

International Census of Marine Microbes:

Open Ocean and Coastal Systems Workshop Discussions: University of Hawai'i at Manoa, May 10th and 11th, 2005

Summary of Workshop:

The Open Ocean and Coastal Systems Working Group discussed options for a community-based, international program to explore marine microbial diversity. A major challenge will be to develop strong consensus about the optimal sampling strategies for short-term, mid-term and long-term objectives. Through extended dialogues between members of the Open Oceans and Coastal Systems working group and leaders of the breakout groups for sampling, technology and databases, the ICoMM secretariat will seek better-defined short and mid-term strategies for consideration by meeting attendees and the general community of microbial oceanographers. Long-term strategies will require more extensive planning efforts that engage the broader community of marine microbiologists and significant increases in funding. A more detailed accounting of the workshop proceedings follows this list of ideas and concepts that most of the workshop participants strongly supported.

1. ICoMM's scientific objectives as it pertains to the open ocean and coastal environments will require high degrees of coordination and significant increases in funding.
2. ICoMM should promote international collaborations. A short-term project would be to map where groups around the world are engaged in ICoMM relevant activities, particularly in coastal waters where time series measurements are tractable.
3. To obtain maximum scientific return on resource investments, ICoMM activities must integrate studies of microbial populations with contextual information (depth, geospatial information, temperature, luminosity, a suite of biogeochemical parameters, etc. from temporal samplings at specific sites) that will inform us about the interplay between microbial mediated activities and oceanic processes.
4. A functional or physiologic census is just as important as a taxonomic census.
5. ICoMM should promote the use of common protocols and techniques that can be calibrated across different laboratories.
6. Scientific questions will drive the sampling strategy and measurement requirements. There was enthusiasm for globally distributed microbial population surveys and for intensive studies of localized ecosystems. The workshop did not reach a clear consensus about optimal sampling strategies, but there was general recognition that single point (as opposed to temporal) samplings are insufficient.
7. The differing ideas about optimal sampling strategies argue for a "nested approach" in which information is sought at multiple scales. Sampling density will depend on the efficiency of measurement technologies and available resources.
8. The ICoMM community must convince the database community about the importance of including contextual data with annotations of gene sequences. Minimal

information includes latitude, longitude, time and depth.

9. Rather than a “monster database”, ICoMM should encourage the development of specialized yet interdependent databases that capture phylogenetic, molecular, physiological and contextual information. These should be federated databases capable of sharing information. If the current MICROBIS strategy of “synchronizing” databases were to be adopted, information would be both shared and maintained in a redundant fashion thus ensuring its availability on the internet for future investigators.
10. There is a need to develop experimental and predictive modeling capabilities.
11. The next generation of microbial oceanographers must cross-train in marine microbiology, molecular biology, biogeochemistry and bioinformatics.
12. There is a need for an informatics-based workshop on molecular ecology.
13. ICoMM should play a pivotal role in linking programs such as the NSF’s ORION and NOAA’s Integrated Ocean Observing Initiative.
14. ICoMM should use the Marine Microbe Forum: http://www.sb-roscoff.fr/marine_microbes/ to exchange ideas about Marine Microbiology.

Invited Workshop Presentations:

Thirty-five marine microbiologists attended a two-day workshop at the University of Hawai’i at Manoa sponsored by the International Census of Marine Microbes’ – ICoMM (<http://icomm.mbl.edu>). The objective was to explore options for developing an international, community-based census of marine microbes in open ocean and coastal systems. On the first day of the meeting, **David Karl** (the Chair of the Open Ocean and Coastal Systems working group) discussed the importance of knowing the genetic and functional diversity of microorganisms in the oceans and **Mitchell L. Sogin** (Co-PI of ICoMM with **Jan de Leeuw** of NIOZ) provided information about the history of ICoMM and its objectives. A series of fifteen-minute talks by eleven other participants offered descriptions of ongoing marine microbiological studies at several different sites using a range of standard and advanced technologies. (see attached Open Ocean and Coastal Systems Workshop schedule for speakers and titles).

