversity across the nodule province. We are not going to be perfect for reasons that Smith earlier mentioned. However, we do need a reasonable level of accuracy or else anything we decide about biodiversity and conservation is going to be nonsense.

Unknown Species

The major problem we face in assessing the deep-sea environment is that many, if not most infaunal species are unknown or very poorly known taxonomically. Infauna are the small mostly microscopic organisms that live in the upper few centimeters of sediment. Let me highlight an example from marine nematodes, the group that I study. When I was working on my PhD on the Clyde beaches, I was working in one of the best-known areas in the world, a northwest European Beach. Almost 60% of all the animals that I worked on, I could assign to species level. I could put a name on them; I knew what they were and everyone else knew exactly what they were. When we did a study for the Irish government in the Irish Sea off Dublin, which was at a water depth of 20 m, we could only name about 30% of the species. In other words, 70% of the species we could not reliably name. As soon as we moved offshore, into the deep Atlantic, we could barely name 1% of the species.

Virtually all the nematodes in the CCZ are unknown having never been described by science. In general, I believe you would expect a very substantial number of unknown species, around 95%.

Our challenge is we can readily assign the animals into a family, sometimes we can put them into genera, but assigning them to species is nearly impossible without significant taxonomic and systematic studies to justify species identification. In practice, we end up labeling the species – as previously described, species 1, species 2 and species 3.

Local Impact versus Conservation Monitoring

Monitoring the environment has two aspects: local impact monitoring and conservation monitoring. Local impact monitoring requires sorting of nominal species while conservation monitoring requires accurate assessment of spatial and temporal distributions of individual species.

First, let us discuss local impact monitoring. What effect does a process have on a particular place? The process can be mining or any other industrial or natural activity. Taxonomically, this is not too difficult a problem. If we don't know what the species are, then, we can collect them, examine them morphologically and sort them pretty reliably into species. If, for example, we find two juveniles that look alike and came from cores only 50 meters apart, we, fairly confidently, can say "I am going to regard them as the same species, they are both species 3". We are probably right. Our degree of error is relatively low.

The second aspect of our monitoring problem relates to conservation issues and is a much bigger problem taxonomically. One of the reasons why we need to know if they are the same species over a long distance is to evaluate questions related to possible extinction of the species. When monitoring local impacts that just require the sorting of species from a relatively small area, the difficulty with taxonomy is not too bad. However, when we start addressing conservation issues such as species loss, we need to know more about the species. Is the species

from this impacted area the same as the species in a core 1000 km or 3000 km away? Are the two juveniles the same species? This becomes a much bigger taxonomic problem. “Yes they look a bit alike under the light microscope”, but all nematodes look alike under the light microscope. Immediately we have a much more difficult question to answer. Are they conspecific? That is, are they the same species? When we are dealing with unknown organisms then these questions are very difficult to answer.

There are two extreme models in this case. If all the same species were distributed everywhere in the deep sea, then we could demolish half the seabed of the Pacific Ocean, and, in terms of extinction, it would not matter.

The other extreme would be if all the species were endemic, if each species was highly localized to a small place and not found anywhere else. It would be a major problem when we started an industrial activity that could cause harm, because we would start losing species. The two extreme models can be characterized as:

Species are universally distributed, and we really don't have an industrial problem, or
Species are very endemic, and we have major problems every time we do anything.

Of course, these models are extreme. The situation in the deep sea is somewhere in between and may vary with different taxa. Some taxa will be more endemic, i.e. more localized, where as some taxa will be globally distributed. This has become a major issue in science. I cannot stress strongly enough what a problem this is when we are dealing with unknown species.

If you are dealing with most mammals or birds, I could go to a library and determine their known range and distribution. We know the distribution of the European fox, for example. However, when we examine unknown species where we don't even have names for the animal, how are we going to know their distributions? Unfortunately, for virtually all deep-sea fauna we just don't have this basic type of biological information. This was a huge problem when we first started discussions with the ISA. Essentially the scientists were being asked a question that they could not answer. The biological information does not exist. We were asked to give advice we could not give.

Biologically, species are the most important taxonomic level. Generally, legislative and regulatory efforts tend to focus on species, as it is a concept that can be readily grasped legally. For example, one of the functions of the Natural History Museum is to act as expert witness in court cases in Britain for the import of illegal species or endangered species. For instance, black coral is illegal to import into Britain. An expert from the Natural History Museum will provide a professional opinion as to whether it is black coral or not. For many legal cases, you actually need to know what species you are dealing with.

Consistent Taxonomy

A basic premise for good taxonomy is consistency. Taxonomic consistency is essential for both local impact monitoring and conservations tasks. This is not the same as accuracy. In traditional taxonomy based on morphology, consistency can be a major problem. Essentially, morphology is the body shape. Many of the animals of interest to deep-sea biologists are very small. We utilize high-powered microscopes and work at the very limits of optical technology. Two very well qualified experienced people could give different opinions. It is an art as much as a science. This is a major problem especially when more than one laboratory is doing the analysis. We need consistent results. We need consistency from laboratory to laboratory. Can you imagine if you are dealing with chemistry and different analytical laboratories had different opinions about what was chlorine or what was sulphur? We would never get anywhere. But that is exactly the position we are in with biology. Therefore, coordination of taxonomy is extremely important. It is particularly important when we are talking about conservation issues such as the need to know whether the same species are from different areas. Contractors working in different areas face this dilemma.

Molecular Approach

For various reasons in our deliberations in March, we came to the conclusion that we should be developing molecular methods. These methods would be for identification of unknown species. We have been discussing this idea for about five years. I have been working with my colleague Alex Rogers who will present more about the nuts and bolts of molecular methods during this workshop.

In moving from morphology, namely, body shape, to a molecular approach to taxonomy, it is not that the molecular approach is scientifically better. In many ways, the molecular techniques have the same problems as traditional morphology-based taxonomy. Decisions as to what represents a species still need to be resolved. The difference is that molecular techniques are consistent. If two laboratories properly carry out molecular work, they will come to the same conclusion. It's not a matter of opinion; it's a matter of chemistry. This is analogous to chemists identifying chlorine; the chemists know what chlorine does and know how to detect it. Similarly, in using molecular methods, every laboratory will yield the same answer.

Advantages of the Molecular Approach

Theoretically, molecular methods offer the advantage of speed, cost, consistency and availability, but they are in their infancy for these purposes and calibration with traditional methods will be essential.

How long do you think it takes for someone like me to examine under a high power microscope, 10,000 microscopic animals and draw them? It's about three months work per sample. A molecular analysis of the same sample will be completed in about 48 hours.

Another advantage of molecular techniques is cost. One of the problems with morphology-based taxonomy is that it requires quite experienced scientists. They tend to come from countries that have high labor costs; so morphological research actually costs more in many ways than molecular techniques. The molecular approach is mistakenly considered expensive because expensive chemicals are consumed in the analysis. However, if you factor in the cost of labor, then molecular analysis offers a less expensive alternative.

Another advantage is availability. One of the problems with taxonomy is the limited pool of experts. For nematodes, I probably run the largest deep-sea nematode laboratory in the world, which consists of a staff of three. Out of three, one has been seconded for 5 years to manage a contract for the UN to study the effect of the Gulf War in Iraq on the Gulf fauna. There are probably less than a dozen people in the world that could reliably work on deep-sea nematode taxonomy. Most of them are busy doing other things. It is not a question of money. Even if you had the resources, the talent is not available. We could train people, but it would take about three years to train someone. There is also the question of who would provide the training out of the limited pool of experts, most of who have other jobs as well as other functions within their institutions. Availability is a huge advantage for molecular techniques because technicians can be trained quickly and there exists a larger pool of trained personnel. I think you will find that universities from every country represented at the workshop have molecular biology or molecular chemistry departments.

Co-ordination Issues

From this brief overview, we notice that coordination will be a major issue. We need to cooperate to produce reliable data for ecological impact and conservation monitoring. Taxonomy is only a tool to reach our goal. We will require reliable data, not necessarily perfect, but consistent and adequate for inter-comparison between contractor areas.

There is no point in each contractor and research lab producing data that is not comparable. It would be completely worthless. The tendency with commercial data is that traditionally companies regarded it as a commercial secret, which is completely understandable. They treat it like other commercial figures such as their balance of payments, their cash flow or their product secrets. Scientists often are just as bad; they tend to see data as power.

Sampling

For a project like the Kaplan Project, data inter-compatibility is critical. To insure that data are inter-compatible, we must begin with sampling. We require the same sampling programme. If you change the sampling method, you change the biodiversity data. To achieve inter-comparability, we have to standardize sampling protocols. For researchers, this can be a nightmare. There are literally hundreds of data sets that could be used, yet, in practice; we discard the majority of these datasets because they are not comparable.

Sorting

Sampling involves removing a chunk of the sediment, bringing it to the surface, and for infauna, getting the animals out of the sediment. With smaller animals, the mechanics of extraction from the sediment is important and depends on type and size of filters, and how samples are treated whether by mechanical or chemical techniques. If different sorting protocols are employed, then the results are not comparable. We need standardization on sorting.

Identification

Identification will need to be standardized as well as the analysis of the taxonomic data. There is not one way to do this. In terms of commitment, 90% of the time is undertaking morphology analysis. The sorting, which partially takes place on a boat, is not so time consuming, as it is expensive. The taxonomic data analysis takes up about 1% of the time. It would be useful if we also standardized how we analyze taxonomic and ecological data.

Role of Research Organizations

What would be the role of research organizations, like the National Museum, in assisting the ISA perform their functions, which also means assisting the contractors? Our objective is to establish taxonomic and biodiversity patterns in the putative mining zones prior to industrial use.

New Sampling Programme for DNA

We need a new DNA-friendly sampling programme. The programme would be able to check for cryptic species; those species that are not detected by morphology. Morphology, based on body shape is almost certainly not detecting all the species. Complexes of species are known to exist.

One of the most famous examples is from shallow water where a polychaete worm called *Capitella capitata*. Its nickname is “the pollution worm”, because it is a pollution indicator in shallow water and was believed to have a cosmopolitan distribution. Work in Chesapeake Bay discovered that it is actually more than one species, what is called a complex of species. At last count, there were 16 species in this complex. Not only are these different species, but also they have different genetics, different physiology, and respond to pollution significantly differently. This worm has been known for over 100 years and has been used for the last 50 years as a pollution-monitoring worm. It turns out to be more than one species. Each species have slightly different physiological responses to pollution. We thought we knew this species as well as we know the domestic rabbit!

Cryptic Species Test

In the deep sea with its unknown species, our problem is compounded. Cryptic species are a major issue. We need DNA material to check for cryptic species. The problem is that we need collections. The Natural History Museum collection holds about 80 million scientific specimens. The Paris Museum has about 100 million. The Smithsonian Institution has a similar holding. The trouble is that the museum collections focus on morphology. Specimens have been fixed in formalin and other methods that destroy or alter the DNA.

Validate Molecular Identification

We are in a situation where we require new samples to use the new technology. We also need to test the utility of molecular identification. This new methodology is advancing at an incredible pace. Alex Rogers will cover this in some depth in his presentation. In order to test molecular data, we are going to have to need inter-comparison with previous morphological data. We don't want to discard existing data.

We will need a programme of inter-comparability to compare the DNA method with the old traditional morphology methods; attempting to make the data compatible so that we can use old data and compare it to new data. Alternatively, we can scrap the last 80 years of work, which would be ridiculous.

Establishing Species Ranges and Gene Flow

Establishing species ranges and gene flow can only be achieved with DNA methods. Species range is estimated because that is the only way we can really monitor conservation of species, but we also need to establish gene flow. Species range and gene inter-connection between species are not the same thing. A species could have a very wide distribution because its individuals have a wide dispersion, which means they can move a long way in their life history. Species like that will breed and inter-breed so that the genes can flow across the whole range of the species. Assuming we clear a strip between two clusters of these species with a mining machine, if they can disperse widely, they can jump the cleared strip. They will still be able to communicate with each other. On the other hand, we could have a widely distributed species for historical reasons, which actually doesn't have a wide dispersion. There isn't gene flow throughout the range of the species. In this case, clearing a strip between two groups will result in them ceasing to communicate genetically. There will be no gene flow between the two groups. Once this happens, the species is in trouble. Its genetic diversity may be reduced. With reduced genetic diversity, species are more vulnerable to being made extinct for a wide variety of reasons, including very natural ones like disease.

An example from human populations, when the populations of the Americas first came into contact with Europeans, they were decimated by disease because the human population of the Americas had been out of contact with the Eurasian–African population for too long. They

had no protection against the diseases that were commonplace among Asians, Europeans and Africans. So they were vulnerable to the effect. Once you can't hop over obstacles that are artificially created, then there is a danger that a group could go extinct and not be able to re-colonize. Once you restrict the genetic flow between populations of an organism, you are putting it in danger of extinction. This is a very big issue in rain forests with roads. At first site, road development seems harmless; it actually destroys very little land. However, if the road network divides the rainforests into fragments, then one could find extinction taking place on a huge scale because the organisms can't jump over the roads.

Required Resources

At the March meeting, we identified two primary needs: ship time and funds for training.

To get deep-sea samples, you need a boat! Research vessels are expensive. We specifically need to collect new samples with DNA friendly techniques. The only way to accomplish this is by new ship time.

We also need monetary resources for training. By training, we are referring to three different post doc positions in key institutions. We are also referring to workshops and scientific exchanges between trained people. The aim is to standardize the knowledge, information and technology around the word.

Taxonomic Standardization

One of our objectives is to ensure taxonomic standardization of contractors' data. This will ensure that work done by contractors is most efficient; they get the most value for their money. We have identified three ways to accomplish this, namely:

- Incorporation of existing biological collections and metadata from contractors into a taxonomic database,
- Development of taxonomic expertise via workshops, scientific exchanges and traineeships, and
- Designation of taxonomic co-ordination centres.

Metadata means the descriptive data covering the collection such as where it was collected, when it was collected, what type of sampler that was used, who was the taxonomist that identified the specimens and what was the weather on the boat. Metadata is anything that might have influenced the data.

We have got to develop taxonomic expertise, particularly with the contractors. The way to accomplish this is through workshops, exchanges and traineeships. By traineeships, I am referring to new young people. There are a variety of sources of funding we can explore. In discussions earlier with our colleague, Dr. Desa from the National Institute of Oceanography of India, we discussed using the British Council administered by the Foreign Ministry to provide

support for trainees from India to the Natural History Museum for training in taxonomy. We are currently using Royal Society funds to train two South Africans in taxonomy. South Africa is interested in building capacity to monitor pollution in shallow water.

Most of the scientific advisers tend to be clustered in Northern Europe and America, which are actually expensive places to visit and live. They are also a long way from the contractors.

Exchanges are for experienced scientists to move from one place to another. My museum is keen to play its role in exchanges. Again, we should look as far as possible for additional financial support and it does exist. At any one time, my group will have three or four or five senior scientists a year coming to visit. For example, the Museum is a recognized "European Strategic Facility". We have a European grant to bring European scientist to the museum to work on our collections. There are other sorts of money to facilitate exchange like NATO, various types of political money, and UN programmes.

How many species in the CCZ?

A representative from a contractor asked, "How many species are we dealing with in the CCZ?" In March, my guess was between 30,000 to 300,000 unknown species for marine nematodes and a similar number for the polychaetes and other macrofauna -- this is based on a single data set.

I will use this question to illustrate the limits of our knowledge. A very famous deep-sea biodiversity scientist, Grassle researched the question of how many species in the deep sea. For bathyal depths (>2000 m) along the coast of America, he looked at the spatial distribution of infauna. He found 1 to 10 new species for every kilometer he traveled. Terrestrial science had come up with an extrapolation technique known as the Erwin method by which they had worked out there was 100 million species in the rain forest. So Professor Grassle did a similar sort of study in the deep sea and he came up with an estimate of around a 100 million species. However, these figures are based on massive extrapolation from the local to the global.

Craig Smith and I worked on the ECPAC and HOTS stations, which straddle the CCZ. We studied the nematodes with a student of mine named Caroline Brown. When I came to the meeting in March, my best guess was about between 30,000 to 300,000 nematodes species. In Figure 1, each dot is actually a core of nematodes across a 3000 km transect which effectively straddles the CCZ north to south¹. Over the 3000 kilometre transect, we collected 2000 animals. I fitted an exponential growth model to the species accumulation curve. There is a very close fit, as indicated by the r-value of 0.9999 (*Table 1*). From this type of curve fit estimates, we can derive an estimate of how many species there would be in the CCZ. In the Central Equatorial Pacific across the 3000 km north south transect of CCZ we estimate only about 360 species, not the 30,000 – 300,000 species originally projected.

¹

See Figure 1 in Chapter 1

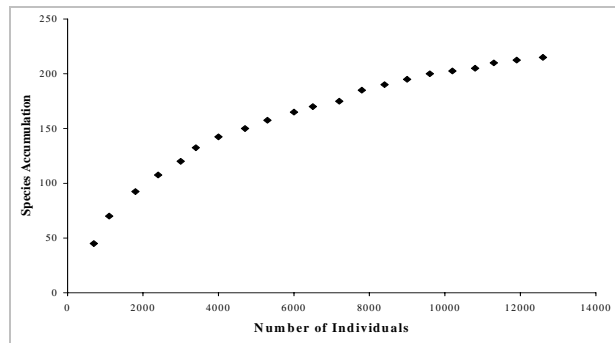


Figure 1: Species accumulation curve for a 3000-km transect in the central equatorial Pacific

Table 1: Estimation of marine nematode species richness for various deep-sea locations.

			Location				
Number of			Maximum	Water	CFE	R2	ICE
Samples	Individuals	Species	Distance between samples (km)	Depth (m)			
<i>Central Equatorial Abyssal Plain</i>							
21	1877	218	3195	4301 - 4994	360	0.9999	281
<i>HEBBLE North West Atlantic Abyssal Plain</i>							
18	2432	174	1	4626	623	0.9999	238
<i>Porcupine North East Atlantic Abyssal Plain</i>							
6	1211	119	1	4850	306	>0.9999	160
<i>Madeira North East Atlantic Abyssal Plain</i>							
6	576	71	1	4950	287	0.9999	106
<i>HEBBLE Core 1</i>							
9	1139	131	*	4626	209	>0.9999	190
<i>HEBBLE Core 2</i>							
9	1293	128	*	4626	236	0.9998	183
<i>San Diego Trough, Bathyal East Pacific</i>							
6	1381	98	0.5	1050	102	0.9989	100

Notes:

*Single Subdivided Box Core

CFE: curve-fit estimation

ICE: incidence based coverage non-parametric estimator prediction

Another approach to estimating how many species are in a region is called ICE, Incident Based Estimator, which is a non-parametric estimator. ICE gives estimation, based on the data, of only 281 species of nematodes in the CCZ. Estimations of marine nematodes have been made for other sites. The HEBBLE site is a high-energy system. HEBBLE was based on some samples that were only 1 km apart. Estimates for the number of species in the HEBBLE region of the northwest Atlantic are similar to the estimates for the equatorial Pacific.

Madeira is a turbidite site where a huge undersea landslide, the size of France, fell across the sea floor. In some ways it's not the same as a mining effect but it is perhaps the nearest thing in the natural world. The lowest species estimates are for the San Diego Trough but this is an unusual location in various ways. How does this data compare with shallow water (Table 2)? In shallow water areas, we have lots of different habitats very close together and each habitat tends to have a different suite of species so high estimates for species richness are obtained. This work was only done because the ISA asked us the question. It never would have occurred to me to go and try and do this. How accurate is it? What conclusions do we draw? We are on the cutting edge and maybe pushing the data too far.

Table 2. Estimation of marine nematode species richness for various shallow water locations.

Location:	Number of			Maximum Distance between samples	Water Depth	CFE	R2	ICE
	Samples	Individuals	Species	(km)	(m)			
English Channel	12	1200	320	465	?	2103	0.9987	922
New Caledonia Lagoon	7	700	155	10	10	1571	0.9999	458
Mersey Estuary	77	68426	323	22	Littoral	384	0.9987	327
Guadeloupe	10	997	140	?	?	871	0.9972	303
Central Equatorial Pacific Abyssal Plain	21	1877	218	3195	4301-4994	360	0.9999	281
Irish Sea	15	9113	158	10	39-56	287	0.9997	221
Thames Estuary	40	6769	152	76	Littoral	230	0.9999	152
Fiji, Ono Reef	7	700	95	4	30	244	0.9997	138
Clyde Inland Sea, Sandy Beach	16	8896	113	50	LW	129	0.9988	133
Mangrove Swamp, KAW Guyana	30	2997	65	2	?	131	0.9992	76
Moorea, Polynesia	7	700	42	0.1	1.6–3.1	56	0.9998	56
Mudflat, La Rochelle, France	36	3600	43	0.1	Littoral	65	0.9986	49

Note: LW: Low Water Spring / CFE: curve-fit estimation / ICE: incidence based coverage non-parametric estimator prediction

There are problems. The second Central Equatorial Pacific data is based on a morphology study that was undertaken as part of an ecological study. How accurate is our taxonomy? Well it was done in the Natural History Museum, but it wasn't a taxonomic study.

Secondly, the data are all based on morphology. There could be cryptic species that could raise that estimate significantly because these estimators are based on extrapolations. It could be that nematodes are different from other organisms. We have no reason to think that nematodes should be more globally distributed than other organisms. But then we know so little about the natural history and biology in some ways of these groups.

Craig Smith noted that he had quite a different perspective. He believes we are pushing the envelope of what we know. I acknowledge that the Kaplan Project is going to test this conjecture using molecular methods. The take home message is that in just three months I have completely changed my mind by two orders of magnitude about how many species there are out there for the group that I have worked on for 25 years.

In conclusion, we need inter-comparability. Taxonomy is the building block to address the ecological issues. Taxonomy depends on sampling for accuracy and consistency. The solution to many of our taxonomic problems is probably the new molecular methods but they are brand new and they haven't been fully developed yet. We are in the process of developing them right now with projects like Kaplan. We have such limited data sets; we can't give you good answers at the moment.

SUMMARY OF DISCUSSIONS

An observer noted that there was a need for a major morphological component in the Kaplan Project and was curious as to what level of morphological investigation would be carried out.

Dr. Lamshead responded that he and Alex Rogers had a PhD student looking at the molecular phylogenetics in nematodes in cooperation with other groups. A major grant was being used to compile all molecular phylogenetic information on nematodes.

On the question of the number of samples required, Lamshead replied that he had not made that determination. At a previous workshop on standardization, he suggested calculating the minimum area to cover the number of species. From the data contained in Table 1, it appeared a good estimation of the number of species could be gotten from a small area. In comparing the 3000 km Pacific transect with those two 1km-apart box cores, there was not much difference in estimations of species richness. More samples and more rigorous molecular techniques were needed to get a handle on what the levels of biodiversity were.

Dr. Lamshead also added that there were two advantages regarding the molecular approach: one was being able to test for cryptic species, and the second was that this approach was fast, relatively cheap and available, so samples could be processed quickly.

He suggested that biodiversity centres could be located in developing countries and not necessarily in the usual places (London, Paris and so on). He added that taxonomic workshops should also be in developing countries although the taxonomic centre would have to be in Paris, London or Washington for several reasons.

A participant asked about stock structure in Cod in the North Atlantic and whether the situation was any better in terms of species differentiation for the deep-sea species?

Dr. Lamshead responded that the molecular methods were not better in a qualitative sense as they did not eliminate ambiguity. However, with the molecular methods, it was consistent and objective

Alex Rogers said that molecular methods were better in many cases and gave better resolution although there were limitations. Often only a part of one single gene within the entire genome of an organism was examined and looking at a gene relationships based on one single gene could be problematic. He added that recent sequencing studies on microorganisms showed that different isolates of the same species of bacteria had genomes made of different sizes. Another problem was whether a species was absolute. There were cases of incipient speciation where apparently separate biological species could still exchange genetic material and still breed with some degree of success.

Chapter 3

New molecular approaches to biodiversity assessment in species rich environments

Dr. Alex Rogers, British Antarctic Survey, United Kingdom

Current methods that are in development to identify species using molecular methods are outlined in this chapter as they relate to the deep sea. Why analyze species diversity and gene flow in the context of deep ocean mining? There are several reasons. First, we have to know what species exist at a locality in order to assess the impacts of mining activities on the seabed. Secondly, analysis of the genetic structure of common species will identify the geographic distribution of within-species (intraspecific) genetic diversity. Intraspecific genetic variation is the basic unit of biodiversity. In addition, intraspecific genetic variation amongst geographically separated populations of an organism can be used to estimate gene flow. This can give us an approximation of the likelihood of migration between different geographic locations. When a swath of the seabed is damaged, through extracting manganese nodules, we need to know whether or not species can actually re-colonize the disturbed area through dispersal. Gene flow data can give us a rough idea of the ability of a species to migrate between geographic locations.

Finally, we know very little about the way in which the fauna in the deep sea has evolved. From a purely biological and evolutionary perspective, the opportunity to go and sample the deep Pacific may yield data towards developing a picture of the evolution of deep-sea fauna. This is important because we suspect that past climate change events have had a major impact on the evolution of deep-sea animals. Knowledge about the biological impacts of these past events may provide clues about the effects of present-day climate change on the deep ocean.

Intraspecific Genetic Variation

To illustrate what information genetic data can provide, I will use an example from part of the coast of Brazil. This is a very long coastline, approximately 8,000 kilometers in length and is characterized by tropical conditions in the north to a warm temperate climate in the south. We examined gene flow between populations of commercially trawled species of shrimp. These shrimp are fairly large animals 15 to 20 cm long.

We expected high levels of gene flow between geographically separated populations of these animals, as they are strong swimmers as adults. However, the South Equatorial Current actually flows from the east and impinges more or less directly onto the coast of Brazil and then splits into two. One branch of the current flows north, the other southwards. It may be expected that even if shrimp are efficient swimmers they may have difficulty crossing this zone and maintaining migration between populations. This was not found to be the case; genetic data from variable regions of the shrimp genome called microsatellites indicated that shrimp populations across the South Equatorial Current were well mixed.

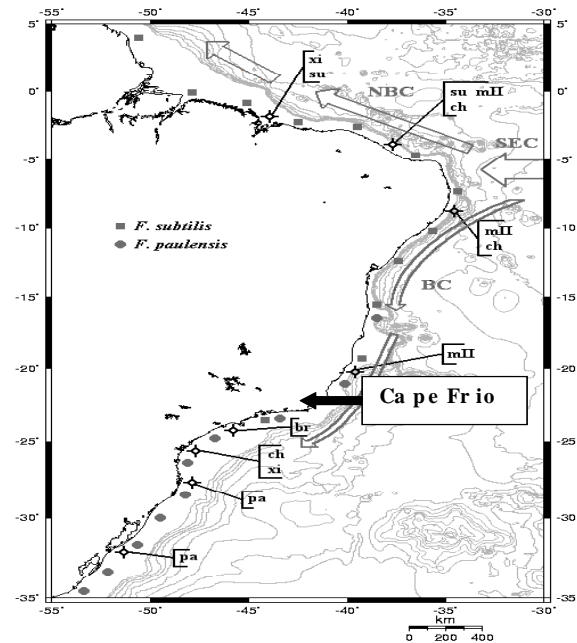


Figure 1: Brazil coast showing the flow of the South Equatorial Current (SEC), the North Brazil Current (NBC) and the Brazil Current (BC).

The symbols show where shrimp were sampled in studies by Maggioni et al. (2001, 2002). Squares and circles show the distribution of *Farfantepenaeus subtilis* and *F. paulensis* according to Perez-Farfante (1969) and D’Incao (1995). Codes for sampled specimens = su, *F. subtilis*; mII, new species; br, *F. brasiliensis*; pa, *F. paulensis*; ch, *Litopenaeus schmitti*; xi, *Xiphopenaeus croyeri*. Bathymetry and circulation patterns from Johns et al. (1990).

However, we did find evidence for genetic differentiation much further south on the Brazilian coast, about 23°S. This turned out to be an area where this strong subtly flowing current actually gets diverted off the shelf by topography, and in this zone there is a strong upwelling of water from the deep sea. There is, therefore, a drop in temperature and the 20° C isotherm runs roughly along this area. This area is known as Cape Frio and it is well known as a biogeographic break. This is an area where there is a major change in the fauna. In this species of commercially trawled shrimp, there is a barrier to gene flow across this area.

These are big animals with a hard exoskeleton with lots of features for a taxonomist to work with. Most people would think that species of these animals are very easy to recognize. When the genetic data was examined a previously unrecognized species of shrimp was found (Figure 2).

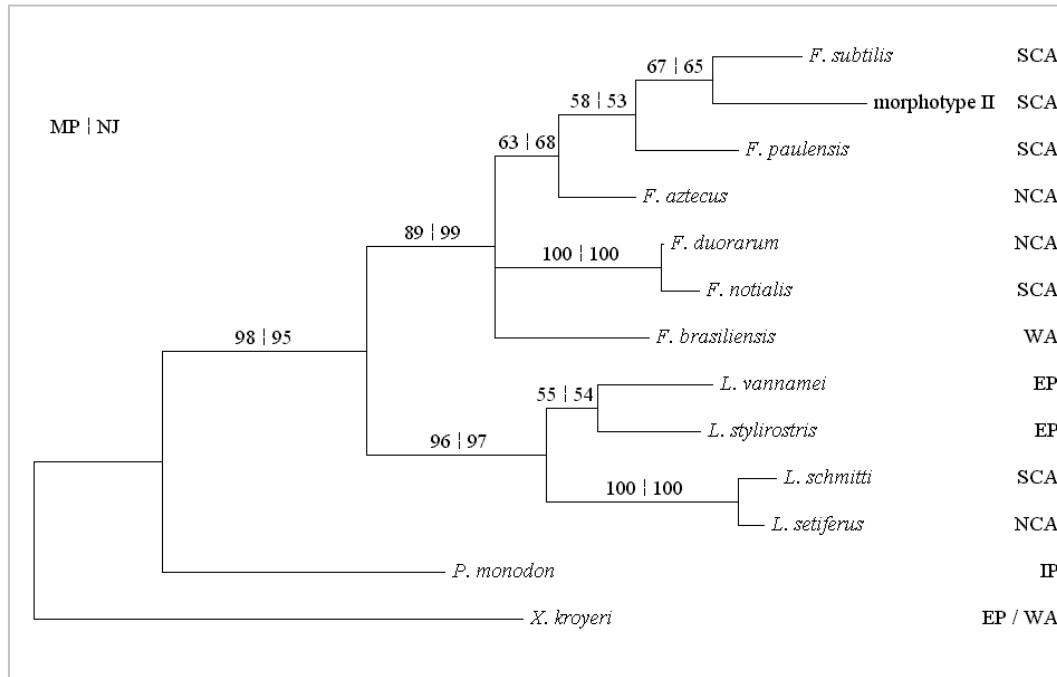


Figure 2 Phylogenetic tree for penaeid shrimps. Tree is constructed by maximum parsimony. Distribution codes are: SCA – Caribbean and/or South America; NCA – Gulf of Mexico and North America; WA – North to South America; EP – Eastern Pacific; IP – Indo-West Pacific. Figure from Maggioni et al. (2001). See this reference for details of study. New specie is indicated as “morphotype II”.

This type of analysis is done by comparing the DNA sequences of samples of the shrimps and is like constructing a genealogy tree. At the tips of the branches of the tree we have different species and essentially the distance on the genealogy tree from tip to tip is a rough measure of how related different species are. All of the species were known apart from the new one, which was closely related to two known species. These are commercially fished animals and biologists in Brazil have been looking at these shrimp for a very long time. Yet, this species remained undiscovered up until the point where we employed genetic methods to actually look at the relationships between species along the Brazilian coast. In the deep sea, we are looking at organisms with far fewer morphological features and which are a lot smaller than these large king prawns, which are fished off the coast of Brazil.

There are other reasons for looking at genetic structure of populations as well. Genetic analysis can provide information about the reproductive strategies of marine organisms. One example is the recently analyzed populations of the sea anemone, *Nematostella vectensis*, using genetic fingerprinting. This is a tiny sea anemone that grows to a height of about 5 mm high and lives in very special lagoon habitats in the United Kingdom and the United States. It has been known for some time that *Nematostella vectensis* is able to reproduce by an individual splitting in half to form two new individuals (clones). Evidence from studies of the reproduction of these animals suggested that this type of reproduction was occurring in the U.K. Analysis of genotypes using fingerprinting showed that this was indeed the case. In some localities, the entire population consisted of a single genotype or clone. This single clone actually dominates many of the populations of the sea anemone in the United Kingdom (Figure 3).

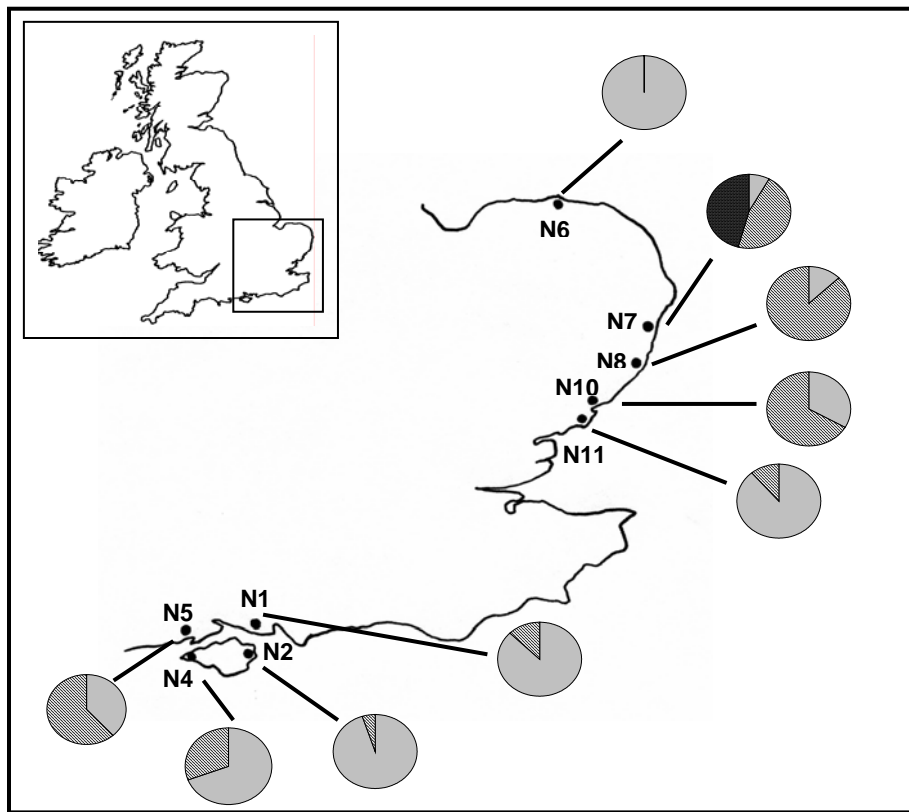


Figure 3 *Nematostella vectensis*. Pie charts show proportion of population represented by different clones. Grey area = most common genotype. Banded area = genotypes occurring in <1% of the total sampled individuals and Black area = the only other genotype occurring in >5% of total sampled individuals. N1 = Fort Gilkicker Lagoon; N2 = Bembridge Harbour Lagoons; N4 = Yar Bridge Lagoon; N5 = Normandy Farm Lagoon; N6 = Half Moon Lagoon; N7 = Reedland Marshes; N8 = King's Marsh Lagoon; N10 = Seafeld Bay; N11 = Naze Lagoon. Figure is from Pearson et al. (2002).

This clone may be what genetic theorists call a “general purpose genotype”. In a very harsh environment, which has low species diversity, a species which is very adaptable can occupy many different niches. A clone with such a flexible genotype and which reproduces asexually can come to dominate whole populations.

These are a few examples of the information that gene flow data can provide. Studying the intraspecific genetic variation of a species is extremely complex and may provide a picture of what is happening to a population in the present day or what has happened in the past. Such studies require specially trained scientists working in dedicated laboratories and using highly complex methods of data analysis. The rest of this paper will concentrate on the simple concept of species identification.

Species Identification

How do we identify species using molecular methods from environments that have very high species diversity? Why do we need to go to the species level of identification? According to the Convention on Biological Diversity, one of the most important levels for monitoring and assessing biodiversity is at the species level. There are several other reasons as well. There are many methods, which are being employed to study biodiversity, which actually act above the species level. There is a loss of resolution when we move to higher taxonomic levels to assess biodiversity in the environment.

Higher systematic levels are not equivalent in evolutionary terms between taxa. Biodiversity using higher systematic levels such as the diversity of genera or the diversity of families does not treat all groups of animals equally. It is, therefore, not appropriate for assessing the impacts of mining in the deep sea.

Systematic organization based on morphology is problematic. As illustrated by the shrimps from Brazil, morphological-based systematics has serious flaws (see also Lambshead, this volume). The genome size and patterns of gene expression, even between closely related species can be dramatically different.

To illustrate these points, Table 1 provides the genome size or the number of nucleotide base pairs in the genome of different species of nematodes. Even between species in the same genus, there is a very large difference in the size of the genome. This means that closely related species potentially have a very different repertoire of genes to respond to the environment.

Table 1 Genome size in different species of nematodes.

Species	Genome Size
Caenorhabditis elegans	96,700,000 bp
Caenorhabditis briggsae	128,300,000 bp
Acrobeloides maximus	237,800,000 bp
Acrobeloides nanus	126,300,000 bp
Oscheius dolichuroides	92,800,000 bp
Oscheius sp.	72,000,000 bp
Oscheius myriophila	137,200,000 bp

Source: Data from the Genomes Online Database ([http:// wit.integratedgenomics .com/GOLD/](http://wit.integratedgenomics.com/GOLD/)).

Another point about the instability of morphological taxonomy is represented in *Figure 4*, which is the latest scheme for nematode taxonomy. This tree was constructed from DNA sequence data and shows that the Chromadorida, previously a single group, are actually comprised of two different groups. Re-organization of higher nematode systematics is based on 18S rDNA sequences (Meldal, unpublished data). The Orders are not monophyletic. Superfamilies are unsupported. Some genera formed mixed clades and species were in the wrong Order. As we go to higher taxonomic categories or use morphology, the taxonomic classification becomes unstable.

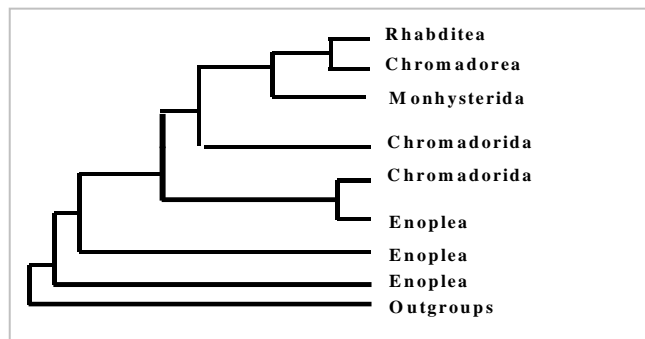


Figure 4: Nematode phylogeny based on Neighbor-Joining analysis of 18S rRNA coding DNA sequences (SSU rDNA) (Meldal, unpublished data).

Highly Diverse Fauna

The challenge we face in the deep sea is the enormous number of unknown species. Nematode diversity at a deep-sea site off New Jersey is illustrated in *Figure 5*. The density of individuals that were collected from their samples is about 8000 per 10 cm². The number of individuals of small organisms like nematodes from deep-water sites can be absolutely enormous. This implies that assessing biodiversity and monitoring changes in communities can be an extremely time consuming and expensive business.

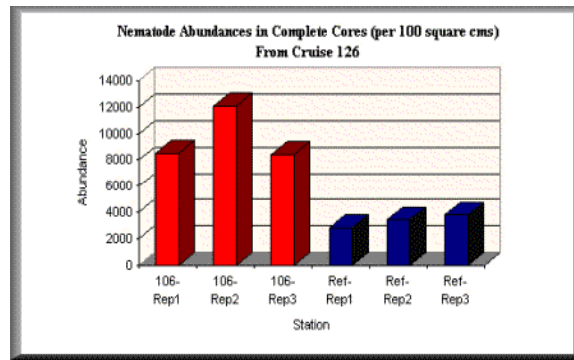


Figure 5. Marine nematode diversity. Samples from deep-sea disposal site off New Jersey (Lamshead and Debenham, pers comm. Natural History Museum, London).

To further emphasize this point, using marine nematodes as an example, there are approximately 4000 species that have been described. Apparently, nematodes are the most abundant metazoans in marine sediment and probably the most diverse metazoans in marine sediments with densities of individuals ranging from 105 to 106 nematodes per square meter up to 107 nematodes per square meter for coastal mud.

Species diversity is on the order of 106 to 108 species per square meter. We are dealing possibly with very large numbers of species.

Global coverage of nematode taxonomy is uneven. For example, in the U.K, about 65% of nematode species are known; whereas somewhere less well studied, like the Venezuelan Basin, we might find that only 1% of species are actually known from that area (Table 2).

Table 2. Proportions of nematodes identifiable to the species level in marine studies located in different geographic areas.

Location	Identifiable Nematodes	Source
Clyde Sea	65%	Lamshead, 1986
Irish Sea	38%	Ferrero, pers comm.
Norwegian Sea	4%	
Venezuela Basin	1%	Tietjen, 1984

There are a lot of problems with identifying nematodes using morphology. It is an exceedingly time-consuming activity requiring highly trained specialists. Nematodes must be sorted from a sample, they have to be mounted on a microscope slide, and then examined by

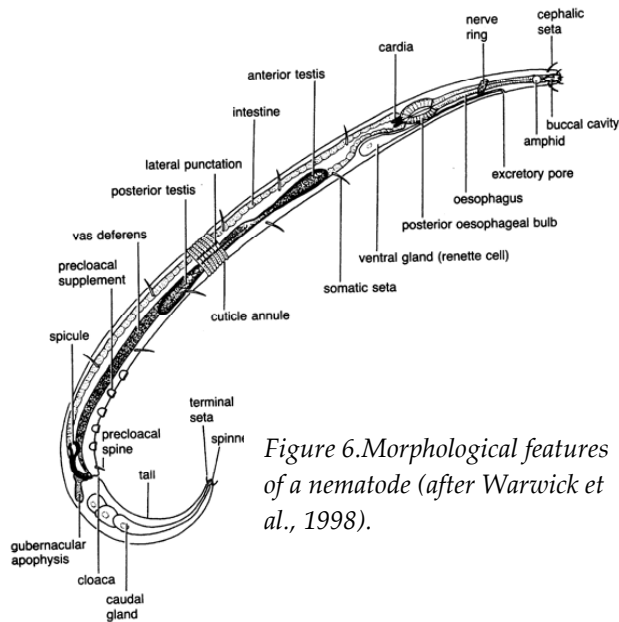


Figure 6. Morphological features of a nematode (after Warwick et al., 1998).

specialists in nematode taxonomy. The numbers of features that a morphologist can use to identify species is limited (Figure 6). In order to sort and identify a single sample of marine nematodes may require up to 3 months by a team of specialist taxonomists. A typical ecological or environmental impact assessment survey requires taking samples at many stations and several replicates at each station.

Another big problem is that nematodes are very variable animals with a high degree of intraspecific variation in morphology. This makes defining species based on morphological characteristics a very difficult job and

can lead to significantly underestimating diversity (e.g. Pontonema, Warwick and Robinson, 2000).

Molecular Identification Methods

Molecular identification methods offer real advantages when it comes to identifying marine organisms like nematodes. They offer a dramatic increase in speed of identification of large numbers of nematodes from environmental samples. DNA sequence information can be used to objectively assess the identification of species regardless of developmental stage, environmentally induced morphological variation, or taxonomic expertise of operator.

These molecular methods do have limitations. They only provide information on species presence or absence and overall information on community structure. Although some methods under development may provide semi-quantitative data (e.g. phylogenetic chips – see below).

At present there are a number of molecular methods which are used to identify organisms from environmental samples. Basically they can be classified into three groups of methods: environmental clone libraries; separation of Polymerase Chain Reaction (PCR) products by electrophoresis and a new method called terminal restriction fragment length polymorphism. All these methods have several common features. Generally, the techniques require extraction of samples using conventional protocols. Another common feature is that DNA is extracted from the organism and amplified for a region that gives species level

resolution. A third common feature and one which applies to most methods for biodiversity assessment, is that extensive ground truthing is required to prove the effectiveness of the method.

What makes a suitable region of DNA? Why do we select one tiny part of the genome? A number of features must be present to ensure part of a DNA sequence can be used to identify to the species level. First, the region of DNA must be present among all species that are potentially in the sampling area. The regions flanking this small section of DNA should be highly conserved; the so-called “primer sites”. The reason we want them to be conserved is that we want to be able to copy the region of sequence that resolves species millions of times using a method called the Polymerase Chain Reaction (PCR) for as wide a range of species as possible.

The region of DNA must be homologous in all the species; that is it must have been inherited from a common ancestor. Essentially, the short stretch of DNA must have the same evolutionary origin in all species that we want to examine.

It is also important that this stretch of DNA has the same function in all the species we want to look at. If this is true then the selected DNA fragment is expected to exhibit a similar mode of evolution in all species examined. If genes have different functions, then they are likely to be subject to different selection pressures and are likely to evolve in different ways. This would complicate interpretation of results.

The selected fragment must be stable; it must be a vital functional gene not subject to lateral transfer. Additionally, it should be sufficient in length to be useful at the species level.

In other words, this region of DNA has the same function in almost all organisms you care to look at. All Eukaryotes (animals and plants – organisms with a distinct nucleus) have very similar sets of genes and they are often in the same arrangement.

