

NOTES OF ODP DEEP BIOSPHERE PPG MEETING.  
13-15 DECEMBER 1997  
SAN FRANCISCO, CA, USA

Present: J. Parkes (Chair)  
Gert de Lange  
Steve Giovannoni  
Kim Juniper  
Jody Deming  
Tommy Phelps  
Lisa Robbins  
Herbert Cypionka  
Martin Fisk  
Takeshi Nagunuma  
Karsten Pedersen  
Daniel Prieur  
Evert Shock

Guests Joris Gieskes  
Kim Goodman  
David Goldberg

Agenda

- Introduction of individual Committee Members and their research areas.
  - The ODP Management and Panel Structure
  - Brief Reports on progress in Deep Biosphere Research
  - Members research planned or future on Deep Biosphere
  - Status of microbiology facility on board ship
  - Key objectives and how they can be achieved.
  - Initiatives RIDGE, EC/EMaPS, CONCORD
  
  - Detailed discussion of strategy and approaches for deep biosphere research including
    - drilling and sample handling
    - pressure core barrel
    - relevant techniques and approaches
  - Requirement for some routine microbiological measurements?
  - Facilities required on board ship - "minimum requirements"
  - Possibility of using a microbiology container - availability, cost, etc.
  
  - Funding and how can we stimulate greater involvement in ODP.
  
  - Microbiology involvement in planned Legs.
  - Gas Hydrates "a unique deep sediment bacterial habitat"
  - Future plans by Deep Biosphere PPG
1. Members of the PPG introduced themselves and their areas of interest.

2. ODP Management, Panel Structure and how to get a drilling proposal through the system. Judith McKenzie.

3. Deep biosphere research conducted by members (and others) were summarized includes (Legs 148 (basalt), 160, 164, 168, 169S, 175. Hence, there already is a significant amount of microbial research being conducted.

4. The outcome of the RIDGE Deep Biosphere Initiative was outlined by Kim Juniper for the next five years.

- vertical and horizontal distribution of deep biosphere both on and off the axis of hydrothermal systems
- temperature as a barrier to migration
- physiological and molecular activity
- oxidants available
- eruptions events
- fate of deep sourced biosphere and source of this biomass -active deep production or old biomass?

5. The outcome of EMaPs/EC Meeting was summarized by John Parkes.

Exploring the Deep Sub-Sea-floor Biosphere Summary of the report of the EMaPS & MAST Workshop July 1997, Bristol, UK

Thirty eight scientists from a range of disciplines and European Countries participated in the meeting which resulted in the following recommendations.

1. Research Objectives

1.1 To determine the extent, distribution and diversity of the deep biosphere in marine sediments.

1.2 To explore the potential energy sources for the deep biosphere and their impact on geochemistry, mineralogy (inc. palaeomagnetism) and ultimately oil and gas generation (inc. gas hydrate deposits).

1.3 To evaluate the biotechnological potential of the deep sub surface biosphere.

2. Research Strategy.

2.1 European scientists have played a major role in the discovery of the deep marine biosphere and have the multidisciplinary expertise to make further major advances in this area. However, European effort needs to be coordinated and effectively funded to fully exploit this new research area.

2.2 Considerable technological advances need to be made to facilitate research in this area.

### 3. Action Plan.

3.1 A Feasibility Study Group should be established under EMaPS to further develop this initiative and to prepare a proposal for an ESF Network.

3.2 This group, in conjunction with EMaPS, have propose input into the 5th EC Framework Program.

6. Takeshi Naganuma described the facilities of JAMSTEC in Japan, Recommendations of the Concord Meeting which included "searching for new species of ancient, but still living bacteria that lie deep within marine sediments and oceanic crust".

This was the highest priority of Working Group 1 which also emphasized the importance of the microbiology of Gas Hydrate and associated sub-hydrate (Free Gas).

7. There was considerable discussion of the goals for deep biosphere research and the role of the Deep Biosphere PPG. This was a wide ranging discussion which demonstrated the broad significance of the deep biosphere and the diverse range of potential research initiatives. It was decided to confirm the broad remit given to the PPG by ODP :

#### Earth's Deep Biosphere Pilot Project Program Planning Group

##### Overall Goal

To develop a plan of drilling and downhole sampling to investigate:

- the distribution, depth extent, and genetic range of the sub-seafloor biosphere living in rocks and sediments;
- the nature, ecology, and contribution of the sub-seafloor biosphere to global biogeochemical budgets.