John Heidelberg of TIGR headed off the talks with a presentation on what TIGR and the Venter Institute are doing towards marine metagenomics. Metagenomics is very much an exploratory science and does not test theories or models. John described the results of their shotgun sequencing work off of Bermuda that consisted of 4 samples and 1.6 million sequence reads. In this work, they detected 1,100 16S sequences. There is a second expedition going on called the Global Ocean Survey that in part aims to trace the steps of the Beagle and Challenger Expeditions. It began in Bermuda and is currently in Australia. John and colleagues Bill Nelson and Ed DeLong are developing a database to make the data from these voyages available to the scientific community. They are searching for ways to make the data they have collected useful to other researchers. Researchers would probably be happier with metabolic maps but these are still a long way from becoming readily available. So far they have 7.7 million reads, 3 million assemblies and 4.5 billion base pairs of DNA.

Forest Rohwer shared his work on uncultured marine viruses that typically occur at 10^7 /ml in the surface seawater. Most of these are eating bacteria – bacteriophage. They play a major role in global carbon cycles and can affect microbial diversity by killing off particular strains of microbes. Forest detailed some of the methods his lab is using to study viruses in the sea. He presented the concept of a “metagenomic species” as being defined by assembly parameters of metagenomic data. He has been using Monte Carlo Analysis to predict the numbers and relatedness of viral genomes. For example, a marine sediment sample has around 10,000 viral genotypes for 1 kg of sediment and 200 liters of seawater contains 5,000 genotypes. Forest has been working on making dynamical programming available to everyone. He has also been exploring how the Power Law appears to be a better predictor of the number species in a sample than other models. Forest concluded by sharing some his work on corals where he has discovered a lot of host-specific microbial populations.

Heidi Sosik's research took the workshop beyond the traditional tools of bottles, nets and filters to *in situ* flow cytometric methods that allow her to ask questions about what regulates coastal phytoplankton populations. She and colleagues Rob Olson and Alexi Shalapyonok have developed the FlowCytobot – a flow cytometer that detects individual particle properties such as fluorescence and light scattering and works underwater in an autonomous mode to provide real-time access to data. FlowCytobot is deployed at the Martha's Vineyard Coastal Observatory, a facility that is available to any users who want to test new sensors in a setting with high power and data bandwidth. Most recently, Sosik and colleagues have developed a second-generation instrument, Imaging FlowCytobot, that combines aspects of FlowCytobot capabilities with in-flow single cell imaging techniques. Using these tools, they have been asking questions like: What causes inter-annual variability and what processes lead to this kind of variability? Some cell identifications can be made down to the species level. She is also interested in what is the ecological entity, how does it change and when does it matter.

William Li reviewed some of the recent reports in the literature that address issues surrounding microbes and spatial scaling. He pointed out that a compilation of extant microbes listed in textbooks might fall short of a true census because the concept of species is problematic. Bill outlined recent studies on soil fungi and salt marsh bacteria in which the taxa-area relationships were used to extrapolate from local to regional scales. The slopes of these relationships were low, indicating that taxonomic richness is not greatly dissimilar at different scales, suggesting a ubiquitous distribution of many microbes. However, more recent studies of bacteria in water-filled treeholes and of phytoplankton in limnetic and marine systems indicate that the slopes are much higher in non-contiguous habitats. In other words, diversity at local scales may not be easily extrapolated to the global scale. The taxa-area issue remains unresolved for marine microbes. Bill suggested that Alan Longhurst's concept of the biogeochemical provinces in

the ocean might be one way to focus our census efforts. This approach has been used to scale up primary production from the regional to the global scale. For the census of marine microbes, it therefore seems worthwhile to understand the patterns and mechanisms that relate microbial diversity to primary production.

Carlos Pedrós-Alió described ways that we might use remote sensing to ask questions relevant to the census. In illustrating this point, Carlos summarized some of the work of Rafel Simó and colleagues who are using remote sensing to test some parts of the CLAW hypothesis formulated by Charlson, Lovelock, Andreae and Warren (Nature 326:655-661, 1987): that dimethylsulfide (DMS) plays a role in regulating the temperature of the planet.