Comparison on 18S Sequence between Marine Nematodes

We have been investigating the 18S region in nematodes from the United Kingdom. Nematodes have been extracted from sediment samples from estuaries. Samples were then fixed in 95% ethanol. Individual nematodes were picked and mounted in glycerol on microscopic slides for identification. Once identified morphologically, the nematodes were subject to DNA extraction, PCR amplification and sequencing.

A very short stretch of this region from four closely related species of nematodes is shown in *Table 3* for illustration. We display about 55 nucleotides of DNA sequence from the 18S region. This entire gene is roughly 1500 nucleotides long, so this represents a very short stretch of this gene. Even in this very short region, DNA actually differentiates between 3 of the 4 species. We are simply comparing sequence between different species and using this information to differentiate between species. These species-specific stretches of DNA are called DNA barcodes or Molecular-Operational Taxonomic Units (M-OTUs).

Table 3: Partial sequence of 18S rDNA for *Daptonema* spp.

Species	Sequence
<i>D. hirsutum</i>	TTACAACAGCCGTTGTTTCTTGGATCTCCGCTTTTACTTGGATAACTG~TGGCAA
<i>D. normanicum</i>	TTACAACAGCCGTTGTTTCTTGGATCTCCGCAAT~ACTTGGATAACTG~AGGTAA
<i>D. oxycerca</i>	TTACAACAGCCGTTGTTTCTTGGATCTCCG~TTTTACTTGGATATGTGCTG~CAA
<i>D. setosum</i>	TTACAACAGCCGTTGTTTCTTGGATCTCCGCTTTTACTTGGATAACTG~TGGCAA
	*** * ** * * * *

By analyzing DNA sequence data a tree of relatedness can be estimated for these species as shown in *Figure 7*. The 18S region differentiates between these nematode species, which have been morphological identified.

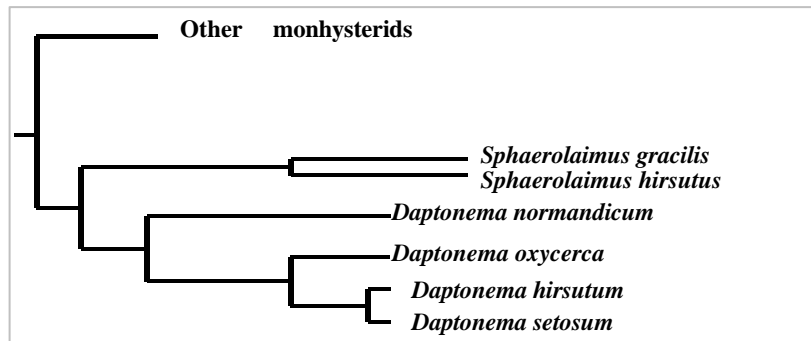


Figure 7: Phylogenetic tree showing relatedness between different species of Daptonema spp.

Our subsequent studies have shown that the 18S rDNA sequence region can separate approximately >95% of nematode species. However, to be absolutely certain of identifying all species from a sample it is likely that two or more sequence regions have to be studied simultaneously. Several other regions of the genome have now been put forward as suitable for molecular bar-coding for species identification (e.g. COI, Hebert et al., 2003).

Clone Libraries

In molecular techniques, the bottleneck is in sequencing. If every single individual nematode in a sample had to be sequenced, then it would be an incredibly expensive and time-consuming process. Building a clone library can shorten this process. Instead of processing just one individual, all the species in a sample are processed simultaneously. These result in a very large number of fragments of the region of barcode DNA amplified for the species that were in the original sample.

All the fragments of DNA amplified by PCR are then inserted into a plasmid, which is a circular DNA molecule. These plasmids are then introduced into bacteria, which are then farmed. Each bacterial colony will hopefully have one type of plasmid with one particular type of this DNA from one species in the sample.

For each colony of bacteria that is grown, the plasmids inside the bacteria are fingerprinted. A restriction fragment length polymorphism (RFLP) fingerprint is created for each clone. Fingerprint patterns are compared with a programme and clones representative of each individual pattern are sequenced. This reduces the number of samples to be sequenced while multiple sites can be compared rapidly. Using an automated DNA sequencer, we can manage a high volume of samples.

Data analysis of the clone library sequences involves searching DNA databases (such as Genbank) to identify the most similar sequence that have already been observed by scientists around the world. We can use phylogenetic analysis to identify the relationships between the sequences in a clone library. Rarefaction curves can be drawn to estimate the number of taxa that were actually in the original environmental samples.

An example of this type of analysis from Antarctica where we have used these methods to analyze microbial and meiofaunal communities in terrestrial and freshwater systems is shown in *Figure 8*. The diagram shows the number of fingerprint patterns, which have been detected versus the number of clones that have been examined from clone libraries. A rarefaction curve can be estimated and the relative estimated biodiversity at each site compared.

These molecular methods are not without a few problems. As Lamshead suggested in his presentation, molecular methods are not the absolute answer to everything. The first thing is that clone libraries provide a huge amount of very detailed data but require a significant effort to establish and maintain. Sometimes there may be biases during PCR amplification because primers do not match exactly for all species. Artefacts can occur where two sets of sequences combine to form a chimaera during the PCR reaction. Special computational methods can be used to identify chimaeric sequences.

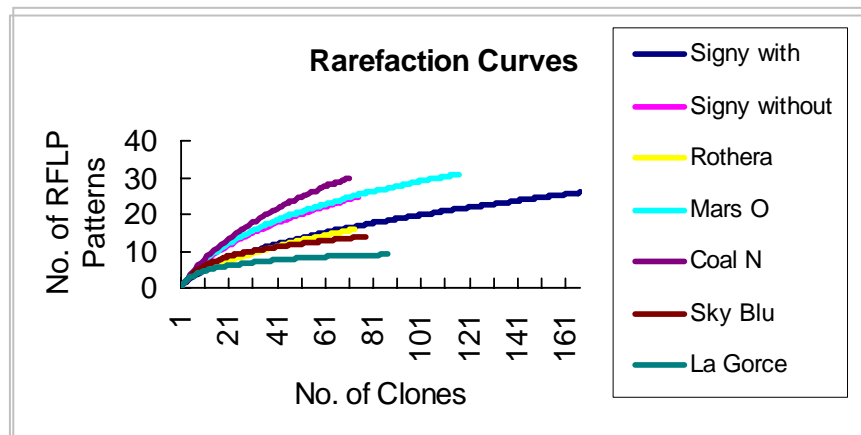


Figure 8: Rarefaction curve for Antarctic microbial Eukaryotes (Lawley et al., In submission).

Another disadvantage of clone libraries is the selection of certain types of sequence during the amplification process. Not all sequences are amplified with the same efficiency resulting in an underestimate of biodiversity.

Electrophoresis-based Methods

The next sets of methods using electrophoresis are slightly different. They share some similarities in that animals are extracted from the environment in the same way and the DNA barcode region is amplified again using PCR. But instead of actually cloning these entire fragments, they are run on a gel matrix, in an electric field and this separates the different types of sequences that we have in the original sample. The distance these fragments of DNA move on the gel, indicates how many different types of the original fragment are present in the sample; in other words, how many species. These methods do this by separating amplified fragments based on sequence composition. These bands can also be cut out of the gel and then sequenced for identification as outlined for clone libraries. This is a much less sophisticated method than the clone library, but it is a very useful method in some contexts.

This method has limitations but it does have some advantages and it is very quick. In general, the technique has an optimal size of DNA fragment that it can separate between about 200 and 1000 base pairs in size. So for the 18S rDNA region it is fine if we are just looking at a part of the sequence. There are several related and complimentary methods, such as single stranded conformational polymorphism analysis (SSCP).

Terminal Restriction Fragment Length Polymorphism Analysis

The final method that will be discussed is terminal restriction fragment length polymorphism analysis (T-RFLP). Again samples are collected, DNA extracted and the barcode region of DNA amplified by PCR. However, instead of sequencing with gel electrophoresis, a fluorescent label is attached to one end of the DNA fragment (*Figure 9*). This fragment is then digested with restriction enzymes that cut at a specific sequence motif. Fragments are then separated by size and the different sizes of the fluorescently labeled terminal fragment are estimated.

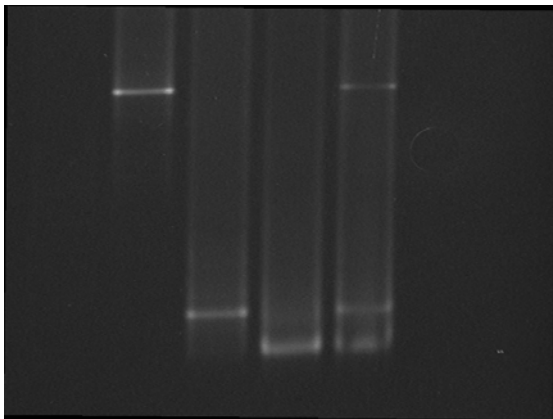


Figure 9 Denaturing gel electrophoresis (DGGE) of three species of nematodes (Lanes 1-3) and all three species in the same lane (Lane 4). Data from Cook et al., in preparation.

By analyzing the fluorogram for brightness, a semi-quantitative value for abundance of species can be obtained with this method. An advantage of T-RFLP is that it is fast and accurate because of the use of an automated sequencer for identification of the size and magnitude of terminal restriction fragments. The taxonomic groups in a sample can be identified from T-RFLP patterns. A real advantage of this method is that, in some cases, it can give us a semi quantitative estimate of abundance of the organisms in the sample.

One of its major disadvantages is that several species can produce the same sized restriction fragment. This can cause an underestimate of species diversity in the sample.

In summary, the methods described are currently being used to identify species from environmental samples. In the next section, emerging technologies will be addressed that will be enormously important in species identification from environmental samples in the near future. There are two classes of these technologies; mass spectrometry and microarrays. Mass spectrometry will not be discussed because this technology is in its infancy. However, microarray technology, the so called DNA chip is an emerging technology that holds promise for addressing the type of scientific questions that the Authority and contractors will need to answer.

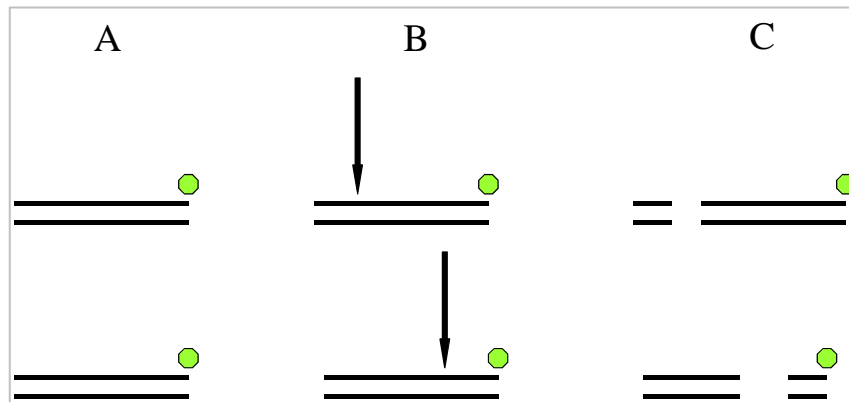


Figure 10. *Simple Schematic of Terminal Restrictive Fragment Length Polymorphism Analysis. A. PCR reaction with one primer fluorescently labeled. B. PCR product is digested with one or more enzymes. C. Terminal digestion fragment is labeled and can be separated by size on an automated sequencer.*

Emerging Technologies

Microarray Technology

In order to use microarray technology to identify species from environmental samples, a clone library or a library of DNA sequences must first be created. DNA sequences from the library correspond to individual species in the environment we are looking at. Pieces of DNA called the probes are spotted on to coated slides using a robot. Spots are about 200 microns

across and each one may correspond to a single species in the original sample. Arrays may consist of 30,000 or more spots arranged in grid-like patterns.

DNA from an environmental sample may then be amplified. The PCR products are fluorescently labeled during amplification either through amino-allyl dye coupling or by labeling the primer with Cy3 (green) or Cy5 (red) labels. The target DNA, from species in the environmental sample, is allowed to hybridize with the probes on the microarrays. Using a laser confocal microscope the slide is then scanned for fluorescent labels. Every bright spot is a positive hit with a species in the environmental sample corresponding to one in the sequence library.

This technology may be taken further in the future by labeling different environmental samples with different fluorescent labels. This would allow estimation of relative changes in abundance of species from environmental samples taken at two different times. In this case, one set of environmental samples could be labeled with a green dye, and another set with a red dye. By scanning the composite image, a red dot occurs when the abundance of that species has increased from time A to B. A yellow dot indicates where abundance has roughly stayed the same and a green dot when the abundance has decreased. Thus, this method may be capable of semi-quantitative analysis of changes in species diversity.

This is a powerful technology, and up to 30,000 or 40,000 spots can be arrayed onto a single glass microscope slide. This technology is widely used at the moment to look at expression of genes across the entire genomes of particular organisms. Microarrays or similar technologies have already been used to identify viruses and pathogens (Lévesque et al., 1998; Wang et al., 2002).

There are some potential problems with this method as well. First, a good clone library or sequence library is required for the environment, which is under study. A significant amount of effort is required to set up the sequence database from which probes can be designed.

The other problem is cross hybridization of sequences where sequences are quite similar. An analogy is that different locks or different keys corresponding to different species fit the same lock. This is called cross hybridization, and may confound the results of this type of approach. However, this may be solvable by use of stringent hybridization conditions and new technological developments especially in slide coating chemistry are making the whole process much more specific.

Another disadvantage of this technology is the cost. The cost of the robot that spots the slides is upwards of £75,000 and a scanner can cost on average about £30,000. However, you can actually build your own robot if you have got a good electronics shop and bring down the cost to about £30,000 for a spotter.

Affymetrix, a private company, markets a microarray technology, which utilizes silicon chip technology to manufacture highly stringent DNA chips. Light is used to mask the array, allowing the chip to hold roughly 18 locks per species on each spot on the specialized chip.

The beauty of this technology is that the whole process becomes a reliable industrial process. Hybridization has a very high affinity so the method is very good at detecting sequences which have very small differences. Once the mask is built from the gene library, it lasts forever. Affymetrix can manufacture the arrays at a cost of US\$150 – US\$660, depending on the numbers of spots or the number of species that are on the chip.

Similar to a standard microarray technique, the Affymetrix DNA chip may be washed with a solution of DNA from the environmental sample. The principle is the same except this specialized DNA chip is a much more sensitive in detecting specific sequences of DNA. Tens of thousands of spots of DNA can be arrayed on a single chip. This type of technology may provide the power to design a probe for every single species in a sample from the specific geographic area. In the case of nematodes, a chip could be designed for every described species on the planet. As with standard microarrays, a very large number of samples can be examined easily and rapidly.

To summarize, for many purposes, it is desirable to identify to species level. Conventional morphological systematics is slow, it requires trained personnel, and is subject to many problems such as the cryptic speciation. Molecular techniques offer objective and very rapid methods for assessment all sorts of biological communities – not just the meiofauna. The new technologies, especially the DNA chip technologies, are going to revolutionize the way we do ecology and monitor species biodiversity.

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SUMMARY OF DISCUSSIONS

One of the participants acknowledged the very comprehensive introduction to this new technology and added that he believed there was a lot of basic work yet to be done before this method would be applicable to deep-sea fauna.

Dr. Rogers responded that work was underway and that the majority of laboratory processes had been refined although there was still need for field work and sample collections to build a library from those samples.

A biologist asked if there was a need to identify all the species in the CCZ in the old way by morphological analysis before a molecular library could be started. Dr. Rogers responded that entire DNA in a sample had to be extracted to build a clone library. The clones are then fingerprinted to find out how many different types of sequences there were. Without specifically identifying the species through morphological analysis, work could be done directly

from the library. The absence of morphological analysis would not restrict identifying more of the species and designing a new chip. The best example was pathogens in soils, where 160 pathogens were sequenced and probes designed for them and arrayed on a nylon membrane.

A biologist inquired about semi-quantitative density information. He said for baseline studies, it may not be as important to get species diversity inventory in the area. But for monitoring after an impact, quantitative data of single species response was critical. Current molecular techniques did not seem to address the issue.

Dr. Rogers replied that if a semi-quantitative approach was used, then it could only be done up to the meiofaunal level. Beyond that, the problem was that individuals in a single species varied greatly in size, so if a specimen of species A was collected, and which was 20 times larger than 100 specimens of species B, the results would see very different quantities of DNA in a sample. Rogers added that it would not work for large organisms on a quantitative basis, and that the best he could hope for, whilst using this technology was a species presence/absence measure.

Dr. Lamshead pointed out that presence/absence data could be used for monitoring if a community had not recovered from a disturbance until the full species list was present in the system. Microarray technology could be used to look at presence/absence of species, and once the full suite of species were present, then an evaluation of the distribution of individuals among those species would be needed to get a better sense of recovery of the population level. The technique, in itself could be a very effective way to look at recovery from impacts.

Dr. Rogers added that the standard shallow water pollution monitoring package Primer which was used worldwide, recommended collecting abundance data then transforming the abundance data using the fourth root, thus making it semi-quantitative. The procedure means that by running the presence/absence data through multivariate analysis, it gives a sharper distinction than if run as raw abundance weighted data through multivariate.

He further commented that the methods of analyzing molecular data were still in development and needed data analysis methodology and tools to cope with the quantity of data that could potentially be derived from species presence/absence using molecular techniques. He added it was possible to have 30,000 or 40,000 microbial or meiofaunal taxa on a single microarray. These could be analyzed at a scale of meters and on a daily basis and no longer limited to sitting at a microscope identifying critters individually or doing biochemical assays to identify the presence or absence of a certain type of bacteria. This type of analysis could start to examine some very interesting and fundamental questions like species quorums in microorganisms and whether the presence of species A could influence the presence of species B. These types of correlations could be evaluated in many samples.

On being asked whether polychaete, for example from the sample, could be ground up with the mixture sprayed on a DNA microarray to see what species were present, Alex Rogers responded that it was not possible to go any further than that of the clone library approach at

the British Antarctic Survey. He said DNA extractions were done directly from the soil without separating individual animals. DNA was extracted out of the whole sample using the clone library approach which works for abundant organisms in the sediment and/or for examining recruitment.

Dr. Paul Snelgrove asked about the large amount of time to set-up the clone library before making routine analysis of samples. He remembered colleagues a few year back, were spending a year designing and preparing a probe for a given bivalves species that wouldn't cross match with other species. He wondered if the clone library should be set up first prior to taking samples.

Dr. Rogers responded that if the approach Snelgrove suggested was chosen, it could be done to a certain extent, but not on a scale similar to what was needed for deep-sea investigations. He said he had prepared a review paper on probe methodologies to identify larvae and with this technology previous methodologies would be redundant. For newer molecular techniques such as microarrays, the rate-limiting step was the process of setting up the library. The difference being that a library could be set up for the entire faunal contents of a body of water rather than just a single species. Although time consuming and expensive, the technology involved a lot of sequencing and required technical expertise in the laboratory. He added that the beauty of some of these chip technologies was that once that step has been completed, a company could manufacture the chips.

Dr. Smith asked the contractors if they would be interested in buying prepared chips standardized for US\$150-\$500 should a research lab set up such a system to allow easy, cheap and quick monitoring.

One contractor's representative responded that although he basically agreed with the method of identifying organisms, he thought the library should be set up first for a full dataset.

Dr. Rogers further explained that part of what the Kaplan Project hoped to accomplish was to go out and create libraries, especially for nematodes and polychaetes. He added that creating an environmental clone library could begin as soon as possible but would require considerable cost and a significant amount of time.

The contractor's representative suggested that samples be collected from monitoring or baseline programmes for use later with new microarray technology after the Kaplan Project had set up the clone libraries. As sampling programmes would exist over several years, it was important for environmental scientists and contractors to think about how DNA-friendly samples could be collected and used with the new technology.

On asked what the phrase "DNA friendly" meant, John Lamshead said the phrase meant that samples were preserved in molecular grade alcohol rather than in solutions with formalin. These same protocols were used by the Natural History Museum.

Dr. Rogers added that DNA friendly was however a little more complicated for organisms like bacteria and nematodes as the material had to be washed and extraction done at low temperatures because warm temperatures between 2-4°C meant the DNA would be denatured and ruined. He added that as part of its approach the Kaplan Project used a chilled sea water system that could wash the samples relatively easily. The system has been designed and built but still needed to be incorporated into some of the sampling programmes. He added that contractors would find this new technology useful in their projects.

On the question of the amount of time it would take for preparatory work in building up the libraries to be able to analyze a community of 1000 species, Dr. Rogers stated that the cost of developing the libraries was totally dependent on the diversity of the samples. If it was with a community of 200 or 300 species of nematodes, then cost per sequence could be in the order of £4 or £5 per sequence. If Affymetrix were to do the work the costs could be basically \$20,000. This was for the design of the specific oligonucleotide probes for each of the sequences in the library and the manufacture of the mask, which are used in the chip manufacturing process. Dr Rogers added that Affymetrix prices varied according to the numbers of slides manufactured. He said that the more slides needed, the lower the cost per unit. He said with all that in mind, developing these libraries for a species rich system could take several years.

A participant noted that the goal of the Kaplan Project was to develop the clone library and set the stage for using powerful and inexpensive molecular techniques for monitoring baseline and impact studies. He said that although the main obstacle would be the cost and time required to set up the clone library, once it was set up however, it would be relatively easy to use in monitoring and impact studies.

Dr. Rogers added that another point to keep in mind was the continuous and rapid development of technology. There were already efforts at a viral chip which after a sample of DNA is squirted onto it could handle the entire process and PCR reaction. He said the direction of development was towards more or less a hand held device. The problem however with this technology at the moment was there were only about 100 spots per chip so the levels of diversity being dealt with could prove inadequate.

A contractor's representative suggested that the meeting think about technology in a realistic, pragmatic way. He wanted to know what part of these methods were developed enough to be implemented, over what time frame could direct benefits be seen in understanding nodule mining impacts and what the benefits could be for the contractors.

Dr. Rogers replied that if the Kaplan Project proves the viability of the technology, it would be a major scientific achievement. Additionally the technology could be further developed to look at gene expression thus opening up the possibility of going down to the molecular level. If a heavy metal was introduced into the environment, technology could possibly look at the expression of stress related genes in the organisms in that system.

A policy analyst suggested that the genetic molecular structure was initially proposed as a measure of species diversity. He asked if comparisons between the molecular structure-based diversity and the taxonomy-based diversity had been performed

Dr. Rogers responded that the British Antarctic Survey was undertaking a study at the moment on nematodes. The level of error depended specially on the type of technology that was being employed, e.g., in comparing the error in a Terminal Restriction Fragment Length Polymorphism Analysis (TRFLP) method with denaturing gel electrophoresis, the levels of error were quite different. All these methods tended to overestimate or underestimate species diversity in certain situations. The only way to get a handle on level of error was to ground truth the method of picking out species diversity. If there was a common organism in the sample that could interfere with detection of other species, then there was a need to know how the method was biased in this case. This was crucial in deploying these methods effectively for monitoring or doing ecological studies.

Chapter 4

Enhancing the Understanding of the Deep-Sea Environment: Examples from the Atlantic Ocean

Dr. David Billett, Southampton Oceanography Centre, United Kingdom

Some examples of work that has been carried out in the Atlantic Ocean, and which are relevant to the discussion of natural variability in the deep sea will be presented in this paper. If we are going to do experiments in the deep sea, then we need to realize the time frame of deep seabed processes. Various methods that we can use to accomplish this task will be reviewed.

In the northeast Atlantic, one approach to studying temporal variability has been to set camera systems on the deep sea floor timed to take photographs at regular intervals (every four hours) and left on the sea bottom for a year or more. We retrieve the camera system by triggering an acoustic release of weights. The camera system returns to the ocean surface, is picked up and the film is processed. We then have a record of events on the seafloor over the period we have been studying (approximately 1 year). Our system records a 2 m² area. By overlaying a perspective grid we can measure processes and features on the seafloor.

Based on time-lapse videos lasting between 9 months and a year, we examined two quite different areas in the Northeast Atlantic: The Porcupine Abyssal Plain (48° 50' N, 16° 30' W), at 4850 m water depth which is a highly seasonal area. Where the overlying surface waters experience large changes in the productivity of surface phytoplankton and changes in the flux of organic matter to the seafloor; and the other area is the Madeira Abyssal Plain (31° 06' N, 21° 11' W, 4,994 m) where polymetallic nodules are found. In this area, we do not see large seasonal changes.

For those who have not seen the deep-sea environment, the contrast between these two sites shows how different these natural systems can be depending on the amount of organic matter, or phytodetritus that is deposited from the sea surface and on how it arrives on the seabed. Note that the non-seasonal environment is thought to be more like the polymetallic nodules area that is being investigated in the Pacific Ocean, in the Clarion-Clipperton fracture zone (CCZ).

In time-lapse photography, the life on the seabed flashes before your eyes -- one year in one minute. For the non-seasonal environment, where there is not much of a seasonal change in surface productivity, the flux of organic matter into the deep sea does not fluctuate widely, very little changes on the seabed; the picture at the start of the year is almost the same as the picture at the end of the year. However, in a seasonal environment, many major changes happen. There is much activity. Many animals seem to dart about. Detritus arrives from above in the spring and summer. A large echiurida worm migrates into the picture frame and plows up the seabed.

From the photographic records, we observe two quite different systems. The deep sea is not the same in all areas. The deep sea varies and it varies depending on how organic matter is conveyed to the seafloor.

When examining an area the size of the CCZ, which covers a wide latitudinal change, we need some idea of the natural processes that are occurring, so we can start to design and deploy experiments in the proper context. Experiments are valuable, but they need to take into account our knowledge of the natural system including time scales of ecological processes.

In recent years, there has been a dramatic change in the abundance of animals living on the abyssal seabed over a vast area of the NE Atlantic. Increases in the abundances of sea anemones, polychaete worms, brittle stars, sea spiders, sea squirts and sea cucumbers have been noted. *Figure 1* shows the increase in the abundance of these animals as determined from trawl catches. In particular, one sea cucumber, called *Amperima rosea*, has increased in abundance from about one individual per 10,000 m² (before 1996) to over 6000 per 10,000 m² (1996-present), as determined from time-lapse photography. The sea cucumber is about 3 cm long. Before 1996 it was very difficult to find. Post-1996 it would have been very difficult to walk on the seabed without squashing one.

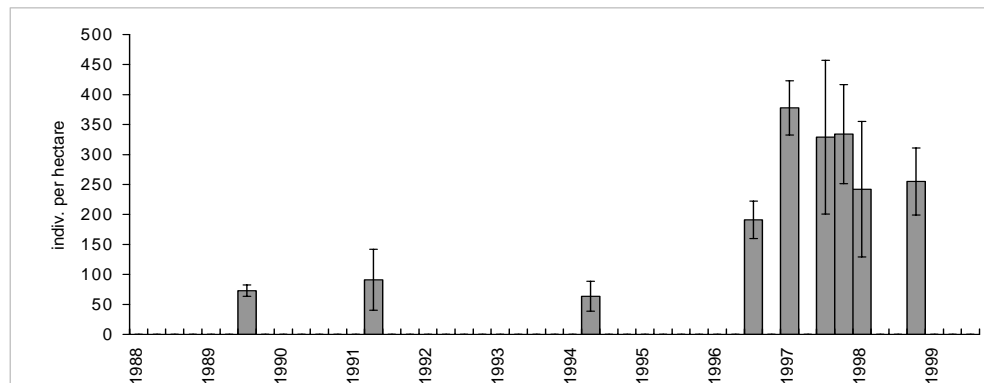


Figure 1. Abundance of Megafauna on the Porcupine Abyssal Plain: an environment with large seasonal changes and interannual variability (Billett, et al., 2001, *Progress in Oceanography*, 50, 325-348).

From recent work, we think that the driving factor is a change in the type of organic matter deposited on the seafloor. Changes in the type of organic matter may be affected by:

1. the type of phytoplankton forming the organic matter at the surface, and
2. the zooplankton that feed on the phytoplankton and create the larger particles that sink to the seabed.

Therefore, natural variability in the surface waters seems to be driving natural variability on the seafloor. In order to understand environmental impact on the deep sea floor, we need to consider this type of natural variability.

After discussions with Nagender Nath about his study area in the central Indian Ocean basin, we find that *Amperima rosea* is one of the holothurians that are found in the zone of polymetallic nodules. In discussions with Virginie Tilot, I found out that *Amperima* is also found in the CCZ. The types of processes I have outlined are likely to occur in the area of interest to the International Seabed Authority. Obviously, this needs to be taken into account in designing field sampling and environmental monitoring programmes. Not only are there some seasonal changes to consider, but there are changes over a period of a number of years. We must be aware of those processes when we are drawing up our plans for environmental management. Environmental Impact Assessments are often made in comparison with a baseline study, but what happens when the baseline changes naturally with time?

Variability with time is not restricted to the large animals that scurry about on the sediment surface. They also occur in smaller animals living within the seabed. Even minute animals, such as protozoan Foraminifera, show sharp changes in the Atlantic Time series described above (*Figure 2*). The shift in these small (meiofaunal) species may be associated with the same environmental changes that have caused the “*Amperima Event*” or *Amperima* may be having a direct affect on the small infauna.

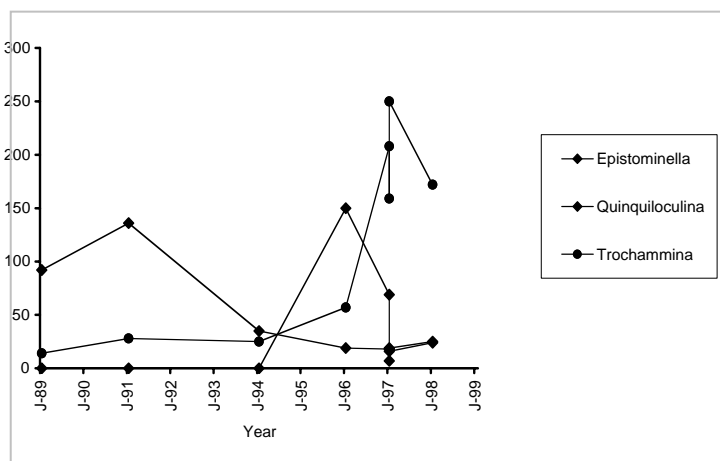


Figure 2 Changes in Foraminifera Meiofauna Dominance with time on the Porcupine Abyssal Plain. (Goody, Pers. Comm.).

When we look at the abundance of meiofauna in a typical small core, about 5 cm in diameter, the uppermost centimeter of the sediment may have in the order of 800 specimens of Foraminifera, representing at least 125 species (*Figure 3*). Only a few species are abundant. Most species occur in densities of 1 or 2 per sample. Many species appear to be rare and on the verge of extinction. However, during the “Amperima Event” those rare species (e.g. *Trochammina*) suddenly became the most abundant protozoan meiofauna. This illustrates the dynamic influence of the surface productivity and the ability of the deep-sea biological assemblage to respond. We need to be aware of the temporal factors in designing and analyzing environmental data from the deep sea.

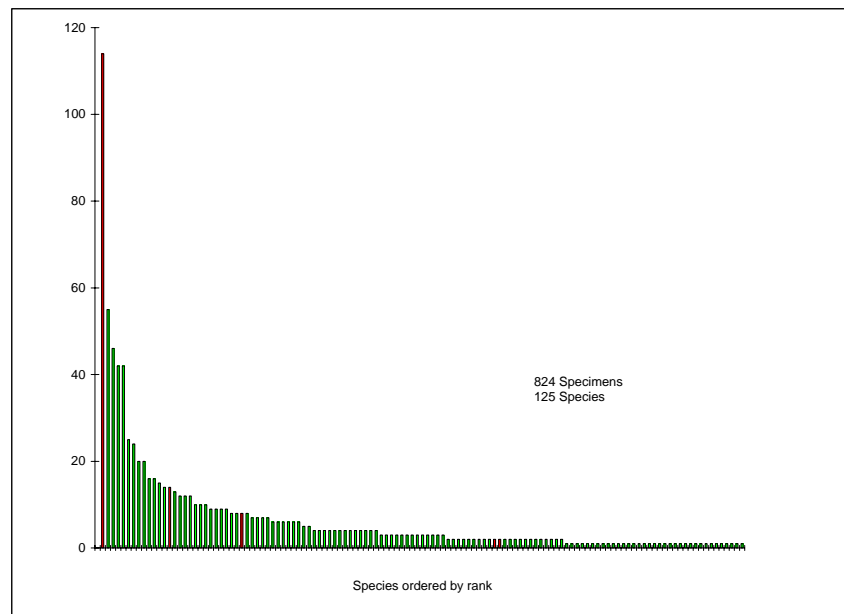


Figure 3. Live Foraminifera species by rank order for the Porcupine Abyssal Plain site.

In planning future work on natural processes in the nodule province, we need to be aware of the natural variability inherent in the system, especially changes over seasons, changes over tens of years and changes in relation to surface productivity in the waters above.

SUMMARY OF DISCUSSIONS

A scientist asked, what kind of organic matter was conveyed to the deep sea and whether it was the total amount of organic matter or the chemical form of the organic matter that was important and which leads to an increase in the holothurian population.

Dr. David Billett responded that the type of organic matter reaching the seafloor appeared to be influencing the biological assemblage. He said he had looked at carotenoid

pigments within the guts of the various species and found that in the species that responded, certain pigment chemistry signatures were the ones that increased significantly in abundance. In particular, *Amperima* appeared to select pigments that were associated with cyanobacteria. These cyanobacteria were unable to get to the seafloor unless they were packaged into larger particles in some way. He added that it was a known fact for example, that salps feeding in the surface waters could make large particles sink very rapidly to the bottom.

The scientist further asked whether any decrease in other kinds of megafauna was found to compensate for the holothurian population explosion.

Dr. Billett replied that in the Porcupine Abyssal Plain time series, there was also a decrease in another holothurian species called *Peniagone diaphana*, a species that swam. It used to be quite abundant, but was hardly ever found (post 1996) leading to suggestions that there has been a decrease in other holothurian species.

On the question of whether the deep ocean affected the upper ocean, Dr. Billett said it was very difficult to answer that question without looking at processes that would be returning organic matter back up into the water column, such as reproductive processes. He said large-scale physical upwelling of material back to the surface was extremely unlikely.

Dr. Smith advised that there was a paper by Ken Smith in *Nature* magazine where Mr Smith had studied the vertical particle flux from the surface ocean to the deep abyssal sea floor using sediment traps. For most deployments, Smith had oriented the traps in the normal position to collect the raining particles. He also installed a trap upside down to collect particles upwelling into the trap. Roughly 40% of the surface flux was collected in the downward facing traps. Apparently particles were transferred vertically upwards. In summary, there was evidence that there may be an upward flux of particles from the bottom to the upper ocean. There were still problems with interpretation of downward looking sediment traps and whether the trap catching particles were from a particle exchange mechanism or water exchange mechanism but there was still some evidence of a limited flux upwards.

Chapter 5

Megafauna Biodiversity as a Tool for Monitoring Manganese Nodules: Proposal for Converting Available Data into a Digital Database

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Introduction

In this paper, we present data on the biodiversity and abundance of megafauna gathered over a seven-year period of monitoring the German DISCOL Experimental Area (DEA) in the South Eastern Pacific Ocean. The megabenthos comprises a major fraction of the deep-sea benthic biomass. They play a key role in abyssal ecosystems (Smith and Hamilton, 1983), and therefore have to be included in environmental studies. Megabenthic organisms are defined as species large enough to be determined on photographs (Grassle et al., 1975; Rex, 1981). They are difficult to study using conventional sampling methods because of their low density, and in areas with polymetallic nodules the animals are typically badly damaged when collected with trawls (Bluhm et al., 1995).

The data we present here are elaborated by our colleague, Dr. Hartmut Bluhm, and published in a paper in 2001 (Bluhm, 2001).

Additionally, we wish to introduce you to a project called FADE – Face of the Deep Ocean, as part of an international collaboration, which was developed in cooperation with Dr. Michael Tuerkay from the Senckenberg Research Museum at Frankfurt, Germany. Perhaps this project can be adjusted or incorporated into the Kaplan Project to examine megafauna.

DISCOL

The DISCOL project (Disturbance and re-Colonisation experiment in a manganese nodule area of the deep South Pacific) became the first large-scale impact assessment study. Originally, ecologists conducted the DISCOL experiment with the assistance of physicists measuring currents (Klein, 1996) in the experimental area. In a later stage, sedimentological

and geochemical studies were implemented, and the succeeding ATESEPP (Impacts of potential technical interventions on the deep-sea ecosystem in the Southeast Pacific) programme combined research groups from various German institutions. During the DISCOL experiment in the South East Pacific Ocean (Figure 1), the surface sediments of the DISCOL Experimental Area (DEA) were intensively treated with the "plough harrow" (Thiel and Schrieffer, 1990), a specially constructed disturber (Figure 2) designed for the simulation of some of the potential disturbance effects of a mineral mining collector (Foell et al., 1990, 1992; Thiel and Schrieffer, 1990; Borowski and Thiel, 1998).

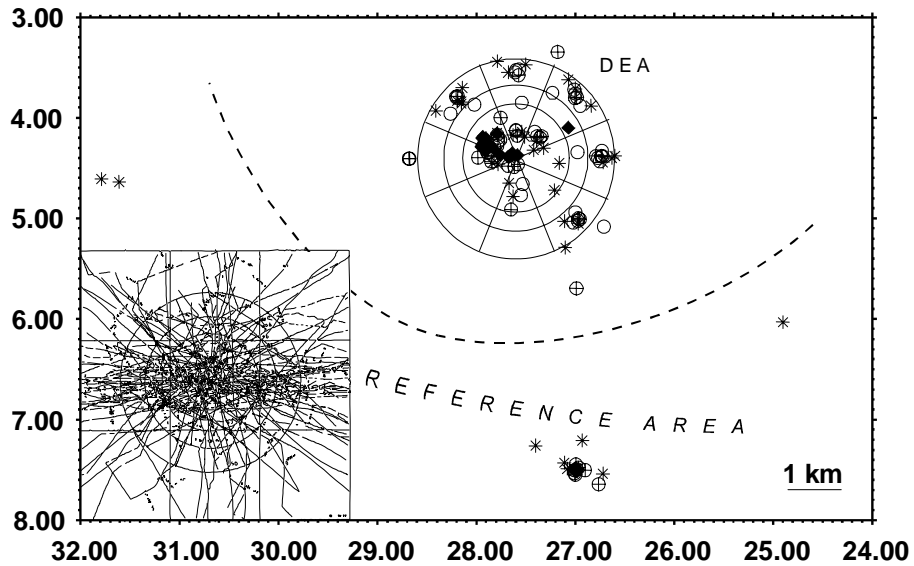


Figure 1: DISCOL Experimental Area (from Borowski, 2001).



Figure 2: The plough harrow.

In February and March 1989, the 8-m wide disturber was towed on 78 radial transects across the DEA (see Figure 1). The device penetrated 10-15 cm deep into the sediments and the resultant plough tracks (8 m wide by 10 - 15 cm deep) covered approximately 20% of the DEA.

Video observations after the experimental treatment demonstrated heavily disturbed areas with high track densities alternating with lower impacted areas and undisturbed regions (Foell et al., 1992; Bluhm et al., 1995). The semi-liquid surface material had been nearly eliminated from the tracks, where the disturber had left behind lighter coloured and sharp edge contoured clay ploughed up from deeper layers. Polymetallic nodules were ploughed under.

Borowski and Thiel (1998) described the re-establishment of the semi-liquid surface in the tracks over the subsequent three years. After this period, many tracks were filled with soft material and their surface contours were more or less smoothed. Nevertheless, the tracks were still recognisable in video observations and in box core samples. Light coloured patches at the surfaces still indicated the presence of sediments originating from deeper layers (Borowski and Thiel, 1998). During the following four years, the shape of the tracks hardly changed and the tracks continued to be distinguishable when the site was revisited during the ECOBENT programme in early 1996 (cf. Bluhm, 2001).

Results from DISCOL

Assumed Mining Impacts

Various mining techniques have been developed and some were tested (Thiel et al., 1991) in the 1970s. However, there was no decision when DISCOL was in its planning phase as to which type(s) might be used one day. We have assumed a vehicle carrying a collector at its front end, to either be towed by the mining ship or self-propelled, moving on chains. This assumption seems to still be the most probable. A design that moves the vehicle by two Archimedes screws and disturbs the sediment in two broad tracks more than one meter deep seems to have no future, particularly under environmental aspects. The vehicle predicted to be employed would move along the seafloor with a speed of about 1m/s. This hybrid (combining mechanical and hydrodynamic principles for nodule take up) collector would be 6 m or more in width and would gather nodules out of the sediment surface layer by mechanical means and with the help of water jets. An unknown percentage of the watery surface sediment, the semi-liquid layer, would be whirled up and partly entrapped during the collection process along with the nodules. Deeper sediment layers may be broken up into lumps, which would be transported in a conveyor belt collection system. The vehicle would move with tank-like tracks along the seafloor, certainly penetrating the semi-liquid layer, and, most probably, the upper stiff sediment layer. At least, the track spines would penetrate this harder material and would break it up into lumps of various sizes. Behind the vehicle a disturbed field would remain, uneven with an irregular distribution of the sediment lumps (compare Figure 5 in Oebius et al., 2001), and to some extent with aggregated sediment particles filling in the gaps and valleys between the lumps. Some of the sediment would also drift away, blanketing nearby regions (for details see Thiel and Forschungsverbund Tiefsee-Umweltschutz, 2001).

For effective mining, the vehicle would meander up and down a mining block along roughly parallel tracks, probably leaving some small patches unmined. But these, depending upon ambient current velocity and direction, should receive a strong cover from re-sedimentation of the plume. We predicted that a typical mining block, covering between 10 and 100 km², would be nearly totally mined out, leaving only some small unmined but sediment blanketed patches. These assumptions, upon which the DISCOL experiment was based, are still valid today.

Material and Methods[†]

The plough-harrow was towed on diametric courses through the centre of the DEA, a total of 78 times, so that the inner circle of the DEA was disturbed to a larger degree than the outer ring. This was confirmed by the results of a combined video and side-scan sonar analysis (Bluhm et al., 1995). During DISCOL and ECOBENT the DEA was sampled five times:

- during the pre-impact study, February 1989,
- directly after the disturbance, March 1989,
- half a year later, September 1989,
- after three years, January 1992, and
- after seven years, February 1996.

The megabenthos were studied using the photo/video system OFOS (Ocean Floor Observation System; Bluhm, 1993). OFOS was towed approximately 3 to 3.5 m above the seafloor (Bluhm et al., 1995). It was equipped with real-time black and white television coupled with a 'photo-on-command' still photography camera loaded with Kodak Ektachrome 200 film. The towing speed varied between 0.5 and 1.3 nautical miles per hour.

The results of the analyses of 23 OFOS transects have been published with a detailed description and discussion of the image analyses methodology (Bluhm et al., 1995). For this study, OFOS transects 7, 9 and 10 were ignored because they were made at a time when not all ploughing had been completed. Five ECOBENT transects were added to the DISCOL data. A total of 14,000 slides and 6,000 minutes of video recordings were used in this study (*Table 1*), representing approximately 700,000 square meters of the seafloor.

[†] Adapted from Bluhm (2001).

Table 1 Analyzed Ocean Floor Observation System (OFOS) images.

Expedition OFOS deployment	Slides	Duration of Video recordings (min)
<i>DISCOL 1/1 (pre-impact study)</i>		
2	518	236
3	551	236
4	243	136
5	717	364
6	561	238
8	556	344
<i>DISCOL 1/2 (first post impact study, 0 years after the ploughing)</i>		
12	375	206
13	471	231
15	659	305
16	724	146
17	505	215
<i>DISCOL 2 (second post impact study, 0.5 years after ploughing)</i>		
19	140	62
21	517	225
22	763	240
23	546	211
24	572	229
<i>DISCOL 3 (third post impact study, 3 years after ploughing)</i>		
30	757	176
31	769	345
32	456	188
33	715	267
<i>ECOBENT (fourth post impact study, 7 years after ploughing)</i>		
36	481	270
37	199	90
38	483	271
41	509	636
42	730	234
Total	13517	6101

Source: Bluhm (2001).

Megafauna Taxa

The megafauna found in the image material of the ECOBENT project was typical for the DEA (Bluhm et al., 1995) and for other polymetallic nodule areas, although there were some

differences in the taxonomic compositions between the Clarion Clipperton Fracture Zone (CCFZ) and the Peru Basin (Bluhm, 1994).

Crustacea, Porifera, Ophiuroidea, and Holothuroidea were the most abundant animal taxa. Sponges are typical hard-bottom community elements and grow on polymetallic nodules in the DEA. They will be significantly affected by future commercial mining operations because their hard-substrate environment will be reduced.

Crustacea consisted mainly of the hermit crab *Proboscidea mirabilis* and to a lesser degree of *Aristaeomorpha*-type shrimps. During image analyses swimming specimens were ignored (Bluhm et al., 1995). Therefore, the shrimps might be underestimated in abundance data. *P. mirabilis* have high densities in the DEA. This species was not found in the North Pacific polymetallic nodule claim areas.

Ophiuroids are often difficult to detect in images of the seabed because the majority of these animals occur within the upper centimetres of sediment. Generally, only the tips of the arms are visible in the photographs, emerging above the sediment. At least two types of ophiuroids were found in the DISCOL material. A whitish-coloured ophiuroid was often seen resting under manganese nodules or crawling through the sediment while an orange-coloured species was frequently observed attached to stalks. It was not possible to identify these brittle stars to species.