##### Mandate

1. To develop a plan for the integration of microbiological sampling and analysis into drill sites with other objectives, where appropriate.

2. To organize the development of drilling proposal, if specific drilling sites or transects are required.

3. To provide advice on the need for, and specification of, a shipboard microbiological facility, development of sampling procedures, analytical techniques and curation of samples.

4. To actively collaborate with other appropriate international science initiatives.

8. Microbial contamination of deep sediments and rock during drilling. After some debate it was decided that assessment of contamination as a result of drilling needs to be addressed, this is particularly important as even deeper samples are obtained, so that we can be sure that we are dealing with indigenous organisms and activities.

(a) Joris Gieskes described the range of drilling systems used on ODP and the associated potential for contamination.

(b) Tommy Phelps described the range of tracers for contamination assessment that have been developed for microbiological sampling on land.

Contamination will depend on sediment type, drilling technology used and sea conditions. QA/QC for flow through systems can use perfluoro-carbons ( 8 types) and fluorescent beads (various dyes and colors). Not all contamination tests are suitable for all types of microbiological study: requirements would be different for molecular genetics and bacterial ecology.

Plan

a. Assess contamination with technology and tools that are currently used and available.

b. Design new drilling technology that may lower contamination or provide better contamination checks (3rd party development tool).

c. Contamination studies are required for porewater chemistry sedimentology as well as for microbiological samples.

d. A liaison is required with drilling engineers and ODP lab staff at College Station , Texas. Action John Parkes to organize a meeting between the PPG and drilling technicians at ODP

e. There may be some money available in the drill budget for 1998 to finance contamination studies. An initial finance of \$30,000 is required for this initial study.

f. Staff will be needed to run this program a? ODP Tech ? cost of staffing.

g. Leg 179 (16 April - 30 May 1998) an engineering test Leg may be suitable for running a QA/QC pilot study followed by some use on Leg 180 where there is some planned microbiological sampling.

9. The requirement for conducting some routine microbiological measurements on Legs was discussed. This would be important to achieve one of the main aims to quantify "the distribution and depth extent of the deep biosphere", in addition the ship may never go back to the same location ! There was a considerable difference

in opinion on this subject with strong support both for and against. Consideration was given to the significant amount of microbiological data already obtained from cores, what type of analyses should be conducted, how realistic it was for non-microbiologists to obtain these samples (whether they would be "trusted"), the value of stored samples and contamination assessment.

The following was recommended as an initial position:

Microbiological measurements on ODP legs 'Routine' microbiological samples should only be taken when there is a shipboard microbiologist is sailing or strict protocols are followed by one of the ODP technicians. Propose to start 'routine' sampling for Leg 183, after meeting with ODP drilling engineers and scientific staff early in 1998.

Suggested routine microbiological measurements:

- direct counts
- QA/QC
- Frozen archive, frozen immediately in liquid nitrogen
- H<sub>2</sub> measurements (gas void, headspace).
- Additional chemical analysis (e.g. dissolved organic compounds and volatile compounds).

10. Microbiological Facilities required on the ship. The present situation of no microbiological facilities on board the ship can not continue as it severely restricts the microbiology that can be conducted and forces detailed analysis and handling to be conducted on stored cores which increases problems with contamination, can result in changes in the dominant bacterial populations and probably loss of sensitive deep sediment bacteria etc.

This involved detailed discussion of the broad range of different microbiological measurements that individuals might want to conduct on the ship. Recommendations were based on "core facilities" which most microbiologists would require and which would encourage more microbiologists to become involved in ODP. These basic facilities would still require microbiologists to bring specialist equipment onto the ship, and would subsequently require to be supplemented to provide comprehensive microbiological facility (see Microbiological Container).

Prices in US Dollars

- |  |       |
|--|-------|
| 1) High power epifluorescent and phase contrast microscope with digital imaging facility | \$60k |
| 2) Laminar Flow Hood   | \$12k |
| 3) Freezer -80 degree C, plus liquid nitrogen storage and transport                      | \$10k |
| 4) Anaerobic gas manifold system   | \$ 3k |

5) Autoclave	\$4.2k
6) Anaerobic Cabinet with core handling capacity	\$15k
7) Two Gas Chromatographs for analysis of contamination tracers and reducing gases	\$65k
8) Three cooled and heated incubators	\$24k
9) Refrigerator	\$ 2k
10) Initial set-up costs	\$20K
Total	\$ 215.2k

This is the minimum requirement/package and we strongly recommend that all these items are funded. If this is not possible the committee will require notification of the total amount of finance that is available and will make further recommendations on this basis.