Dimethylsulfoniopropionate (DMSP) gets converted to DMS (a volatile compound), the main source of biologically formed sulfur in the atmosphere above the oceans. Phytoplankton produce DMS that escapes into the atmosphere where it is oxidized to sulfuric acid, acts as a nucleus for the condensation of water and ultimately contributes to the albedo of the planet. When albedo increases, less solar radiation reaches the microbial plankton populations resulting in less photosynthesis and less DMS production creating a feedback loop that contributes to the regulation of the Earth's temperature. These scientists found that if the mixed layer depth is very shallow, then almost 100% of DMSP is converted into DMS, and as the mixed layer depth increases this value goes down. Using the mixed layer depth, chlorophyll concentrations and the DMS relationship, these investigators were able to show that predicted DMS concentrations were nicely correlated with the real DMS concentrations. Carlos challenged the workshop participants to see if there are other scientific questions that can be answered using remote sensed data beyond the chlorophyll and DMS examples. In summary, Carlos emphasized that you don't have to be an expert to take advantage of remote sensing data and that even though what you are measuring may not be directly related to remote sensing, there may be ways of correlating your data with remotely sensed data to enhance your area of research.

Oswaldo Ulloa presented his work on low oxygen minimum zones off of Peru and Chile. Oxygen minimum zones (OMZ) are defined as those zones with oxygen concentrations of $0.5 \text{ ml O}_2/\text{L}$ or $22 \mu\text{M}$. They are typically distributed off the eastern coasts of the ocean. Some reports indicate that they are areas of low diversity but no one has looked at microbial populations in detail. Some of the questions facing this environment include: How do intermediate waters vary in OMZ's, and how stable are they? Oswaldo's group has found that OMZ's are not stable and that they vary over large scales and geological time scales. OMZ's need to be studied in relation to the physical forces that shape them. Oswaldo's group has been examining the nitrogen cycle in OMZ's. He has been detecting high rates of nitrification, a high diversity of denitrifiers, and the presence of anaerobic ammonia oxidizing bacteria that may be a significant sink for oceanic nitrogen.

Gordon Taylor presented his work on Microbial Processes in the Cariaco Basin. The Cariaco Time Series is a collaboration between three U.S. institutions (U South Florida, U South Carolina and Stony Brook U) and three Venezuelan institutions (Fundacion La Salle de Ciencias, U de Oriente and U de Simon Bolivar) that has been ongoing since 1995. The Cariaco's setting is along a productive coastal margin, prone to strong seasonal upwelling that makes it a very dynamic portion of the coastal ocean. Annual primary production is three times that found at the subtropical BATS and HOTS stations and carbon fluxes exported from the epipelagial are twice as large. This is the world's largest truly marine anoxic basin and the only US-sponsored time series in the tropics. The Cariaco Basin is considered a natural laboratory for studying the biogeochemistry and microbiology of an anoxic system, where organisms may have novel metabolisms and physiologies. It may also contain evolutionarily significant phylotypes. Two Microbial Observatory programs have recently been added to the Time Series Program, representing collaborations between scientists from Stony Brook University, University of Louisiana at Lafayette, Northeastern University, Universidad de Simon Bolivar (Caracas, VE), WHOI and MBL. These projects are examining both prokaryotic and eukaryotic (protist) community dynamics using a variety of molecular, cultivation and manipulative experimental techniques. Their goal is to better understand how geochemical gradients organize microbial communities in this sulfidic, oxygen-depleted environment. Methods being exploited include CARD-FISH, MICRO-FISH, DGGE, T-RFLP, SSU rDNA libraries and oligo-FISH combined with SEM for protistan molecular and α -taxonomies (Stoeck, Fowle & Epstein 2003). They are finding many prokaryotes related to organisms isolated from hydrothermal vents, cold seeps and sulfur-dominated habitats. Many novel protistan 18S rDNA sequences have been recovered from anoxic waters, including protozoa related to known anaerobes from animal guts and shallow anoxic habitats as well as a novel deeply-branching clade with no known close relatives. In addition to novel thiosulfate disproportionating prokaryotes, cultivation studies have yielded the first cultivable thiosulfate-oxidizing manganese oxidizing chemoautotrophic bacterium.