The Holothuroidea observed on the photographs have been described (Bluhm and Gebruk, 1999) and a few specimens were taken in the DISCOL trawl material. This taxon appears frequently in the images. Within the Actiniaria, some larger specimens were observed rolling on the seafloor (Foell and Pawson, 1986) within the plough tracks. Some species of Actiniaria appear to have limited mobility and are able to leave less desirable locations for new areas.

A number of fish are present including *Ipnops* sp. (fam. Ipnopidae). These fish rest for hours on the seafloor, but should be able to escape the moving plough-harrow. In addition zoarcids (Anderson and Bluhm, 1997), synphobranchids, ophidiids, and macrourids were also observed in the DEA. Ascidia, Cnidaria, and Crinoidea have limited or no mobility and fragile body structures. In the disturbed areas these taxa were absent or showed low densities in the post-impact studies. The Lophenteropneusta (Lemche et al., 1976) and different stages of their decaying faecal loops were observed frequently in the images; the life cycle of this taxon is still unknown. A high percentage of the image material was classified as 'Indeterminable'.

Density Data

Eighteen different taxa were identified in the DISCOL and ECOBENT data (Table 2). Specimens that could not be attributed to any particular taxonomic category were classified as 'indeterminable'. Only a few taxa showed high abundances. Fourteen taxa were

missing from one or more of the transects. The taxa were divided into four groups depending on their mean abundances and variances.

- Group 1: Crustacea, Porifera, Ophiuroidea, and Holothuroidea
- Group 2: Actiniaria, Asteroidea, Osteichthyes, and indeterminable
- Group 3: Cnidaria (other than Actiniaria, Pennatularia, Gorgonaria, Ceriantharia, and Antipatharia), Ascidia, Hemichordata, and Crinoidea
- Group 4: Rest (Pennatularia, Gorgonaria, Ceriantharia, Antipatharia, Cephalopoda, Polychaeta, Echinoidea)

Group 1 taxa included animals with the highest abundances and variances. In all cases, the minimum mean density was greater than zero. The mean abundances decreased from Group 2 to Group 4. The latter group contained only rare taxa.

Table 2 Descriptive statistics of records from the 'reference' areas, combined for all expeditions. (Total 18 transects for all taxa).

Taxon	Mean	Confidence Limit		Median	Minimum	Maximum	Variance	Standard Deviation	Standard Error	Skewness	Kurtosis
		- 95%	+ 95%								
Porifera	372,45	243,60	501,29	293,30	134,58	1150,65	67134,29	259,10	61,07	1,96	4,24
Cnidaria	13,23	0,91	25,55	3,56	0,00	95,82	613,84	24,78	5,84	2,77	7,71
Actiniaria	39,25	21,10	57,40	28,12	6,41	162,26	1331,90	36,50	8,60	2,55	7,54
Pennatularia	0,83	0,20	1,46	0,00	0,00	3,93	1,61	1,27	0,30	1,59	1,64
Gorgonaria	4,72	2,13	7,32	3,35	0,00	18,07	27,23	5,22	1,23	1,33	1,40
Ceriantharia	1,70	0,04	3,36	0,00	0,00	11,14	11,11	3,33	0,79	2,42	4,99
Anthozoa	1,04	-0,42	2,50	0,00	0,00	10,56	8,57	2,93	0,69	2,87	7,50
Cephalopoda	3,24	1,05	5,43	0,86	0,00	14,69	19,34	4,40	1,04	1,46	1,63
Crustacea	523,79	337,72	709,85	436,28	125,43	1560,09	139996,28	374,16	88,19	1,87	3,23
Polychaeta	6,16	2,02	10,31	3,09	0,00	29,38	69,48	8,34	1,96	1,70	2,66
Asteroidea	18,65	10,01	27,28	12,33	0,00	70,14	301,49	17,36	4,09	2,01	4,20
Ophiuroidea	224,16	165,99	282,32	192,61	69,08	549,77	13680,00	116,96	27,57	1,36	2,41
Echinoidea	3,62	0,48	6,77	1,36	0,00	25,63	39,93	6,32	1,49	2,82	9,01
Holothuroidea	157,41	120,04	194,78	155,51	72,20	348,95	5647,25	75,15	17,71	1,22	1,52
Crinoidea	7,70	2,85	12,54	4,09	0,00	38,45	94,88	9,74	2,30	2,21	5,40
Hemichordata	6,60	3,28	9,92	5,03	0,00	24,18	44,53	6,67	1,57	1,42	1,84
Ascidia	12,42	5,72	19,12	10,49	0,00	44,08	181,61	13,48	3,18	1,01	0,43
Osteichthyes	14,02	4,95	23,08	5,51	0,00	51,45	332,59	18,24	4,30	1,25	0,23
Indet.	47,57	30,82	64,32	38,54	0,00	146,45	1134,52	33,68	7,94	1,43	3,60

Source: Bluhm (2001)

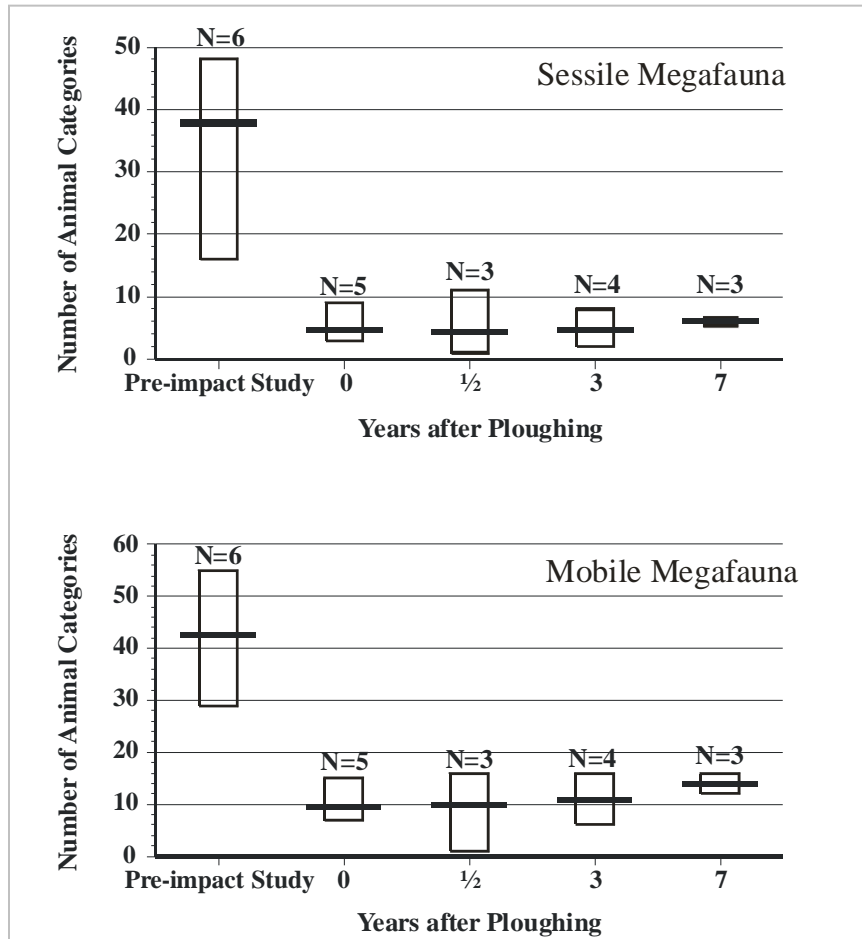


Figure 3: Disturbed area. Number of animal categories found during the experiment: arithmetic mean, minimum-maximum range and N = number of records used.

Figure 3 presents the numbers of animal categories found in the disturbed areas during the experiment. Sessile, as well as semi-sessile and mobile megafauna categories declined after the impact, and remained at the same level seven years after ploughing. A similar decrease was observed in animal densities (Figure 4), but while the densities of sessile animals remained approximately the same throughout the post-impact studies, the densities of mobile megafauna almost reached the pre-impact level three years after ploughing. However, a decline in total density was apparent seven years after the impact.

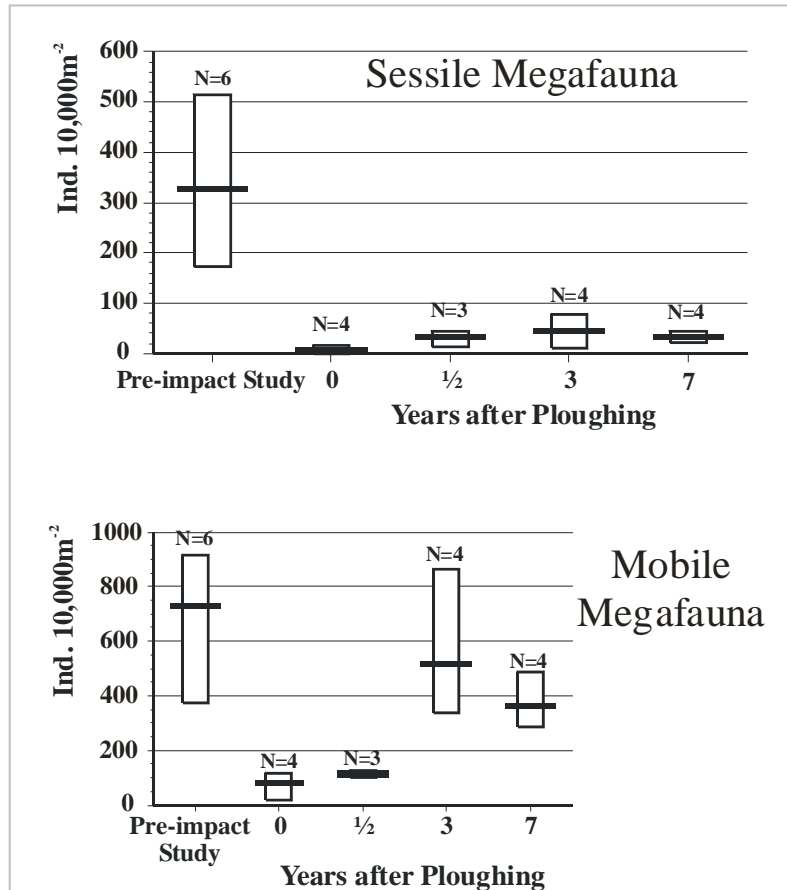


Figure 4: Disturbed area. Densities (Ind. 10,000 m⁻²): arithmetic mean, minimum-maximum range and N = number of records used.

Megabenthos from polymetallic nodule areas is difficult to capture (Bluhm et al., 1995) and only a few specimens could be determined at the species level. The protocols created during image analyses therefore had to use higher levels of taxonomic classification, although the lowest level was used wherever possible, e.g. family, order, or class. The 'animal category' (Bluhm, 1994) employed therefore encompasses a variety of taxonomic levels. These were used for diversity comparisons.

Physical Disturbance and Resedimentation

Physical disturbance of the seafloor is not an uncommon phenomenon. For instance, the carcasses of large animals fall onto the seafloor (Stockton and DeLaca 1982; Smith, 1985, 1986; Priede et al., 1991) and the activities of scavenging animals change the physical and chemical structure of the sediment surface. On a larger scale, turbidity currents, debris flows, and benthic storms modify benthic communities (Nardin et al., 1979; Hollister and Novell, 1991). Commercial mining will impact the surface sediments to an even greater degree.

The DISCOL experiment successfully showed what effects commercial mining might have on the seafloor. Plough tracks were evident seven years after the impact. The plough device changed the structure of the sediment from a flat smooth surface to a mosaic of lumps, valleys, and ridges. The sediment structures affected the composition of the meiofaunal and macrofaunal communities (Ahnert and Schriever, 2001; Borowski, 2001; Vopel and Thiel, 2001), and it is likely that this change in landscape also affected the megafauna because some of these animals feed on the meio- and macrofauna, and organic matter for deposit feeders tends to collect in hollows and around mounds.

The plough harrow worked effectively but left patches of sediment untouched so that some manganese nodules and the associated fauna remained within the tracks. Although the plough marks created look very similar to those of the Benthic Impact Experiments (see below), the collecting gear used during future commercial mining will probably penetrate the sea floor to a much higher degree.

Sediment plumes were created during ploughing, which could be observed drifting in the water column as long as several hours after the impact. Depending on speed and direction of the local bottom currents they were driven away from the point of origin. The sediment particles resettled in the surrounding areas. X-ray analyses of nine multiple corer samples showed different degrees of resedimentation and suggested that the predominant bottom currents during the experiment were oriented in a northerly direction (Schriever and Thiel, 1992). OFOS deployments were made mainly in a northwest to southeast or west to east direction because it was easier to manoeuvre the photo/video system with the research vessel orientated into the surface wave field. As a result, areas affected by high resedimentation rates were only partly monitored by OFOS.

Depending on the geographic locations of the undisturbed sub-areas monitored within the DEA, different amounts of sediment particles had resettled to the bottom. The hypothetical model of Bluhm et al. (1995) assumed that this process would negatively affect at least filter feeding animals. Therefore, a mosaic of slightly different megafauna associations should inhabit the undisturbed areas. This assumption was supported by the high values for the minimal areas, which were found to be highest at undisturbed areas, indicating an increased spatial heterogeneity within the DEA after the impact. A small decrease in total animal densities was evident at the undisturbed locations directly after ploughing when compared to the temporal development at the reference areas, confirming the initially negative effect of the physical disturbance on the megafauna.

The sediment plume created by the collector vehicle during future marine mining will also impact undisturbed areas (Bluhm et al., 1995) to a large extent. The effects of resettled sediment on the fauna are currently being studied in detail within the US - Japanese, IOM (InterOcean Metal Consortium), and Indian Benthic Impact Experiments (Barnett and Yamauchi, 1995; Fukushima, 1995; Sharma et al., 1997; Tkatchenko et al., 1996; Trueblood and Ozturgut, 1997).

Temporal Development in the Plough Tracks

Manganese nodules together with attached fauna were buried by the plough-harrow. This device worked effectively resulting in reduced megafauna densities directly after impact (Figure 4). In Hemichordata and Cephalopoda (mainly Octopoda), this negative effect was not noticeable, but the abundances of these taxa are low and variable and, therefore, it is not known if the differences are significant.

The ploughing activities left undisturbed patches, so that in the disturbance records hard bottom fauna also occurred. Therefore, the minimal area values were comparatively large and exceeded the values of the areas observed. The reverse is the case when the taxa list is reduced to only five soft bottom taxa (Crustacea, Asteroidea, Ophiuroidea, Holothuroidea, and Hemichordata). The results of the nonmetric MDS considering five or twelve taxa looked very similar.

The re-establishment of the megabenthic community was a long process (Bluhm et al., 1995) and even seven years after the impact, the megabenthos of the disturbed area showed a different abundance composition compared to the pre-impact study. The disturbed areas formed distinct clusters in the nonmetric MDS plots (Euclidean distance) of all different functional groups because of the low numbers of individuals found. The disturbed areas are very distinct from the pre-impact record.

The greatest difference in taxa composition compared to the pre-impact situation was found directly after the impact. During the experiment the similarities between the disturbed and pre-impact records increased and were greatest after seven years for total megafauna, carnivores, deposit feeders, suspension feeders and soft bottom taxa, and after three years for omnivores and mobile taxa. The results are influenced by additional factors, but a trend in abundance composition to the pre-impact situation is noticeable.

In total, the different soft bottom taxa and abundance compositions found seven years after ploughing indicate that the recovery process is still in progress.

Natural Variability

The DEA is located approximately 450 nautical miles away from the high production zone off the coast of Peru (Cowles et al., 1977; Zuta et al., 1978). Its surface production was classified to be mesotrophic (Weikert et al., 1993). Surface currents originating in the coastal upwelling areas transport phyto- and zooplankton into oceanic waters where they die and/or pass through the food web. Eventually their remains reach the seafloor as detritus or 'marine snow' and serve as food resources for the abyssal benthos.

In the video recordings of one OFOS deployment, during the exploration phase of DISCOL prior to the pre-impact study of the experiment, patches of greenish soft-structured material were detected which were to a lesser extent and with lower frequency also occasionally

found in the image material of this study. Borowski and Thiel (1998) observed patches of degraded pelagic diatoms within some of the third post-impact samples. Both observations indicate that the DEA is influenced by periodic food impulses. The variability of the particle flux to the sea floor should have a large effect on the composition of the benthos.

The differences in the composition of megafauna communities at the undisturbed and reference sites were small. During the experiment, animal densities in both areas showed the same temporal development, an increasing difference from the faunal composition of the pre-impact sites up to three years after ploughing and a gradual approach to the pre-impact situation again seven years after impact. In the nonmetric MDS plots using Cosine distance, the undisturbed sites were distributed randomly around the pre-impact record. The same is true of the reference site. This is an indication that variation in the composition of the megafauna community during the experiment depended mainly on changes in the abundances of individual taxa.

During the DISCOL experiment, the total megafauna abundance in undisturbed areas increased to a level three to four times higher (three years after the impact) as compared to the pre-impact situation. This temporal development was similar to that observed at the reference areas and indicated a natural oscillation of megabenthic densities in the DEA. High abundance of bacteria, meio-, and macrofauna three years after impact were also observed during the other DISCOL investigations (Foell et al., 1997). Macrofauna total density increased significantly during the experiment at the reference and undisturbed sample sites (Borowski, 2001). Maximal total abundance of Nematoda was found three years after ploughing (Schriever et al., 1997; Ahnert and Schriever, 2001). The values observed were double the pre-impact values. No drastic increase in population density could be found within the Harpacticoidea, the second most populous taxon within the meiofauna of the DEA. Seven years after impact, nematode abundance again equalled pre-impact values (Schriever et al., 1997; Ahnert and Schriever, 2001).

The reason for the strong increase of the benthos three years after the impact is still unknown. The ploughing activities may have led to imbalances of the fauna that occurred over a wide area of seabed. The natural stochastic variability in the populations might also be responsible for the phenomena observed.

The particle flux into the deep-sea at the DEA seems highly variable. Seasonality has been observed several times in abyssal regions (Billett et al., 1983; Rice et al., 1986; Thiel et al., 1989) and probably also occurs in the Peru Basin. Variations in the weather system over the Pacific ('Southern Oscillation'; Wyrski, 1982) and El Niño events (e.g. Philander, 1989) might additionally influence the flux of particles into the deep sea of the DEA. In the literature, only one reference exists with regard to the relationships between surface and abyssal benthic productions in association with upwelling systems. Ingram and Hessler (1987) related an alteration in the recruitment and standing crop of a population of *Eurythenes gryllus* in the North Pacific to an El Niño event. Further data exists on the reaction of the upper shelf benthos to El Niño (Philander, 1989) events (Rosenberg et al., 1983; Arntz, 1986; Thiel, 1982; Arntz, et al., 1991; Thompson et al., 1993). Arntz et al. 1991 analysed benthic assemblages up to 400 metres

depth off the coasts of Peru and Chile. Dependent on the geographic positions of the samples taken, the authors reported a positive effect of El Niño on some taxa found deeper than 40 m. An increase in mollusc numbers one and a half year after this event was also attributed to El Niño. Thompson et al. (1993) analysed the megabenthic assemblages of coastal shelves, slopes, and basins off southern California from trawl samples. The authors detected increased biodiversity at the lower slope stations (478-780 m depth) after an El Niño event and observed a time delay in the benthic effects attributable to El Niño. In the occasionally anoxial sub-sill basins (715 – 878 m depth), no observable effects of El Niño were found.

Conclusions

The results of the DISCOL and ECOBENT surveys show that photo/video image systems are useful in detecting changes of epibenthic communities resulting from physical disturbance impacts. The megafauna play an important role in the deep-sea ecosystem and, therefore, should be monitored in future environmental studies.

During the experiment, natural variations in the taxonomic and abundance compositions of the megafaunal communities were observed. Clear relationships to the flux of organic matter into the deep sea could not be perceived due to a lack of long-time series data, biomass data, and detailed information about life strategies of individual species, of the structure of the food web, and of transport processes into the deep-sea off the South American coast.

Due to the removal of hard substrata, the re-establishment of the megafaunal community in the disturbed area is limited to soft bottom species. Seven years after experimental impact, the megafaunal community still differed in the taxonomic and numerical composition of soft bottom animals although the difference from the pre-impact situation was found to be small. Further post-impact studies should be done to clarify the time interval request for the community in the disturbed areas to become comparable to the baseline.

Due to time restrictions within the present study, only higher taxa were taken into account and, therefore, information about the relationships between the different faunal size classes is limited. In the future, with the help of modern technologies and higher resolution image materials, more detailed investigations should be possible which will enhance our currently limited knowledge of the abyssal megafauna.

Large uncertainties in the taxonomic determination still exist in deep-sea research and knowledge of the ecology of deep-sea species is limited. To obtain more precise information there is a need to identify suitable indicator taxa for this type of environmental impact. Future research should continue to test and develop the Holothuroidea as an effective indicator taxon.

For detailed information on Hartmut Bluhm's results of the DISCOL/ECOBENT project from 1989 to 1996, see Bluhm (2001).

Introduction to the FADE Project

Our knowledge about megafaunal life and its diversity in the deep-sea is still very limited. All our knowledge is based on samples covering a total area of not more than 5 km² from different locations - an extremely low coverage compared to the vast extension of the deep sea. A little more information is available based on stationary photo and video systems or deep towed systems. Hundreds of thousands of slides and hours of video images exist and are in archives of institutes and companies engaged in deep-sea research and deep-sea mineral exploration. Most of the slides and videos show megafauna and could be used to demonstrate the faunal diversity, to identify deep-sea communities, and to show local distribution, lebenspuren and variability in faunal composition. Unfortunately, this information is only available for those on whose shelves these items are resting - and not available for interested scientists or other users. If the person working on this material retires or changes position, often this information will be forgotten and perish forever. Under the auspice of climate change or future commercial use of the deep sea (e.g. manganese nodule mining, CO₂-discharge) and taking into account the time and investment to gather this information, it would be irresponsible not to set up a global data base containing all the information and access for those who are interested in the available information.

Project Outline

The existing slides about deep-sea megafauna from as many sources as possible should be digitized, put in a central database and accessible for all potential users via the internet (Figure 5).

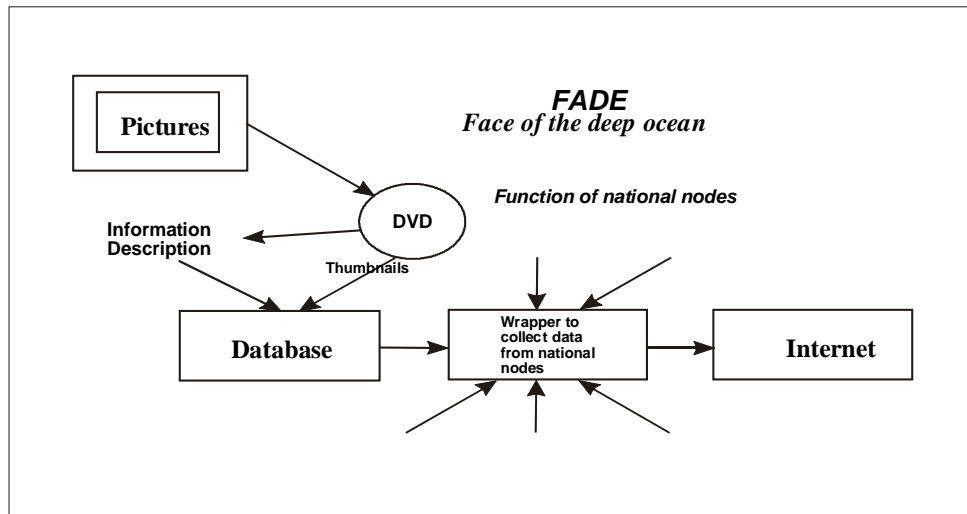


Figure 5: Schematic flowchart of FADE database.

Therefore, the existing information has to be digitized, stored on DVD and to be prepared for the internet. A database has to be created providing the following information of each photograph::

- What kind of organisms or organism groups are visible (taxonomic category determined as best as possible)
- Information about the substrate
- Geographical data including water depth, date, time, expedition
- Equipment used
- Name of the author and rights on the photograph

Additionally, the possibility for links to further information about the cruise, oceanographic or biological data from the area should be given as far as available. The user will be able to add comments on the existing data, to discuss comments with the original describer and so enlarge the knowledge base

Within the suggested project, a specialist has to describe every picture in detail so that all organisms can be safely categorised to the highest taxonomic level possible. This enables the database to be used for scientific work and to identify deep-sea megafaunal communities. Once the database is created, we suggest extending the project as follows:

Within the Kaplan Biodiversity Project, the following steps to increase our knowledge about megafauna within the CCZ are suggested:

- Find out the available information on photos and video images from the contractors and scientific resources from manganese nodule areas in the CCZ and other nodule areas.
- Identify the available information and in what format it can be delivered by institutions/contractors. Digitize all available pictures and store on hard disk and on DVDs. (To be done by contractors or via the project).
- Set up a database including the name of the collector/describer and a description of the species to some taxonomic level as far as possible.
- Add all available information about distribution, place of collection, water depth, biology, ecology, and references as far as possible.
- Adjust database to the Internet requirements with links to the providing contractors/institutes and ISA.
- Make information available for science and the public on the Internet.
- Provide the possibility for interactive discussions between the describer/collector of the image and/or between scientists interested to work on the image.

Nearly no DNA-data exist from deep-sea fauna. As an addition to the Kaplan Project, it is planned within the FADE Project to look for available deep-sea megafauna specimens

collected and stored in formaldehyde in scientific museum collections and find out if samples for DNA-analysis can be taken.

Additionally, new species from further Kaplan cruises will be collected. A co-operation and co-ordination with the Kaplan group is planned. It is planned to try to fish megafauna within the CCZ during one of the cruises with the help of an experienced fishermen. Nets will be set in a way that only the organisms are caught and the nodules are kept out of the net. This should prevent the grinding effect normally occurring during hoisting the net back to the ship.

Benefits for Pioneer Investors and Future Contractors

The common deep towed systems used in deep-sea research allow information to be converted about deep-sea megafauna from large areas within short periods of time. These systems can provide real time images about bottom topography, nodule coverage, megafauna density, and biodiversity on board ship. With knowledge about the megafauna from the FADE database, the possibility of identifying categories or classes of organisms can be realised rapidly. In case further information is desired, access to the FADE database can be accessed aboard the ship. Responsible persons on board can react at once and decide about a detailed sampling programme on faunal size classes identify impacts because the first impression from megafauna images does not replace the necessary work on other size-classes at all.

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SUMMARY OF DISCUSSIONS

An observer who noted that 3 years after the impact, there was an increase in the abundance of a number of megafaunal components asked whether this was a reproductive or a functional response by the megafauna. Dr. Schriever answered that he believed it was a functional response.

Dr. Smith questioned the assumption on “undisturbed” areas as the areas still showed an effect on megafauna. Dr. Schriever responded that this was dependent on the extent of the plume. He said that models from German colleagues suggest, and that results from BIE experiments confirm that sediment is redepositing faster than expected. He also said that very

fine particles were moving far away from the area that mining was taking place. Dr. Schriever said he believed that the Japanese had obtained the same result in their JET experiments. Dr. Smith inquired about the extrapolating from DISCOL results to actual commercial-scale mining. He said that mining would result in many orders of magnitude and greater production of a sediment plume which could affect suspension feeders for example, because there were going to be particles of no nutritional value traveling and being intercepted by the suspension feeders. Dr. Smith asked the meeting to think about the plume and its local effects.

Another point pressed by Dr. Smith was that other individuals, for example, Lauren Mullineaux, a scientist at the Woods Hole Oceanographic Institution, had studied the distribution of manganese nodule fauna and found very similar species, based on morphology, from the Clipperton Clarion Fracture Zone to the Central North Pacific – several thousand kilometers away. Based on her morphological study, there is now some evidence that megafauna are very widely distributed.

Another observer asked if there was fauna specific to deep seabed polymetallic nodules. Dr. Schriever replied that there was not a fauna specific to polymetallic nodules. Dr. Smith, however, raised the point that rocks were extremely rare in most of the abyssal claim sites. Most of the hard substrates were nodules and the animals that lived on nodules, which were not found, were associated with sediment so fauna was specific to hard substrates.

An ocean policy expert relayed to the participants' details of an expedition focused on biodiversity within the context of the South China Sea. He said more than 1000 species were collected in the southwest part of the South China Sea and scientists were in the process of preparing a book on this material within the context of collaboration on the South China Sea's littoral processes. He added that the expedition cost about 128,000 Singaporean dollars, which was equivalent to approximately US\$ 80,000 with airfare included as well as 12 days at sea with 4 days in Singapore for consolidation meeting. The only difference he said was that the expedition was restricted to much shallower water than those depths discussed at the current workshop.

The expert continued by asking if there was any relationship between the bottom species and the species which lived much higher in the water column. Dr. Schriever stated that the German scientists did not study the animals in the water column. He noted that this was one of the topics of the workshop. He reiterated Dr. Koslow's point that more information on the faunal components and processes in the water column was needed. According to information on the structure of the water column at the German site, he recommended discharging tailings from mining operations in water depths greater than 1000 m. This would be below the oxygen minimum zone. He said the general migration zone of mid-water animals, however, could extend from 1200 m water depth closer to the surface.

Dr. Smith said that the general view of oceanographers was that there was coupling between the water column and the benthos. Processes in the water column such as intercepting particles on their way to the sea floor did affect the seafloor biological community although it

was not as clear what impact the sea floor processes had on animals living in the water column. He said the general opinion was that the direction was from the water column to the benthos rather than the opposite.

A participant asked if there was a link between the fauna associated with polymetallic nodules and the fauna associated with the sediment. Dr. Schriever stated that fauna associated with nodules needed hard substrate to attach themselves to and to live. He said that there was a whole associated ecosystem living around the nodules. He also said that meiofaunal organisms were also known to live inside nodules and showing nodules that he said were from the (South Pacific), he pointed out that they have an exterior texture like a cauliflower and were up to 20 cm diameter in size. He also showed small nodules that were attached together and arranged with crevices and holes that provided living spaces for nematodes and harpacticodes.

Dr. Smith mentioned that much has been learnt from experimental studies in the deep-sea and that the quality of disturbance was very important in effecting a community response. In future studies, he said that it was very important to duplicate as precisely as possible the disturbance effects that could be created by mining. That was why he was advocating a controlled deposition thickness, which allowed quantitative examination of the relationship between the amount of sediment accumulation and mortality. Additionally, duplicating the sediment scraping effect of mining by actually removing the top few centimeters of sediment on various scales was as close an analog to mining as possible to investigate the spatial scale effect. Smith believed large-scale studies with test mining and very controlled studies in which the nature of the disturbance were duplicated precisely was critically important in assessing impacts from commercial mining activities.

A workshop participant who interpreted the megafauna data from DISCOL as indicating that a small number of taxa responded to the disturbance, suggested that if after 7 years the system had not returned to the variety of organisms observed before the disturbance, it meant that there was a need to understand the natural change in the system in order to detect and understand how it was going to recover from anthropogenic impact.

Another highlighted aspect was the need for good samples of megafauna from ROV or by other methods, through collection of reference samples, planning collaborative investigations, and identifying opportunities to collect megafauna.

Regarding FADE, a participant suggested that it was a worthy idea analogous to the geophysical data that was not yet digitized and sitting in paper records. He said a programme was now underway to gather this data and scan the paper charts into a standardized format. It could mean being able to take the technology on the road and visit the institutions to extract the archival information.

Dr. Schriever mentioned that the megafauna atlas project had started about 10 years ago and although contributions were very slow, he believed that it would benefit all contractors with the ISA.

Chapter 6

Disturbance and Recolonization Processes

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This chapter addresses issues related to disturbance and recolonization processes at the seafloor following mining.

Rationale

Why do we need to study disturbance and re-colonization processes? This is because various options of marine mining technology is expected to cause a number of major disturbances on the sea floor that will impact various biological processes.

One of the major disturbance impacts of mining activity will be the creation of a sediment plume near the sea floor. It is impossible to collect nodules near the sea floor without re-suspending substantial amounts of sediment, given current technology. Plume creation will result in re-deposition of suspended sediment over some substantial areas of the seafloor, both within the mining tracks themselves and but also in areas removed from the direct path of the mining device.

A second major disturbance impact of mining will almost certainly be removal of the top few centimeters of sediment, and its associated biota and nodules, in the path of the mining apparatus. Essentially, the top few centimeters of sediment, where most benthic organisms are found, will be removed to expose subsurface sediments. The exposed subsurface sediment will initially contain very few animals.

Elucidating the biological response, especially faunal disturbance and re-colonization following re-sedimentation and surface-sediment removal, are critical to predicting and managing the impacts of nodule mining. In the March 2002 workshop, we proposed two topics for collaborative studies that could address mining disturbance and re-colonization processes:

- Burial sensitivity of the CCZ benthos; i.e., elucidating the single-dose response function, and

- Time and space scales of benthic community recovery following simulated mining-track disturbance.

In other words, we divided biological responses to mining into two components. The proposed studies are designed to begin to address disturbance and re-colonization processes. The experimental approach is not going to tell us everything, but it is a logical next step in improving our state of knowledge. The first component is studies looking at burial sensitivity of the CCZ benthos, i.e., an experiment to clarify the single dose-response function.

Burial Sensitivity of the CCZ Benthos

From a variety of perspectives, it appears that the macrofauna, in particular, will be especially sensitive to sediment burial. The natural rates of sediment accumulation, sediment transport, and bioturbation in the CCZ nodule province are very low. Typically, this habitat is physically very stable. Sediment accumulation rates are on the order of 1 cm per 1000 years. Sediment transport events in many areas of the CCZ and the Indian Ocean nodule province are rare on ecological time scales. Moreover, the movement of sediment by animals, through the process of bioturbation, also occurs at low rates.

Another reason we suspect that these systems may be very susceptible to sediment burial is that most of the animals, particularly the macrofauna, are very small and fragile compared to other environments, and appear ill adapted to withstand rapid sediment burial.

Typical macrofaunal polychaetes are about 3 mm long and live in the sediment. Many of these polychaetes have long appendages for feeding on surface sediments, which contributes to their fragility and inability to burrow through sediments.

The third reason we predict the benthos in the nodule provinces may be especially vulnerable to sediment re-deposition and burial is that many of them are surface deposit feeders. Surface deposit feeders use appendages to collect and feed on particles, recently deposited organic material, on the very surface of the sediment. They extend tentacles, which often have mucus and ciliated grooves to gather small particles and transport them to the mouth. They feed on a thin veneer of recently deposited organic material. During re-deposition event, this thin veneer of organic material is likely to be buried or diluted.

To illustrate how susceptible this kind of material might be to burial, *Figure 1* is a photograph taken near the Clipperton Clarion Fracture Zone. The sediment surface is covered with a thin veneer of green phytodetritus. In the path behind the burrowing urchin, the green veneer is absent. In the process of overturning ~1 cm of surface sediment, this food material has been buried and potentially lost to the surface deposit feeders living in the wake of this urchin. This example illustrates how easily this thin veneer of material might be diluted by re-depositing sediment and potentially lost to surface-deposit feeders following plume re-sedimentation from mining activity.



Figure 1: *An irregular urchin plowing across the sediment surface at a depth of ~4400 m at 2 °N, 140 °W. For scale, the width of the track is approximately 10 cm (Smith et al., 1996).*

A number of studies have explored disturbance impacts of mining on the deep-sea floor. These include DISCOL and Benthic Impact Experiments (BIE), such as the JET programme. In BIE-type experiments, a simulated mining apparatus is dragged along the sea floor to create a plume of re-suspended sediment. Disturbance effects are examined in areas around the track to evaluate the faunal response.

In most BIE type experiments, scientists were able to compare areas of no deposition, where no visible re-sedimentation occurred, to areas where re-deposition did occur (*Figure 2*). Using megafauna, for example, Fukushima et al., (2000) found a significant decrease in abundant deposit feeders in a disturbed area even after 2 years. Studies have also examined various infaunal components, contrasting areas of no deposition versus areas of light and heavy deposition.

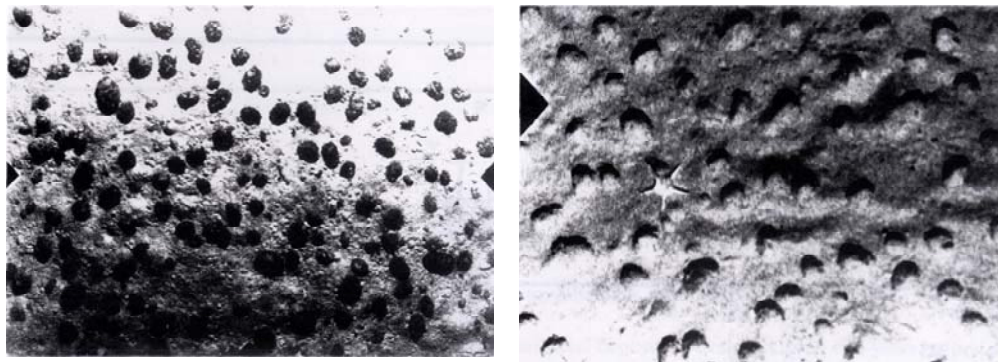


Figure 2: *Redeposition effects on megafauna from the JET experiment (Fukushima et al., 2000). Left image is from no redeposition area. Right image is from redeposition area.*

These studies have contributed some useful insights into disturbance and recolonization processes. Yet, one facet that has been surprisingly difficult to quantify in all of these studies is the re-deposition thickness resulting from the simulated mining. In other words, there is only an ordinal or semi-quantitative comparison between areas of deposition and no deposition, rather than a quantitative comparison between actual amounts of deposition and the amount of disturbance or mortality in the benthos.

In other deep-sea environments, there have been quantitative studies on re-deposition. These experiments were explicitly controlled and disturbance effects evaluated. One such experiment examined the effects of burial disturbances associated with biogenic mounds in the Santa Catalina basin. In this study almost 2 decades ago, explicit amounts of sediment were visualized with stereo photography or other controlled means, and disturbance effects on the benthos were quantified. One of the surprising results from this study was that bathyal animals at ~1200 meters depth in Santa Catalina Basin were quite resilient to burial beneath 5 cm of sediment deposited in the form of an artificial mound (Smith et al., 1986).

Somewhat disappointingly, even decades later, we still cannot rigorously predict the impacts of re-sedimentation from mining plumes on abyssal seafloor communities. To predict the spatial impacts of mining plumes, it would be extremely useful to elucidate dose-response functions between re-sedimentation thickness and seafloor community mortality.

Knowledge of burial dose-response functions would allow scaling up of results from small-scale (e.g., pilot-mining) studies to the prediction of full-scale mining impacts. Such prediction of the spatial scales of mining impacts is essential to assess the potential for nodule mining to cause species extinctions.

Dose-Response Function Project

Scope

Conduct an experiment in the CCZ to elucidate dose-response relationships of sediment biota (microbes, meiofauna, and macrofauna) and habitat characteristics (e.g. sediment geochemical properties) to acute re-sedimentation thickness.

Proposed Experimental Design

- Treat controlled replicated areas of seafloor with 5 treatment levels of burial (0, 5, 10, 20 and 40 mm of re-sedimentation).
- Use a ROV-mounted injector and sediment fences (existing technology).
- Create ~8 replicate treatments per treatment level (each treatment ~0.6 m on each side).
- Resample experiments after one month (i.e., long enough for mortality and decay to have occurred, but short enough to preclude substantial recolonization).

Output

Dose-response function of benthic-community mortality following rapid redeposition, to be combined ultimately with plume models to predict the spatial extent of mining impacts.

Duration

Twelve months for equipment design, fabrication and shipping; 2 months for field programmes; 24 months for processing and publication preparation. Total project duration = 3.5 yr.

Approximate Funding Resources RequiredShip time

To implant and sample experiments, two ROV cruises are required each ~24 days long (assuming use of Atalante and the ROV Victor)

Total ship time costs 50 d@ \$44,000/day = \$2,200,000.00

Equipment

Sediment delivery system	\$60,000.00
25 corers at \$4000 each	\$100,000.00
30 fences - \$15,000; miscellaneous	\$15,000.00
Total equipment costs	\$190,000.00

Personnel

Macrofaunal – (sorting and identification) –	3 yr
Meiofaunal – (sorting and identification) –	2 yr
Molecular technician –	1 yr
Geochemical technician –	1 yr
Microbial technician –	0.5 yr

Travel

Planning meeting	\$25,000 .00
Cruise participation	\$25,000.00
Synthesis meeting	\$30,000.00
Collaboratory visits and presentation of results at conference	\$15,000.00
Total travel	\$95,000.00

Supplies \$50,000.00

Shipping \$20,000.00

Total 3.5 yr project costs

Ship time	\$2,200,000.00
Direct support costs	\$885,000.00
Overhead at 40% of direct	\$348,000.00
Total	\$ 3,432,000.00

Benthic Community Recovery

There are two major biological issues associated with the anticipated effects of deep-sea mining. The first of these is mortality, which was briefly addressed in the first part of the paper. The second issue is biological community recovery. Assuming that there are going to be negative biological effects, how long will it take for the faunal community to recover?

In general, we are interested in characterization of sediment geochemistry and various other geotechnical properties associated with sediments and how they influence biological recovery. For biological response variables, we are specifically interested in the response of microbial, meiofaunal, macrofaunal and megafaunal communities – i.e., in the responses of a broad range of taxa.

In manipulative field experiments, it is often necessary to directly control the experimental deployment to produce, quantitatively, the desired treatment. For the controlled experiments, we envision that an ROV or research submersible is essential to conduct some aspects of the work.

One set of experiments would create mining tracks of different widths and then monitor biological recovery in the tracks over time. Swaths of 1 m, 3 m, and 9 m are proposed. We view these experiments as important because patch size may substantially influence recovery. Big patches are likely to have longer recovery times than small patches.

We must create realistic mining tracks in terms of the area, length, and depth of disturbance. Mining can dramatically influence residual geochemistry near the sediment-water interface. From a biological perspective, geochemistry is very important, in that it may affect rates of biotic mortality as well as recovery. Our experiments will be designed to address some of these issues.

In nature, rates of natural disturbance are often positively correlated with the resilience of the biological community - the ability of the community to rebound. The deep sea has very low rates of natural disturbance, and resilience is therefore predicted to be quite low. The recovery process is consequently expected to take a long time.

For insight into how slow recovery processes in the deep sea might be, some perspective can be gained from experimental work published by Fred Grassle in 1977. His experiment was simple; he collected natural sediments, froze them to kill organisms, and then used those sediments to fill trays that were deployed in shallow water in Buzzard Bay, Massachusetts, and at a deep-water site off the coast of New England (*Table 1*).

Table 1: Grassle's Experiment

Station		Time Exposed (months)	Density of ind. (m ⁻²)	No. of species	No. of Ind.	Sample area (m ²)
Buzzards Bay	Control	—	48,325	61	1935	0.040
(10 m)	Exp	2	35,714	47	704	0.021
DOS 1	Control	—	5,189	>103	454	0.0875
(1,760 m)	Exp# 1	2	160	14	43	0.250
	Exp#2a	26	564	31	141	0.250
	Exp#2b	26	536	10	15	0.028

Source: Grassle (1977).

The natural communities in the shallow-water system have high densities, whereas densities in the deep sea were much lower. In shallow water, after two months of exposure of azoic sediments, 35,000 individuals per square meter and 47 species were found. Thus, the macrofaunal community in the disturbed sediments had recovered significantly, even in just a few months.

In contrast, after 2 month and 26-month periods, the recovery in the deep sea was far less advanced. Neither the number of species nor of individuals was anywhere near natural background levels. Based on Grassle's (1977) and other deep-sea studies, rates of colonization in the deep sea are generally considered extremely slow, indicating a very long time for communities to recover from mining disturbance. In contrast, highly resilient communities have the capacity to rebound quickly. Ecological communities that have high levels of chronic disturbance (e.g. storms, fishing) can often bounce back fairly quickly. In the deep sea, however, recovery will probably require years because resilience is so low.

Over the last few decades, a number of small-scale experiments have been done that provide insight into how species are likely to respond to disturbances (Table 2). Typically opportunistic species invade very quickly, and diversity is reduced relative to undisturbed areas. In a few instances, enhanced diversity is observed within disturbed sites.

Table 2: Studies on Disturbance

<i>Source</i>	<i>Site</i>	<i>Depth (m)</i>	<i>Impact</i>
Wood Island	NW Atlantic	1630	Increased abundance of
		3506	Rare (opportunistic) species
	Bahamas	2066	
Unenriched, azoic Sediments in trays	NW Atlantic	1880	Colonization by unusual and
	NW Atlantic	3600	Common species
	NE Atlantic	2120 - 4150	Diversity < background
	NE Pacific	1240	
Fieberling Guyot		585, 635	
Nekton falls	NE Pacific	1300	Reduced abundance/diversity,
	NW Atlantic	3600	A few common and rare species increase
Artificial mounds	NE Pacific	1240	Higher diversity, lower numbers
Enriched sediment	NE Pacific	1240	Reduced diversity, different
Trays/depressions	NE Atlantic	2120-4150	Rare species attracted to
	Virgin Islands	900	Different types of enrichment

Source: Snelgrove and Smith (2002).

Recolonization results from artificial mound experiments cannot be directly extrapolated to mining disturbance on the abyssal plains. To elucidate the mortality effects of re-sedimentation, working at a very small scale is appropriate, but recolonization studies conducted on small scales may yield very different results than would be expected over large scales. The scales of experiments on colonization conducted to date are very small relative to the scenario expected with seabed mining.