There is planned microbiological participation on Legs in 1998 (e.g. 180 & 183) and hence there is urgent need to get these facilities installed.

11. Microbiological Container facility ('Advanced Microbiology Laboratory'): Container is required as it seems the only way that radio- isotopes could be used onboard ship and they are essential for bacterial activity measurements. At present WRC have to be stored and transported back to shore laboratories for these measurements to be conducted and this introduces some uncertainty in how these rates reflect in situ conditions. Installation of container during dry-dock. Roof will require strengthening to take weight of container.

ODP could hire container or purchase.

Should container be used just for radioisotope work or other microbiology also? Is a second container necessary for other microbiology facilities?

This would be in addition to the basic microbiology facilities permanently placed in main lab stack.

12. Downhole experiments including legacy holes Again there was a range of opinion on this. The view after discussion was that although this would be an artificial environment it could be an invaluable natural laboratory -"Window on a Deep Biosphere" enabling experiments at insitu temperatures and pressures. Microbiological experiments would have to be coordinated with other experimental programs. Legs near Japan it might be possible for the JAMSTEC deep submersibles to sample these experiments.

13. Facilities and involvement in future ODP Leg's. The legs planned for 1998 to 2,000 were described by John Parkes, and planned and possible microbiological research considered along with facilities which would be required.

Leg 177: Bristol group samples being taken for direct counts and pore water acetate.

Leg 180: Bristol group hope to be involved, awaiting award of research grant.

Leg 183: Bristol group hope to be involved, awaiting award of research grant.  
Leg 185: (Iza Mariana, April-June 1999): First Leg 'targeted' for deep biosphere work. Very old sediments and basement. Re-entry cone being laid. 6000m water depth. Possibility that ODP may pay part of costs of bringing a ship alongside for radiotracer experiments.

**Action** Everet Shock and Marty Fisk to approach the Proponents

Leg 186: Yoshiti (co-chief) will be contacted by Takeshi Naganuma re: incubation experiments in holes. Nautilus will be in the vicinity at this time.

Leg 187: Australia-Antarctic Discordance (Oct-Dec 1999) First leg post-dry-dock. 1200m sediment to basement. sampling on and off ridge axis. Good leg for deep biosphere studies due to temp profiles.

**Action** Fisk to contact proponents Leg targeted for deep biosphere studies. Possibility of capped- off hole over aquifer. ? WSTP water sampling in aquifer.

**General Action** Judith McKenzie to provide details of proposals in system for assessment of potential for microbiological involvement.

14. Initiatives to increase microbiological involvement in ODP.

a) Advertise the new microbiological facilities on the ship in EOS etc Action Kim Juniper. and the Deep Biosphere Web Site at Bristol

**Action** John Parkes.

b) Presentations at The Third European Marine Science and Technology Conference in Lisbon in May 1998 (**Action** John Parkes) and the ISME 8 Nova Scotia

**Action** John Parkes & Karsten Pedersen, 4th International Sub-surface Microbiology Symposium a Deep Marine Sediment session

**Action** Karsten Pedersen. Deep Biosphere Gordon Conference Action Everet Shock

c) ODP should have its own Deep Biosphere Web Page with links to other Web Sites. **Action** John Parkes

d) FUNDING NASA's Exobiology Program **Action** Everet Shock, NSF Extreme environments program **Action** Everet Shock, European Space Agency Action Judith McKenzie, EC MAST & EMaPS, there might be a possibility of some European Funding for a Microbiology Container **Action** John Parkes.

Timescales

1) Contamination assessment (QA/QC) program needs to start as soon as possible, should be undertaken at least once during 1998, Leg 179 for pilot study. Then run on ideally on Leg 180, or 183.

2) The 'basic' microbiology facility should be set-up ideally by Leg 180 (3rd June 1998).

3) The 'advanced' microbiology facility to be installed during dry dock in 1999.

Future Meetings and Immediate Action

1. List of equipment with costs to Joris Gieskes as soon as possible (Measurements panel meets 2nd week in February). **Action** John Parkes.

2. Arrange meeting in College Station for end February with drilling engineers to discuss QA/QC. **Action** John Parkes.

Subsequent Deep Biosphere PPG meeting in Townsville?

3. SCICOM meet 3rd week in March.

4. Recommend that a member of this PPG becomes a member of the Gas Hydrate PPG (Phelps) and that there should be close liaison between these PPG 's (Parkes) .

**Action** John Parkes contact Susan Humphris.

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