David Caron spoke of his joint Microbial Observatory (MO) with Jed Fuhrman that has worked cooperatively with SPOTS (1997). SPOTS has provided a wealth of contextual information for the MO. The MO started in 2000 and emphasizes prokaryotic and eukaryotic discovery based on diversity studies sampled on short and long time scales. The San Pedro basin is hypoxic below 150 meters. Samples are taken at four depths from the surface through this hypoxic zone. They have used a combination of flow cytometry, epifluorescence microscopy, and a combination of SSU clone libraries and ARISA, TRFLP to explore aspects of microbial diversity from viruses to eukaryotes. They have discovered 800 distinguishable types of the SAR 11 bacterial clade. They are exploring the relationship between prokaryotic and eukaryotic presence in relation to

predator/prey and symbiosis relationships or other associations. Although many protistan taxa are morphologically defined, they still represent a large diversity. Dave and his group have been trying to determine a proxy of similarity that can be used to separate most species of protists in GenBank – they have settled on 95%. Of 1200 clones they found 488 OTUs with 95% similarity cut-off – so most of the species are present but rare. It may be that what's out there is present but is rare. Dave pointed out a couple of myths that are not true, 1) we have a good estimate of protist diversity, 2) we can forget about the species concept. We have to keep in mind that species are the unit of evolution. The definition of species is a matter of perspective.

Mike Zubkov presented work on the Atlantic Meridional Transect (AMT, NERC UK funded consortium) that extends from the British Isles to the Falkland Islands. Zubkov and colleagues have been using flow cytometric methods to sort cells, followed by PCR, sequencing and subsequent phylogenetic probe construction to return to the environment and identify the organism of interest to couple their identity with biogeochemical processes. They have been using tracers followed by cell-sorting to estimate the rates of amino acid uptake at ambient concentrations – this allows evaluation of microbial population growth in situ. Questions being asked include: Do *Prochlorococcus* populations in the surface waters rely on organic nutrients (such as amino acids) more than those at depth? On the contrary they found that *Prochlorococcus* living in the deeper waters was consuming 50% the amount of methionine tracer given compared to 25% of the methionine given to those *Prochlorococcus* living in the surface waters. Deep cells are taking up more of the methionine than average prokaryotic cells in these waters. This approach allows us to learn about the physiology of these cells in situ. They also found higher *Prochlorococcus* cell activity in temperate waters than inside the Gyres. In a different study they examined the mesoscale spatial variability in picoplankton in the Celtic Sea and found that cell concentrations of *Synechococcus* and heterotrophic bacteria vary up to 50-fold over distances as short as 12 km. Advection of such spatial variability through a time-series site would therefore constitute a major source of 'error'. Consequently, attempts to model and to investigate the ecology of globally important microorganisms in situ must take into account and quantify the hitherto ignored local spatial variability as a matter of necessity.

Matt Church reviewed the on-going efforts and progress at the Hawaii Ocean Time series (HOT) program. Based on the nearly 17 year record of monthly observations at the field site for the HOT program, Station ALOHA, investigators are beginning to assemble information on temporal variability in plankton processes and biogeochemical cycling spanning diurnal to decadal time scales. Some of the questions that have guided research at Station ALOHA include: “What are relevant time scales for us to study phytoplankton diversity?” and “Are these time and space scales the same as ones that control biogeochemical cycles?” In 1988, Dave Karl and Roger Lukas initiated observations at Station ALOHA

under the auspices of the JGOFS (Joint Global Ocean Flux Study). The central objectives of the HOT program are to characterize time-dependent dynamics at the Station ALOHA. The following points summarize the findings to date: 1) prokaryotes dominate the system, 2) oceanic biology regulates nutrient stoichiometry and carbon export, 3) the surface ocean appears chronically oligotrophic, 4) nitrogen fixation plays an important role in nutrient dynamics and carbon export, and 5) the organization of plankton populations seems to be controlled by longer-term oceanic teleconnections. Church urges the microbial oceanographic community to consider time series stations in future census of marine microbe efforts. The existing data sets available from time series stations provide us with a framework for a census. If we are interested in examining how diversity maps onto biogeochemical cycling we need to sample at the appropriate time scales to capture both short term population dynamics and longer term (decadal and inter-decadal time scales) ecosystem transitions.