Colonization Trays

Two types of experiments are envisioned. The first involves use of colonization trays, in which sediment is collected from the site, defaunated, and then returned to the environment in trays to monitor recovery over time. This approach has positive and negative aspects. On the down side, the process of faunal removal from sediments, then substantially alters the sediment geochemistry. On the up side, biological interactions between resident fauna and settlers are eliminated. For example, in a simulated mining disturbance followed by settlement of sediment particles, some fauna may survive and influence the subsequent colonization process. Teasing out such effects can be quite messy.

Another advantage of colonization trays is that they can be easily manipulated. For example, geochemical gradients could be created within them. Using this approach, one could test, for example, whether recolonization differs into surface sediments versus exposed subsurface sediments. Exposed subsurface sediment may be less attractive to potential

colonizers. Geochemical cues could potentially be very important, especially in determining whether larvae settle or do not settle.

Colonization trays focus on recruitment by juvenile and larval stages because adults cannot migrate through the walls of the tray. In this sense, their recolonization processes may mimic those occurring in the center of a large-scale, mining type disturbance. Trays may be elevated above the sediment surface or placed flush with the sediment-water interface. The colonization tray experiments are quite different from experiments in which sediment is either added or removed from the natural seafloor.

Sediment Addition/Removal

In sediment addition or removal, the sedimentary geochemical gradients are more natural than in colonization trays because the sediment is less disturbed. Resident (adult) and settler (juvenile) interactions can occur. Sediment can be either defaunated or not defaunated before it is injected onto the natural seabed to explore the effects of faunal survival in the sediment plume on recovery time. In sediment addition/removal experiments, adults surviving near the experimental site can be quite important, because they may migrate into experimental patches and influence recovery trajectories. The sediments themselves are more difficult to manipulate than in colonization tray experiments. For example, it would be difficult to create defined chemical gradients using sediments that are onto the seabed.

Response Time

The response time (or sediment community recovery time) following mining may depend on the spatial scale of the mining disturbance. In the case of a large disturbance patch, we predict that larvae are probably the most important colonists. In the case of a small patch of disturbance, adults migrating horizontally may colonize more quickly than larvae. Larval response tends to be much slower than adult response. For simulating the scales of mining disturbance, a large patch analogy is probably more relevant than small patches.

A central question is how does the biological community respond to mining disturbance as a function of time? For example, over what times scales will the sediment community recover following mining disturbance? Response variables could include diversity, densities of dominant species, or other community parameters. The nature of the response will depend on the specific community examined (e.g. meiofauna vs. macrofauna) and on the nature of the patch size. We therefore recommend returning to the experimental site at several time intervals. One scenario is to create the experimental disturbance (e.g., by plowing) with sampling immediately pre- and post-disturbance, and additionally after one year, three years, and perhaps nine years.

The outcome of this work will provide useful information to help predict (and potentially minimize) the impacts of deep-sea mining activities. The types of questions likely to be answered with such experiments include:

- Which species are most vulnerable to burial and scouring disturbance, and which species recover most quickly?
- What aspects of disturbance create the largest problems? For example, will exposure of subsurface sediments reduce larval settlement for an extended time (e.g., years)?
- Will adults or larvae lead the recovery, and is the outcome highly scale dependent? We believe community recovery will be scale dependent and that larval recruitment will play a critical role for the macrofauna following large-scale disturbances.
- What sort of mining strategy might allow the most rapid recovery?

In the initial experimental scheme, plowed swathes of 1 m width, 3 m width and 9 m width were proposed to explore the effects of mining-track width on recolonization. In addition, during mining it may be possible to leave gaps between the mining tracks. Would the presence of gaps (and a surviving faunal assemblage) between tracks increase the speed with which the community could recover from mining impacts? There are many interesting questions that could be addressed by carefully varying the scale and spacing of simulated mining disturbance.

In terms of ship-time commitment, the initial set up of the experiment would take ~ 20-25 days. There would also be a transit time of 12-16 days for the ship to travel to and from the study area in the CCZ. We would propose to return one year later and would require about 6-8 ROV days for sampling. Three years later we would repeat the sampling effort. The estimated costs of ship-time would be ~ \$3.7 million.

There are some other relevant topics of study that we have not fully developed. One idea is to examine the relationship between larval supply and recolonization response. One approach to evaluating this relationship is to use a plankton pump (e.g. modified version of a Hardy-Longhurst plankton recorder) to collect periodic samples at times when a ship is not present for later recovery and analysis.

A second idea is to look at natural variability in sediment and organic-matter (e.g., phytodetritus) deposition and how that might affect rates of recovery.

The resources required for this series of experiments include ship time already mentioned. The total cost for this series of experiments would be ~ \$5.3 million but subsets of the research could be completed at a lower cost.

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SUMMARY OF DISCUSSIONS

One of the scientists pointed out that in mining activities, it was not only the redeposition thickness that was important, but also the chemical composition of the deposited materials and asked if there were any methods of estimating thickness.

Dr. Craig Smith responded that one approach to estimating thickness greater than 1 cm would be to use radiography on core samples because the redeposited layer had a different x-ray density. He noted that in the DISCOL experiment and in the American BIE experiment, this technique was used but because the re-deposition thicknesses were too thin (on the order of 1 mm); the technique was not very useful. He said there were recent applications of medical CATSCAN technology to sediment structure that could also be explored although the remote location of the site did create problems with this particular approach.

Dr. Smith continued that other techniques, such as the use of particle-associated radionuclide thorium 234 (Th²³⁴), were available and when tested on BIE redeposition, the approach proved to be semi-quantitative. One of the appeals of doing an experiment where re-deposition thicknesses precisely controlled (e.g., from a 1 mm to 10 cm) was quantitative knowledge of the re-deposition thickness. He said it was also possible to alter the chemical composition of the material in the sediment delivery reservoir, if that was deemed desirable, and even add organic material. He said the experimental approach was a powerful tool for understanding many of the processes.

One of the participants noted that it was very difficult to generalize how any individual species was going to respond because it depended on its natural history. He said using a generalized species that had a sedimentary adult and a pelagic larval stage was realistic as most metazoans in the deep sea do not utilize that life history strategy.

Dr. Snelgrove responded that there was indeed a lot of direct development in the deep sea, which could decrease the role of water column processes.

A scientist asked about mining changing sediment structure with coarser particles settling faster and finer sediments being transported away resulting in altered sediment size structure and whether this factor would influence re-colonization?

Dr. Snelgrove replied that it was an area where colonization trays would be an ideal manipulation because trays could contain different sediment types, allowing direct comparison of colonization response to grain-size differences.

A scientist questioned proposed experiments that would look at leaving swaths unmined over scales of 1 m to 9 m. He said contractors may be unwilling to leave unmined swaths for economic and operational reasons and on the scale of the proposed mining, biological responses were probably going to be very scale dependent. He cautioned on extrapolating from experimental scales of 1 m to 9 m to proposed mining scales on the order of km.

Dr. Snelgrove responded that based on current knowledge of conservation biology, undisturbed strips within the mining areas would likely expedite biological recovery.

Dr. Smith added that when thinking about scale, it was important not to just think in linear terms. Certain processes, such as the migration of adults into the middle of the patch, were very non-linear. He said on the one hand, if patches were 9 m on each side, there was a very good chance that distance would preclude adult immigration into the patch. On the other hand, difference in larval recruitment between a patch 9 m on each side and 1 km on each side were likely to be small. In short, very useful generalizations could be drawn from working at scales smaller than the mining disturbance, although scale dependence should be considered carefully.

Dr. Snelgrove further pointed out that if all of these experiments were deployed at one site (particularly with colonization trays), it would be possible to quantify available larval supply. A preliminary look at a second site using sediment trays could then give insight into the vulnerability of the mining area.

An observer reported that recent datasets suggest that in some circumstances the deep-sea community may be dominated by a single settlement event that occurred even decades

earlier. He suggested careful design of the investigations as occasional recruitment events could obscure the experimental outcome, if not considered at the outset of planning.

Chapter 7

Mining Plume Impacts on Water Column Ecosystems

Dr. B. Najendar Nath, National Institute of Oceanography, Dona Paula, India

Introduction

The open ocean water column is a complex ecosystem with very low biological productivity. The water column ecosystem will be affected by mining operations. The exact nature of the impact depends on the design of the selected mining system, the composition and volume of discharge, and the depth of discharge.

A schematic of a nodule mining system is presented in Figure 1. How the discharge characteristics will behave depends on the type of mining collector and lift system. A surface discharge will affect the turbidity, photosynthesis and biological productivity. Subsurface discharge will alter the physical, biological and biogeochemical characteristics of the water column. The selection of mining and transport equipment will control the kind of discharge that would be introduced into the water column. Depending on the mining system, the composition and the volume of the discharge will vary.

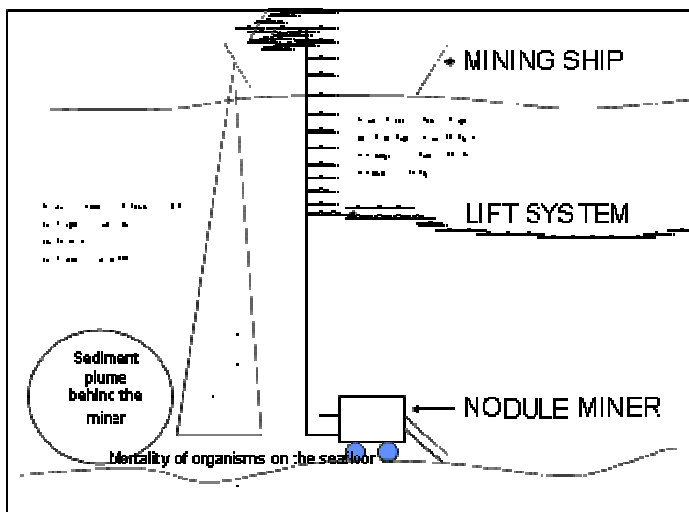


Figure 1 Generalized mining system.

Composition of Discharge

The discharge would have nodule particles. How much and the size of the particles released would depend on the type of on-board processing. In addition, if a hydraulic lift system is employed; cold water would be introduced as a byproduct. The cold nutrient-rich water could enhance productivity in surface waters and cause the waste stream to sink. A third, more likely, alternative involves discharge of nodule waste plus cold water plus sediment particles. This complication would increase from the first stage to the last stage. Sediments characteristics are not as simple as the nodule characteristics. They may effect the concentration of suspended material.

Volume of Discharge

What volumes of discharge are anticipated? At an annual production of 3 million tons per year, economic mining is expected to harvest about 10,000 tons of nodules per day as determined from calculations made by Yamazaki of Japan.

The ratio of nodules in the intake slurry at the mining platform would be approximately 20% of the total mass brought to the surface. So, for every ton of nodules, there would be approximately 4 tons of water incorporated into the lift system. That same amount of water must also be discharged, so the total discharge is probable around 40,000 tons per day.

Additionally, there would be some loss after processing the nodules. There will also be some sediment brought up with the nodules. A rough estimate of this quantity is about 1000 tons per day. If we process the nodules at sea, then there would be addition nodule waste too. Approximately 80% of the nodule material will be discarded, as the principal target metals are Cobalt, Copper and Nickel. Nodules processing loss could be as high at 8000 tons per day. This information gives us a perspective on the volume of the discharge.

Depth of Discharge

Discharge of mining waste at different levels in the water column would have varying effects depending upon the physico-chemical conditions at that level and the composition of the material being discharged.

The discharge depth depends on the kind of discharge system as well as the energy requirements. Deeper discharged depths may require more energy, whereas a surface discharge would require less energy, so economic considerations may dictate the terms of depths of discharge. Water column chemistry, physics and biology vary from the surface to the seafloor.

There are three possible depths of discharge:

- the surface or mixed layer,

- mid-depth in low oxygen waters, and
- near the benthic boundary layer.

Each zone has its own diverse oceanographic characteristics. The surface is generally where primary productivity occurs. Primary productivity, even in remote low productive water, is the key biological driver for the entire oceanic ecosystem. At mid-depth, ranging from 200 m to 1200 m, low oxygen water drives chemical and biological interactions. In abyssal basins of the Central Indian Ocean basin as well as of the equatorial Pacific, a zone of different chemistry exists, known as the carbonate compensation depth. Additionally, the benthic boundary layer is dynamic (*Figure 2*).

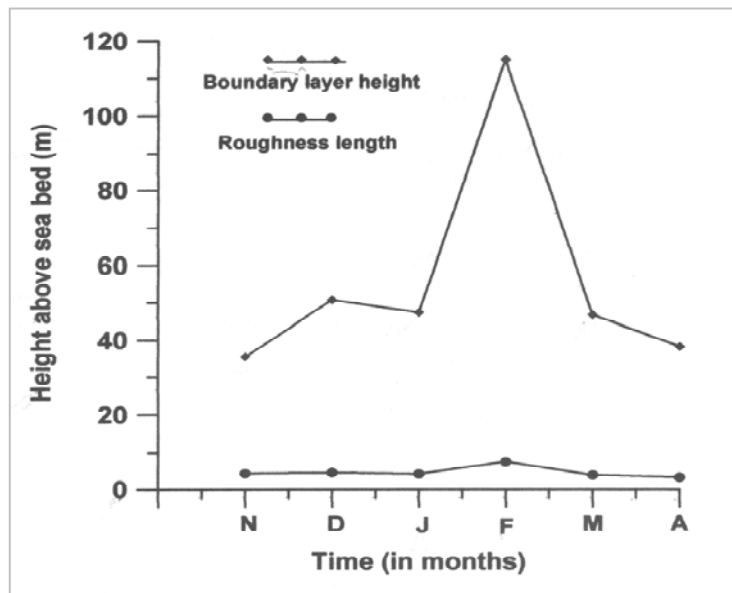


Figure 2: Benthic boundary layer height in the north Central Indian Ocean Basin.

Impacts on the Water Column

Impacts on the ecosystem depend on the composition of the discharge and the depth of discharge. To predict long-term impacts, you can examine what would happen on a smaller scale. Once the composition and discharge depth have been defined, we may be able to predict long-term impacts.

Surface Water Impacts

The effects are presented in tabular fashion in *Table 1*. There may be more effects than are listed. Three possible components of the discharge are identified.

Table 1: Impacts on the surface ecosystem.

Parameters	Components of the Discharge		
	Nodules	Water	Sediment
Particle flux	++	++	+++
Release of metals	++	++	++
Absorption of metals	++	++	++
DOM ¹			
Biogeochem changes	+	+	++
Temperature	-		
Turbidity	+	+	++
Light transmission			+++
Vertical transport	+	++	++
Lateral transport			+
Residence time			+
Nutrients	-	+	++
pH ²	-		
Bacterial activity			+
CO ₂	-		
Oxygen consumption			
Photosynthesis			
Dominance of diatoms	-		+

¹ Dissolved organic matter

² A term used to describe the hydrogen-ion activity of a system

Release of Metals

Laboratory studies on crushed nodules in filtered seawater demonstrated that iron would increase in seawater by a factor of 2 within 24 hours (cf. Robertson and Rancitelli, 1973). Of course, the results depend on the pH of the seawater and the composition of the seawater. What would happen if crushed nodules were released into the water column? We do not know if the released metals will be available for new biological production. In the case of iron released from the nodules or sediments, whether it will lead to fertilization is unknown. On a continuing basis, if 10,000 tons nodules are collected, approximately 8,000 tons of material would be discharged. Will the effect be the same?

Metals are richer in the sediments and the cold water compared to the crust and surface waters. So there would be release of metals during the transport and discharge.

Metals may have a positive effect on primary productivity. On the negative side, metals released during the discharge may enter the food chain and possibly biomagnify, creating ecological harm.

Absorption of Metals

Both nodules and sediments have iron oxides and manganese oxides. The iron and manganese exist in oxide form so they have very good absorption capacities. If the manganese nodules and crushed manganese oxides and iron oxides from the sediments are released in the water column, then these oxides have a good capacity for metal absorption. The nodules could scavenge metals from seawater.

Lateral Transport

The lateral transport would be larger if the sediment is included in the waste stream. Deeper depths have sluggish currents, so you will find that lateral transport would depend on the depth of discharge. For surface discharge, the lateral movement would be largest. Adjoining geographic areas may be affected, not only the mining area.

Nutrients

The chemical effects of nutrients need to be considered. Nitrate is higher in pore waters in both the Central Indian Ocean basin as well as the CCZ. Values can be as high as 32 to 35 μM per liter. Release of nitrates, in simple terms, might stimulate biological productivity. The surface waters are typically denitrified, so nitrification is would take place.

Bacterial activity will be higher if sediments are present resulting in the concentration of carbon dioxide increasing and oxygen being consumed. Photosynthesis will be affected by these changes.

Photosynthesis

Photosynthesis is totally dependent on light. Downwelling solar radiation is attenuated by particulates. Attenuation is in proportion to the number of light scatterer or particulate concentration. This degree of light reduction is a function of three elements:

- nature and concentration of sediment particulates,
- planktonic particulates, and
- dissolved organic matter (DOM).

Light transmission would be decreased with the mining discharge into the water column, thus reducing photosynthesis. The extent of the reduction would depend on:

- rate of discharge,
- degree of mixing,
- rate of sediment particulates settling out of the euphotic zone, and
- light adaptation characteristics of phytoplankton.

Dominance of Diatoms

Porewaters from the Central Indian Basin sediments are rich in silica (300 – 700 μM) compared to surface waters. Release of silica would probably increase the diatom production. The net effect would be diatom production dominating over coccoliths, which may affect carbon dioxide uptake.

Mid-Water Impacts

Impact on the ecosystem if waste streams are discharged into mid water is tabulated in Table 2. For mid water, we are using water depths in excess of 200 m. Deeper down is an environment low in oxygen. Metals may be released from the particles in this zone. In addition, certain elements may be altered chemically when oxides are encountering the low oxygen environment of the oxygen minimum layer. Because of the oxygen minimum zone, absorption of metals will not occur and there may be release of metals. Biological changes also need to be considered.

Table 2: Impacts on the mid-water ecosystem

Parameters	Components of the Discharge		
	Nodules	Water	Sediment
Particle flux			++
Release of metals			+
Adsorption of metals	-	-	-
DOM ¹	-		+
Biogeochem changes			+
Temperature	-		
Turbidity			+
Light transmission	-	-	-
Vertical transport		+	
Lateral transport			
Residence time			
Nutrients			
pH ²			
Bacterial activity			
CO ₂			
Oxygen consumption			
Photosynthesis			
Dominance of diatoms			

¹ Dissolved organic matter

² A term used to describe the hydrogen-ion activity of a system

In a small experiment we conducted last year, sediment collected with box cores was used to experimentally examine introduction of sediment into the surface waters. The characteristic of the discharge was a specific gravity of 2.2. Water content was 86%. Observations were recorded on the primary productivity, the zooplankton, the chlorophyll content and the bacterial numbers. The chemistry of the water column was also evaluated including nitrate and other nutrients in the water column, the pH of the water column and the suspended materials in the water column. There were changes noted after a series of observations over one day (Figure 3).

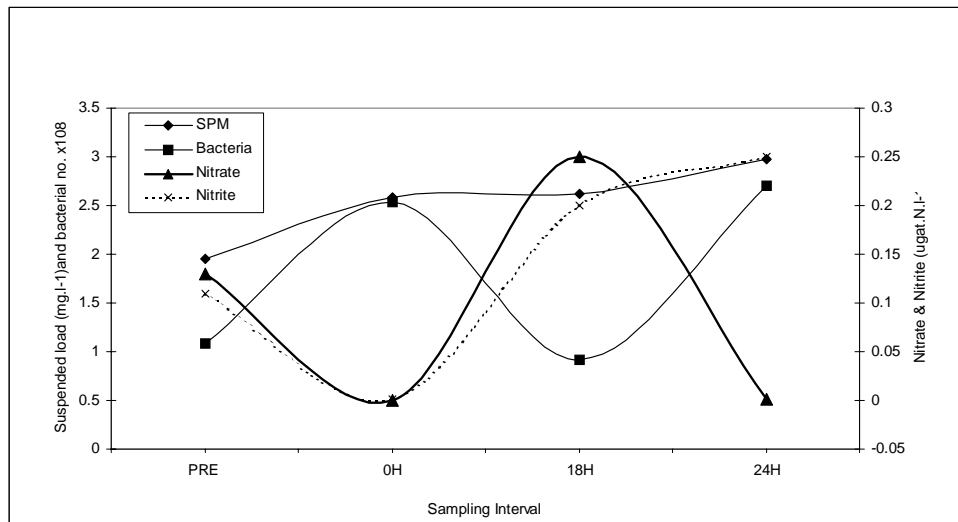


Figure 3: Changes in suspended matter, bacterial numbers, nitrate and nitrite concentration at the surface (Data from SEDEX).

After 18 hours, the nitrate increased and the bacterial numbers went up after 24 hours. These are very miniscule experiments. These observations are very small-scale observations just to show the effect on the surface discharge and what would happen on a very short time scale. These are the short terms effects where we have seen an increase in the diatom production.

Benthic Impacts

Potential benthic impacts are summarized in Table 3.

Table 3: Impacts on the benthic ecosystem.

Parameters	Components of the Discharge		
	Nodules	Water	Sediment
Particle flux			++
Release of metals			+
Adsorption of metals	-	-	-
DOM ¹	-		+
Biogeochem changes			+
Temperature	-		
Turbidity			+
Light transmission	-	-	-
Vertical transport		+	
Lateral transport			
Residence time			
Nutrients			
pH ²			
Bacterial activity			
CO ₂			
Oxygen consumption			
Photosynthesis			
Dominance of diatoms			

¹ Dissolved organic matter

² A term used to describe the hydrogen-ion activity of a system

Long-term Impacts

On a longer-term time frame, several impacts are probable. Discharges into the water column would definitely affect the entire water column chemistry. In the long-term, the bacterial population would be different. Grazers would shift from smaller forms to the larger forms as the pelagic community adapts to the changing nutrient loads.

The size and species composition of the pelagic community would vary with the introduction of discharge on a long-term basis.

Heavy metals would become available to the food web. They may go into the food chain and affect the food web.

Oxygen levels will change because of the productivity changes.

Export of carbon from the surface mixed layer due to increased productivity may affect the level of carbon in deeper waters.

Future Research Needs

The parameters to be measured will be discussed, as well as types of special experiments to elucidate the response of the water column ecosystem to the discharge of mining waste. The requirements and the kinds of scientific deliverables or products that could be anticipated from future research will also be discussed.

Eventually, international collaboration will be required for carrying out these projects.

Parameters

The parameters to be measured would be:

- Geological parameters of interest including particle fluxes, nodule chemistry and sediment characteristics.
- Biological parameters of interest including a whole range of studies in biological processes, such as chlorophyll concentration, biological productivity and the whole chain of the biological cycle (phytoplankton diversity, zooplankton diversity). Additionally, biochemical properties and bacterial abundance and activity would be examined.
- Water column chemical parameters of interest including characterization of trace or heavy metals in the water column, nutrients, oxygen levels, and pH and carbon dioxide levels in the water column would be useful for study.
- Physical parameters will be measured including temperature-salinity profiles, water-mass structure, currents and optical properties, all of which will aid in determining the scattering and the effects of the suspended material on light transmission, and turbidity.
- Meteorological conditions including surface characteristics are important in lateral migration of the plume (like wind direction and the surface water temperature).
- Remote sensing techniques would be employed to study sea surface conditions such as sea surface temperature, surface winds, currents and chlorophyll patterns. These factors may affect the plume direction or dispersion.
- Modeling efforts would include development of physical, biogeochemical, ecological and risk assessment models.

Special Experiments

What kind of experiments could be conducted on a micro scale in bottles to experimentally characterize the processes and interactions between primary and bacterial productivity and grazing?

What is the dose response of sediment introduced into the water column? In the open ocean, experiments with large bags over a period of one month will be used to evaluate the community over a range of experimental treatments. Initial treatments include adding nodule powder or nodule pieces and nodule plus cold deep water, and finally, nodule plus water plus sediment. To assess the long term as well as large-scale impacts of mine discharge in the water column after conducting these small experiments, we will initiate modeling studies.

Work Plan

A work plan arranged over five years was developed. In the first year, protocols would be established through workshops. Methods would be streamlined taking into consideration international standardizing of methods. Baseline studies in the test area would be undertaken. Most studies to date have been conducted in the benthic environment. A baseline is needed specifically looking at the water column processes.

Additionally, the first year plan includes assessing the physical and chemical characteristics of the nodule wastes and sediments. Physical and biogeochemical models will be initiated.

In the second year, bottle and bag experiments on nutrient additions and sediments additions would be instigated at sea. Modeling efforts would look at the impact of enhanced nutrients and sediment loading on the ecosystem.

For the third year, we plan to undertake experiments on the interaction of high nutrients and sediment loading. Bottle and bag experiments on nodule waste additions will also be undertaken. Modeling efforts would continue to look at the impact of enhanced sediment and nodule waste loading incorporating field results where applicable.

In the fourth and fifth years, we could test the interaction between the nutrients, sediments and nodule waste. We also intend to develop risk assessment models for alternative mining strategies.

In the fifth year, we plan on compiling the data and results, organizing a workshop to discuss the results and provide inputs to the International Seabed Authority on mining discharge.

Requirements

Annual research cruises will be required; whether these are conducted by pioneer investors or others is unsettled. We also require experienced manpower in geology, biology, chemistry, physics, modeling and remote sensing.

The anticipated funding requirements are outlined below. There are eight cruises proposed; four cruises each in the Central Indian Ocean Basin (CCIOB) and the CCZ. There will be devices like sediment traps, current meters and all the gear going onto sediment traps and equipment for the analysis. An estimate is provided below.

Ship (4 cruises of 40 days each to CIOB and to CCZ)	\$ 3,200,000
Equipment for sample collection and analyses	\$ 4,000,000
Analyses	\$ 2,000,000
Workshop	\$ 100,000
Travel & subsistence of scientists	\$ 1,000,000
Total	\$ 10,300,000

Deliverables

What kinds of deliverables are expected from the proposed project? The primary outcome will be data and models to evaluate impacts, different mining techniques and mine-waste disposal strategies. Which depth would be good for discharge? We will also provide input for models to test during pilot mining.

The results will be used to assess pelagic-benthic coupling. A scenario envisioned is to add nutrients to the water column which will lead to new biological productivity, eventually raining down to the sea floor. This new productivity might be available for the benthos, so there would be a coupling of the pelagic and benthic communities. On a long-term basis, this coupling will have to be investigated.

Another consequence of the research will be an assessment of impacts on the carbon sequestration as siliceous diatoms are anticipated to dominate over calcareous Coccoliths. Questions on CO₂ uptake will need to be resolved.

Obviously, databases, reports, and publications will be produced to provide valuable input to assist the ISA in formulating policies.

SUMMARY OF DISCUSSIONS

On the question of whether the best discharge point would be as close as possible to the bottom, Dr. Nath responded that based on the assessment outlined in the presentation at depth there would be reduced effects.

An expert asked what the constraints or concerns were in discharging waste streams that close to the bottom to which Nath replied that there were two constraints: one was the cost of the piping in excess of 4000 m long and the other was the energy required to pump the discharge to the deep.

Dr. Koslow noted that the German proposal discharged below the oxygen minimum layer which suggested that the particles were out of the euphotic zone and below the point where they would interact with the oxygen minimum layer before going through chemical transformation. He added that for the CCZ, depth would be approximately 1000 or 1200 m. There would be the cost of the pipe, but the waste stream would probably sink by gravity, because the sediment plus cold water was denser than surface water. He added that both economic and environmental considerations needed to be assessed. Part of the purpose of the proposed research is to look at the environmental impact so that risk assessment studies could be undertaken to examine the environmental risk and economic choices prior to the decision-making made through the Seabed Authority and with the Contractors.

Another scientist mentioned that calculations for the pipe were prepared by Texas A & M University in the 1980's and was an insignificant component (0.0001%) of the total cost of activities.

Dr. Smith indicated that there were other issues that needed to be considered in this appraisal of environmental impacts. Currently, there were quite a few groups discussing the idea of iron enrichment to mitigate global warming for the purpose of earning carbon credits. He said it may seem like a wild idea but one possible scenario could be to use the plume discharge in the surface water to encourage iron enrichment of surface water, thus supporting carbon sequestration. He added there could actually be financial motives for mining operations to discharge in the surface water and understanding the impact in the surface waters would be relevant to this choice.

Dr. Koslow responded that one of the reasons the proposal was set up in a particular way was to enable a number of different options to be looked at because there were an awful lot of uncertainties in terms of how the material would be processed. There were a number of different options as to how it would be disposed of, what was going to be disposed, to what degree processing would be done on board and to what degree things will be taken back to shore. Until there was a consensus, Dr. Koslow said a variety of options and processes for environmental impact could be weighed.

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Chapter 8

Interannual Variability of Deep Sea Ecosystems and Environmental Impact Assessment of Potential Seabed Mining

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Introduction

Polymetallic nodules were discovered in the abyssal sea floor by the famous survey vessel “H.M.S Challenger” in 1873. It is estimated that the total reserves of polymetallic nodules in the world seabed is about $15 - 30 \times 10^{11}$ tons. Since these deposits have significance as a mineral ore containing great quantities of nickel, cobalt, copper and manganese, more and more countries and organizations are showing a greater interest in their research, prospecting and exploration. Seven entities, including the China Ocean Mineral Resources Research and Development Association (COMRA), have become contractors with the International Seabed Authority. With plans of work approved by the International Seabed Authority, contracts were concluded with all seven pioneer investors in 2001 for exploration of the polymetallic nodules in the Area under the UNCLOS regime. At present, research on polymetallic nodules in the world is concentrated in three areas as follows: the Clarion-Clipperton Fracture Zone (CCZ) in the Pacific Ocean, the central part of the Indian Ocean and the Peru Basin in the Pacific Ocean.

While taking into account the great commercial value to be obtained from mining polymetallic nodules, the protection and preservation of the ocean environment, especially the deep-sea environment impacted by potential deep-sea mining, has to be seriously considered. Some studies show that deep-sea sediments appear to be a major reservoir of biodiversity, by some estimates, harboring 10 – 100 million species of worms, crustaceans and mollusks. These estimates do not include microbes and also are thought to underestimate the number of species in deep-sea sediments because of limitation in sampling and analysis methods. Therefore, it is obvious that the deep-sea ecosystem plays an important role in regional ecosystems and is a significant part of the global ecosystem. Protecting the deep-sea environment and evaluating

the threat of potential nodule mining to biodiversity becomes a significant problem even in nodule prospecting and exploration.

In 1982, an international legal framework was established by United Nations Convention on the Law of Sea (UNCLOS) for the usage of the oceans and their resources. In the law, resources of the seabed and ocean floor are defined as the common heritage of mankind. The exploration and exploitation of resources in the ocean must be accorded with the plan of work approved by the International Seabed Authority. Subsequently, "*Regulations on Prospecting and Exploration for Polymetallic Nodules in the Area*" was adopted and published by the International Seabed Authority. Part V of this document focuses on the protection and preservation of the marine environment. The abovementioned regulations provide the basic legal guarantee for protection of the deep-sea environment.

Significance of the Natural Variability of the baseline in Deep Sea

What can we do scientifically to protect the environment impacted by potential deep-sea mining? As the International Seabed Authority is responsible for developing and establishing guidelines for the assessment of environment impacts of exploration and exploitation, we need to provide a scientific basis for this assessment. The degrees of seabed mining impact can be classified as no obvious impact, obvious impact or serious impact. At present a key problem is that it is difficult to classify these impacts scientifically in the deep-sea environment. Some standards for shallow water and coastal oceans have been established, known as EIA system. There are no standards or experience for assessing deep-sea mining impacts. Establishing conditions that are typical in order to classify the degree of impacts and determining standards that have social, economic and scientific significance are still questions that we cannot answer. Therefore, to effectively assess and protect the deep-sea environment, first of all, we must establish scientific standards for assessing mining environment impact.

Since the beginning of the large scale exploration for polymetallic nodules in the 1950s to 1960s, some industrial countries and international consortia have carried out a series of environmental research projects, such as "CLB", "DPE", "DOMES", "DISCOL", "BIE" and "JET", etc. These impact experiments helped establish standards for assessing the impact of mining. However, due to lack of knowledge of the natural variability of the baseline in spatial and temporal scales, some of the results of these experiments cannot be explained reasonably. In other words, to establish effective deep-sea environmental standards, parameters must be based on total and exacting understanding of the natural baseline (including biologic, chemical, hydrological, and geological baseline) over reasonable changes in temporal and spatial scales (*Figure 1*).

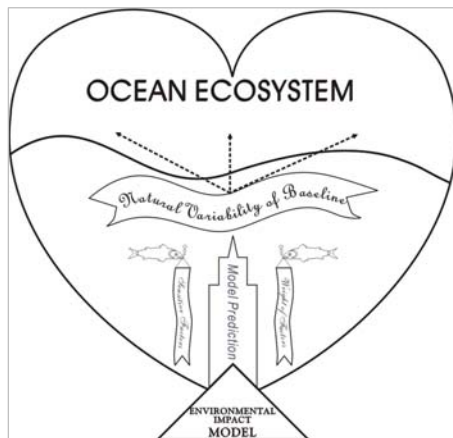


Figure 1.: Deep-sea environment impact model.

Until now, there are some basic questions relative to the natural baseline that have not been answered. For example, we do not know the precise number of biological species and the exact geographic range of biological species in the polymetallic nodule areas. Moreover, we also lack enough knowledge of the natural variability on spatial and temporal scale. In fact, the deep-sea ecosystem is not as calm as imagined several decades ago. Many biological, chemical and hydrological, and geological activities actively and dynamically take place even at 5,000 m water depth. Recent expeditions have shown that the natural variability of the baseline in the deep-sea environment can be unexpectedly large temporally and spatially. Particularly important scientific questions in the CCZ concerning this variability include the following:

- To what degree does variability in the upper ocean ecosystem result from processes such as El Nino-Southern Oscillation (ENSO) events?
- What is the influence of laterally derived deep ocean water such as the Antarctic Bottom Water (AABW) on benthic ecosystems?
- How tightly are the upper ocean and abyssal benthic ecosystems coupled?
- What are the spatial scales of variability in biological communities, geochemical cycling, sediment types and topographical units?

Therefore, data obtained at one time or at one site and used as the baseline of the deep-sea environment to assess non-natural disturbances may mislead the international community in its understanding of the deep-sea environment and in the establishment of standards for environmental assessment. In order to effectively distinguish the non-natural disturbance influencing deep-sea ecosystem from the natural variability and to also accurately assess the impact of non-natural disturbance on deep-sea ecosystem, research on natural variability of baseline is necessary.

“NaVaBa” Project in China

Since registering as a Pioneer Investor in 1991 with the International Seabed Authority, COMRA has paid great attention to the protection of the deep-sea ecosystem. While surveying for polymetallic nodules in its Pioneer Area (in western part of CCZ) in the Pacific, COMRA simultaneously developed an environmental protection study. In 1993, COMRA investigated and collected certain baseline data. In late 1995, the programme for deep-sea environmental

research and protection became independent from the programmes for resource prospecting. Taking into account the results and experiences gained by previous impact experiments, COMRA recognized that it was less valuable to carry out an additional impact experiment similar to DISCOL or BIE. As a substitute, and as a contribution to deep-sea environmental protection, a project called “Natural Variability of Baseline”, for short “NaVaBa”, was initiated by COMRA in 1995. This project plans to collect biological, hydrological, chemical and geological baseline data in the same stations during the same season interannually to establish an environmental baseline in COMRA’s pioneer area and to determine the range of natural variability of selected environmental parameters both temporally and spatially. The NaVaBa project will help to isolate the parameters that are sensitive to natural variability and non-natural disturbances to the ecosystem. More than forty parameters are measured in China’s NaVaBa project.

Natural Variability of the Baseline in COMRA’s Contract Area

Biological Baseline

Chlorophyll-a and primary production

Chlorophyll-a concentrations from 0 to 200 m water depth ranged from 17.35 - 26.50 mg/m² (average 22.91 mg/m²) in the eastern area and 27.05 - 37.52 mg/m² (average 31.58 mg/m²) in the western area in COMRA’s contract area, respectively. The interannual variation of the vertical distribution of chlorophyll-a was determined (Figure 2). The sub-surface peak of Chlorophyll-a concentration appeared at 75 m during surveys in both 1997 and 1998, while the peak was observed at a shallower depth of 50 m in 1999. Size fractionation of chlorophyll-a showed that the pico fraction (passing through 2 µm filters) comprised 80% of total chlorophyll-a. The average primary production in 1999 was obviously higher than that in 1997 and in 1998 (Table 1).

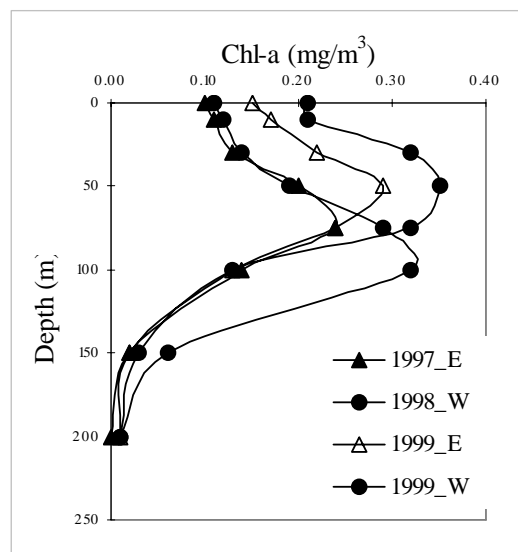


Figure 2: Interannual variation of vertical distribution of chlorophyll-a concentration in COMRA area (E: Eastern area in COMRA pioneer area; W: Western area in COMRA pioneer area).

Table 1: The interannual variation of primary production (mgC/m²•d) in COMRA area.

	1997	1998	1999
Western area		119.0	222.3
Eastern area	89.0		185.6

Fish egg and fish larva

The density of fish egg and larva has obvious spatial-temporal variations. It was higher in the eastern area than in the western area and higher significantly in 1999 than in 1997 and in 1998 (Figure 3).

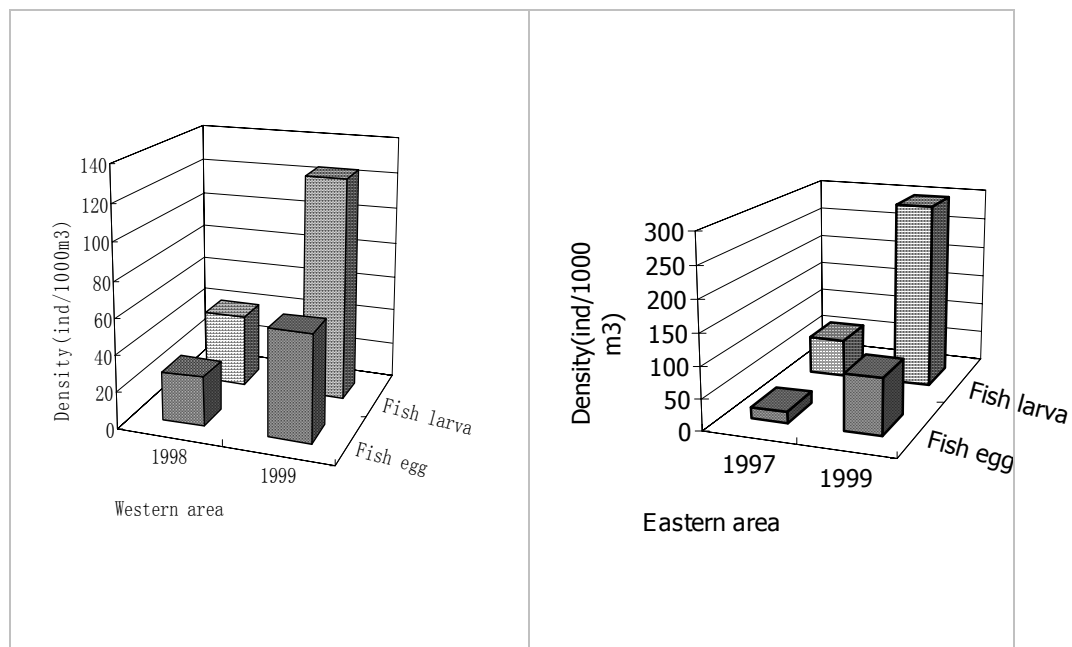


Figure 3: The interannual variation of the density of fish egg and larva.

Megafauna

There are 48 taxa of megafauna based on the preliminary analysis of nearly 30 hours of videotapes and 2000 bottom photographs from the COMRA's Pioneer Area. Among them, holothuroidea, ophiuroidea, porifera and osteichthyes are the main taxa of the megafaunal community. The density of megafauna was 114 individuals per 10,000 m² in the eastern area and 146 individuals per 10,000 m² in the western area, respectively. It is obvious that the megafaunal density in the western area is higher than that in the eastern area. The density is mainly contributed by small brittle stars in the western area, whereas it is mainly composed of bigger sea cucumbers in the eastern area

Macrofauna

The average density of macrofauna collected by box corers was 92.4 individuals/m³ in the eastern area and 136.0 individuals/m³ in the western area, respectively.

Meiofauna

The spatial-temporal variations of meiofauna density were analyzed on the basis of samples collected by an 8-tube multicorer. *Figure 4* shows that meiofaunal density in the eastern area is much higher than in the western area and significantly higher in 2001 than in 1998 and in 1999. The spatial distribution of density within an individual reference zone was also quite uneven.

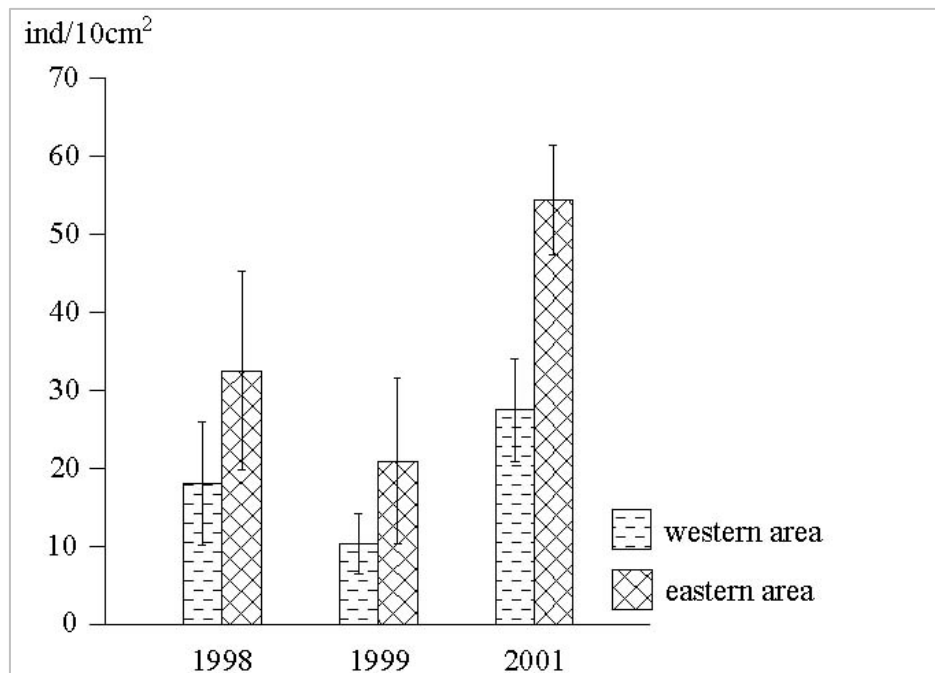


Figure 4: Spatio-temporal variations of meiofauna density in COMRA's area.

Chemical Baseline

Nutrients in seawater

The distribution characteristics of nutrients are different in the eastern and western areas (*Figure 5*). The pH value, dissolved oxygen, nitrite and nitrate in the western area were higher than those in the eastern area at the same level in water depth. But the concentrations of phosphate and silicate in the western area were lower than that in the eastern area. The interannual variation of the nutrients was also very obvious. Based on the results of nutrients and hydrographic parameters during 1997 – 1999, we found that the El Niño event, which

occurred in 1997 – 1998, remarkably affected the COMRA contract area in the Northeast Pacific. Because of the influences of El Niño and La Nina events, the concentrations and distribution patterns of nutrients in the seawater column correspond to the variability of the thickness of the mixed layer in the upper ocean. Nutrients concentrations in seawater decreased during the strong El Niño event from 1997 to 1998, and increased again in 1999.