Craig Carlson reviewed progress on the Oceanic Microbial Observatory, a microbial observatory associated with the Bermuda Atlantic Time Series (BATS) station. The BATS program, on their 200th cruise this year, provides the relevant biogeochemical backdrop for this observatory. The work represents a collaborative effort between Carlson's group at UC Santa Barbara, Steve Giovannoni's group at Oregon State University and some of the research staff lead by Rachael Parsons at the Bermuda Biological Station for Research (BBSR). Objectives of this observatory were to 1) identify spatial and temporal patterns in specific bacterioplankton and prokaryotic populations and 2) to initiate experiments to investigate potential linkages between microbial processes, community structure and biogeochemical processes and events (mixing events, changes in nutrient fields, etc.) with an emphasis on discovery. Giovannoni's role has been to bring some of the uncultured bacteria into culture through low-nutrient, high-throughput extinction culturing methods. Giovannoni and Rappe recently succeeded in bringing SAR11 into culture. There are also ongoing outreach efforts geared at education in the form of summer courses at the BBSR – John Heidelberg and Steve Giovannoni offer a course in Marine Genomics and Bob Morris and Craig Carlson offer a course in Marine Microbial Ecology. BATS is located 80 km southeast of the island of Bermuda – in the Northwestern Sargasso Sea and is characterized by seasonal oligotrophy and annual patterns of temperature availability and mixing. Carlson and his colleagues are using the BATS long time series data to provide focus to questions on microbial diversity. Their carbuoy mesocosm experiments wherein deep water was inoculated into surface water media produced interesting trends of significant bacterial production and removal of DOC (3 to 5 μM). This suggested a physical separation of a zone of DOM production from a zone of DOM remineralization that may be related to the microbial community present. They are also getting quantitative data using FISH for specific clades of bacteria – SAR11, *Cytophaga* and *Roseobacter*. Differences exist– SAR11 makes a major contribution and *Cytophaga* can be up to 10 to 15 % depending on the time of the year and position

in the water column. Giovannoni has >2,000 strains of bacteria that won't grow on agar – 18 were selected for genomic sequencing by the Moore Foundation.

Comments about ICoMM goals and relevant issues:

The second day of the meeting provided opportunities for participants to raise additional questions and issues that potentially impact ICoMM and its goals. **Daniel Vaultot** discussed the formation of an electronic Marine Microbe Forum at the address: http://www.sb-roscoff.fr/marine_microbes/. **Lucas Stal** presented data about distribution and measurement of N₂ fixation by marine microbes and **Ricardo Letelier** discussed the role of remote sensing and the need to integrate very large data sets of remote sensing of physical and biological data. In the near future we can look forward to even richer satellite data sets with hourly observations. **Oscar Scholfield** summarized a series of new initiatives that would benefit from the microbiological activities within the ICoMM community. Most are tied to the Ocean Observing Initiative and recognition that we need to develop a nested observing network strategy to look at overlapping scales. New initiatives such as the ORION program will provide support for development of infrastructure. A partner program NOAA's IOOS Integrated Ocean Observing systems together with ORION may have as much as 500 million dollars/year to invest and ICoMM could play an important role in linking these communities together. Some of these programs will support research on high power moorings distributed on a global scale. There will be regional Arrays i.e. fiber optic nodes. Neptune will provide access to the deep ocean and there are proposals for Coastal networks that will facilitate long-term time series measurements. Scholfield will provide to ICoMM a more detailed summary of new opportunities in the Ocean Observing Initiatives including contact information for lead scientists who might facilitate collaborations and new research opportunities in microbial oceanography. Finally, Dave Karl reminded the workshop that we would soon be celebrating the International Polar Year and that ICoMM should be involved. This could be the year of the microbe!

Before adjourning to individual working groups, **Grieg Steward** challenged the meeting participants to think in terms of a real census of microbes so that we might understand their ecology and interpret genomic data etc. Rather than an exercise in stamp collecting, the census represents a phase of discovery. We need the census to fully take advantage of new information from genome sensors, gliders etc. The new tools coming on line are very powerful but sometimes inefficient. Until recently, surveys were limited to 16S rRNA sequence comparisons. Yet, if we want to address functional diversity, this is not enough to make progress. Metagenomics and the hope of inferring metabolic potential is both promising but inefficient science. One day single cell genome projects may partially circumvent this inherent inefficiency, but it will still be necessary to first "tease apart" microbial community structures before we study their DNA at the genomic level. This initial characterization must include morphology, biochemistry, analysis of lipids and detailed information about microbial population structures. We must be able to sample molecular diversity at levels required to detect minor members of populations. This will require technology developments that reduce the cost of molecular diversity surveys. The minor population members must be important for survival of the community otherwise they would not survive over the long term.

Specific science questions will dictate the details of sampling and measurement methods. It would be highly desirable to carry out time course studies of spatial grids at a large number of sites, but given available resources, such a scenario would not be attractive for funding. Difficult decisions must be made through workshops and community meetings, ICoMM can contribute its collective wisdom towards identifying the most important priorities. For example, questions about global change will require more ocean sampling on global scales. Alternatively, if the goal is to understand how microbes are catalyzing transformations, it becomes necessary to study a particular system at greater levels of detail in order to understand likely emergent properties.

Goals for working groups in the workshop:

The participants formed three break-out groups including: Group 1 - **Sampling**, Group 2 - **Measurements**, and Group 3 - **Data analysis and training**. In addition to the general questions assigned to all of the ICoMM working groups (Benthic, Open Ocean and Coastal Systems, Technology and Scientific Advisory Council) each breakout group addressed a specific set of questions. Dave Karl and Mitchell Sogin emphasized the importance of focusing discussions on “**HOW**” to collect, organize and analyze relevant data, as opposed to arguing more philosophical questions such as “What is a microbial species?” The organizers also reminded the participants that Big questions in science demand community-based efforts similar in scope to those used in the Physics, Astronomy and Genetic community for tackling seemingly impossible tasks such as the construction of high energy Accelerators, large earth-based and orbiting observatories, and the full sequencing of the human genome. Microbial Oceanography is likely more complex than any of these disciplines because it requires massive amounts of data (genetic, physiological, biogeochemical), major computational capabilities, extensive modeling and BIOLOGY. Any strategic plan developed through this workshop should consider resource constraints available in the short term (immediately available capabilities within the community), the mid-term (resources that can be competed for from existing funding sources) and long term – (large scale resources that will require broad-based support from an international constituency of scientists and policy makers at the highest levels of government and the private sector.) Finally, ICoMM’s goal extends far beyond the mere identification and counting of different kinds of microbes in the sea. To obtain maximum scientific return on resource investment, ICoMM activities must integrate studies of microbial populations with contextual information that will inform us about the interplay between microbial mediated activities and oceanic processes.

Plenary Discussions:

The general plenary discussion prior to reports by the breakout groups asked the question “**What is the goal of the census?**” Is it a description of all organisms in all marine environments? (Genotypic and phenotypic diversity) Is it a description of microbes at a selected latitude, longitude and depth? (Descriptions of microbial populations at one or more geographical locations) or Is it a description of organisms that are associated with a particular process? (Functional diversity). Should the census include direct counts of microbes and measurements of chlorophyll? Does it correspond to enumerating subgroups and clades of *Prochlorococcus*? All of these issues are important

topics for marine microbiology and each will contribute to an International Census of Marine Microbes. There was strong agreement that the science questions will drive the sampling strategy and requirements for specific measurements. There was enthusiasm for both a globally distributed survey of microbial populations and for intensive studies of localized ecosystems. Key questions include “Are all microbes everywhere? How is the microbial world connect to circulation patterns? What roles do microbes or microbial activities as defined by the genome play in major biogeochemical processes? One of the strongest rationales for supporting ICoMM research initiatives relates to the development of tools for understanding emergent properties of marine ecosystems. The task that this workshop should address is to set realistic priorities for allocating existing and future resources.