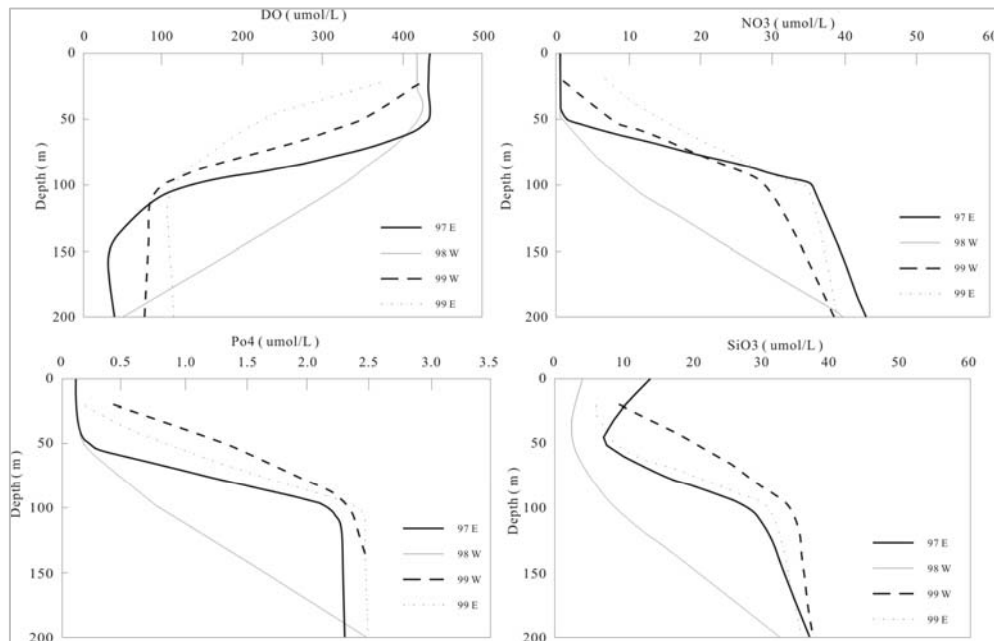


Figure 5: Interannual variation of dissolved oxygen and nutrients in COMRA's area during 1997-1999 ("E" and "W" represent eastern area and western area, respectively).

Compared with the large-scale variation in surface seawater, the nutrients in bottom waters also show some variability to some extent with the same trends as in the surface water. It is suggested that there seems to be some relationship and interactions between surface water and bottom water.

Chemical Elements in Porewater

The geochemical behavior of major elements in pore-water is controlled by the interaction between sediments and seawater trapped in sediments. In COMRA's contract area, relative to seawater, the porewaters are enriched in potassium, sodium, and depleted in calcium and magnesium. The nutrients concentration in porewater is the results of organic matter decomposition in early diagenesis, and is controlled by the redox conditions in the sediment. The trace metal distributions in porewater are also controlled by the redox conditions in sediments, coupled with organic matter degradation. Mn, Ni and Cu showed their maximum

concentration gradients just at the sediment/water interface. Based on calculation using Fick's first law modified for sediment, the direction of Mn flux was mainly from overlying water into the sediment, and the seawater was the main source of Mn in the sediments. The Cu and Ni fluxes were from the sediment into overlying water. Compared to the Cu and Ni fluxes from surface water to bottom water, the maximum concentration gradients of Cu and Ni at sediment/water interface was sustained by the early diagenesis of organic matter, which settled probably from the surface water.

Physical Baseline

During the 1997 – 1998 El Niño events, seawater in the upper ocean warmed and the thermocline became depressed. The thermocline increased again in 1999 when the El Niño event came to an end and cold La Niña conditions were established in this area. The bottom temperature recorded by the mooring system also varied periodically within the time period (Figure 6).

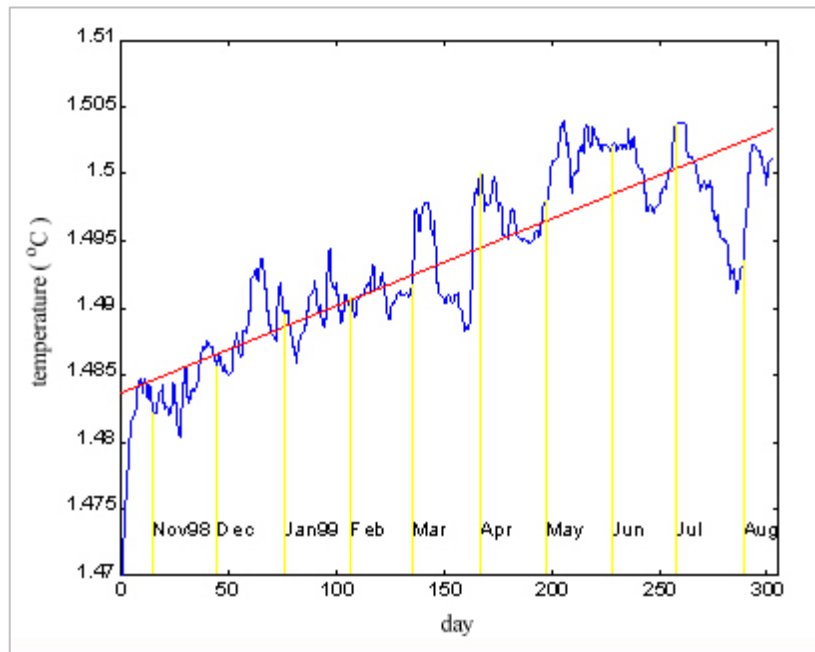


Figure 6: Daily variations of bottom temperature in COMRA's Eastern area.

Geological Baseline

In the open ocean, the sedimentation rate is usually very slow. In the CCZ, the sedimentation rates are variable from less than 0.5 mm/ka to several mm/ka. In COMRA's pioneer area, the predominant sediments are siliceous clays, siliceous oozes and pelagic clays, and can be divided into two units based on distinctive color and bio-disturbance properties (Figure 7). In the Eastern area, the sediment is characterized by homogenous yellow to yellowish brown sediments (Unit A). This homogenous yellowish brown sediment overlies dark brown sediments (Unit B) at Western area. The thickness of the dark brown layer is

different among stations. In some cores, these two types of sediments alternate in the lower part of the core. Based on radiolarian fossils, Unit A is assigned to Quaternary and Unit B is assigned to Tertiary. The color boundary might be the hiatus between Quaternary and Tertiary. In the Eastern Area, a record of low temperature hydrothermal mineral assemblage, which is composed of autogenetic quartz, albite and barite was observed. This perhaps suggests that there was a thermal spring or geothermic anomaly in the geologic history of the Eastern area.

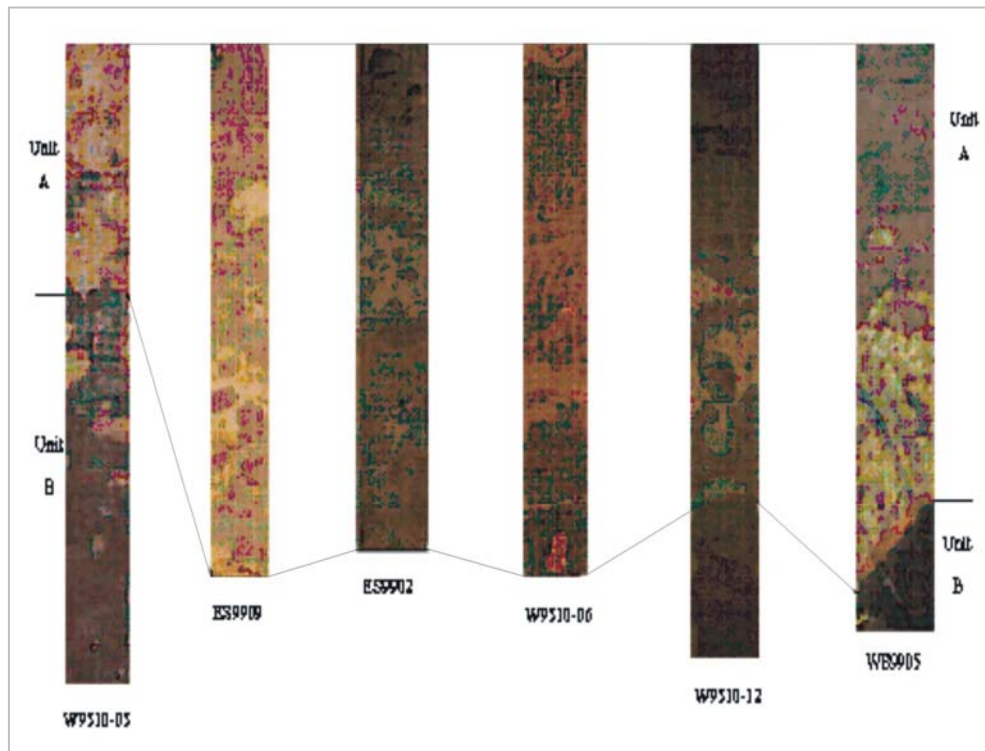


Figure 7: Surface sediment profiles in COMRA's Area.

Part II

ACTIVITIES OF CONTRACTORS IN THE MEDIUM
TERM AND PROSPECTS FOR COLLABORATION
IN IDENTIFIED RESEARCH PROJECTS

In view of the fact that results of research topics will be of direct benefit to contractors in their efforts to protect and preserve the marine environment from their activities, the workshop sought to obtain an idea of the research that is planned in any of the above subject areas, cruises that each contractor has planned for the next five years (study sites, cruises, duration of cruises, ports of call, general goal of cruises), berth space and wire time available to collaborators, and cost to collaborators of adding days to a cruise. It was also useful to have each contractor inform the workshop of what it would like to obtain from collaborations.

Chapter 9

Introduction to the Environmental Research Programme of COMRA

Bin MAO, China Ocean Mineral Resources R & D Association

Huaiyang ZHOU, Second Institute of Oceanography, State Oceanic Administration, PR China

Preliminary Results of NaVaBa Project

In order to identify the effects of natural variability and non-natural disturbances on the marine ecosystem, the China Ocean Mineral Resources R & D Association (COMRA) launched an environmental research project named as “Natural Variability of Baseline (NaVaBa)” in 1996 to investigate and study the natural spatial and temporal variability in the deep-ocean ecosystem in COMRA’s contract area. This project has been carried out during four cruises over the past five years (1997 – 2001). The results obtained from the biological, chemical, physical and geological surveys in the area show that the project is critical to the marine environmental study in the deep-ocean.

Impacts of 1997 – 1998 El Niño Event on the Upper Ocean Ecosystem

A significant inter-annual variation was found in the chlorophyll *a* concentration, primary production and fish larval density, and their values were considerably higher in 1999 than in 1997 and in 1998 (*Figures 1 and 2*). There is also an inter-annual variation in the values of concentrations of nutrients, and values obviously decreased in 1997-1998 and returned to normal level in 1999. These were considered inter-annual variation responses to the 1997–1998 El Niño Event.

Figure 1: Interannual variation of vertical distribution of chlorophyll a concentration.

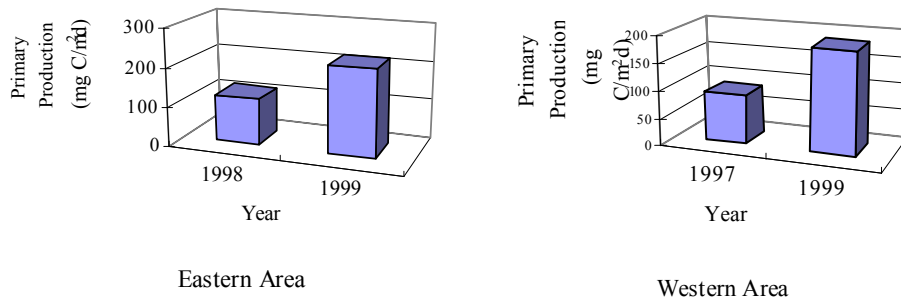
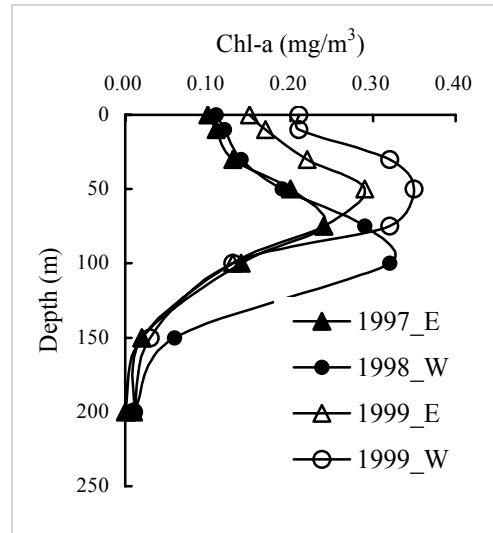


Figure 2: Interannual variation of primary production.

Impact of Antarctic Bottom Water (AABW) on the benthic Environment

The bottom temperature in the contract area gradually rises from the western area to the eastern area (Figure 3), while dissolved oxygen content gradually decreases. Their spatial variations are related to Antarctic Bottom Water. The western area is situated upstream of a diffuse axis of Antarctic Bottom Water, so the bottom temperature is lower in the western area than in the eastern area.

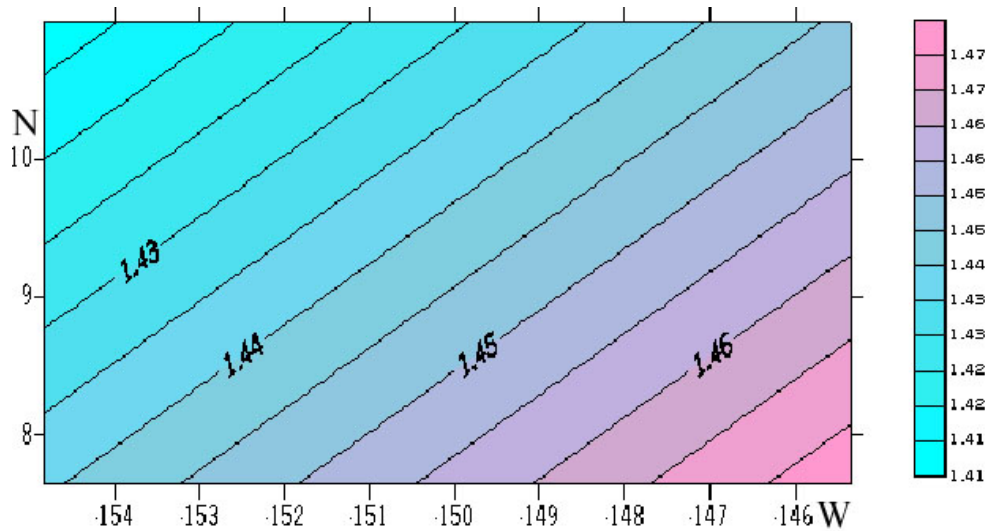


Figure 3: *Spatial variation of bottom temperature (°C) in COMRA's contract area.*

However, the above results are very preliminary. In order to further understand the natural variability of the deep-sea ecosystem and its mechanism, COMRA will continue to carry out NaVaBa research over the next five years. Considering the large spatial and temporal scale of the project, international cooperation in this field is essential.

Five Year Plan of the NaVaBa Project

Cruise Plan

During 2003 to 2007, at least three cruises are planned for environmental research in COMRA's contract and adjacent areas, on board R/V "Da Yang Yi Hao" equipped with the appropriate instruments for ocean expedition. Generally, the duration of each cruise is about six months divided into five cruise legs, among which there is one leg dedicated to the NaVaBa Project. The NaVaBa leg is usually arranged for the summer and the ship calls at Honolulu harbour.

General Objectives of NaVaBa Cruises

The general goal of the NaVaBa cruises is to understand the natural variability of deep-ocean ecosystem and its mechanism. The investigation will focus on the following four topics.

- The spatial scales of variability of environmental baseline.
- Degree of natural variability in the upper ocean ecosystem caused by oceanographic processes such as the El Niño-Southern Oscillation (ENSO) and the La Nina events.
- Impact of laterally derived deep ocean water such as Antarctic Bottom Water (AABW) on benthic ecosystem, and

- Coupling between the upper ocean ecosystem and the benthic ecosystem

Survey Areas and Sampling Stations

The NaVaBa project is carried out in COMRA’s contract area in the western part of the Clarion-Clipperton Fracture Zone (CCZ) of the northeast Pacific Ocean basin. The contract area is divided into the eastern area and western area on the boundary of 150°W. The eastern area and western areas are located at approximately 141° to 149° W, 7° to 10° N and 151° to 155° W, 8° to 11°30’ N, respectively (Figure 4).

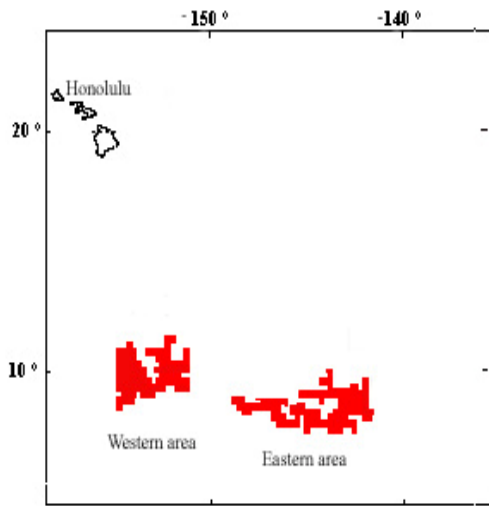


Figure 4: Location of the survey areas.

A reference area and a south-north section are designed in the eastern and western areas respectively (Figure 5). Each section and each reference area have 6 and 7 stations respectively. The two reference areas are located at approximately 145° 21’ to 145° 24’ W, 8° 20’ to 8° 24’ N and 154° 00’ to 154° 05’ W, 10° 00’ to 10° 05’ N, respectively.

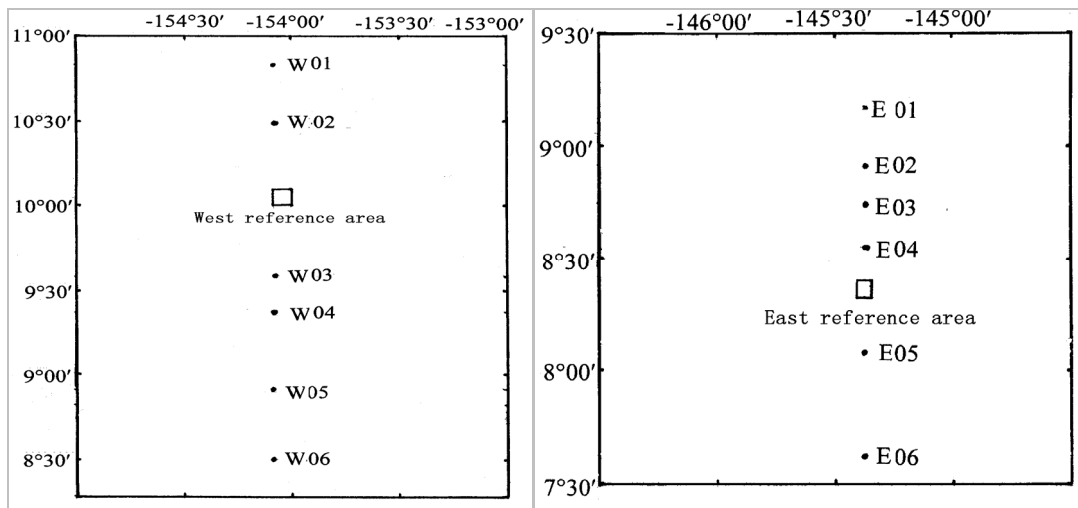


Figure 5: Location of sampling stations.

Features to be investigated

The following basic oceanographic parameters will be investigated:

Biological Baseline

chlorophyll *a* concentration, primary production, species composition and biomass of phytoplankton, zooplankton and fish larvae, species composition and abundance of megafauna, macrofauna, meiofauna and bacteria in the deep seafloor

Chemical Baseline

Sea water: pH, DO, PO₄³⁻-P, SiO₃²⁻-Si, NO₃-N, NO₂-N, NH₃-N, total alkalinity, TOC, DOC and POC

Surface sediment: DO, pH, PO₄³⁻-P, SiO₃²⁻-Si, NO₃-N, NO₂-N, NH₃-N and trace elements

Physical Baseline

Sea current, temperature, salinity and concentration of suspended particulate

Characteristics of Surface Sediment

Geotechnics

Penetration resistance (Pc), shear strength (Sc), water content (ω) and density (ρ) of soil, *etc.*

Meteorology and Ocean Wave

Wave height, wind speed, wind direction, air temperature, air pressure, relative humidity and surface water temperature

Research Vessel and Equipment

The deep-sea research vessel “Da Yang Yi Hao” is designed to engage in multi-disciplinary survey in the oceans. Its main particulars and equipment are as follows:

Principal Particulars

Gross tonnage: 5600 ton

Service speed: 11 knots

Endurance: 45 days

Complement: crew 38, research personnel and others 37; Total 75

Laboratories

Name of Laboratory	Area (m ²)
Geology laboratory	26.8
Chemistry laboratory	28.0
Biology laboratory	15.0
Hydrology laboratory	10.0
Deep-tow laboratory	31.0
Seismic laboratory	15.0
Magnetic laboratory	9.2
Underwater (AUV) laboratory	35.0
Seabeam and sub-bottom profiler laboratory	46.7
Network and data center	48.5
Biological sample storage	13.0

Main Survey Equipment

Seabeam
 Deep-tow system
 Deep-sea camera
 CTD + ADCP
 Box corer
 Multiple corer
 Piston core sampler
 Deep-sea current meter chain
 Sediment trap
 Dredger
 Sub-bottom profiler
 AUV

Survey Support Equipment:

Dynamical positioning system
 Heavy-duty winch with 10,000 m wire
 Armored cable winch with 10,000 m wire
 Armored optical cable winch with 10,000 m wire
 Hydrographic winch with 10,000 m wire

Resources Available for Collaborative Studies

Ship Time

Assuming that three survey areas distributed from East to West in the CCZ are investigated, 45 days of ship time is available for collaborative studies every cruise, more

specifically, 24 days of transit time for round trip between Honolulu and survey areas in CCZ (Figure 6), and 21 days of working time.

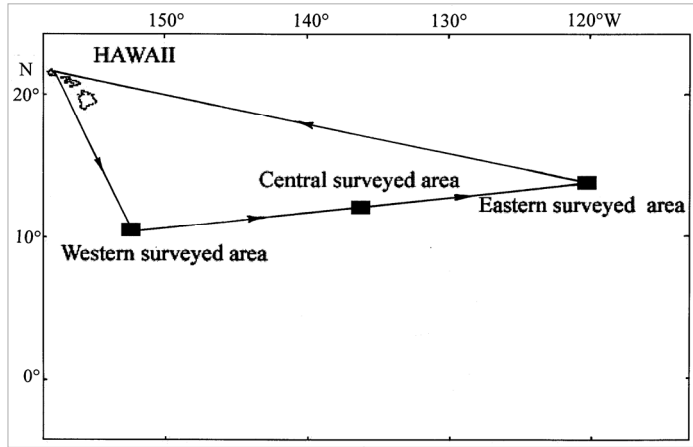


Figure 6: Cruise tracks and surveyed areas of collaborative studies in CCZ.

Equipment

The survey equipment onboard R/V “Da Yang Yi Hao” can meet investigation of natural spatial and temporal variability in deep-ocean ecosystems.

Personnel

At present, there are 20 environmental scientists and technicians engaged in NaVaBa research in China. They are 9 marine biologists, 8 marine chemists and geochemists, and 3 physical oceanographers.

Collaborative Cost

Ship time costs \$30000 per day.

Recommendations for International Cooperation

COMRA has made great efforts in investigating the natural spatial and temporal variability in the deep-sea ecosystem since 1997. Although COMRA has collected a series of data in this field and obtained some preliminary results over the past five years, the natural variability in the nodule province ecosystem and the processes controlling this variability are still poorly understood. Thus, international cooperation in this field is needed. COMRA will continue its efforts in carrying out this project.

COMRA appreciates the effort made by the International Seabed Authority in preparing the *Recommendations for the ISA workshop in July 2002*. The four research topics made in the recommendations are of great importance in addressing *prospects for international collaboration in marine environmental research to enhance understanding of the deep-sea environment*. However, these four research tasks may not be fulfilled at one time because of limited

resources. To share the limited resources so as to cost-effectively conduct these tasks, the best way is to integrate these four research proposals into a scientific programme to be carried out step by step. In this regard, the International Seabed Authority should play an important role.

COMRA will take an active part in the international collaboration in marine environmental research

SUMMARY OF DISCUSSIONS

Dr. Smith voiced his agreement with the observations and the goals of NaVaBa adding that it dovetailed well with the goals of the Kaplan study of biodiversity. He continued that it would be productive for both Kaplan and COMRA to study biodiversity issues at the western end of the CCZ in collaboration with the NaVaBa cruises.

In answer to various queries about the ship, Mr. Mao advised that the ship had modern labs with low temperatures which was available on every cruise and AUV survey equipment operating at depths up to 6,000m. He added that the ship had dynamic positioning and was currently deploying time series sediment traps at depths of 200m above the seafloor and hoped that COMRA would support more sediment traps in the future.

On the question of inviting non-contractors to participate in the cruise, Mr. Mao said there was some bilateral cooperation from foreign scientists but on a very small scale.

When asked about collaboration and whether ship time would be made available, Mr. Mao acknowledged that it could be done and that the cruise was generally 45-days long but could be longer.

Chapter 10

Japan's Nodule Development Environmental Impact Study

Masao Someya and Tomohiko Fukushima, Deep Ocean Resources Development Co., Ltd

Introduction

The Metal Mining Agency of Japan (MMAJ) and Deep Ocean Resources Development Co., Ltd. (DORD) initiated “the environmental study for manganese nodules development” in 1989, under a cooperative agreement with the National Oceanic and Atmospheric Administration of the USA (NOAA). This project was carried out in the Clarion – Clipperton Fracture Zone in the central Pacific Ocean, Toyama Bay in the western part of Japanese coastal region and on seamounts in the western Pacific (*Figure 1*). The goals of these studies were to obtain environmental baseline information, to evaluate the magnitude of impact and to predict the effects of commercial mining on the ocean environment.

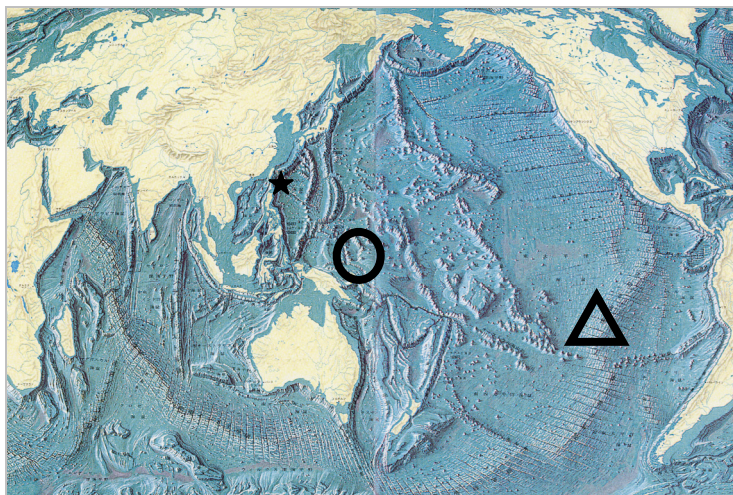


Figure 1: Location map of the Environmental Studies conducted by Japan. **Triangle**: Japanese claimed area in the central Pacific Ocean. **Circle**: Western Pacific where field test “DIET” was conducted. **Star**: Toyama Bay where deep-sea water discharge test was conducted.

Potential Impact of Deep-Sea Mining

With regard to environmental impact concerns, the following issues were addressed as the most significant for potential impacts caused by commercial mining (*Figure 2*):

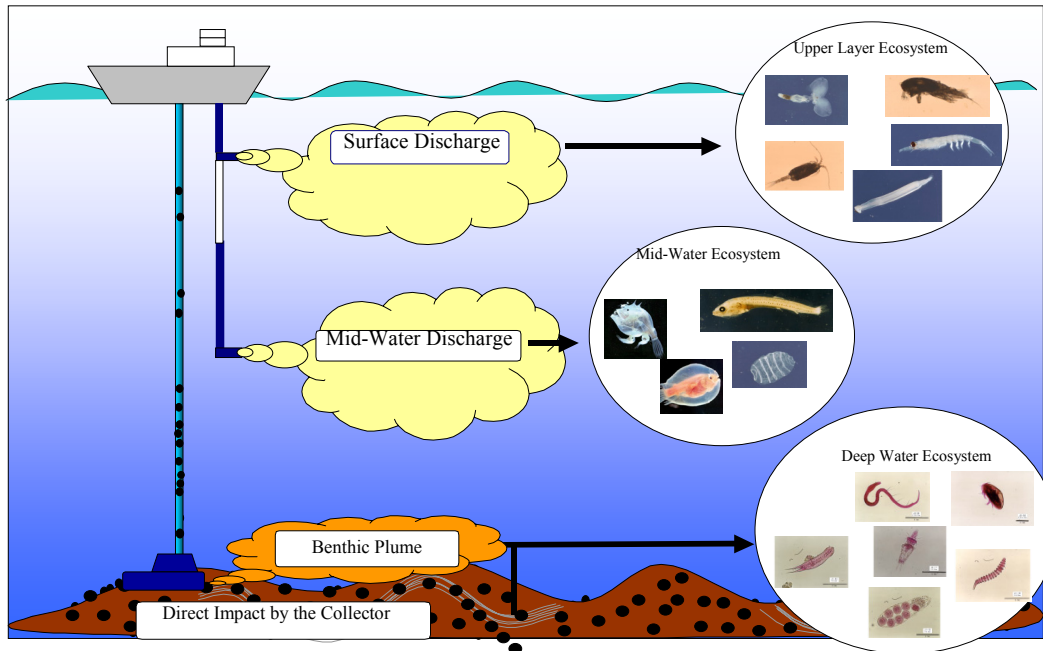


Figure 2: Potential impact of deep-sea mining.

Surface Discharge

A hydraulic collector system would be inevitable with water discharged into either the surface or mid-water. Discharged water is anticipated to be cold, nutrient-rich, highly turbid and rich in heavy metals. Discharged water may affect the phytoplankton or zooplankton in the upper layers of the ocean.

Mid-Water Discharge

Similar to the case for water discharged into the surface, water discharged in the mid-water might have an effect on the zooplankton or micronekton living in the mid-water depth.

Direct Impact

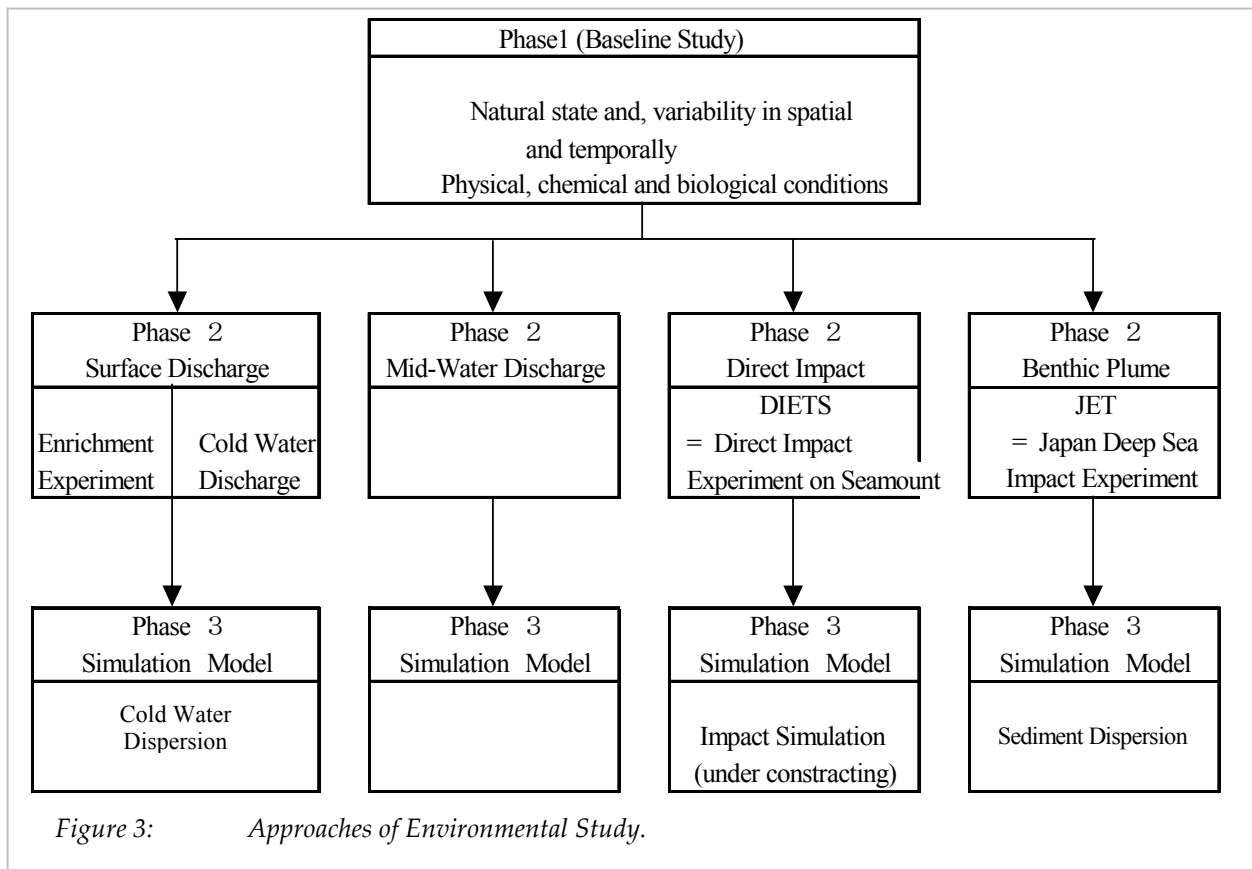
A nodules collector moves across the seafloor, it may destroy or compress the benthic organisms, which are the main component of the benthic ecosystem.

Benthic Plume

The collectors may create a benthic plume, which would change the concentration of organic materials on the seafloor – a principal food source for benthic organisms.

Approach to Study

The environmental study for manganese nodules development consist of three phases as outlined below (Figure 3 and Table 1):



Phase 1. Baseline Study

The baseline study was conducted to understand the natural environmental conditions in the mining area, which includes spatial and temporal variability.

Phase 2. Impact Assessment Study

The impact assessment study was conducted to evaluate the potential impacts by deep-sea mining

Phase 3. Impact Prediction

Based on the results of baseline study and impact assessment study, the possible harmful effects caused by larger scale of mining activities will be predicted.

Table 1: Time Schedules of the Environmental Study by Japan.

Upper Layer Environment Study														
Phase 1 Fiscal Year	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Phase 1 (Baseline Study)														
Physical Environment (SD)				→	→	→	→	→	→	→				
Physical Environment (MD)				→	→	→	→	→	→	→				
Water Quality (SD)			→	→	→				→	→				
Water Quality (MD)			→	→	→				→	→				
Biological Environment (SD)			→	→	→				→	→				
Biological Environment (MD)			→	→	→				→	→				
Phase 2 (Impact Assessment Study)														
Enrichment Experiment (SD)			---O---	---O---										
Cold Water Discharge (MD)	-----	---O---												
Phase 3 (Impact Prediction)														
Cold Water Dispersion Model (SD)	→	→	→	→	→	→	→	→	→	→				
Ecological Model Development (SD)													→	→
SD: Surface Discharge, MD: Mid-Water Discharge, O: timing of the artificial impact														

Benthic Environment Study														
Phase 1 Fiscal Year	1989	1990	1991	1992	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002
Phase 1 (Baseline Study)														
Geological Environment (DI)			→	→	→					-----	-----	-----	-----	-----
Geological Environment (BP)			→	→	→					-----	-----	-----	-----	-----
Chemical Environment (DI)			→	→	→					-----	-----	-----	-----	-----
Chemical Environment (BP)			→	→	→					-----	-----	-----	-----	-----
Biological Environment (DI)			→	→	→					-----	-----	-----	-----	-----
Biological Environment (BP)			→	→	→					-----	-----	-----	-----	-----
Phase 2 (Impact Assessment Study)														
JETS (BP)						---O---	→	→	→					
DIETS (DI)										-----	-----	---O---	-----	-----
Phase 3 (Impact Prediction)														
Dispersion Model of Resuspended Sediment (BP)						→	→	→	→	→				
Ecological Model Development (DI)													→	→
DI: Direct Impact by the mining collector, BP: Benthic Plume, O: timing of the artificial impact														

Surface Discharge

Studies on the surface discharge are summarized in *Table 2*.

Table 2: Output related to the study of effects of surface discharge.

Subject	International Journal or Proceedings	Technical Reports
Concept or Scheme	12, 13, 14, 21	T1, T2, T3, T4
Phase 1		
Temperature & Salinity	19, 21	T5, T6, T7, T8, T9, T10
Chemical Properties	19, 21	T5, T6, T7, T8, T9, T10
Organisms		
Pico-, nanoplankton	18	T5, T6, T7, T8, T9, T10
Microplankton	18	T5, T6, T7, T8, T9, T10
Macroplankton	18	T5, T6, T7, T8, T9, T10
Phase 2		
Enrichment Experiment	9, 21	T7, T8, T9, T10
Cold Water Discharge	19	T3, T4
Phase 3		
Numerical Model	27, 30	T3, T4, T5, T6

Note: Number indicates literature number, see Chapter Appendix.

Phase 1. Baseline Study

Phase 1 study was conducted in the central Pacific Ocean.

Study subject: physical and chemical profiles in the water column (water temperature, salinity, nutrients), planktonic organism (bacterial-, pico-, nano-, micro-, macro-plankton), chlorophyll and phaeopigment.

Methodology: CTD observation, water sampling by the Rosette sampler, net sampling by the ORI and NOPAC

Phase 2. Impact Assessment Study

Two approaches were attempted in Phase 2 of the study: experiments on the effects of deep-sea water on growth and composition of phytoplankton communities (hereafter “enrichment experiment”) and field tests conducted in Toyama Bay to address the dispersion process of deep-sea water discharged into the surface of the sea (hereafter “cold water discharge”).

Study subject:

Enrichment experiment: water temperature, planktonic organism (bacterial-, pico-, nano-plankton), chlorophyll and phaeopigment.

Cold Water Discharge: water temperature monitoring (spatial and temporal).

Methodology:

Enrichment experiment: water bottle methods.

Cold Water Discharge: thermistor-chain system, etc.

Phase 3. Impact Prediction

The impact of water discharge on the sea floor was predicted by numerical model. Results of enrichment experiment have not been reflected in simulation model.

Mid-Water Discharge

Studies on mid-water discharge are summarized in *Table 3*.

Table 3: Output related to the study of the effects of mid-water discharge.

Subject	International Journal or Proceedings	Technical Reports
Concept or Scheme	12, 13	T22, T23
Phase 1		
Temperature & Salinity		T22, T23
Chemical Properties		T22, T23
Organisms		T22, T23
Pico-, nanoplankton		T22, T23
Microplankton		T22, T23
Macroplankton		T22, T23
Phase 2		
Phase 3		

Note: Number indicates literature number, see Chapter Appendix.

Phase 1. Baseline Study

Phase 1 study was conducted in the western Pacific Ocean.

Study Subject: physical and chemical profiles in the water column (water temperature, salinity, and nutrients), biological profiles (micro plankton, micronekton)

Methodology: CTD observation, water sampling by the Rosette sampler, net sampling by the ORI.

The impact assessment study and the impact prediction study in mid-water depth have not been carried out yet.

Direct Impact by the Mining Collector

Studies on the impact of the mining collector are summarized in *Table 4*.

Table 4: Output related to the study of the direct impact by the mining collector.

Subject	International Journal or Proceedings	Technical Reports
Concept or Scheme	21	T22 to T25
Phase 1		
Water Column		
Sedimentation		T24
Current		T24
Sediment		
Chemical Properties		T22 to T25
Geological Properties		T22 to T25
Organisms		
Sedimentary Bacteria	26	T22, T23, T24
Nanobenthos	26	T22, T23, T24
Meiobenthos	21,26	T22 to T25
Macrobenthos	26	T22, T23, T24
Megabenthos		T22, T23, T24
Phase 2		
Concept or Scheme	21, 36, 40	T22 to T25
New Methods	39	T22, T23
Disturbance Process	21, 37, 38, 39	T22 to T25
Sediment		
Chemical Properties		T24, T25
Organisms		
Sedimentary Bacteria	26	T24, T25
Nanobenthos	26	T24, T25
Meiobenthos	26	T22 to T25
Macrobenthos	26	T22 to T25
Megabenthos		T24, T25
Phase 3		
Numerical Model		T24, T25

Note: Number indicates literature number, see Chapter Appendix.

Phase 1. Baseline Study

Phase 1 study was conducted in the western Pacific Ocean.

Study Subject: Chemical composition of the sediment (total organic carbon, total nitrogen, calcium carbonate, biogenic silica), geological properties of the sediment (grain size, shear strength, penetrate strength), physical profiles (current, sedimentation rate), and benthic animals (sedimentary bacteria, nano-, meio-, macro- and megabenthos).

Methodology: Sediment sampling by multiple corer and box corer, mooring system observation using current meter and sediment trap, video / photograph observation.

Phase 2. Impact Assessment Study

For Phase 2 studies, a field experiment named the Direct Impact Experiment on Seamount (DIETS) was conducted.

Study Subject: Chemical compositions of the sediment (total organic carbon, total nitrogen, calcium carbonate, biogenic silica), geological feature of the sediment (grain size, shear strength, penetrate strength), physical profiles (current and sedimentation), benthic animals (sedimentary bacteria, nano-, meio-, macro- and megabenthos)

Methodology: Sediment sampling by multiple corer and box corer, mooring system observation using current meter and sediment trap, video / photograph observation.

Phase 3. Impact Prediction

For Phase 3, a numerical model, which simulates the ecological changes by the direct impact, is under development.

Benthic Plume

Studies on the benthic plume are summarized in *Table 5*.

Table 5: Output related to the study of the benthic plume.

Subject	International Journal or Proceedings	Technical Reports
Concept or Scheme	12, 13, 14, 21, 28, 30, 33	T9 to T14
Methods	21, 22, 29	T9 to T14
Phase 1		
Water Column		
Sedimentation	8	T9 to T14
Current	30	T9 to T14
Sediment		

Chemical Properties	7, 10, 11	T9 to T14
Geological Properties		T9 to T14
Organisms		T9 to T14
Sedimentary Bacteria	15, 22	T9 to T14
Meiobenthos	15, 16, 21, 22, 24, 25	T9 to T14
Macrobenthos	6, 22	T9 to T14
Megabenthos	5, 17, 22	T9 to T14
Phase 2		
Concept or Scheme	4, 21, 36	T9 to T14
New Methods	32, 33, 35, 41, 42	T13, T14
Disturbance Process	2, 21, 31	
Water Column		
Sedimentation	8, 29	T15 to T22
Currents	30	T9 to T14
Sediment		
Resedimentation	1, 32, 33, 35, 38, 41, 42	T15 to T22
Chemical Properties	7, 10, 11	T15 to T22
Geological Properties		T15 to T22
Organisms		
Sedimentary Bacteria	15, 22	T15 to T22
Meibenthos	15, 16, 22, 25	T15 to T22
Macrobenthos	6, 21	T15 to T22
Megabenthos	5, 17, 22	T15 to T22
Phase 3		
Numerical Model	3, 19, 27	T9 to T22

Note: Number indicates literature number, see Chapter Appendix.

Phase 1. Baseline study

Phase 1 study was conducted in the Japanese contract area in the central Pacific Ocean.

Study Subject: nutrients in the pore water, chemical compositions of the sediment (total organic carbon, total nitrogen, calcium carbonate, biogenic silica), geological feature of the sediment (grain size, shear strength, penetrate strength), physical profiles (current, sedimentation rate), benthic animals (sedimentary bacteria, nano-, meio-, macro- and megabenthos).

Methodology: sediment sampling by multiple corer and box corer, mooring system observation using current meter and sediment trap, etc.

Phase 2. Impact Assessment Study

For Phase 2 studies, the field experiment named the Japan Deep-Sea Impact Experiment (JET) was conducted to evaluate the magnitude of the resedimentation impact.

Study Subject: resedimentation thickness, dispersion of sediment, changes in the sediment properties by the disturbance, biological response to the disturbance.

Methodology: Sediment sampling by multiple corer and box corer, mooring system observation using current meter and sediment trap, benthic disturber, video / photograph observation etc.

Phase 3. Impact Prediction

For Phase 3, a numerical model, which simulates the dispersion process of the sediment, was developed.

Future Prospect

The Japanese Government, the Metal Mining Agency of Japan (MMAJ) and the Deep Ocean Resources Development Company Limited (DORD) (both of which are hereafter referred as "Japan") believe it is important to maintain the balance between the implementation of activities at the mining site and the protection and preservation of the marine environment. In this context, the environmental study for the manganese nodules development was carried out for thirteen years.

As mentioned before, Japan has carried out the environmental baseline studies from 1991 to 1993, and 1997 to 2002, impact assessment studies including JET, DIETS and etc. in 1990, 1991 to 1993, 1994 and 1999, and monitoring to follow up the experiments from 1995 to 1996 and 2000 to 2002. It should be noted that, for the study in the Japanese contract area in the central Pacific Ocean, Japan already invested more than 20 million US Dollars.

Japan believes that it has so far contributed in no small measure to the preservation of the environment in the contract area, by conducting with large investment, environmental research such as those studies and experiments described above, making available the results of such research in the form of reports and papers, and hosting, in Tokyo in 1997 (Metal Mining Agency of Japan), an international symposium on the results of the above.

Japan wishes to reiterate the view that it is important to maintain the balance between the implementation of activities in the contract area and the protection and preservation of the marine environment. From this viewpoint, Japan is planning to further contribute internationally in the field of the environment, by making available, the data and information regarding the results of research done from 1989 to 2002.

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Chapter 11

Korea Deep Ocean Study: KORDI's Environmental Programme

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Introduction

The future plans for environmental research conducted by the Korea Ocean Research and Development Institute (KORDI) are presented in this paper. A brief introduction to the history of our environmental studies is discussed below.