Breakout Group 1. Sampling. (William Li - Group Leader, David Kirchman - Rapporteur, Matthew Church, David Caron, Gordon Taylor, John Waterbury, Osvaldo Ulloa, Daniel Vault, James Cowen, David Karl, and Lucas Stal).

The scale of sampling (spatial and temporal) and the ability of analytical techniques to discriminate between different kinds and numbers of microbes will define the resolving power of an International Census of Marine Microbes. The “Sampling” working group considered the following issues:

*1) To address questions of diversity and distribution of different kinds of microbes: An important goal of these investigations is to address the question **Is everything everywhere?***

Should we explore many sites in a superficial manner?

or

Should we focus upon two or three intensely studied oligotrophic sites?

2) What is the optimal sampling strategy for an intensely studied site?

3) What is the optimal global sampling strategy?

4) How shall we address the issue of temporal sampling?

5) What is the relevance of quantitative measurements and what is the best available methodology?

6) What is the known role and what might be the role of viruses in shaping microbial population structures? – What measurements are needed and how can they be obtained?

Discussions in the sampling group initially considered the enormity of the task at hand both in terms of total volume of the oceans (estimated to be $\sim 1-4 \times 10^{18} \text{ m}^3$) with a potential population of 10^{29} microbial cells. Strategic efforts to sample microbial populations in the water column according to a selected measurement criteria must consider tradeoffs between high density studies with many data elements from a small number of sites versus the cursory analysis (fewer data elements) of many samples that are either globally distributed or that span smaller scales at one or a few sites. The group also considered the effect of collecting large sample volumes versus a larger number of small samples that can provide increased levels of spatial and or temporal resolution. If

the goal is to understand the relationship between microbial populations and global models, investigations of local processes orchestrated by smaller scale distribution patterns of microbes may be of minor importance.

Importance of temporal measurements: The working group used *Synechococcus* studies to examine some of these tradeoffs. For these organisms, transects from South American to the North Atlantic were not informative because snap-shot measurements inherent in transect sampling will miss temporal variation. The studies of seasonal cycles off Woods Hole were more instructive and diel cycles provided a great deal of information about *Synechococcus* biology and ecology. These results argue for the temporal sampling of a limited number of sites instead of broad-scale transects.

Limitations of single site studies. Analysis of *Synechococcus* populations only in Woods Hole waters provides a counter example because such a geographically restricted temporal sampling scheme will miss *Prochlorococcus*. This suggests that a global description of a few key microbes would be of value, but it must include a temporal component. Comparisons of time-series studies of microbial populations at multiple sites are important and if a sufficient number of sites are included it becomes easier to establish international collaboration.

These differing points of view argue for a “nested sampling approach” in which information is sought at multiple scales. Sampling density will depend on the efficiency of measurement technologies and available resources. For example, cytometric analyses can be adapted to time series investigations whereas the expense of metagenomics restricts the application of this technology to snap shot studies.

An important role for ICoMM would be to “help establish international collaborations”. This would mitigate regulatory problems that foreigners encounter when working in territorial waters. A short-term project would be to map where groups around the world are engaged in ICoMM relevant activities, particularly in coastal waters where time series measurements are more tractable. As part of this survey a questionnaire could determine where and the frequency of sampling, the target organisms, the methodology and willingness to share data and engage in collaborations. This could be the first step in carrying out a global census of marine microbes. ICoMM should lead this effort and it should take advantage of existing organizations and networks such as Antarus for South America, and POGO. This effort would benefit from a contact person in different geographical regions. ICoMM would collect this information and collate results on its web site. It could also promote pilot projects to prime the pump for mid-term and long term projects.

ICoMM should also promote the use of common protocols and techniques that can be calibrated across different laboratories. For example it is possible to calibrate methods for obtaining direct counts, DOC and nutrient measurements in different experimental environments.

Mid-term objectives: The working group endorsed a mid-term objective of using commercial shipping for obtaining survey data from surface waters and the development of centers that could carry out technologies not available in some marine biology laboratories. The community should also take full advantage of the International Polar Year (2007-2008) to work in the Arctic and Antarctic waters.