We started the Korea Deep Ocean Study (KODOS) programme in 1991. Major efforts have been directed at determining the abundance and distribution of manganese nodules. In 1991, we collected nodules from 27 stations using free-fall grabs. In the subsequent year, we spent about four months surveying and sampling nodules at 245 stations. At that time, we did not have enough ship time for extensive environmental studies, so we just collected benthic organisms using a box corer. In 1993, we also spent about four months collecting nodule data as well as data on the megafauna, plankton and water column characteristics using a CTD at 33 stations. In 1994 and 1995, we spent about two months at sea, so we could obtain more data on the environment, but most of the effort went toward manganese nodule research.

For the period 1996 to 2000, we further developed our environmental programme as well as increased the sampling intensity for manganese nodules distribution and abundance. During this time, using box corers, piston corers, and the multiple corers, we collected sediment to characterize sediment properties. We also collected organisms such as phytoplankton and benthic animals. We measured chlorophyll and other water column characteristics.

Last year (2001), we had two cruises. Each cruise was 30 days in duration. We spent considerable time collecting data on manganese nodules. We had very limited time for environmental data.

Scope of Environmental Studies

The environmental studies examine the temporal and spatial variations of the water column structure. Another aspect of our study was to forecast natural fluctuations in physical, chemical and biological parameters. Results from 1995 to 2000 will be highlighted below.

Our study area is between the Clarion and the Clipperton Fracture Zones (*Figure 1*). Depending on the purpose of our studies, the sampling stations were not the same for each year. In some years, we examined latitudinal variability, so we set up sampling stations from 5°N to up to 13°N. In other years, we sampled stations to determine longitudinal variability, so we set up stations along 10.5°N.

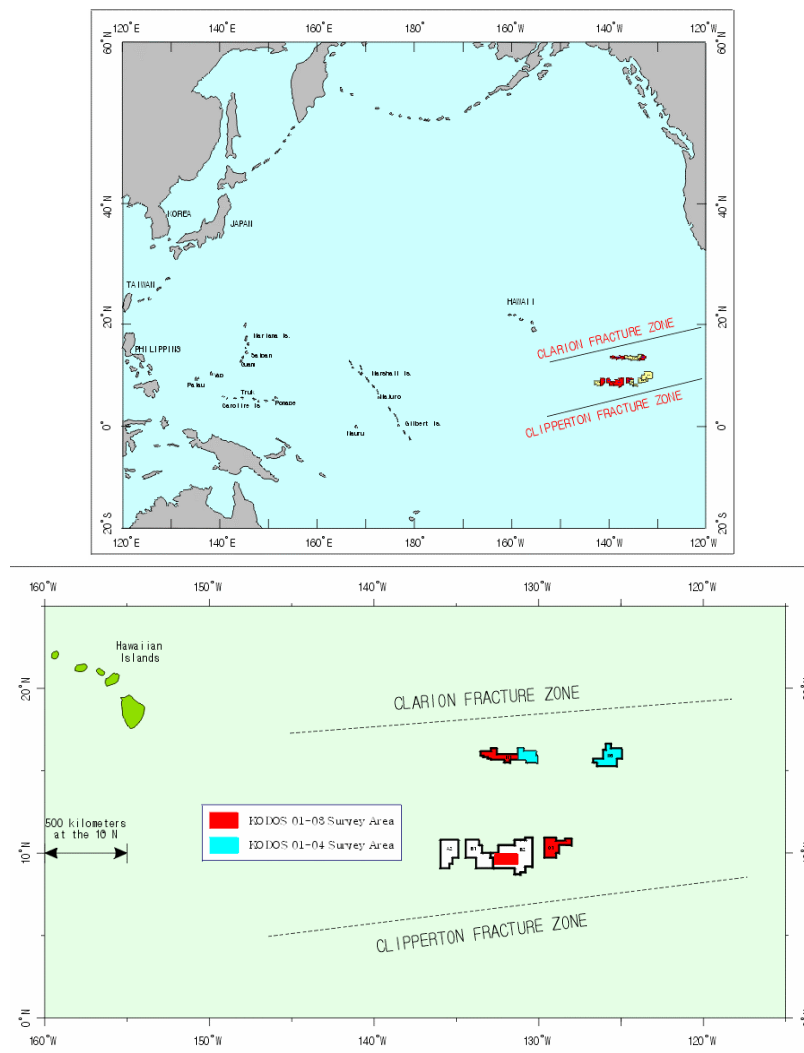


Figure 1: Map showing sampling stations in the northeastern Equatorial Pacific.

In 1999, stations for latitudinal variability were sampled from 5°N to 13°N. Geophysical surveys were conducted along survey lines depicted in *Figure 2*. A deep-sea camera was towed along tracks marked in *Figure 2*. The dots in *Figure 2* indicate stations where free-fall grabs were used to collect manganese nodule samples.

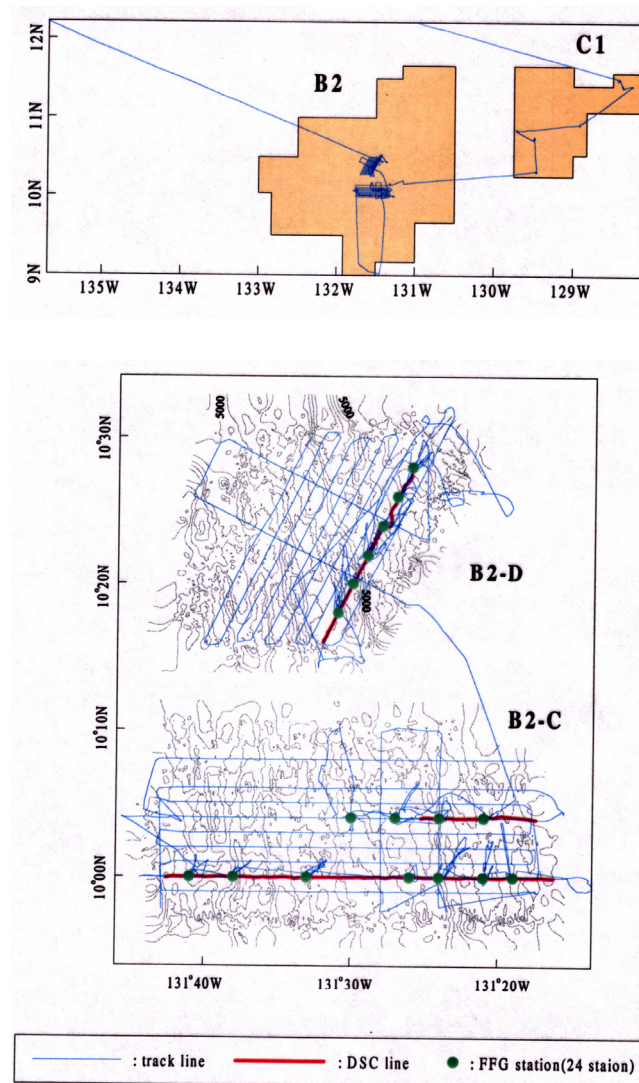


Fig. Survey Line of KODOS 99-3

Figure 2: Survey Lines

Seawater Chemistry

Materials and methods for seawater analysis are outlined in *Figure 3*. To characterize the water column, we measured several physical chemical parameters.

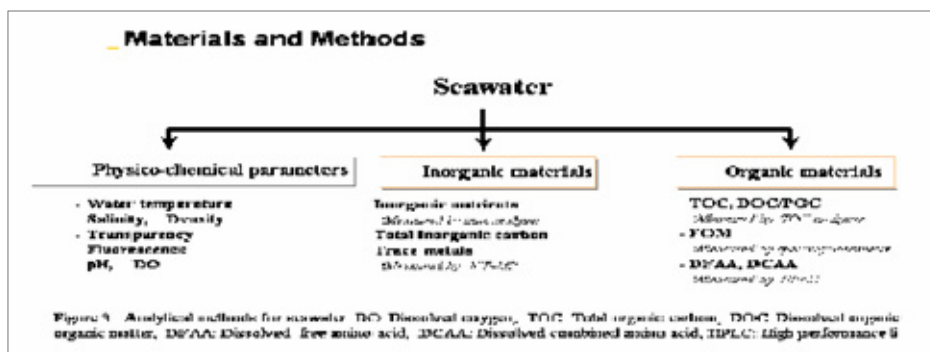


Figure 3: Materials and methods for seawater analysis

We measured the water temperature, salinity, density, transparencies, and fluorescence. For the inorganic materials, we measured the inorganic nutrients such as nitrate, nitrite, and phosphates, total inorganic carbon and trace metals. For organic materials, we measured total organic carbon, dissolved organic carbon, particulate organic carbon, fluorescence organic matter, as well as dissolved free amino acids and dissolved combined amino acids using high performance liquid chromatography (HPLC).

Results indicate a typical vertical profile of water temperatures. In our study area, generally, the surface mixed layer extends between 50 m and 75 m. There is a noticeable seasonal change in the upper thermocline depending on year.

You may recall that in 1998, we had an El Niño event and at that time, the warm surface waters extended down to about 100 m. Immediately after the El Niño event, we recorded a La Niña event in 1999 and at the time, the warm surface water was very shallow extending to less than 20m. This record indicates the natural variability in terms of surface water temperatures from 1995 to 2000. For salinities, there were no large differences, but during the El Niño years, salinities were slightly lower.

Latitudinal changes in terms of temperature, dissolved oxygen and chlorophyll-a concentrations were sampled. Data from 5 °N to 13 °N show a divergence at 8 °N. For the KODOS areas, there is not a large difference in surface water temperatures during the El Niño years in terms of latitudinal changes. In 1999, when we experienced a La Niña event, especially in our KODOS areas near the divergence, the warm water was very shallow. A large latitudinal change in surface water temperature was observed. The natural variability of the upper water

column can vary greatly depending on the El Nino and La Nina events. For chlorophyll-a concentration, the latitudinal pattern is similar to those patterns observed with temperature.

Latitudinal variation in various nutrients was also collected. For nitrate, the latitudinal patterns were similar to water temperature patterns. The distributional pattern differs between the three years. Phosphate and nitrite also shows the same trend with latitude.

Additionally, latitudinal variability in our areas is greater than longitudinal variability. In terms of longitudinal differences, salinity showed a similar trend to water temperature.

Biological Surveys

For biological data, we collected planktonic protozoans. Their distribution patterns are related to physical parameters. The protozoans were most abundant between the 7 °N to 11 °N. In surface waters, the protozoans were more abundant than in the deeper waters (*Figure 4*).

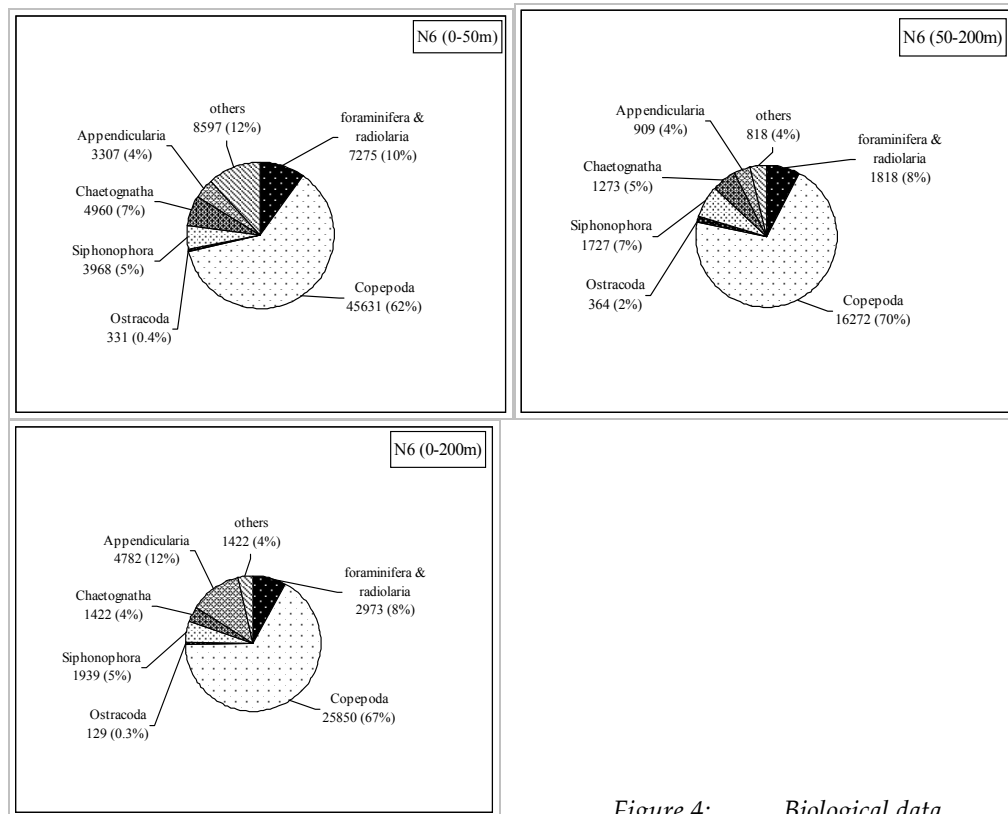


Figure 4: Biological data.

Zooplankton data collected in the area are presented in *Figure 5*. The most abundant taxonomic groups of zooplankton were copepods, foraminiferans, radiolarians, chaetognaths,

and appendicularians - There were no observed differences between the upper two layers: from surface down to 50 m and from 50 m down to 200 m.

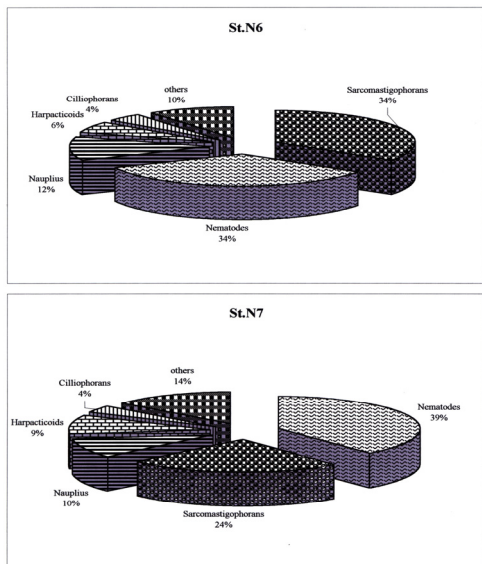


Figure 5: Biological Data

For meiobenthos, the most dominant taxonomic groups are foraminifera, nematodes, polychaetes, and benthic harpacticoid copepods. In terms of the latitudinal differences of the meiobenthos, in general, they are more abundant in the lower latitudes and less abundant in the higher latitude.

The relative abundance of meiobenthos with respect to the vertical distribution within the sediment was also determined. Typically, most of the meiobenthic animals are in the upper 1cm of sediment. Their distribution decreases sharply as we go further into the sediment. The foraminiferans were the most dominant animal group. The nematodes and polychaetes are also relatively dominant.

Figure 6 shows our study areas and the areas that were relinquished by KORDI.

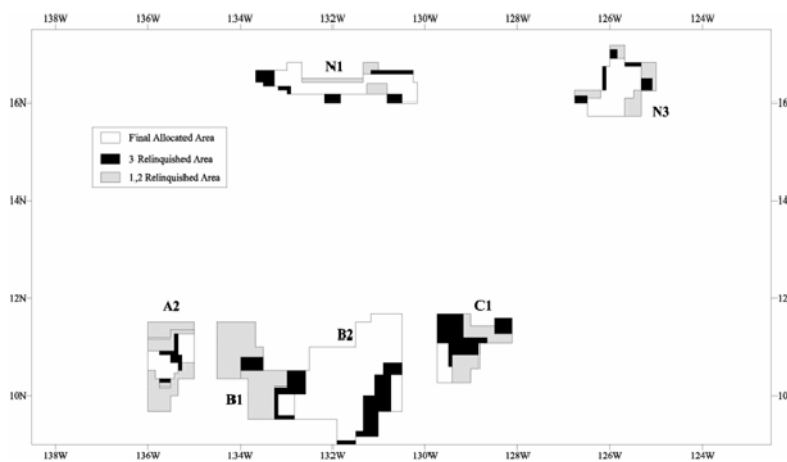


Figure 6: Study and relinquished areas

Future Studies

Future environmental studies are summarized below (Table 2). For physical and meteorological surveys, we plan to measure water column characteristics such as temperatures, salinity, dissolved oxygen, etc. We also anticipate measuring water currents and meteorological characteristics such as air temperature, wind speed and directions. For chemical surveys, we plan to measure organic materials and to investigate the sediment–water column interactions. We will examine temporal variations in various chemicals constituents.

Table 2: Future Environmental Research Plan of KORDI

Field	Items
Physical & Meteorological Survey	<ul style="list-style-type: none"> - water column characteristics; temperature, salinity, D.O. etc. - current; mooring and ADCP - meteorological characteristics; air temperature, wind speed and direction, etc.
Chemical Survey	<ul style="list-style-type: none"> - organic materials, nutrients, etc. - sediment/water column interaction - temporal variation of chemicals
Biological Survey	<ul style="list-style-type: none"> - phytoplankton; species composition, cell number and horizontal and vertical distributional pattern, temporal variation, chl-a concentration, primary production - zooplankton (metazoan); species composition and abundance, horizontal and vertical distributional pattern, grazing rate, temporal variation - zooplankton (protozoan); species composition and abundance, horizontal and vertical distributional pattern, temporal variation - microorganisms; bacterial biomass, bacterial productivity, - macrobenthos and meiobenthos; species composition and abundance, horizontal and vertical distribution, temporal variation, - demersal scavenger - bioturbation
Geological Survey	<ul style="list-style-type: none"> - characteristics of sediments; gravity, shear strength, etc. - pore water chemistry - vertical flux of sediment

For biological surveys, we plan to examine the phytoplankton in terms of species compositions, cell number and horizontal and vertical distribution patterns. Also, we plan to study temporal variations in parameters such as chlorophyll-a concentrations and primary productions. For the zooplankton, both metazoans and protozoans, we plan on studying their species compositions, abundance, and horizontal and vertical distribution patterns. Their grazing rates and temporal variations will also be investigated. We are planning studies on macrobenthos, microorganisms and bacterial biomass and bacterial productivities. For

macrobenthos and meiobenthos investigations in terms of species composition and abundance, horizontal and vertical distribution and temporal variations are in the planning stages. We also plan to investigate the scavenger and bioturbation on the ocean floor.

For geological surveys, we plan on investigating characteristics of sediments in terms of gravity and shear strengths, etc. Pore water chemistry and particulate flux to the sediments will also be examined.

Future Environmental Research Plan of KORDI

Our study area is definitely in the CCZ. Our government has some funds for future environmental studies so we plan to visit our site once or twice a year for the environmental studies. Each cruise would be about 30 days in duration. The primary goal is to fulfill the contractor's obligations toward the ISA and to develop environmentally safe technology for mining. In order to do this, we need to investigate the natural variability of the ecosystems in the final allocated area. We hope to predict the anthropogenic impact on our study area and also to understand the deep-sea environment better.

SUMMARY OF DISCUSSIONS

A scientist asked if photographs taken were for exploration (nodule coverage) or for the identification of megabenthos. Dr. Kim replied that KORDI had different camera systems – the systems on the free fall grabs captured pictures of manganese nodule coverage whereas the towed deep-sea camera system with still and video cameras were used for macrobenthic animal identification.

On the question of whether the pictures would be available for biodiversity studies should the Kaplan Project include megafauna investigation, Dr. Kim responded that a book was planned to commemorate the tenth anniversary of the KODOS programme (2003) and would include pictures of macrobenthic animals.

When asked how the foraminiferans were counted in the sediment, Dr. Kim replied that although he was not a macrobenthic biologist, as far as he was aware the foraminiferans in the sediment were counted by colonies on the manganese nodule and not individually. He added that the KODOS programme began in 1991 and may continue for 10 years.

Dr. Smith asked if KORDI had a trawling approach that allowed for collecting megafauna without grinding them up through the nodules. Dr. Kim indicated that there was a good chance macrobenthic animals were damaged in the KORDI deep water trawling procedure, but that KORDI also used box corers to collect animals. The primary purpose of the dredge was to collect the manganese nodules rather than the macrobenthic animals. However, bottom trawls also collected megafauna – most of which were damaged in this sampling

procedure. Nevertheless, some organisms were still recognizable, even to species level and tissue for DNA analysis could be possible from this material.

When asked if Korea had plans to carry out a benthic impact experiment in the future, Dr. Kim replied that Korea was planning a disturbance experiment, but that they were still developing their collectors and wanted a similar-type disturber in benthic impact experiments.

Dr. Smith asked if KORDI could also be able to collect DNA friendly samples in the KORDI area to which Dr. Kim replied that KORDI would be willing to cooperate. Dr. Kim continued, saying that he had discussed his concern about using DNA-friendly techniques to name animals with Dr Rogers and it was his understanding that molecular techniques could only monitor the fluctuations of the animals but not identify the animals down to species level.

Dr. Smith replied as that part of the Kaplan Project, he would be very interested in collaborating with the Korean scientists. He said polychaetes, for example, could be identified morphologically to the species level as well as through DNA analysis by using washing samples with chilled sea water and then preserving them with ethanol. He added that the Project planned to merge classic taxonomy with the DNA friendly techniques.

Dr. Kim responded that Korea had very limited taxonomic expertise in polychaetes and usually could identify organisms to species levels. In his opinion, there would be an opportunity to participate on board KORDI's research vessel because the Kaplan Project's central sampling stations were very close to KORDI's study area. Korea would also welcome experts on board who could train their taxonomists, if berth space on the research vessel was available.

Dr. Rogers added that genetic methods could also identify species. He said they could be looked at across the nodule province without attaching names to species. Another point was that only a tiny piece of tissue was required for the molecular techniques. In work with Dr. Billet of Southampton Oceanographic Centre on holothurians, for example, Dr. Rogers said a tiny piece of muscle was dissected from the body wall of the holothurians. The taxonomist was then left with the rest of the animals for morphological analysis. So actual sampling of the animals for genetic analysis did not preclude using the same specimens for morphological taxonomic work and furthermore genetics could be quite useful if you are bringing up specimens, which have been pulverized by grinding with the manganese nodules. Dr. Rogers concluded that genetic methods on fragments of organisms could also be used.

A biologist mentioned that Korea's central claim area was very close to the location of the Kaplan Project's station although the Kaplan Project had not yet decided on an exact location. He added that it would be of great benefit to coordinate the sampling stations to allow for combining of results from different studies while saving time and money. Dr Kim agreed, adding that the collaboration would be ideal.

Chapter 12

IOM Comments and Recommendations on Collaboration

Ryszard Kotlinski, Interoceanmetal Joint Organization

Introduction

The Interoceanmetal Joint Organization (IOM) was set up in 1987 by a number of governments to prospect for, survey, explore, and prepare commercial development of polymetallic nodules in the Clarion-Clipperton Fracture Zone. Hence, by definition, IOM has been from its very start committed to international cooperation and collaboration. This commitment, beginning with cooperation and collaboration between the IOM sponsoring states, was extended to cooperation with other contractors in 1992. In the course of time, the cooperation and collaboration has taken various forms, from exchange of scientists and observers during cruises to workshops and seminars to joint research. Remaining faithful to its tradition, IOM is open to any new form and format the ISBA-supported international cooperation and collaboration may take and is willing to contribute to it.

The IOM scientists have read the Workshop's background document with great interest. The interest has stemmed from the fact that IOM's research so far has made it possible for the Organization to contribute to all the four major topics identified by the preparatory meeting of experts, although IOM pursued issues related to those topics with varying emphasis and various successes. Before venturing any further, however, let it be said that the Natural Spatial and Temporal Variability in Deep-Ocean Ecosystems is at the present state of knowledge the most important one, essential for understanding recolonisation processes and long-term mining plume impacts on the water column ecosystems.

IOM has prepared some specific comments addressing the potential research focuses as outlined in Section C of the background document.

Biodiversity, Species Ranges and Rates of Gene Flow in Abyssal Nodule Provinces

This topic addresses fundamental biological aspects of the deep-sea environment; IOM has made important inroads into gathering relevant knowledge by collecting unique taxonomic information (at the genus level) on nematodes and harpacticoid copepods, the most abundant

and diverse meiofaunal major taxa that seem, in addition, to respond differently to environmental disturbance; most of the data acquired pertain to nodule-free sediment of the IOM BIE test site, summarised in a number of papers (Radziejewska, 2002; Radziejewska et al., 2001 a, b). Those studies have demonstrated a very high diversity – at the genus level – of the two taxa mentioned: representatives of about 260 nematode and about 60 harpacticoid genera were collected from the uppermost 3 cm sediment layer in a small (1.5 km x 2 km) nodule-free patch of the surficial sediment which had been experimentally disturbed.

In this work, IOM has relied on collaboration between members of the general scientific communities of the sponsoring states; the collaboration helped to establish data bases on taxonomy (genus level) of the two major taxa mentioned and specimen collections. The nematode collection is maintained at the Russian Academy of Sciences' Zoological Institute, in St. Petersburg, Russia, while the remaining part of the meiobenthic collection is housed at Department of Oceanography, Agricultural University, Szczecin, Poland. In addition, specialists interested in other taxa (e.g., the Loricifera) may, and in fact have already consulted the collections. It can be proposed here that, wherever such collections of specimens from the nodules provinces exist, ISA could be instrumental in providing support for curation and appropriate documentation (including digital imagery-based databases).

In my opinion, development of a common taxonomic framework among the contractors and other groups is essential, particularly with respect to traditional morphological approach, supplemented – time frames and resources allowing – with genetic and molecular studies. IOM is willing to encourage and facilitate sharing of taxonomic expertise. Should ISA become involved in supporting the “Kaplan Project”, relevant experts from IOM sponsoring states should be a part of it, contribute to it, and benefit from it.

IOM is of the opinion that the same taxonomic criteria and procedures are necessary to ensure consistency and comparability of the results. To this end, taxonomic intercalibration should constitute the first step, involving intercomparison of the existing data and collections of specimens. Such activities should draw from expertise of scientists from both the contractor and non-contractor parties; among the latter, Dr. Richard Warwick's group at the Marine Laboratory in Plymouth (UK) and Magda Vincx's group at University of Ghent (Belgium) have gained international prominence in taxonomic studies on invertebrates, which are most important from the standpoint of exploration in the Area.

It should be remembered that, in addition to meio- and macrobenthos, information gathered from research on megabenthos as observed on video- and photographic imagery provide additional materials for studies on biodiversity and on responses to natural events and man-made disturbance (cf. Radziejewska and Stoyanova, 2000). However, deep-sea megabenthos is seldom amenable to investigation involving molecular and genetic techniques for the simple reason of the lack of specimens. Therefore, more emphasis should be placed on collaborative research towards publishing joint collections of quality images as an aid in broadening the scope of biodiversity and species range research. Research along these lines is at present in progress at IOM.

Disturbance and Recolonization Processes

Burial Sensitivity of the CCZ Benthos: Elucidating the Single-dose Response Function

Results of dose-response experiments carried out on the seafloor would be extremely valuable, provided the desired burial levels could be ensured and controlled with reliability against natural physical processes on the sediment-water interface (e.g., near-bottom currents of different intensity, lateral advection, horizontal sediment transport, episodic sedimentation events). However, this cannot be guaranteed, hence the cost-effectiveness of such exercises is questionable. This type of research is, at the present stage, more suitable for laboratory testing (e.g., in flume tanks) than under *in situ* conditions. The project mentioned in the document, although theoretically interesting, is not applicable under the sole umbrella of a body such as the International Seabed Authority (ISA); on the other hand, experts nominated by ISA contractors could participate in such projects arranged (and funded, with ISA's partial involvement) by large scientific bodies.

Time and Space Scales of Benthic Community Recovery following Simulated Mining Track Disturbances

In my opinion, this is the topic in which IOM, like other contractors (China, Japan, Russia, India) and non-contractor states (USA, Germany), has already acquired a considerable amount of data. The IOM Benthic Impact Experiment (BIE) has armed IOM with a large body of information regarding a field disturbance-oriented experiment (cf. Kotlinski and Stoyanova, 1998; Tkatchenko and Radziejewska, 1998; Radziejewska, 2002) and awareness of the pitfalls and constraints of the hitherto-followed approach. At the same time, this topic presents an ample area for a tight, well-planned, rewarding cooperative and collaborative effort on the part of ISA and contractors, where the participation of the international scientific community at large could be particularly necessary and desirable. The project presented in the background document follows the footsteps of the series of Benthic Impact Experiments (BIE's). However, instead of performing a number of separate experiments, the approach presented in the background document would allow the pooling of resources for an efficient and cost-effective activity. Particularly worth recommending is the proposal to use a submersible for precision sampling. In IOM's experience, accurate targeting of some disturbance zones such as mining tracks, while especially desirable, would be difficult to attain; cf. Radziejewska, 2002.

However, it ought to be remembered that results of a sampling scheme to study effects at a temporal scale as proposed in the background document (e.g., 1 y, 3 y, etc.) can be interpreted with reliability only when the level of natural variability in the area is known. As shown by IOM's research, natural hydrodynamic processes can rapidly aid in levelling off the mining tracks (cf. Tkatchenko and Radziejewska, 1997), while episodes of phytodetritus sedimentation may be crucial for changes in animal communities (cf. Radziejewska et al., 2001 a, b; Radziejewska, 2002).

Mining-Plume Impacts on Water Column Ecosystem

Assessment of mining plume impacts on water column ecosystems should be incorporated into the overall design of the experiment aimed at creating the plume. In this context, the experience and expertise (including the modelling efforts) accumulated by the German scientists centred around the TUSCH group [cf. Thiel, H. (ed.): "Environmental impact studies for the mining of polymetallic nodules from the deep sea", Deep-Sea Research Part II, Vol. 48, 2001] is invaluable and should be made use of at the planning and implementation stages.

Natural Variability in Nodule Province Ecosystem

As already said, this is the most important area of environmental research, as the understanding of natural variability at different spatial and temporal scales is a prerequisite of a meaningful interpretation of results of any field experimentation, particularly if the experimental design calls for sampling at time intervals of a year and more. Various aspects of natural variability both in the water column and on the seafloor may profoundly affect and distort conclusions drawn from monitoring the anthropogenically disturbed area (e.g., the responses of meiobenthos to phytodetritus sedimentation over a disturbed area; cf. Radziejewska, 2002). In its environmental research, IOM has been paying a good deal (but perhaps not enough) of attention to natural variability in its area of exploration (cf. Kotlinski et al., 1996; Tkatchenko and Stoyanova, 1998; Tkatchenko et al., 1997). The fine-scale spatial variability of the physical and chemical sedimentary environment was fully appreciated after the preliminary results of the cruise IOM 2001 became known.

The topic offers an ample space for international collaboration; in fact, international cooperation is indispensable in appreciating the extent and scales of natural variability. Collaborative survey stations or transects (similar to that studied jointly by COMRA and IOM; cf. Tkatchenko et al., 1997) in areas of interest seem to be a good starting point, provided agreement is reached with respect to parameters to be monitored and the spatial (both horizontal and vertical) resolution of sampling.

IOM's Plans for Ocean-Going Work

In the context of its 15-year Plan of Work for Exploration, in the next 5 years IOM plans a cruise to its pioneer area in 2004 or 2005. The cruise will be aimed at:

- mapping the largest and most productive nodule-bearing areas;
- assessing average quality and composition of nodules;
- identifying natural ore types present in the exploration area;
- studying major physical and mechanical properties of nodules and bottom sediments in individual deposits;
- assessing general mining conditions in the largest nodule deposits;

- collecting data contributing to a recognition of natural variability in abiotic and biotic (meio- and megabenthos) components of the nodule province ecosystem;
- complementing side-scan sonar imagery of the seafloor with sediment sampling; and
- evaluating nodule resources by means of the major criteria.

The duration of the cruise will be 5 to 6 weeks. The ports of call considered include Manzanillo (Mexico) and San Diego (USA). Once the cruise programme and schedule have been finalised, IOM should be in a position to offer 2-3 berths to collaborators; the cost of adding days to the cruise for the work required/envisaged by collaboration programmes will be approximately US \$ 25,000 to US\$30,000 per day.

In conclusion, let me reiterate that IOM has been and is committed to international cooperation and collaboration towards a better understanding of the complexity of the deep-sea ecosystem so that our ultimate goal – sustainable development of oceanic resources – can be safely reached in the future.

SUMMARY OF DISCUSSIONS

When asked whether the standardization and methodology would be an issue in making the datasets comparable, Mr. Kotlinski said that prior to beginning the BIE investigation, the standardization and unification method was a priority which BIE has always tried with its partners and contractors in its sampling methods which produced results comparable to that of Japan, COMRA and Russia.

Mr Helmut Beiersdorf, member of the LTC, queried the sampling pattern shown by the BIE and why only the NW locations of the disturbed area were shown and not the SE areas. Mr. Kotlinski replied that the track was shown after current analysis to provide information on the source and the track area before accumulation of material in the deep sea. Additionally, the materials were too small quantitatively to show possible mining impacts. However, he added that results from JET, NaVaBa, COMRA, Russia and USA were a good start for future investigations.

A participant asked whether contractors, for the benefit of potential collaborators were willing to identify planned cruises for which there was space for collaborators. Mr. Kotlinski replied that it was not possible to prepare a cruise a year in advance as there were organizational and logistical problems to consider.

A participant observed that what was being discussed was not competition between contractors but competition in viewing the work. He added that there seemed to be a consortium of contractors and scientists with two types of membership – contractor and scientist. He suggested that the Authority form a secretariat for this type of consortium so it could coordinate, invite different scientific groups and liaise with contractors, their cruise plans and scientific institutions for better cooperation within the consortium itself.

Mr. Kotlinski agreed that the idea was a good one but that he thought the most important factor was to formulate a main objective to solve the issue.

Dr. Smith added that he was not sure how a consortium was defined but that he saw the working groups as the initial first stage of a consortium focusing on different research areas and promoting collaboration. He said one model would be for the working groups to exist beyond the workshop with continual interaction and with a point person or leader to keep track of plans, and exchange of ideas etc. One theme that could result from the workshop, Dr. Smith said, was specific collaboration and continuity in carrying the plans forward.

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Chapter 13

Perspective from the “Yuzhmorgeologiya” Experience

Mikhail F. Pilipchuk, State Scientific Center “Yuzhmorgeologiya”

Introduction

The State Scientific Center Yuzhmorgeologiya considers the following topics related to this Workshop of particular importance:

- The influence of physical oceanographic properties on the velocity of sedimentation,
- Post-disturbance effects of geochemical barriers on bottom ecosystem in the water-sediment boundary,
- Creation of local reduction zones during nodule extraction, and
- Geological and ecological problems of mining buried nodules.

Influence of Physical Oceanographic Properties on the Velocity of Sedimentation

The destruction of almost all living organisms in the way of the mining machine is an inevitable fact of mining. However, in view of the complex relief in the ore zone, mining machines will leave untouched areas from which benthos can migrate to the disturbed areas, thus restoring (or increasing) their abundance.

A much greater problem is the resedimentation of suspended sediments, which will affect both the disturbed and undisturbed areas. Settling sediment of different thicknesses will bury not only benthos, but also their food resources.

The distribution of suspended sediments in the area will depend on the bottom relief and structure of near-bottom current. Special attention in this workshop should be paid to the question of the re-suspended sediment. The distribution of re-suspended sediment in the area according to physical oceanographic properties includes vectors of currents, bottom gravitation flows, and vortices from turbulence, their structure, horizontal dimensions and energy.

A second important factor is the deposition velocity of suspended sediments. In this connection, one should consider the role of vertical component (up, down) of current velocity

vector by cyclonic and anticyclonic vortices. The vertical component of the velocity vector by an anticyclonic vortex will be directed downwards and thus promote quicker deposition of suspended sediments to the seafloor. The vertical component of the velocity vector by a cyclonic vortex will be directed upwards and this will delay sediment settling by reducing deposition velocity of sedimentary particles. If the “up” component of current velocity exceeds the magnitude of “down” velocity of sedimentary particles, the deposition on the seafloor will not exist and the particles are transported by horizontal strength of the currents.

Post-disturbance Effect of Geochemical Barriers on Bottom Ecosystem in the Water-Sediment Boundary

Experimentally, it can be shown that the upper sediment layer has the maximum adsorption, which decreases with depth into the sediment. So, when the mining collector removes the surface sediments of this “barrier zone”, the risk of dissolved metals passing into the bottom water increases.

At the same time, a zone of oxygen minimum is formed in the upper section of the water column below the thermocline at depths of 100 – 800 m. Surface discharge of sediment-laden water will cause an increase in sedimentation, which carries along with it, living and dead organic matter. This may lead to the suppression of the oxygen minimum.

Creation of a reduction zone below the thermocline (or pycnocline) is a very dangerous precedent for the existence of the ecosystem in the photic zone. Study of this phenomenon was carried out before, during, and after discharge of sedimentary material during natural experiments.

Creation of Local Reduction Zones during Nodule Extraction

In any method of deep-sea mining, the collector will scrape off 10 to 20 cm or more of the upper layer of sediment (for example, dredge-bucket method).

Measurement of Eh shows that the upper sediment layer has the highest redox potential. In this zone, Eh ranges from +550 to +600 mV. In such cases, one can consider that after scrapping off the upper sediment layer, the flow of dissolved metals will be increased from the pore water into the bottom layer. Therefore, the disturbed geochemical barrier at the sediment-water boundary promotes the increase concentration of mobile forms of ferric, manganese and other accompanying heavy metals, which are easily assimilated by filtering organisms.

Geological and Ecological Problems of Mining Buried Nodules

A serious problem in mining ferromanganese nodules may be a change in the structure of geochemical equilibrium systems, which were established over periods of geological time. The nodules are accumulators of high concentrations of highly oxidized ferric combinations, especially manganese (up to 30%). In an equilibrium system, manganese is the high oxidizer, the so-called "fire", putting out the reduction processes (reactive organic matter, microorganisms). Mass extraction on the order of millions of tons of this component from a natural system in equilibrium will result in decreasing redox potential and in the creation of a reduction zone where life for bottom organisms will be impossible. It is necessary to study the scale of this danger with the help of natural experiments in the areas of high nodule accumulation.

During the last cruise to the Russian license area with high density of nodules, we found many buried nodules, may be up to 40% of the surface density. This has relevance to commercial activities, but also presents an ecological problem, as more aggressive methods of mining may be required for extraction. A monograph on our efforts and previous collaborations with Americans, Interoceanmetal, India and Japan is forthcoming. We welcome the opportunity to collaborate on future projects.

During the last two years we prepared a report on our investigations with the Americans and other scientists over the last 5-7 years and this is the first time we are showing the contents of our monograph. The monograph includes complex investigations, hydrophysical results of studies, hydrochemical investigation in the water column and the investigation of sediment, chemical, biological and geological results.

SUMMARY OF DISCUSSIONS

Dr Smith asked if it was possible for Yuzhmorgeologiya to talk about its plans and what prospects there were for the future. Mr Pilipchuk said the monograph was an example of collaborations between themselves and other countries and it was unfortunate that at that time it was only possible to speak to the General Director of IOM to try out the collaboration. He said the report was published in Russian initially but added that it was important that the organizations join with others to publish the material in English.

Mr Pilipchuk said Russia was undergoing various changes. Yuzhmorgeologiya is now under the the Ministry of Natural Resources which is not academic science. Although the Ministry acknowledges the advantages of science, financial constraints play a major role. He added that Yuzhmorgeologiya was planning a March/April cruise in the coming year and he was waiting on foreign interests to submit their proposals.

When asked about plans to study nodule areas, Mr Pilipchuk said he had written guidelines in the previous month which included the mechanism of commercial mining. He said the first variant of the machine has been completed and will be tried out on the next cruise.

The Secretary-General of the ISA said he understood that deep seabed Russian activities had been reduced and that he was encouraged to hear that a cruise was being planned for the following year. He asked about the possibility of cooperation by Russian scientists in the kinds of projects being discussed and also whether data obtained from Russian research could be made available to the Authority similar to that of Japan that has agreed to make available to scientists data from their research. Mr Pilipchuk replied that he could see ISA playing an important role for the kind of work being discussed but suggested that perhaps his chief scientist and other experts could better explain the available resources than he.

Chapter 14

Possible Intergovernmental Oceanographic Commission (IOC) Support for Research and Collaboration

Umit Unluata, Ocean Sciences, IOC/UNESCO, France

Introduction

The Intergovernmental Oceanographic Commission (IOC) is happy to assess the kind of assistance it is able to offer in relation to what this workshop is trying to develop towards improving the understanding of the deep-sea environment. Needless to say, within these areas of which we can be of assistance, we propose to work within the framework of the existing MOU between the Authority and IOC. This MOU was created for promoting the development and conduct of marine scientific research in the deep-sea area.

The following are points IOC would like to note in relation to the deliberation of the working groups.

- IOC is not a funding agency;
- IOC exists for facilitating, promoting and coordinating international cooperative research;
- This is done through, either by IOC's own research programme jointly with SCORE (Scientific Community on Oceanic Research), GEOHAP (Global Ecology and Oceanography

The role of IOC in ocean research has been accomplished through

- Its cooperation with global research programmes such as the IGVP's Global Ocean Ecosystem Dynamics programme and also Land Ocean Coastal Zone etc.
- Through cooperation with SCORE in many areas through working groups etc., and agencies with interest in the oceans, e.g., FAO, ISA, UNEP.

In addition to its ocean sciences area section, IOC has two other major programmes : the Global Ocean Observation Systems (GOOS) and the International Ocean Data Exchange Programme (IODEP). GOOS is the setting up of global observation systems for the purpose of forecasting in the future and IODEP involves data management and the dissemination of information on ocean scientific issues.

I would like to emphasize that the Training, Education and Mutual Assistance (TEMA), is a major underpinning for the IOC activities. This underpinning is mostly geared toward developing nations, least-developed nations and small island developing states sometimes known as the large ocean states.

I would also like to bring to your attention in the context of the UN Convention of the Law of the Sea, that IOC is identified as a competent international organization in matters relating to marine scientific research, technology transfers and continental shelf issues.

Last year IOC formed within its own ocean science section, a new inter-disciplinary programme area, called Ocean Ecosystems and Marine Environmental Protection. The programme is intended to address marine environmental issues and 'ecosystem approach'. What this really means is that whatever you do, you have to do it with the ocean ecosystems in mind. This is following the lines of recommendations by the UN concept in the process in oceans and the Law of the Sea, which has been operating for three years and also was a recent resolution of the 55th and 56th sessions of the General Assembly

All these concepts are in harmony with the evolution of inter-disciplinary ocean science. After listening to your talks and comments carefully, I am of the opinion that we may be of assistance to the activities as the foci concerning the level of biodiversities, species ranges and associated methodologies in taxonomy. It is not only mining, but also mining issues of various other areas that would be useful to consider. A second area that we could pay attention to is the natural variability in ecosystems; which is the last part of our foci because it is concerned with processes such as El Niño and Antarctic water formation and the coupling of the benthic and pelagic ecosystems. Of course there are some selected aspects that may be of interest one of these being the impact of mining on biogeochemical cycles in the water column.

In the context of IOC and ISA cooperation, and in a concerted effort with the Authority, IOC's assistance could be (and we have to discuss this with the Authority) in terms of small sub working groups addressing specific issues in taxonomy and natural variability. Other areas that we can support is capacity building which is essentially the primary mission of UNESCO. We also need to see well-formulated activities by the working groups in this area.

SUMMARY OF DISCUSSIONS

On the question of whether IOC had funds or was able to find funds to support capacity building, Mr. Unluata advised that a significant portion of IOC's budget was dedicated

to TEMA, which was capacity building in terms of training, workshops, providing support for young scientists to attend meetings, fellowships and cooperating with other agencies like UNDP etc. He added that there was money at the IOC and that IOC was also capable of finding extra funds. These extra funds would typically come from agencies like NOAA, French agencies and the Canadian government. He said there was a vacancy at the P5 level for a person to run this show which would also involve a lot of fund-raising activities. He continued that a lot of governments were willing to provide significant financing for this type of activity, provided that they are convinced that it was something sustainable.

When asked by a participant whether IOC was able to assist the limited budgets of scientists from developing countries to participate in practical research on some vessels by the contractors, Mr Unluata said assistance was available for people going on cruises and after-cruise meetings.

Mr Unluata provided examples of TEMA activities - German training courses on *Utilization and Protection of the Seas and Coastal Zone Management* since 1995 where it supported 20 students in each of the courses. He said during the last three years both courses were put together because of financial constraints, but were held every year in Germany especially for young scientists and administrators from developing countries. He said that Dr. Zhou (workshop presenter and participant) was one of the participants in 1996 in the first courses. For 2002, there were 20 participants from Central America, the Caribbean and South America.

Mr Unluata said that in Coastal Zone Management segment, a module about utilization and protection of the deep sea was included because most of the coastal countries had deep-sea areas and resources within their EEZ as it was important to accumulate information about sustainable use within these areas.

He continued that he would like to pick up on a project that IOC was involved with, but which was not biological, which was why it wasn't mentioned. It was in relation to what the workshop had been discussing over the last two days – plain old-fashioned bathymetry. The seafloor itself was where the manganese nodules lived. There was a world-wide project to map bathymetry under IOC (International Oceanographic Organization). The deep oceans were not particularly well known and everyone knew that bathymetric data were very sparse. While data was being collected for the biological purposes and while ships were in transit, scientists could operate their sounding equipment to pick up data for feeding into bathymetric mapping systems.

When asked by a participant whether IOC could have some scientific input in an international cooperative programme that the ISA wanted to set up, Mr Unluata replied that IOC was the ocean science focal point in the UN system and the science arm for agencies who have interest in the seas, like the FAO for fisheries, UNEP for environmental management. He added that IOC would cooperate with anyone within the UN system as it was obliged to do so.

Chapter 15

Legal and Technical Commission

Arne Bjørlykke

As most of you know, these workshops, arranged by the Authority have been going on for approximately three years. During this time we have covered a lot of different topics ranging from technology, nodule collection, resource management and environment. These workshops have been quite successful and they have improved year to year in the sense that more scientists are coming in and we are now able to bring the scientific community, contractors and the Authority together to collaborate and not only have discussions. This is essentially what the International Seabed Authority and the people working in the Secretariat would like to do.

We often get reports that some countries are reducing their activities in this field. With the reductions, one still has to go through and see how we can do things more efficiently. We would also like to profile all the workshops and establish a forum for cooperation in marine research.

The purpose of the ISA is the initiation and organization of marine research related to the international seabed area. It invites participants from contractors, scientists, the Legal and Technical Commission (LTC) members who are then tasked to promote and initiate international cooperation on integrated projects. It is not the work of the ISA to run any project and/or to supervise projects. When such projects are established they live their own life. What happens is the LTC looks at the project for the ISA to see what can be learnt from it and then generates and disseminates research with the ISA as the focal point.

What I would like to hear is feedback from scientists and contractors. This feedback then in turn will be tabled at the LTC meetings which are held annually and precede and overlap with the Assembly, Council and Finance meetings at the ISA Annual Sessions.

SUMMARY OF DISCUSSIONS

Dr Smith asked how the functioning of the LTC could help identify scientific problems and facilitate cooperation in research and whether it could be viewed as a scientific body.. Mr. Bjørlykke replied that the workshop was a good example of a forum where people brought in ideas and suggested projects for cooperation with contractors. He added that the LTC had refrained from being more precise in establishing terms of reference or guidelines to allow more flexibility in the group.

On the question of financial support, Mr. Bjørlykke said main financing was outside the ISA and cited the Kaplan Project as an example. He said all of the projects must seek financing outside the Authority. The objective of the ISA was to try and reduce costs by bringing the contractors and the scientific communities together. Mr. Bjørlykke added that the LTC was not about financing but about bringing people together for an efficient operation. He said formal cooperation could occur within individual projects but with a diverse group of people such as the current workshop participants, building a consortium would be difficult to achieve.

Dr. Smith said the way he saw it was that there were two functions in the forum idea – one was to provide scientific guidance and continuity to the ISA and the other was a meeting place for people to discuss ideas and establish collaborations. He suggested the forum to set up a scientific advisory group that provided advice to ISA on scientific progress made. He said a group of five or six scientists with an overview of oceanography and geology would be most suited to merge the interests of the ISA, the scientific community and the contractors into working groups.

Mr. Kotlinski said what the contractors needed from ISA was its support for activities in the claim area. He added that contractors all had guidelines but if all would be talking about activities at workshops they would never be able to achieve standardization and unification of methods in the open sea to which ISA holds the control. He suggested that discussions at the workshops be converted to recommendations to the ISA who will in turn combine the proposals.

The Secretary-General of the ISA said there was nothing unique about the forum. He continued that he guards very carefully the role of the Authority and the Secretariat. The Secretariat convenes the workshops and everything done under the auspices of the Secretariat is part of the Authority. He said each project should have some kind of an informal body on the operational level. Whatever comes out from the forums and projects were done under the auspices of the Secretariat. Whatever was being discussed, the Secretariat was only there to facilitate discussions and provide the forum - it would not tell scientists what they should be doing.

Mr. Nandan said the Secretariat recognized that there were different structures at national level. He noted too that many contractors were government-supported entities. On the Kaplan Project, he said some funding had been obtained from foundations and other sources

but there was still some funding to be provided. He suggested governments indicate training availabilities (in particular for developing countries) which the Secretariat could co-ordinate to find trainees.

Dr. Lamshead stated his concern that setting up a formal board with the task to promote international cooperation would not augur well with the civil service governmental structure as it was not the job of the scientist but that of an organization's marketing department and may have political reflections. He noted that the ISA was already doing what a formal group would do and creating another bureaucratic layer would only trigger more bureaucratic responses.

Part III

CONCLUSIONS, RECOMMENDATIONS AND
FOLLOW-UP OF THE WORKSHOP AND ITS
WORKING GROUPS

Four working groups, each consisting of at least a Contractor representative and non-contractor scientists were set up to focus on each of the four research topics and to outline specific collaborative project(s) and associated collaborators.

The groups were also requested: to compile a list of resources that may be available for the project(s) including their costs; assess the additional resources required to complete the project; identify potential sources of additional resources and outline a proposal to obtain additional resources required.

The working groups and their composition were:

- Potential Collaborations in Biodiversity, Species Ranges and Gene Flow
- Disturbance and Re-colonization Processes at the Seafloor
- Mining Plume Impacts on Water Column Ecosystems
- Natural Variability

Chapter 16

Report and Recommendations of the Working Group on Potential Collaborations in Biodiversity, Species Ranges and Gene Flow

The working groups were given the following tasks:

- identify working group leader and rapporteur,
- outline specific collaborative projects, collaborators, and required resources,
- compile a list of resources (e.g., ship time) currently available to project(s),
- identify potential sources of additional support needed, and outline a proposal to obtain these resources (i.e., funding, ship time, etc.),
- formulate specific plans for capacity enhancement (e.g., traineeships), and
- prepare and present a report summarizing collaborative research plans.

Working Group Members

The members of the working group were Gerd Schriever, Alex Rogers, Bin Mao, Jin Jiancai, Hasjim Djalal, Jung-Keuk Kang, Frida Armas-Pfirter, John Lambshead (rapporteur), and Craig Smith (leader). Other occasional participants included Virginie Tilot, Woong-Seo Kim and Ryszard Kotlinski.

Scope of Collaboration

The group was charged with looking at potential collaborations in biodiversity and agreed by consensus that these collaborations, in particular the Kaplan Project, were very important, potentially productive and would benefit all parties involved. Specific collaborations were proposed as follows.

Megafaunal Biodiversity

The analysis of megafaunal biodiversity is recommended to complement the existing Kaplan Project's focus on macrofauna, nematoda, foraminifera and microbiota. Knowledge of megafaunal diversity in the abyssal Pacific, particularly nodule provinces, is still limited except for a comprehensive study in the French nodule area (see Appendix A). Nonetheless, there is substantial potential to collect biodiversity data from existing archives of deep-sea photographic images from other nodule areas. Only a small number of species have been described taxonomically because of the low densities of megafauna at the abyssal seafloor. Megabenthos from manganese nodule areas are especially difficult to collect due to the grinding effects of nodules during the hoisting of trawls to the ocean's surface.

As an addition to the Kaplan Project, the following steps to increase our knowledge of megafaunal biodiversity within the CCZ are suggested:

- determine the availability of photographic archives with high quality images of megafauna in contractor and museum collections (e.g., that of the Naturmuseum Senckenberg, Frankfurt, GER, IOM and IFREMER),
- digitize high-quality images of megafauna from the CCZ in archives and store on DVDs. Find out what kind of information and what available format can be delivered by institutions/contractors to accompany each image (e.g., position, depth, date, other environmental information),
- set up a database including the name of the collector/describer and a description of the animal to the lowest taxonomic level possible,
- add all available information about distribution, place of collection, water depth, biology, ecology, and references,
- adjust database to internet requirements,
- make information available for science and public on the Internet,
- provide possibility for interactive discussions between the describer/collector of the image and/or between scientists interested in working on the image,
- look for available deep-sea megafauna specimens collected and stored in formalin in scientific museum collections and take samples for DNA-analysis,
- set up DNA-species library at the British Antarctic Survey,
- opportunistically collect new species, in particular scavenging fish and Crustacea, during Kaplan cruises for preservation using DNA-friendly techniques, and
- evaluate species ranges through merging of photographic, molecular and museum-collection data.

<i>Cost Estimates of Megafaunal Programme*</i>		US\$
1 Scientist	3 years	183,000
1 Technical Assistant	3 years	23,000
Travel costs**	3 years	24,000
DVD-Burner		7,500
Costs to digitise about 100,000 photos		71,600
Database adjustment to the Internet		25,500
Consumables CDs, DVDs, storage etc		18,000
<u>DNA determination and library</u>	3 years	<u>45,000</u>
Total		\$ 397,600
Cost per year		\$ 132,533

Additional Expenses

Participation of an experienced fisherman in a cruise to apply a new method to trawl megafauna in manganese nodule areas to avoid grinding. \$ 10,250

The point person for pursuing the megafaunal component of biodiversity studies is Dr. Gerd Schriever, with Michael Tuerkay as Co-PI, and Dr. Virginie Tilot as a collaborator.

Ship Time – Molecular Training Exchange

It was quickly recognized that the Kaplan Project would benefit substantially from being able to share KORDI and COMRA ship time to the central and western CCZ, while KORDI and COMRA scientists would benefit substantially from obtaining training in molecular techniques in Kaplan scientists' laboratories. The following exchange was tentatively agreed to, pending approval of KORDI and COMRA directorates.

Shiptime

KORDI will provide 3-4 days of wire time and 1-2 scientific berths on one cruise each in 2003 and 2004 to the KORDI area in the central CCZ for Kaplan Project scientists. This wire time (totaling 7-8 days) will be used to collect samples of macrofauna, meiofauna, and microbiota to be processed with DNA-friendly methods for analyses within the Kaplan Project, in collaboration with Korean scientists, (see traineeships below).

COMRA will provide 7 days of wire time and 3-4 scientific berths on a cruise to the COMRA area in the western CCZ. This wire time (totaling 7 days) will be used to collect samples of macrofauna, meiofauna, and microbiota to be processed with DNA-friendly

* The project will be submitted to the German Ministry of Science for funding. It is expected that funding from this source will cover at least labour costs for the scientist and technical assistance.

** In case images are available at institutes or contractors facilities in a non digitized format but cannot be sent to Germany, the responsible person has to travel to that institute to digitize the material over there.

methods for analyses within the Kaplan Project in collaboration with Chinese (scientists, see traineeships below).

Traineeships

The Kaplan Project will offer traineeships in molecular techniques for scientists to come to Dr. Alex Rogers' Laboratory at the British Antarctic Survey in Cambridge, England for 9 months of training in molecular techniques for the study of biodiversity. The trainees will be provided with funds for roundtrip travel to Cambridge, living costs, and supplies for molecular analyses. They will work on material collected with DNA-friendly techniques from the CCZ, and will be full scientific collaborators with the Kaplan scientists. It is anticipated that the results from trainee analyses will be published in the peer-reviewed scientific literature in collaboration with Kaplan Project scientists.

Support for additional traineeships in molecular techniques, classical taxonomy, and quantitative ecology will be sought to allow training of scientists from other contractors and from developing countries. In detail, traineeships will be structured as follows.

The Kaplan Project will offer a number of short-term training fellowships or longer-term Ph.D. studentships. These training placements will be financed through Kaplan or other external funds and will be targeted at young scientists working in contractor or developing countries. Training will directly transfer knowledge from scientists working in institutions involved in the Kaplan Project, specifically the University of Hawaii, The Natural History Museum, London, and the British Antarctic Survey, Cambridge. Training will be concentrated in areas of biological science which are under-represented or absent in institutes belonging to contractors or developing countries, namely seagoing and numerical ecological techniques, systematics and molecular biology. The training fellows will utilize the skills acquired under the programme to directly benefit Kaplan and other projects connected with assessment and monitoring of manganese nodule mining impacts in the deep ocean. However, such skills will be sufficiently generic to be transferable to other areas of environmental science connected with biodiversity. In addition, this will help in the standardization of species identification methods and gene-flow studies amongst members of Kaplan and other participants in impact studies. The training fellows will subsequently develop their own research programmes connected with deep-sea and other environmental research and act as hubs for further dissemination of the skills that have been acquired through the programme. The programme will cause a cascade of training throughout the contractor organizations, their nations and associated regions.

Specifically training in the Antarctic Genomics Laboratory (AnGeL) will encompass methods for DNA extraction, polymerase chain reaction (PCR), DNA sequencing and the use of robotics in molecular biology. Training in analysis of sequence data for phylogenetics and population genetics will also be undertaken. This will include sequence alignment (using Clustal X), phylogenetic tree building (using PAUP and Mr. Bayes) and gene-flow analysis (using Arlequin and TCS). Fellows will be expected to work on Kaplan material and work will be published in peer-reviewed journals when completed.

Training will be given in The Natural History Museum on sampling, sorting and extraction of infauna and the morphological identification of taxonomically difficult but environmentally important metazoa such as polychaetes and nematodes. Such training would include numerical morphological phylogenetic analysis, numerical biodiversity analysis and associated evolutionary and biodiversity theory.

Training will be given at the University of Hawaii in quantitative biological oceanography, stable isotope analyses, and quantitative benthic sampling techniques.

<i>Estimated costs for a 9-month position:</i>	<i>US\$</i>
Subsistence and rent	\$15,000
Travel	\$1,500
Scientific supplies	\$5,000
<u>Bench-fees</u>	<u>\$1,500</u>
Total	\$23,000

Initially funds for fellowships will be found through the Kaplan programme. However, funds for fellowships will be sought from various international bodies concerned with regulation of human impacts in the deep sea, marine biology, biodiversity and other branches of environmental science. Such bodies may include the ISA, Royal Society, British Council, European Union, NSF and national governments and institutes. Additional support may come from other bodies such as the IOC, WWF, and World Center for Biodiversity Monitoring, CBD and ESF.

Future Development and Exchange of Molecular Array Technology

The working group recognized that there might be substantial benefits to contractors and scientists from developing nations from the development of molecular array technology for species identifications from the abyssal deep sea. However, the topic is sufficiently complex that additional explanation and discussion of the technology was considered necessary. Thus, Dr. Alex Rogers has formulated the following description of molecular array technology to stimulate further discussion.

Understanding patterns of species diversity in large marine ecosystems is highly problematical. Identifying species using morphological methods and comparing identifications across large geographical distances, especially when different taxonomists are employed, is extremely difficult and governed by subjectivity. This is because many species are taxonomically poorly known and intraspecific morphological variation arising from environmental influences on phenotype is significant, especially in marine invertebrates. Some stages in the life history of marine animals are also particularly difficult to identify. For example, many larvae have not been attributed to recognized species and, because of conservation of larval morphology this is not likely to become possible in the future using conventional methods. Within the meiofauna, these problems become extremely acute as

reduction in body size and adaptation to interstitial habitats often result in a paucity of morphological characters.

New developments in molecular biology may, for the first time, allow rapid, accurate and reproducible identification of large components of benthic and pelagic (planktonic) marine communities. In particular, developments in functional genomics, utilized to identify expression of large numbers of genes, may be applicable to simultaneous identification of multiple taxa from environmental samples. These technologies are known as microarrays or DNA chips.

Microarrays depend on a process called hybridization. DNA consists of two strands of sugar-phosphate molecules linked by complementary nucleotides. These nucleotides are adenine, guanine, cytosine and thymine (A, G, C, T). Adenine binds to thymine and guanine binds to cytosine and together these four different nucleotides make up the genetic code. When the double DNA strands are split up by heat or by the presence of a chemical (denaturation), they come back together (reanneal) according to the complementary binding of A>T and C>G. This reaction is highly specific and fully complementary DNA strands will bind with the greatest affinity.

Microarrays consist of spots of single stranded DNA (the probe) corresponding to specific genes arranged on a substrate such as glass or nylon. These spots of DNA will bind with greatest affinity only to other single stranded DNA molecules (target) that are perfectly complementary to them, forming a double stranded molecule. If target DNA molecules are fluorescently labeled, binding to the probe DNA is detectable using laser excitation and scanning using a confocal microscope. Binding is recognized by a change in the color of a DNA spot on the microarray.

DNA spots on a microarray are between 100 – 200 μm in diameter and there may be up to 30,000 spots on a single array. Theoretically each spot could represent the DNA from a single species. This raises the prospect of arraying DNA from all the species within an entire community. To achieve this, a region or regions of the genome would have to be selected that occurred in all the taxa to be identified. This region would have to have highly conserved flanking DNA sequence so that PCR amplification of the gene(s) is reliable. In addition, the region(s) would have to encompass sufficient interspecific variation that it could be reliably used to distinguish species. Several such regions are already routinely used to identify species but generally this is within specific groups and for relatively low numbers of species compared to the diversity that may be encountered in a deep-sea environment. Other regions of the genome that could be targeted for such purposes may include taxonomically conserved stress proteins such as *Hsp-70* (heat shock protein – 70) or genes involved in critical cellular functions such as protein synthesis (e.g. the translation initiation factor *eIF-2a/b*-translation initiation factor 2).

What could this technology offer if it was fully developed for species identification? In theory, microarrays could be employed to identify the presence/absence of a very large number

of species, within an environmental sample, simultaneously. The smaller fractions of marine communities, such as the microbial fraction, the meiofauna, macrofauna and the plankton would be particularly suitable for this method. Traditionally, it is in these types of communities that our greatest problems lie in estimation of species diversity and our understanding of its geographical distribution and ecological dynamics. Because array hybridizations are so quick compared to morphological identification (about 6 hours compared to 3 months to identify nematodes from a single sample), communities could be sampled and analyzed at finer spatial and temporal scales than have ever been possible previously. Furthermore, such data would be directly comparable, regardless of the operators employing the method. All life-history stages, from egg, through larvae to adults would be identifiable using such methods, even if individual specimens were damaged or fragmented. Microarrays can be manufactured commercially allowing them to be purchased "off the shelf" once they have been developed from environmental DNA libraries. Our understanding of many dynamic processes influencing the diversity of marine communities has previously been severely limited by our ability to identify species in large sample sets. Using an array approach such limitations would no longer apply as the diversity within an entire sample could be estimated faster than the time to identify a single specimen in some cases. A further advantage of this technology is that identification is standardized and no longer subject to individual opinion.

To enable this technology a large-scale pilot study would be necessary. This project would involve sampling a community of interest, extracting the DNA from all the organisms present within the sample and then amplifying suitable regions of the genome through PCR. Amplified fragments of DNA would then be used to make an environmental library from which clones would be fingerprinted by restriction fragment length polymorphism analysis (RFLP) and then individual clone types sequenced. Microarray probes would be designed from sequence information. Sequences would also be used in a phylogenetic analysis (probably using Bayesian tree-building methods) so that interspecific variation (species) could be resolved from intraspecific variation (genetic polymorphism). Once this is complete, microarrays would be made available to the scientific community for biodiversity investigations (cost \$150 - \$500 for each array depending on spotting density). Such a project would cost between \$600,000 and \$700,000.

Future Work

LTC meeting, 7th August, 2002

A presentation was made to the LTC on the 7th of August. The presentation gave an overview of the Kaplan Project and also summaries of the recommendations of the other three working group projects (Disturbance and Recolonization, Mining Plume Impacts on the Water Column, Natural Variability). Collaborations developed during the ISA workshop, July 2002, especially in respect of exchange of time on cruises for training in molecular biology, systematics and ecology was also being presented. Finally, a summary of new molecular methods for studying biodiversity was discussed.

Workshops to Organize Kaplan Biodiversity Studies Collaborations

A workshop is planned in British Antarctic Survey, Cambridge, to develop and organize the Kaplan Project. This will take place around November 15th, 2002 to January 15th 2003. Prof. Craig Smith will act as the main point of contact for this meeting with Dr. Alex Rogers undertaking practical arrangements in Cambridge. The workshop will have several principal aims:

- define collaborations within Kaplan so that plans for the project maybe confirmed. In particular, it is important to precisely define who is contributing what skills, sea time, laboratory facilities and funds for the project,
- to explore the options for additional funds for training fellowships and to view the training facilities and programme to be provided by British Antarctic Survey,
- discuss the contents and select a location(s) for a website connected to data arising from the programme,
- detailed planning of cruises including the sea time available for sampling, logistics and participants, and
- define the exact scale of sampling, sampling design and sampling protocols (e.g. DNA friendly sampling techniques).

Prepare Proposals for Training Fellowships

Whilst it is planned to provide at least 4 training fellowships under Kaplan for COMRA and KORDI scientists, it was clear that such training was appropriate for other contractors and for scientists from developing countries. Proposals will, therefore, be prepared to fund additional training fellowships to be based at BAS, Cambridge (molecular biology), The Natural History Museum, London (systematics) and the University of Hawaii (ecology). Several organizations will be targeted for such proposals including the ISA, Royal Society, NSF, British Council and European Union. Support may also be obtained from the IOC and CBD. Other organizations such as the Center for Biodiversity Monitoring in Cambridge and WWF will also be approached for such funding. Dr. Alex Rogers will investigate potential sources of funding and will construct proposals, where feasible. It is hoped that funding for an additional 8 - 9 training fellowships will be found over several years.

Megafaunal Project

A proposal to fund the megafaunal study project, based around Kaplan Project will be submitted to the German Government. The Principal Investigator on this proposal will be Dr. Gerd Schriever and Dr. Tuerkay with appropriate contributions from Kaplan and other participants including Prof. Craig Smith, Dr. John Lamshead, Dr. Alex Rogers and Dr. Virginie Tilot. The project will be aimed at addressing levels of biodiversity and species ranges in the megafauna. It will utilize existing photographic material for the development of a comprehensive electronic database of megafauna in the CCZ. This will link photographs and metadata to molecular data acquired during the current Kaplan Project and training

fellowships. It will also allow access to existing formalin fixed specimen collections in various institutions (i.e. KORDI, NHM, London, Smithsonian Institution, Scripps Institute of Oceanography).

Training Fellowships

A detailed description of the training fellowships will be circulated to all potentially interested parties. This will describe benefits to participant scientists and their respective organizations. The potential exchanges of ship time or other scientific services or funds will also be defined.

GEF Proposal

Documentation will be submitted to support a proposal for funding to the GEF. This proposal will be supported by the Secretariat of the ISA and will be aimed at further development of biodiversity studies, including molecular techniques, of the CCZ and Indian Ocean prospective mining areas.

Chapter 17

Report and Recommendations of the Working Group on Disturbance and Re-colonization Processes at the Seafloor

Working Group Organization

The members of the working group were Paul Snelgrove, (group leader), Francis O'Beirn (rapporteur) and the following group members: Arne Bjorlykke, Bob Burrell, Gerd Schriever, and Lindsay Parson.

Background

Any effort to extract manganese nodules from the abyssal ocean will disturb considerable areas of the deep seabed, followed by deposition of resuspended material onto the sediment surface. The effects of these activities on living organisms will be at least two fold. First, the deposition of material will cause mortality of infaunal organisms as organisms are smothered and isolated from their food source. Second, the plowing of the bottom will effectively strip the surficial sediments of the meiofaunal and macrofaunal organisms, which are concentrated in the upper few centimeters.

Several nations have undertaken experiments designed to examine disturbance effects, by disturbing a broad area of seafloor and monitoring the biological response. We propose to build on these studies with experimental manipulations specifically designed to generate a mechanistic understanding of disturbance response in the deep sea. The details of these experiments are outlined in Appendix B (Summary of March 2002 Workshop), but in brief we propose to undertake three types of experiments:

1. Experimentally generate sediment deposition of varying thickness over natural seabed communities on a small scale. This task will be accomplished with a sediment deposition device that can create uniform coverage over a relatively small (~1 m²) bottom. These sediments will be sampled approximately 30 days after sediment deposition in order to determine mortality effects as a function of sediment "dosage".

2. Create “mining” swaths of sediment disturbance of different widths that mimic the disturbances that would be created under a mining scenario. By examining swaths of different widths it will be possible to determine the influence of scale on rates of recolonisation of stripped seabed.
3. Deploy trays of defaunated sediment onto the seafloor in order to determine whether specific sediment characteristics (e.g. surface vs. subsurface sediments) influence rates and composition of community response.

Rationale

Justifications for this particular research programme are:

- An experimental approach is needed in order to provide a mechanistic understanding. An impact assessment does not equal understanding environmental effects of mining. Observational data are insufficient in themselves.
- There is a scientifically defensible argument that mining impacts could persist for more than a decade. If this is true, then there would be considerable risk in waiting until 2-5 years before mining begins to initiate disturbance/recovery experiments. Given this potential time scale, it may be very difficult for ISA to approve any mining activity without a full appreciation for the overall impacts.
- The studies will help in minimizing impacts and may strongly feed into the design of mining apparatus.
- This work would link strongly with other studies, especially those documenting biodiversity pattern and those looking at temporal variability.
- Local scale effects are needed in order to do detailed diversity analyses.

Given that this programme has no identified contractor collaborators, a goal of this working group was to gauge the interest of contractors in this type of research. It was observed that the justification of this research would have to be clear and realistic and must have implications for all parties (ISA, contractors). It must provide information on the effective management of the resource from a conservation perspective (ISA interest) and provide practical advice that would aid in the design of mining equipment in order to minimize the impacts of the activity (contractor interest).

Discussions on Manipulative Field Experiments

Single Dose Response to Sedimentation

This proposal was described in detail in the previous workshop (outlined in *Appendix B*). A critical component of this study is that it directly related to one of the known impacts of

mining activity on the sea floor, i.e. suspension and deposition of sediment. In addition, the technology to set up the experiments is proven.

Collaborators

The inclusion of a geochemist and a marine geologist as co-investigators in this study was considered important. If existing geological and geochemistry programmes could be merged into this programme or vice-versa, it may help mitigate overall costs.

Much of the discussions centered on potential partners and the need for ship time. Though not specifically discussed in much detail with respect to this particular experiment, there was a strong feeling that this project needed to be paired with other projects that have emerged from the recent ISA workshops (e.g. natural variability, biodiversity), if they are to be viable. Those discussions are summarized in the section below.

Resources Required

Previous workshop already defined resource requirements (see *Appendix B*).

Disturbance Experiment

The primary goal of this research topic is to assess the response of benthic organisms to seabed mining activities. However, the technology has not been developed sufficiently to allow economical mining. There are a number of unknowns regarding the commitment of states to the future development of these technological developments. These ambiguities raise a number of questions regarding the design and implementation of experiments. The ability to simulate accurately the effects of the mining activity on the seabed and near bottom water column is limited. Bearing these limitations in mind, there are still several assumptions that can be made about mining impacts:

- material will be removed,
- sediment will be suspended and deposited on the seafloor, and
- compaction of the seabed may occur.

While compression of the sediment on the seafloor may occur, the ability to simulate this effect will be difficult. The group decided that planning of the study should focus primarily on the former two assumptions. As a preliminary assessment, it was felt by the group that it would be important that experience from other sources should be sought and factored into the design and ultimate goals of the project. Such experience may be sourced from:

- Technology advances (e.g. ISA Workshop Proceedings) or literature searches on mining activity on sediment impacts (e.g. compactions, in-situ shear stress measurements, soil mechanics predictions). It is therefore important to

determine the state of development in contractor's countries in terms of the actual mining methodology likely to be employed.

- Researcher working on turbidites and other geologic features that may have some bearing on projected mining impacts. Active involvement of geologists and geochemists is encouraged.
- Other research programmes examining broadly similar themes, e.g. DISCOL.
- Shallow water studies resulting from aggregate extraction and pipeline laying programmes.

Such reviews of previous experiences may direct sampling strategies or protocols. For example, specific conditions to be simulated in trays for the various colonisation studies. They may also broadly direct the experimental design in terms of scale of impact to assess.

Collaborators

As potential collaborators;

- KORDI was identified given their commitment to future cruise events. In addition, it was noted that they had committed to carrying out some disturbance experiments. The group felt that the KORDI efforts may provide a suitable platform for the implementation of the studies – either fully collaborating or “piggy-backing” on cruises where controlled dredging of areas would occur when baseline information could also be gathered. However, clarification was provided by KORDI to the effect that no specific plans for disturbance studies had been formulated and that cruise to the nodule province typically use grabs for sampling with low levels of disturbance.
- The Kaplan Project, which is already funded in part, is strongly linked with the work proposed here. There is a problem of temporal mismatch, in that the initial Kaplan cruise is planned for summer 2003, which is too soon to identify and secure funding for a disturbance/recolonization study. Nonetheless, later Kaplan-related cruises to expand on the initial sampling area could form the basis for strong collaboration links with the work proposed here.
- IFREMER was also identified as a potential collaborator given its stated interest in similar questions relating to the seafloor. However, as no representative from IFREMER was here, contact will be made to assess their level of interest.
- The new ROV at the Southampton Oceanographic Centre was also identified as a potential platform for carrying out experimental procedures on the seafloor. However, there were questions raised regarding the availability as well as the capabilities (in terms of load bearing). Both India and China were known to have developed (to prototype stages) mining platforms – the status of these developments and the potential for utilization in experimental research needs to be assessed.

Resources Required

The required resources were highlighted in the summary of last year's workshop (Appendix B) and in the presentations at the current workshop. The major obstacle is that this study requires a minimum of two ROV/submersible cruises including one cruise to acquire baseline data, given that methodologies need to be consistent across the project.

Tray Experiments

The tray experiments to assess recolonization rates in strictly controlled conditions were outlined in detail in the summary of the first workshop (Appendix B). The tray experiments are designed to focus primarily on larval and settlement dynamics. There are practical considerations involved in this proposed study relating to the impacts on sediment geochemistry that would require the active involvement of geochemists in this experiment. Furthermore, an additional treatment scenario was proposed whereby trays could be deployed with different sediment compaction levels. The implication on resources of this additional treatment was considered somewhat negligible.

Table 1: Evaluation of potential collaborators

Collaborator	Required Resources	Status
KORDI	Ship Time	No current plans, need ROV/submersible
KAPLAN	Ship Time	Schedule tight, need ROV/submersible
IFREMER	Ship Time	Status Unknown
International Exchanges	Personnel Training	Programmes Exist
Pew & Other Foundations	Ship Time, Salaries, Equipment etc.,	Proposal needed for major funding

Collaborators

The inclusion of a geochemist and microbiologist as principal investigators in this study was considered important. On a larger scale the, the interest of IFREMER in this aspect should also be gauged. Scientists from Ireland and Germany suggested several of their colleagues who they felt would be interested in participating.

Resources Required

The costs and availability of ROVs and/or submersibles from Southampton or Woods Hole should be assessed. The estimated costs of ship time and equipment, etc. outlined in the earlier proposal (Appendix B) otherwise remain much the same.

Conclusions

Though some new experimental ideas were suggested (e.g. canopy type experiments to reduce sedimentation, compaction treatments in tray experiments) there were no fundamental changes proposed to the basic experiments. Nonetheless, it was felt that the critical mass of scientists needed to further develop the experiment was not present at the workshop and more work is needed to refine and develop the specific details of the experiments.

Considerable discussion centered on devising means to reduce costs and the cost and effort associated with the proposed research. Suggestions included:

- Using an existing disturbance area, and
- Reduce cost associated with disturbance creation by doing it without an ROV/submersible.

In the case of working in a previously disturbed area, it was concluded, that from a biological perspective, the level of control obtained in previous studies would be insufficient to draw any concrete conclusions regarding the level of impact and recolonization rates. In addition, there were numerous questions relating to the scale of impacts obtained in previous studies and whether they were realistic. For example, the level of disturbance obtained in DISCOL is difficult to quantify and therefore, it would be difficult to apply the results obtained in this study to the specific questions relating to scale and impacts detailed in this survey. In summary, it was concluded that to obtain a robust and statistically relevant experimental design, a very high level of control is needed in order to set the specific levels of disturbance (e.g. swath width).

In the case of creating a disturbance without the availability of an ROV or submersible to subsequently sample the area, it was determined that identical sampling methods were critical in order to avoid confounding sampling gear artifacts with response dynamics. Thus, ROV or submersible sampling gear is required for all cruises, including the initial pre-impact sampling. Therefore, although it may be necessary to use a disturber that is towed by the surface ship (indeed there was some doubt as to whether an ROV could mimic a realistic disturbance), there is no alternative to using an ROV or submersible on the same trip in order to achieve the necessary pre-impact biological sampling.

In summary, it was concluded that to obtain a robust and statistically relevant experimental design, a very high level of control is probably needed in order to set the specific levels of disturbance (e.g. swath width). Perhaps more importantly, the maintenance of a consistent form of sampling device is critical to avoid confounding interpretation. The standardization of sampling methods and scale of sampling throughout all stages of this project is critical to successful implementation and conclusion of this programme of experiments. For example, the use of a box corer to obtain baseline information and a smaller multi-corer at the later monitoring stages would be difficult to justify and would seriously confound subsequent statistical analysis. The standardization of sampling methods has been highlighted as a major

issue in marine science and has been a consistent theme in the workshop as a whole (See ISA Workshop on Standardization).

It is appreciated that this study is an expensive proposition. A number of factors contribute to this expense:

- the experimental, rather than observational, nature of the study,
- the study is process oriented and quantitative rather than descriptive, and
- studies of local scale effects require very careful analysis.

Recommendations

Several recommendations arose from discussions within the group and with members of other groups. In each case, we have identified a point person to spearhead each activity:

- talk to the Kaplan Project and ISA regarding GEF proposal. Contact Census of Marine Life (Sloan) and other foundations (e.g. Pew). Dr Snelgrove and Professor Smith were identified as leaders in this effort.
- assemble literature on disturbance, etc. over the next few months. Dr Snelgrove is to lead this effort.
- contact potential collaborators. Dr Snelgrove and Professor Smith to lead this effort over next few months. Interested parties include Snelgrove, Smith, Lamshead, Nath, O'Beirn, Boetius.
- assemble roster of scientists, organize small workshop (5-8 people) within the next year. Dr Snelgrove, Professor Smith and the ISA to lead this effort.
- talk to IFREMER. Dr Snelgrove and Professor Smith to lead this effort.
- follow up on Russian Federation Plans (Dr Parsons to initiate discussions).
- liaise with next year's workshop on geological modeling to determine available data and interest of other parties. Professor Smith and Dr Snelgrove to lead.

Chapter 18

Report and Recommendation of the Working Group on Water Column Ecosystem Impacts

Working Group Organization

Working group members were Tony Koslow, Narinder Nath and Woong-Seo Kim

Key Issues Addressed

Deep seabed mining of polymetallic nodules may have substantial impacts on water column ecosystems in the Clarion-Clipperton Fracture Zone (CCZ) or in the Central Indian Ocean Basin (CIOB), where mining claims are held. Pelagic ecosystems in these regions are characterized by low nutrient concentrations, low productivity in the euphotic zone, plankton community dominated by small primary producers and micro-zooplankton grazers, with little carbon export to deep water.

There is still considerable uncertainty how deep seabed mining will be carried out and what would be the composition of the wastes that will be disposed off at sea. Hydraulic suction mining will transport large volumes of deep water and sediment to the surface, along with the nodules, in contrast with continuous line bucket mining. A combination of the two processes would also be a possibility. Wastes from nodule processing may be disposed off in surface waters or at sub-surface depth. Given present levels of uncertainty, assessing the risks of future scenarios for deep seabed mining operations will require the development of models based on a detailed understanding of relevant processes and factors.

Transport of large volumes of nutrient-rich cold deep water into the euphotic zone may substantially alter the character of the pelagic community, enhance nutrients, significantly increase biological productivity, shift the biological community toward larger phytoplankton and grazer groups, and increase the export of particulate carbon into deep water.

Oxidation of large amounts of organic carbon will require large volume of dissolved oxygen and this may further intensify the Oxygen Minimum Layer. These changes in turn will substantially affect the deep-sea benthic diversity and populations. Sediment released into

surface waters, on the other hand, will increase turbidity, thereby diminish light penetration, decrease primary biological production, alter microbial food webs and their diversity. In addition, wastes from nodule processing will contain a complex mix of trace metals, which may stimulate or inhibit bacterial and primary biological production, alter community composition and introduce heavy metals into marine food webs.

Considering the great complexity involved in the estimation of the impact, it is appropriate to proceed through a series of observations, controlled experiments, and complex predictive models. To summarize, the following are the key issues that we seek to address in this study:

- What are the characteristics of the discharge that includes deepwater, sediments, sediment interstitial waters and nodule-processing wastes?
- What is the effect of mining discharge on water column chemistry?
- How does the optical profile through the water column change directly from sediment and waste discharges and indirectly from changes in biogenic particle size and concentration?
- How do the sinking sediment and other particulate matter affect bacterial populations and grazers through the water column?
- How are the productivity, size and species composition of the pelagic plankton community affected by mining discharges?
- How are oxygen levels affected by the alterations in productivity?
- To what extent are the heavy metals incorporated into the food web and magnified?
- What are the effects of mining discharges on carbon export to deep waters?

Approach

To address the issues in the initial phase of the project, we propose a linked programme of experimentation and modeling to treat the influence of the three primary factors (nutrients, sediments and trace/heavy metals from nodule processing wastes) both individually and in concert, to test for synergistic or interaction effects. A nested series of experiments will be carried out at two scales.

- micro-scale (bottles) to characterize processes and interactions (e.g. primary and bacterial productivity, grazing) and to parameterize dose responses
- mesocosm (bag) experiments carried out in the open ocean over periods of one month to examine community evolution over a range of treatments

In general, micro and mesoscale experiments will be conducted with replicated controls and three replicated treatments. Ecosystem (trophodynamics) and biogeochemical (carbon budget) models will be developed continuously in conjunction with the experimental programme. The primary collaborators are scientists from the Korean and Indian contractor research organizations KORDI (Korea), NIO (India) and independent scientists from CSIRO and University of Western Australia (Australia). The experimental programmes will be carried

out in alternate years in the CCZ and Indian Ocean basins by teams covering the following disciplines:

Geology	Particle flux, nodule chemistry, sediment characteristics,
Biology	Phyto- and zooplankton community diversity, abundance and interactions, bacterial abundance and activity,
Chemistry	Trace/heavy metals, nutrients, O ₂ , CO ₂ , pH, eh,
Physics	Temperature – salinity profiles, water-mass structure, currents, optical properties, turbidity and meteorological data, and
Modeling	Physical, biogeochemical, ecological, risk assessment.

KORDI and NIO have research teams with skills in most research areas. CSIRO and UWA will contribute expertise primarily in the areas of phytoplankton and zooplankton dynamics and modeling.

Expected Outputs

- Data and models to evaluate water column environmental impacts under several mining and mine-waste disposal strategies (continuous line bucket, suction, or a combination; surface and intermediate water disposal)
- Model deep sea mining impacts on carbon sequestration
- Inputs for Phase II model testing during pilot mining operations, i.e. to test model predictions under pilot mining conditions
- Outputs will be produced in the form of databases, reports, publications, conference reports and inputs to ISA management of deep seabed mining
- Human resource development will be addressed
- Assisting the Authority in formulating policies

Available Resources

KORDI and NIO have ongoing field efforts in the CCZ and Central Indian Ocean basin. Much of the requisite field and laboratory equipment and facilities are available for use in the project. Experienced research teams will contribute their time to the project. Limited shiptime will be contributed.

Additional Resources Required

Additional shiptime will be required to support the field effort. Twelve mesocosms suitable for open ocean deployment will be constructed. Operating expenses will be required and travel for exchange of scientists between the collaborating institutions and for annual workshops to review progress and plan future work. Substantial manpower training will be undertaken as part of the project: PhD students, post docs and short-term training opportunities in the field and laboratory. The project will be carried out over 5 years: 4 years of fieldwork and a final year for analysis, project synthesis and write-up. The estimated cost in

additional resources required for the 5-year project is US \$3 million. The co-authors will represent our respective institutions in developing a proposal with the ISA to be submitted to the GEF. A further workshop comprising project participants may be held in late 2002/early 2003 in Korea, India or Australia to review and coordinate the project and bring this stage of proposal development to completion. Without additional resources, the project cannot be carried out.

Chapter 19

Report and Recommendations of the Working Group on Natural and Spatial Variability in Deep Ocean Ecosystems

Dr. Tony Jones

Introduction

It is increasingly appreciated that deep-ocean ecosystems vary unexpectedly in space and time. Particularly important questions concerning this variability include the following:

- To what degree does variability in the upper ocean ecosystem result from processes such as El Nino-Southern Oscillation (ENSO) events?
- How tightly are the upper ocean and abyssal benthic ecosystems coupled given the variability on time scales of the ENSO events?
- What are the spatial scales of variability in biological communities, geochemical cycling, sediment types and topographical units in the CCZ given that we observe environmental gradients across the CCZ both in the East-West direction and North-South direction?
- What is the influence of laterally derived deep ocean water such as Antarctic Bottom Water (AABW) on benthic ecosystems within the CCZ?

Rationale

In order to examine natural variability, it is important to "know your area" including such things as bathymetry, habitat type, sediment geochemistry and sediment structure, among others.

Natural variability can be divided into variability associated with space and variability associated with time.

Spatial Variability

Spatial variability is of interest especially considering the environmental gradients in the CCZ (i.e. depth, primary biological productivity, nodule geochemistry). Variability of sediment fauna can be best examine by defined size categories such as megafauna which are sampled with photography/ROV, macrofauna which are sampled with box core, meiofauna which are sampled with multicore and examination of geochemistry of sediment.

Temporal Variability

Interannual change is of primary importance. Various types of measurements such as current meter moorings, sediment traps, and time-lapse photography can be used to address issues related to interannual change.

Focus on benthic environment

As the major environmental impact is likely to occur in the areas that are mined, it is suggested that the natural variability study focus on benthic environment, while also considering relationship with the overlying upper ocean.

Existing Capacity

It is recommended that existing deep sea equipment be utilized, such as:

- box corer,
- multicorer,
- CTD and water sampler,
- sediment trap and current meter mooring system with time-lapse camera system,
- benthic trawl
- deep tow (video and still photography)

Note: Visual record of changes between cruises was deemed important to examine temporal variability.

Co-ordination

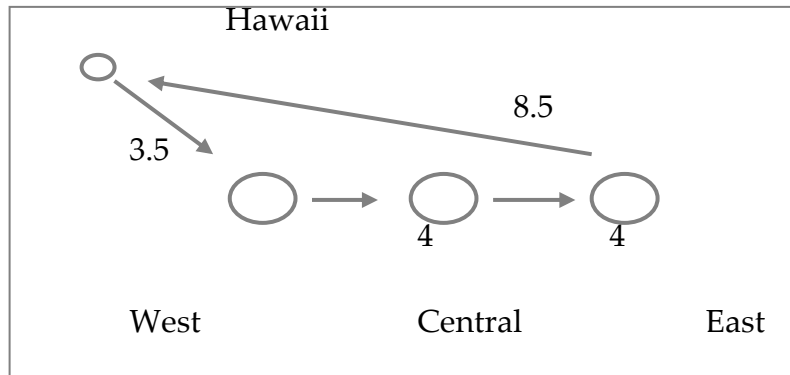
It was suggested that the Proposal be submitted to the International Seabed Authority (ISA). It was proposed that ISA co-ordinate the activity. One idea was to set-up a scientific board.

The science board would:

- identify standard methods,
- select sites or sampling strategies for different disciplines, and
- recommend scientific participants.

Cruise Duration

Cruises are limited to 45 days. Proposed cruise track shown below.



Transit Time = 24 days

Sampling Time = 21 days

One major constraint is the amount of available “wire time”. It was recommended that we need to look at:

- faster ships and
- faster sampling technology.

Approximate Funding Resources Required

Ship time:

Three expeditions would be required during the first three years.

Three survey sites distributed from East to West in CCZ will be investigated.

Every expedition need about 45 days, including 21 days wire time on survey stations in CCZ, 24 days transit time for round trip among stations and between Honolulu and CCZ.

Total ship time for three cruises = $3 \times (21+24) = 135$ days

Total ship time costs

(e.g. *RV “Da Yang Yi Hao”*)

135 days \times \$30k/day = \$4050k

		Time (Hr)
Boxcorer	4.5 hr * 15	67.5
Multicorer	4.5 hr * 10	45
CTD + Water sampling	4.5 hr * 5	22.5
Sediment trap + current meter mooring system		6
Benthic trawl	7.5 hr * 2	15
Deep Tow		12

New equipment required:

Three Sediment trap +current meter mooring chains	\$400k
Time-lapse camera system	\$120k
One Seawater chilling system	\$10k
Miscellaneous equipment	\$100k
Total equipment costs	\$620k

Personnel:

Technicians:

Meiofaunal	sorting	3 yr
	identification	2 yr
Macrofaunal		
	sorting	2 yr
	identification	2 yr
Microbial technician		1 yr
Plankton identification		2 yr
	Chlorophyll and primary production	1 yr
	Remote sensing Specialist	
	Geochemical technician	3 yr
	Water chemical analysis —	3 yr
	Physical oceanographic technician —	2 yr

Total Technician costs = 21 yr × \$44000 per yr \$924k

Investigators:

Principal investigator/coordinator	
2mos/yr × 5 yr = 10 mos	
Five investigators	
1.5 mos/yr × 5 yr × 5 = 37.5 mos	
Total investigator costs = \$10000 × 47.5 mos =	\$475k

Travel:	\$100k
Supplies:	\$ 50k
Shipping:	\$ 20k
PROJECT COSTS:	
Ship time	\$4050k
Direct support costs	\$2199k
Overhead at 40%	\$880k
<i>Total</i>	\$7129k

Three cruises, Three years

It was suggested that 3 cruises over a 3-year period be proposed with a 2003 – 2007 timeframe.

Appendices

Appendix A

Megabenthic Assemblages associated with Polymetallic Nodules in the East Pacific Ocean Clarion – Clipperton Fractures Zone

Dr. Virginie Tilot

The structure of megafaunal assemblages in different areas of the polymetallic nodule province was investigated on a large scale. This comprehensive study over a large area serves as a reference for deep-sea research in abyssal plains and for resource management in the nodule area, where future mining may take place.

On a qualitative level, the identification, ethology, taxonomic richness and faunal composition classified by functional and trophic groups has been assessed within the French nodule provinces in the Clarion – Clipperton Fracture Zones comprising about 430,000 km² (zone NORIA). Comparisons were made using photographic data from the DOMES C site taken by a Deep Tow camera system. An annotated photographic atlas has been assembled with contributions from specialists in each phylum. The basis of the collection is more than 200,000 photographs and 55 hours of videos (*Figures 1, 2*) taken by IFREMER's unmanned free vehicle Epaulard, towed fish "RAIE", free fall grabs and manned submersible "Nautilie" (*Figures 3,4*). The analysis reveals a total taxonomic richness of 240 taxons including 46 Echinodermata. Cnidaria represents the most diversified phylum with 59 taxa. Suspension feeders were the dominant trophic group.

The quantitative study is based on photographic and videographic data in two particularly well-studied areas, NIXO 45 and NIXO 41, within the French nodule mining area. NIXO 45 is about 400 km² lying at a mean depth of 4950 m and including a large array of environmental conditions (*Figures 5,6*). Since 1975, IFREMER has explored its mining concessions by means of Seabeam, side-scan sonar, seismic profiles, box cores, free-fall grabs and cameras. In reviewing various data sets including slope, sediments (Oligocene to Plio-Quaternary), topography, geology, and currents, we have classified different edaphic and nodule "facies" which have variable nodule composition, nodule size, abundance and surface cover (*Figure 7*).

Within NIXO 45, a total of 13 different facies has been identified according to morphological characteristics, nodule coverage and nodule size, topography, degree of burial and sediment geochronology. Among these different facies, the megafaunal density and composition have been assessed according to different classifications, by phyla, by trophic and

by functional groups. A total of 122 taxons including 37 Echinodermata, and 27 Cnidaria have been identified which corroborate with megafaunal taxa richness observed at the Domes C site (Figure 8). The total density of the megafauna in NIXO 45 is estimated at 498 taxa/ha, which is higher than total density of megafauna in Domes C (361 taxa/ha). The primary production is greater (180 gC/m²/yr) in NIXO 45 than DOMES C. The difference in primary production may explain the difference in total megafauna. However, no conclusions could be drawn because the sampling area is smaller and environmental conditions are different in Domes C (Figure 9).

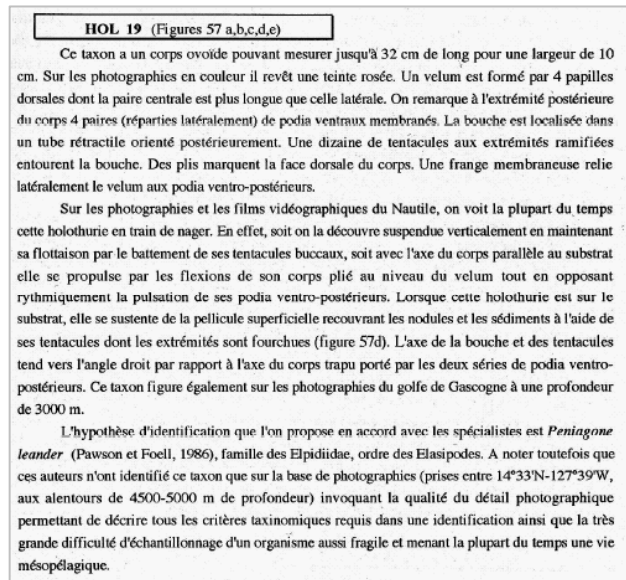
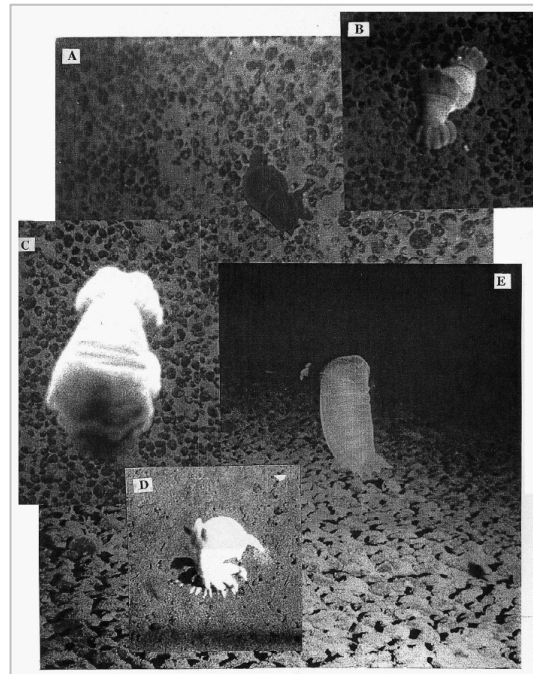
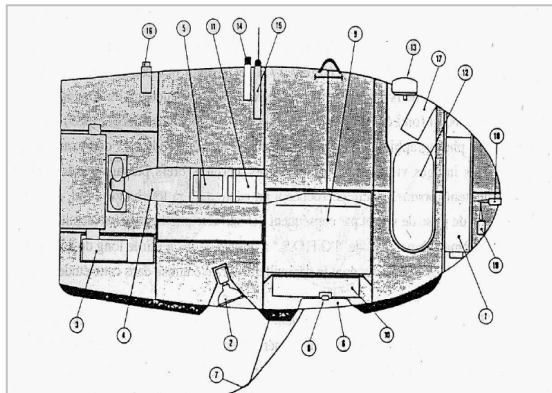
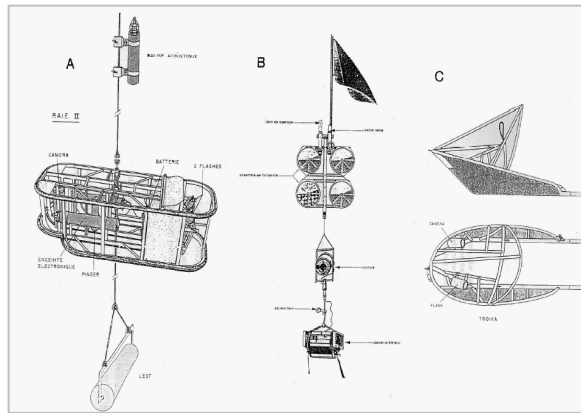
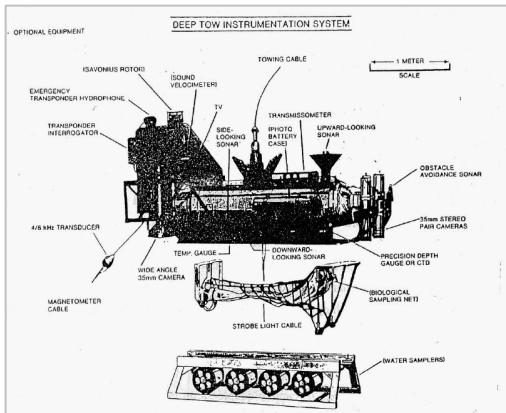


Figure 1: Example of the annotated photographic atlas, comments on the holothurian "Hol 19", identified as *Peniagone leander* (comments in French, not yet translated in English).

Figure 2: Example of the annotated photographic atlas, example: photographs of the holothurian "Hol 19", identified as *Peniagone leander*.





Figures 3, 4: Graphic representation of IFREMER's free unmanned vehicle with cameras "Epaulard", and the American Deep Tow instrumentation system.

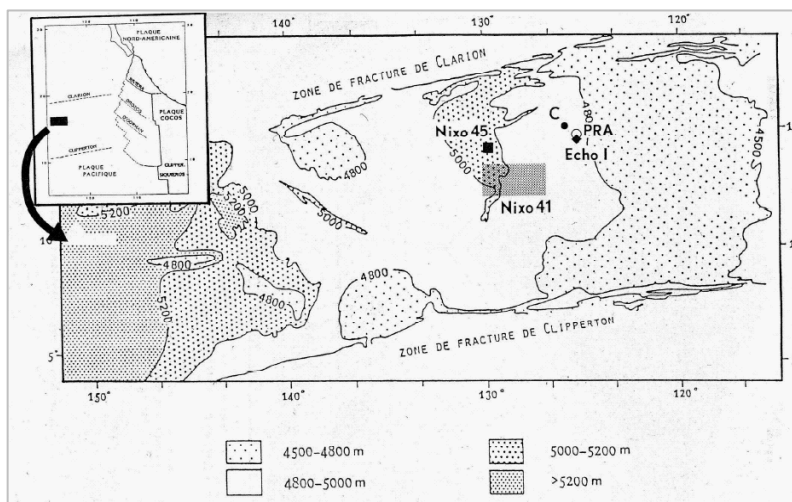


Figure 5: Location of the 3 study sites, Nixo 45, 41, and Echo I in the Clarion Clipperton fracture zones

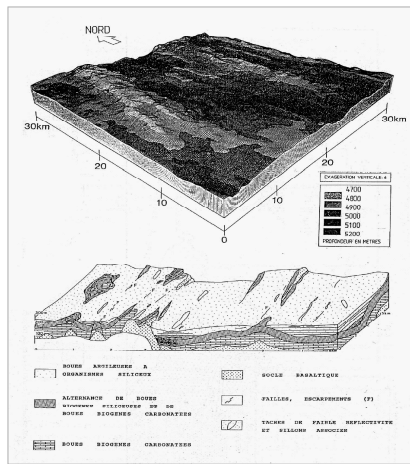


Figure 6: Block diagram of Nixo 45 site with a diagram describing the geological formations.

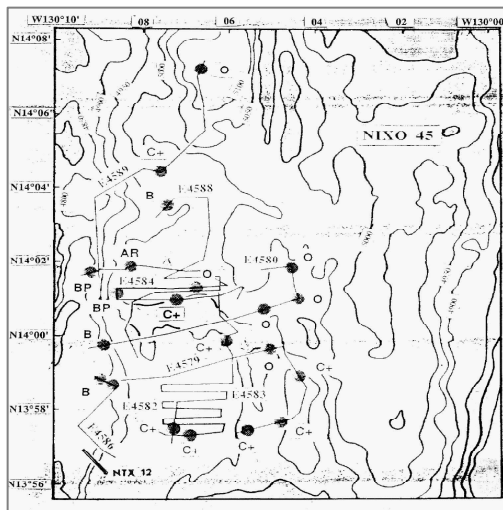


Figure 7: Localization of the photographic surveys taken by "Epaulard" and the "Nautile" along Nixo 45 and identification of the different nodule facies.

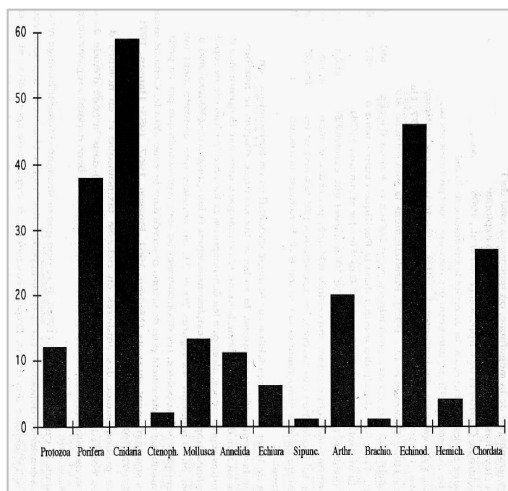


Figure 8: Graphic representation of the megafaunal taxa richness in the CCZ.

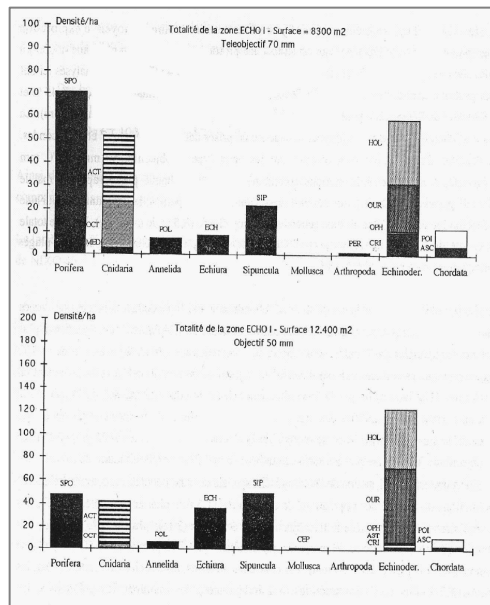


Figure 9: Graphic representation of the abundances of taxa seen by Deep Tow over Echo 1 site with 70 and 50 mm lenses.

The total density of Cnidaria (258 taxa/ha) is greater than Echinodermata (180 taxa/ha) in both NIXO 45 and NIXO 41. These results corroborate with assessments made in an area southwest of NIXO 45 and in DOMES C (Figure 10).

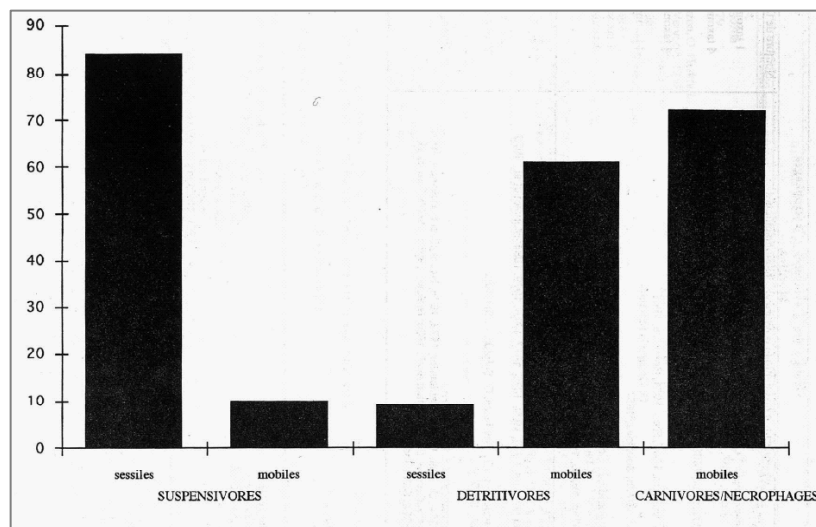


Figure 10: Graphic representation of the megafaunal taxa richness in the CCZ classed by trophic and functional groups.

Results show that suspension feeders are more numerous than detritus feeders, carnivores or necrophagous feeders regardless of the facies. The total faunal abundance is more important on facies C+ (10% coverage, nodules 7.5 cm in diameter) and facies C+ with 20-40% nodule coverage on slopes $>15^\circ$. As for facies BP (smaller, oval, flattened and often conglomerated nodules), the megafauna is more abundant when the nodule coverage is less than 35%. The lowest faunal abundance occurs in facies B (40% coverage). Higher densities of suspension feeders are observed on facies O (no nodules) composed of old sediments (from Oligocene to Middle Miocene) and on facies C+ with a slope $>15^\circ$. Detritus feeders are more abundant on facies C+ (10% coverage with no slope) (Figure 11, 12). On facies C+ (20 – 40% coverage on a slope $>15^\circ$), there is a majority of suspension feeders such as sponges, Hexactinellids sponges, Cladorhizids and polychaetes. The most abundant detritus feeders are echinoderms Brisingids and holothurians. Generally, carnivores are represented by decapod invertebrates and Bythitids. Facies B (small nodules 5 cm in diameter with 50% coverage) is the most interesting commercially. This facies hosts more suspension feeders (mostly Actinians, Antipatharians) than detritus feeders with Echinodermata (mostly holothurians).

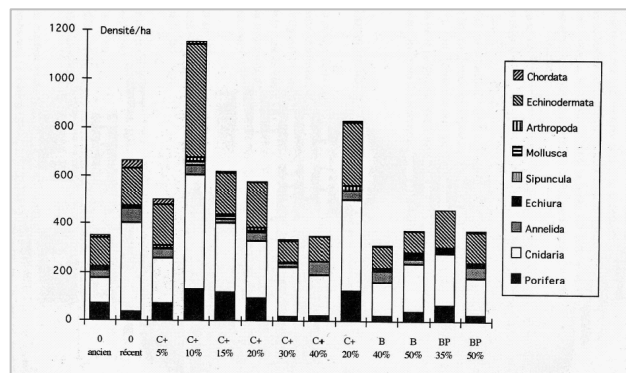


Figure 11: Graphic representation of the megafaunal abundance within each facies in NIXO 45.

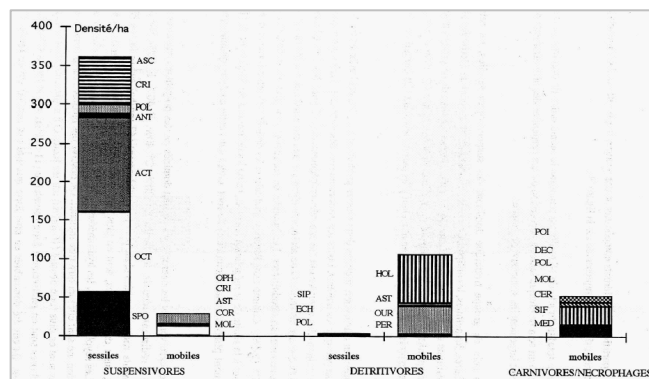


Figure 12: Graphic representation of the megafaunal abundance within each facies in NIXO 45 classed by trophic and functional groups.

The megafaunal assemblages were assessed using multivariate statistical techniques that compare similarities among taxons and edaphic (substrate) facies. This enables us to differentiate preferential habitats and "faunal facies" ranked according to an edaphic gradient.

The quantitative analysis also allows for assessing the spatial heterogeneity of the taxa distributions (Figure 13).

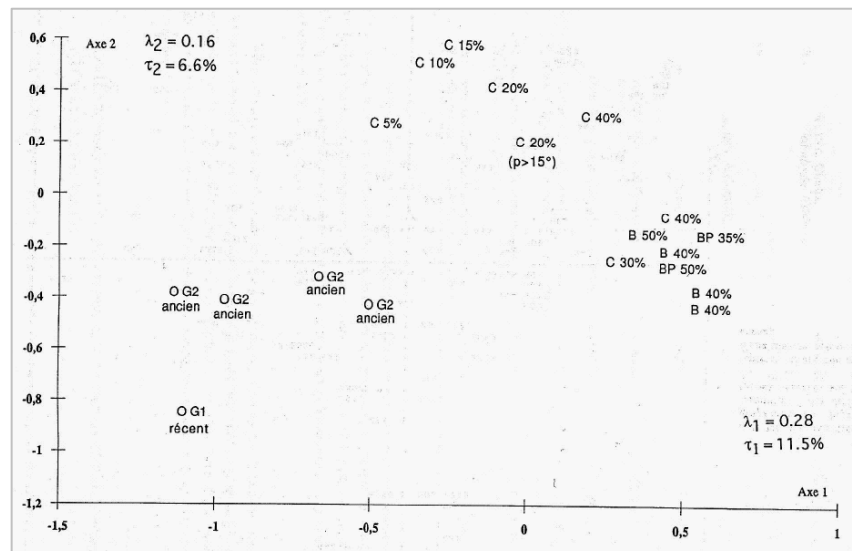


Figure 13: Graphic representation of the degree of similarity of the different facies in NIXO 45 site assessed by multivariate analysis.

For example, Facies C+ 10% hosts a greater abundance of mobile detritus feeders such as sipunculids, echinids, holothurians. The most abundant suspension feeders in this facies were sponges hexactinellids, hyalonematids and octocorallians Primnoids.

Comparisons between different sampling equipment and sampling strategies are drawn from "in situ submersibles observation". IFREMER's manned submersible "Nautilie" explored one of the facies in the NIXO 45 site. IFREMER's towed submersible "RAIE" sampled three facies over the NIXO 41 site and the "Deep Tow camera system" was used to sample pictures of 3 nodule facies identified at the ECHO 1 site (Figure 9). The strategy of sampling with time lapse photography along fixed transects with the free unmanned vehicle Epaulard has proven to be more reliable than photographic sampling by the "Nautilie" along transects. On the other hand, the Nautilie has proven to be better at documenting the ethology of the benthic life. The new IFREMER's ROV Victor has proven, in other cases, to be a better platform for sampling, photography and filming at abyssal depths. The next French nodule programme is planned to use this latest technology in our study sites.

The megafaunal biomass, arranged by trophic and functional groups are assessed and annotated for two study sites (NIXO 45, NIXO 41). The data were compared with the biomass of megafauna, macrofauna and meiofauna from DOMES site C and with data from an Atlantic Ocean site, Meriadzek Terrace, a shallower site (2000 m) in the Bay of Biscay (Figure 14, 15). The total megafaunal biomass of NIXO 45 is lower than NIXO 41 and 6 times higher than at ECHO I. Both NIXO sites have the same faunal composition with a dominance of suspension feeders. The biomass values of Meriadzek Terrace are very different with a dominance of detritus feeders then carnivores over the suspension feeders.

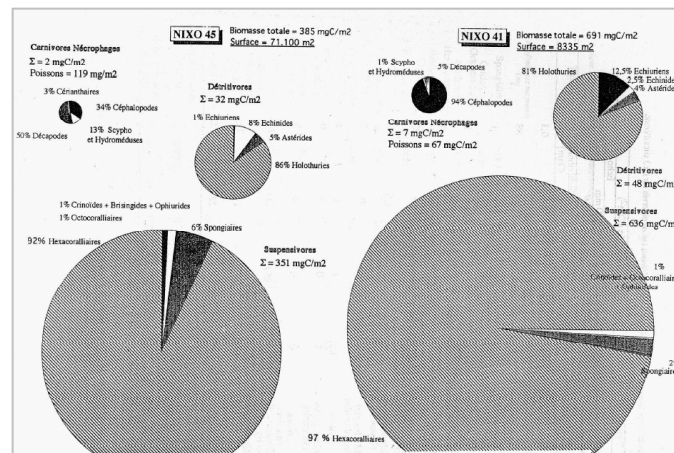


Figure 14: Graphic representation of biomass assessments of the megafauna classed by trophic groups for NIXO 45 and NIXO 41.

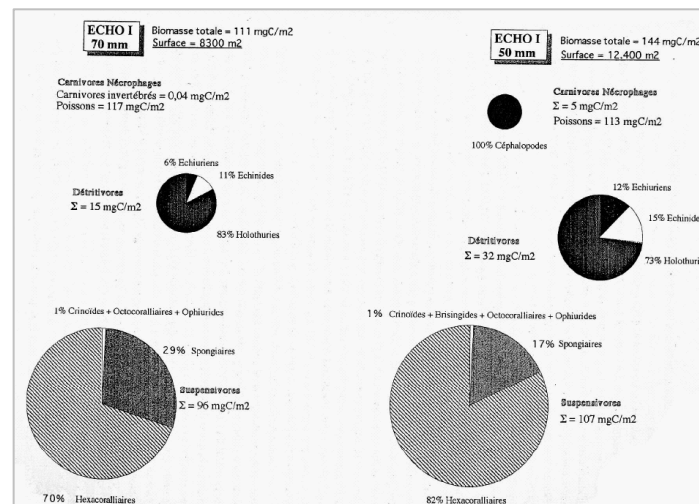


Figure 15: Graphic representation of biomass assessments of the megafauna classed by trophic groups for ECHO 1.

The roles of limiting factors (heterogeneity, geomorphology, currents, sedimentation) and biotic factors (natural microdisturbance of surface layers) are proven to be essential in modeling the epibenthic assemblages in nodule ecosystems. On the basis of these findings, one can assess the impact of the benthic plume created during nodule mining. The groups most likely to be affected are the suspension feeders and the benthic sessile infauna. As competition between suspension feeders (cementing the substrate with particules mixed to sediment) and the detritus feeders (in particular echiurians biodisturbing the sediments) occurs generally on the benthos, one could assess how both communities would interact in areas impacted by the resuspended benthic plume. The importance of biotic factors in stabilizing sediments and in maintaining the nodules at the sediment surface has been emphasized in the literature.

The concept and success of setting up artificial substrates (such as artificial reefs in impacted coastal areas) on the abyssal benthos could be a means of mitigating loss of nodule substrate. Large frame-like devices could favor the heterogeneity and microtopography that epibenthic megafaunal assemblages seem to prefer. This observation can be drawn from the results of our study, the preferential substrate being a larger abundance of nodules as in facies B and C+ and with a slope $>15^\circ$. These frames could trap particles and sediments thus creating an array of microhabitats that would favor recolonization of sessile suspension feeders. Also microtopography may create more turbulent conditions, hinder burial of nodules and favor a more complex hierarchy of faunal assemblages and the diversification of trophic resources. This alternative could be tested in one of the upcoming benthic studies within the nodule province in association with sediment burial experimentation.

Conditions for deep-sea megafauna research are difficult, expensive and time consuming. In the abyss most of the megafauna assemblages are widely distributed and new to science. Morphological identification based on photographs and films is not sufficient. Biological specimens are compulsory for faunal identification. DNA identification will first need to establish a reference collection or library of all living megafauna in the area, an expensive and time consuming procedure. The large collection of photographs and films collected during the exploratory surveys are most valuable for morphological identification considering the large distribution and relatively great diversity of the megafauna. Photographic and videographic sampling along transects should be continued and coupled with DNA sampling in order to establish the baseline reference for megafaunal assemblages. One method does not preclude the other; they complement each other. Also photographic and videographic surveys are generally used as valuable tools for rapid assessments over large areas for different purposes such as exploration, monitoring and impact assessments of different habitats and ecosystems. On the basis of these photographic and videographic assessments, guidelines and recommendations can consequently be drawn for the sustainable exploitation of natural resources.

Appendix B

Summary of Proposed Research from the International Seabed Authority Workshop held in March 2002

Experiment 1.

Burial sensitivity of the CCZ benthos: Elucidating the single-dose response function

Rationale

Despite decades of study, we still cannot predict the impacts of re-sedimentation from mining plumes on abyssal seafloor communities. We cannot say, for example, whether rapid accumulation of 1 cm of sediment will wipe out the benthic macrofauna or have little effect. In order to predict the spatial impacts of mining on seafloor communities, it would be extremely useful to elucidate dose-response functions between re-sedimentation thickness and seafloor community mortality. Knowledge of burial dose-response functions would be extremely useful for scaling up results from small-scale (e.g., pilot-mining) studies to the prediction of full-scale mining impacts. Prediction of the spatial scales of mining impacts is essential to assess the potential for nodule mining to cause species extinctions.

Scope

Conduct an experiment at the seafloor in the CCZ to elucidate dose-response relationships of various biotic components (microbes, meiofauna, and macrofauna) and habitat characteristics (e.g., sediment geochemical properties) to acute re-sedimentation thickness.

Experimental design could be as follows:

- Treat controlled, replicated areas of sediment with 5 treatment levels of burial (0, 5, 10, 20 and 40 mm of re-sedimentation).
- Create ~8 replicate treatments per treatment level (with each treatment being ~0.6 m on a side, created by sediment injection into fences). NB: the technology for conducting such experiments has already been developed.
- Resample experiments after one month (i.e., long enough for mortality and decay to have occurred, but short enough to preclude substantial recolonization).

- Experiments to be deployed and sampled with an ROV (preferably) or a submersible. An ROV is preferred because of the much longer bottom times attainable at 5000 m with an ROV compared to manned submersibles.

Output

Dose-response function of benthic community mortality following rapid redeposition, to be combined ultimately with plume models to predict the spatial extent of mining impacts.

Duration

Twelve months for equipment design, fabrication and shipping; 2 months for the field programme; 24 months for processing and publication preparation. Total project duration is 3.5 yr.

Approximate Funding Resources Required

Ship time: To implant and sample experiments, two ROV cruise would be required. Assuming use of an ROV like the French Victor, the first cruise would require 12 d on station and approximately 12 d transit (round trip between San Diego and the eastern CCZ). The second cruise, occurring ~ 1 month later, would require 10 days on station and 12 transit day. Total ship time costs 50 d @ \$44,000/day = \$2,200,000

Equipment: Sediment delivery system - \$60,000; 25 corers at \$4000 each - \$100,000; 30 fences - \$15,000, Miscellaneous - \$15,000.
Total equipment costs = \$190,000

Personnel:

Macrofaunal	-	sorter	2 yr
	-	identification	1 yr
Meiofaunal	-	sorter	1 yr
	-	identification	1 yr
Molecular technician	-		1 yr
Geochemical technician	-		1 yr
Microbial technician	-		0.5 yr
Total Technician time 7.5 yr X \$44,000 per yr = \$ 330,000			

Investigators and project coordinator: 5 at 1 mo/ yr X 3 yr X \$10,000 per month = \$150,000.

Travel: Planning meeting \$25,000; cruise participation - \$25,000; synthesis meeting - \$30,000; collaboratory visits and presentation of results at conferences - \$15,000.

Total travel = \$95,000

Supplies: \$50,000

Shipping: \$20,000

Total 3.5 yr Project Costs

Ship time	\$2,200,000
Direct support costs	\$ 885,000
Overhead at 40% of direct	\$ 348,000

Total	\$3,433,000

Experiment 2.*Time and space scales of benthic community recovery following simulated mining-track disturbance*Rationale

We cannot predict with any confidence the time scales and mechanisms of seafloor community recovery within mining tracks. Nor do we know how these recovery processes depend on the spatial scale of mining disturbance. Both recovery time scales and spatial-scale dependence of recolonization processes must be understood to predict and manage the environmental impacts of mining. For example, if recolonization is very spatial-scale dependent (i.e., occurring much more slowly in large areas) it may be desirable to leave undisturbed corridors between passes with the collector system to facilitate more rapid community recovery, and to mitigate the chance of extinctions.

Scope

The goal is to evaluate time scales of recovery and evidence of spatial-scale dependence in recovery processes for CCZ seafloor communities in the track of a collector system.

Key points in experimental design are:

- Need to remove at least 5 cm of surface sediment and expose the deeper, compacted sediment layers over controlled spatial scales. Disturbance-creation options to be explored include: submersible or ROV bulldozing (most likely), submersible or ROV thruster resuspension, miniature collector-system operation, and towing of a plow from a surface vessel.
- Key mechanistic insights into recovery processes could be obtained by time-series studies of simulated mining swathes 1, 3, and 9 m wide. Aspects studied would include:
 - sediment geotechnical properties,
 - sediment geochemistry, and
 - the responses of microbial, meiofaunal, macrofaunal and megafaunal communities.

- Development of a mechanistic, conceptual model of geotechnical, geochemical and biological succession in mining tracks would provide a valuable framework for track-recolonization studies and should be pursued.
- It would be useful to concomitantly compare colonization processes in sediment trays and simulated mining tracks to determine whether tray colonization might be useful as an “indicator process” in nodule areas beyond the experimental study site. For example, can trays be used to assess potential recolonization processes in other areas? In addition, colonization trays could be used to explore the effects of different types of disturbance on recolonization processes [e.g., (a) complete removal of the top 5 centimeters, (b) complete removal of the top 10 cm of sediment, and (c) removal of nodules and homogenization of the top 5 cm of sediment].
- Recommended time intervals of sampling are: $t = 0, 1 \text{ y}, 3 \text{ y}$, with fine-tuning of longer interval based on results after three years (for planning purposes, 9 y and 20 y might be considered).
- Numbers of replicates per treatment type and time: ideally 5-6, minimally three. Colonization tray experiments obviously could have substantially higher replication.

Second-Order Process Studies

Although not the central focus of the study, a number of processes could be studied at relatively little additional cost to provide insights into factors influencing recolonization at the abyssal seafloor. These include evaluation of larval availability in the benthic boundary layer using plankton sampling (e.g., net tows or larval pumps) and sediment-trap deployments at various elevations above the seafloor.

Output

A synthesis paper presenting a conceptual model of potential succession processes, including testable hypotheses concerning mechanisms of succession in abyssal mining tracks. A prediction of the minimum time scales, and mechanisms, of community recovery in mining tracks, and the presence of the spatial-scale dependence in these processes.

Approximate Funding Resources Required

Ship time:

To set up the experiment and collect samples at time zero, we estimate that 22-25 dives with a submersible such as Nautilie would be required. In addition, 12-16 days of transit time would be required for the support vessel to get to and from the CCZ study site from San Diego or Honolulu, indicating a ship time requirement of 34-41 days for the first cruise. At \$44,000 per day for the RV L'Atalante (the support vessel for Nautilie):

Ship time cost for cruise one = \$1,800,000.

Each additional sampling cruise would require approximately 6 dive days and 12-16 transit days (a total of 18-22 days at \$44,000 per day), yielding:

Ship time costs for cruises two and three = \$1,940,000.

Equipment:

Plowing device:	\$50,000
12 large Ekman corers @ \$3000	36,000
Miscellaneous equipment	30,000

Subtotal	\$116,000

Personnel Costs:

Technicians:

Geotechnical technician	0.5 yr	
Geochemical technician	1.5 yr	
Microbial technician	1.5 yr	
Meiofaunal sorting technician	2 yr	
	identifications	3 yr
Macrofaunal sorting technician	2 yr	
	identification	1 yr

Total technician time: 11.5 yr @ \$44,000 per yr = \$506,000

Investigators:

Principal Investigator/coordinator 2 mos/yr X 5 yr = 10 mos

Five Investigators at 1 mo/yr X 5 yr = 25 mos

Total Investigator costs: \$10,000/ mo X 35 mos = \$350,000

Travel: ~\$80,000

Supplies: \$50,000

Shipping: ~20,000

Total 5 yr Project Costs:

Ship time	\$3,740,000
Direct support costs	\$1,122,000
Overhead at 40%	\$ 448,000

Total	\$5,310,000

Appendix C

Report on a Workshop to Confirm Collaborations in Research on Levels of Biodiversity, Species Ranges and Rates of Gene Flow

British Antarctic Survey, Cambridge, United Kingdom, 25-27 November 2002

Introduction

At a meeting convened in Kingston by the International Seabed Authority in the summer of 2002, it was decided that research was required into four scientific issues related to the environmental aspects of deep-seabed mining. The four topics are summarized below:

- biodiversity, species ranges and rates of gene flow in the nodule areas,
- burial sensitivity of deep-sea animals,
- impacts on the ocean layers above a mining site caused by unwanted materials from a mining operation, and
- natural variability in deep-ocean ecosystems over space and time.

It was decided that research into the four issues would be conducted mainly in the Central Pacific Ocean where most known deposits of polymetallic nodules occur. A result of the workshop was that some participants voiced a willingness for collaboration as it was deemed that the most expensive component of research schemes was shiptime and reducing the overlap between studies would benefit all concerned. A project was already being planned to study biodiversity, species ranges and gene flow in the deep central Pacific Ocean in the area of polymetallic nodule deposits. This project is called the Kaplan Project and is being led by the University of Hawaii. It was suggested that this would be a good starting point for collaborations. It was decided that a group of scientists would meet towards the end of 2002 in Cambridge, England to identify potential collaborations and to plan the Kaplan Project.

A meeting was convened at the British Antarctic Survey in Cambridge, UK, to address the current understanding of biodiversity, species ranges and gene flow in the deep central Pacific Ocean and to promote collaborations.

The intended goals of the meeting were as follows:

- establish the current understanding of biodiversity in the deep sea, with particular reference to the areas of potential mining operations and determine the major gaps in current knowledge,
- formulate future collaborations in deep Pacific studies between scientists from different institutions and mining contractors,
- finalize the format of traineeships within Kaplan and other deep-sea biodiversity studies, and
- develop a cruise plan for the RV New Horizon cruise for the Kaplan Project.

All of these goals were met.

What is the State of the Current Understanding of Biology in the Deep Pacific?

Initial presentations showed that there is not much data available regarding the biology of the Pacific abyss. Because of the time constraints and the rarity of species in the deep sea, it is very unusual to obtain all species from a particular area and as such any biodiversity measurements are estimates. True diversity values are only obtained when all species from an area are observed. At present, very little is known regarding the ecology of the Clarion-Clipperton Fracture Zone (CCZ) as it is poorly investigated, with only eight sample stations. This means that scientists *“haven’t sampled even close to all the species”*. Even when species are recovered from the deep sea, there are often problems with their identification as a result of missing characteristics or the characteristics required for identification being hard to identify. The material is often *“dirty”* to quote one delegate. Suggestions have recently been made regarding the use of DNA, rather than morphological techniques to identify organisms to negate these problems, but it was stated that certain animals, with particular attention being drawn to polychaetes, do not fix well in alcohol. For morphological analysis fixation occurs in formaldehyde. However, formaldehyde has detrimental effects on molecular techniques. Therefore, molecular grade alcohol has become the standard storage media when DNA techniques are to be used. Species identifications based on molecular techniques alone are not sufficient to describe new species but they can work in compliment with morphological descriptions or can be used on their own for determining species ranges and the presence of previously described species.

The data that is available suggests that the level of similarity between sites is less affected by the distance between the sites in the Pacific Ocean compared to the Atlantic Ocean. Samples within sites are almost as heterogeneous as between sites, which is a reflection of the low abundance of individuals and the high abundance of species. This means that, in the Pacific abyss, communities that are spatially close may be vastly different. It was suggested that cosmopolitan species account for less than 50% of the total number of species, implying that even impacts on small areas could reduce global diversity. Other studies have suggested that there is less than a 30% overlap of species between sites in the EQPAC study carried out in the CCZ. Nematodes show *“hyperdiversity”*, which implies endemism, meaning that they have small distributional ranges and are, therefore, at risk from mining activities. All of this data indicates the need for careful selection of mining areas. Apparently cosmopolitan species

may be cosmopolitan or may be sibling species, which, whilst being separate species, are indistinguishable morphologically with current techniques. This could be addressed by better resolution of existing morphological data or the use of molecular techniques.

It was noted that current morphological techniques should not be abandoned, as some places cannot afford the equipment required for molecular analyses. There was debate about whether morphological or molecular techniques were best suited for monitoring resulting in agreement that a combination of both molecular and morphological analyses is ideal. Whether species identifications are required or whether species richness (species count) would be sufficient depends on the question being asked. Obtaining species richness, using morphological or molecular techniques, takes a great deal less time and money than obtaining species identifications. Of more interest than species diversity, when determining the potential impact on the environment, is a measure of genetic exchange that occurs between communities and the range of genetic dispersal. It was suggested that the most useful result of the projects currently being planned would be to *“sidestep traditional ideas to create a species atlas of the deep sea”*.

One delegate stated that *“the deep sea harbors a high proportion of diversity of the globe”* and as such nodule mining has potential for significant impact on global diversity. It was declared that to predict the risk of extinction of species there were two things that were required to be known:

- the number of species living in the area of impact, and
- the geographic range and scale of gene flow of these species.

Collaborations

Next in the programme came the discussion of collaborations of the scientists with each other and the mining consortiums. The first project to be mentioned was the Kaplan Project. This is the first collaboration and research will commence on the *RV New Horizon* in February 2003. This cruise will have 7 scientists on board, including a representative of the ISA. Samples will be taken with both box corers and multiple corers. Data will be obtained for macrofaunal analysis, meiofaunal analysis, microbial analysis, and chemistry. Meiofaunal analysis will mainly concern nematodes and foraminifera but also a molecular analysis of the rest of the meiofaunal animals will occur if samples allow. Radiochemical analyses will be performed if sufficient samples are collected. A section of the meeting was devoted to planning the sampling strategy and allocation of samples from this cruise.

The projects being organized by KORDI and COMRA are tests of the effect of mining with pre-, during, and post-disturbance cruises. Three cruises were carried out between October 1994 and April 1997 in the IOM area using this strategy to observe the potential impact of mining on the marine environment. The first cruises of both KORDI and COMRA are planned for summer 2003. KORDI plans two cruises each year thereafter until 2008, each cruise lasting approximately 30 days. COMRA plans one cruise each year until 2005, each cruise

lasting 35-50 days. It was expressed that there was very limited space on board the KORDI cruises, but if training occurred, KORDI representatives could collect samples in such a way as to be useful to all parties. COMRA was also interested in scientific collaborations with the Kaplan Project, particularly with aim of standardizing techniques. KORDI cruises will accommodate up to 18 scientists, whilst the COMRA cruises will carry 35 scientists. There was some debate as to how many of the berths would be allocated to Kaplan scientists.

IOM has cruises planned for 2004 and 2005 and samples may be made available to Kaplan scientists in exchange for traineeships. An agreement had recently been signed between IOM and COMRA such that each would make space available on cruises for researchers from the other consortium.

IFREMER has a cruise planned to the eastern (~15°N, 130°W) and western (~10°N, 150°W) claim areas for the spring of 2004 and will use the French submersible *Nautilus*. Submersible studies are the main focus of the research, but sampling using box corers and multiple corers will be feasible at night when submersible operations are not permitted. Collaboration with Kaplan scientists, particularly taxonomists, was encouraged with 3 berths potentially available for collaborating external scientists.

The Indian consortium plan to visit their claim area in the Indian Ocean between 2003-2005 to investigate environmental variability. It was agreed that, if training was given, samples suitable for DNA analyses could be taken on these cruises. The availability of space for external scientists was unknown. Collaboration was requested including participation in the Kaplan cruises to carry out sedimentological analyses, sediment geochemistry, radiochemistry and enzymatic activity. This was not possible because of space constraints on the ship but it was agreed that, wherever possible, additional samples would be taken on the Kaplan cruise to be sent to Dr. Nath for analysis.

The capabilities of the German Center for Marine Biodiversity Research (DZMA) and the Census of the Diversity of Abyssal Marine Life (CEDAMAR) were outlined. Whilst neither was directly useful for the Kaplan Project, the potential of both were realized for future reference.

Japanese scientists have several cruises planned to the CCZ from November 2003 onwards, including one using the submersible *Shinkai 6500* which will make approximately 10 dives in the CCZ in 2004. There may be berths available to Kaplan scientists on all cruises with the possibility of wire time for benthic sampling equipment on the cruises that do not involve the submersible.

A Korean project (KODOS) has investigated latitudinal variations in biological and environmental variables. Field experiments into the impact of mining on the environment are planned. A plan and proposal for work is currently being formulated.

The potential of GEF as an “*excellent source of funding that could solve all our problems*” was discussed further. Up to US\$10 million of funding is available and it was suggested that a small team write a concept paper of approximately 5 pages. The suggested team was Alex Rogers, Myriam Sibuet, Craig Smith, John Lamshead or Gordon Paterson, and a representative from the ISA. If this concept paper were successful then the team would be assisted in writing the full bid by the GEF secretariat. Any resultant project would be an extension of the Kaplan Project. In addition to the research planned for the Kaplan Project, the GEF bid would incorporate ecological studies as well as species richness. It was also suggested that it include potential for collaborations between the Pacific and Indian Ocean claim areas. The aim was to have the first draft of the concept paper circulated by the end of 2002.

Another area of collaboration suggested was the idea of different groups swapping sample residues to allow for quality control and intercalibration.

Discussion then progressed to other collaborations. The potential for a postdoctoral researcher to organize and facilitate IFREMER-Kaplan collaborations was discussed and this will be investigated further. The formation of a database containing data from previous research, which may be of interest to future collaborations, was discussed. This database would also contain any newly synthesized data and would be constantly updated. It was decided that maps of the claim areas in the CCZ and the Kaplan Project would be very useful for presentations and use by the ISA.

Training and Standardization

The Secretary-General of the International Seabed Authority addressed the importance of traineeships, particularly for scientists from developing countries. Potential sources of funding for such training were discussed and included the Global Environment Fund (GEF), The Royal Society of the UK, Marie Curie Fellowships (a European-based funding source), the British Council, the Fulbright Association and the Natural History Museum in London. Alex Rogers was proposed to investigate this further, with Craig Smith investigating American sources of funding.

It was observed that there is a lack of standardization with regard to the type and size of samples when investigating the deep-sea environment. This led to the suggestion that a shipboard techniques standardization workshop would be the ideal forum with which to standardize sampling protocols. Adopting standardized sampling strategies would allow all future data collected by scientists and contractors to be comparable. The Secretary-General agreed such a venture would be useful and agreed to provide possible support. The aim of this workshop would be to have 3-4 scientists from the Kaplan Project jointly training 1-2 field biologists from each contractor in the chosen sampling strategy for each group of organisms. It was also suggested that each technique is videoed and made available in various languages so that a video protocol is available for those not able to attend the training workshop. The training would be carried out over approximately two days split between a small research vessel, for training in sampling, and a laboratory, for sample-sorting and identification

instruction. Training would cover techniques that would facilitate both morphological and molecular analysis so that all future studies are comparable. The workshop was suggested for May 2003 and two locations were suggested, Friday Harbor (Washington, USA) or the University of Hawaii.

Results of projects completed or currently underway were presented. The sampling strategies of projects currently planned were also proposed. There is a reasonable amount of research either being planned or carried out into the effects of mining on the marine environment. This emphasizes the need for a standardization workshop at the earliest convenience.

Where samples have already been taken, it was decided that methods to convert the results of one method to another should be investigated wherever possible. This is important as different sampling strategies have been shown to bias both abundance counts and measurements of community structure. Some work has been carried out with regard to this where four contractors investigated ways of converting to a standard. Pedro Martinez Arbizu planned to carry out a pilot study in February 2003 to compare the effect of different sized corers on meiofaunal measurements.

To summarize, the major gaps in current knowledge appear to be:

- under sampling,
- lack of standardization and, hence, intercalibration between studies (90% of the polychaetes from the EQPAC study were new to science), and
- lack of molecular data.

The ISA agreed that it could help in the following ways:

- the collection of a photo library,
- collaborate databases of environmental variables,
- a workshop about mid-water processes in Goa, India, and
- supporting a standardization techniques workshop.

It was noted that this workshop was just the beginning and that there had been good international agreement between scientists and contractors. It had shown the importance of international co-operation and there was hope for free exchange of information between all those involved for everyone's benefit.

List Of Cambridge Participants

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