Long-term objectives: The working group discussed long term projects and the need for remote “microbial sensors” which currently have very limited capability for monitoring biological parameters. There is also a need to develop experimental and modeling capabilities that are predictive. Current models define rates but lack ability to differentiate activities of different kinds of microbes. They also explored how many biomes would be suitable for detailed analyses. The number of recommended sites ranges from 2 to 200 with approximately 20 representing sites that already have historical information including biogeochemical data. A list of candidate sites includes: 1. HOT, 2. BATS, 3. KNOT, 4. Station P, 5. EAST, 6. 48 N, 7. Canary Island, 8. COPAS, 9. SEATS, 10. CATS, 11. Antarctic, 12. Leo, 13. ORION - part of the coastal ocean observatory work, South Pacific -off Easter Island-currently not under investigation. Cost estimates for each site includes on the order of \$1 million/site for ship time (assumes monthly sampling) and at least \$1 million for laboratory work but potentially much more if the studies include large scale molecular investigations. Each of these sites would operate for ten years but at least 10 of the sites would continue on a longer term.

The needs for such an ambitious program would include developing an appropriate labor force and securing resources for ship time. The working group did not recommend specific sampling frequencies beyond setting a minimum of biannual collections at each of these international sites.

Challenge to the Open Ocean and Coastal Systems Working Group plan for sampling.

The sampling group recommended a tractable, long-term strategy that will require increased funding levels. It could be part of a larger oceanography program such as ORION. However, the short and mid-term objectives lack concrete ideas that will lead to new data. The short-term recommendation (1-2 year time horizon) of *mapping the location of marine laboratories and who is doing what* is valuable but this activity will not demonstrate the collaborative strengths of the microbial oceanography community. If the ICoMM community does not believe that it is possible to collect new data over the short-term horizon, then at a minimum its members can work together to organize existing data sets and derive a new synthesis that will point the way towards new projects. If this is to become the short-term goal, we must identify data sets that are most worthy of integration.

We had aspirations that it would be possible to identify a community-based project that might be accomplished for something less than 1,500,000 dollars. Such a project would require identification of an important site or perhaps up to three sites and the analysis of samples with some agreed upon technology. The alternative would be to select a larger number of sites that are under investigation by different laboratories and to contribute samples or data for analyses using a community-based resource such as sequencing power or DNA micro-arrays in concert with the collection of contextual data. It might be possible to distribute a common set of tools to individual laboratories for making measurements at many sites. The question is should we be more restrictive in our scale of sampling by increasing the frequency and/or density of sampling? Further discussions are necessary to make decisions that will allow us to go forward with a credible short-term plan.

Group 2. Measurements. (Jonathan Zehr- Group Leader, John Paul-Rapporteur, Heidi Sosik, Claire Mahaffey, Michael Zubkov, Grieg Steward, Michael Rappé, Robert Bidigare, Valerie Franck, Karen Selph, Craig Carlson)

To make informed recommendations about optimal measurements for ICOMM, the Measurements breakout group considered the questions that ICOMM should address. An important goal would be to gain new insights about how the ocean works, information about evolution, and discoveries of novel microbial forms. In addressing the seven specific questions outline below, they considered the counterpoint position that knowledge about diversity might not lead to improved understanding of biogeochemical cycles and underlying processes. Perhaps the concept of a functional or physiologic census is just as important as a taxonomic census. The working group did not elaborate on this thread, but the inference of metabolic function from genomic surveys provides a way to infer general physiologic and functional properties of a microbial population. At the same time, the genetic basis of these phenotypic traits often reveals phylogenetic affinities. A census based upon sequence data offers a means to organize and interpret information from functional and physiologic investigations. Taxonomic or genomic surveys versus a physiologic census may require disparate kinds of measurements. The density and efficiencies of measurement techniques coupled with the sampling plan will ultimately determine the cost of ICoMM related activities. The Measurements breakout group offered answers to each of seven specific questions.

1) When is it important to quantify numbers of a kind of organism and what is the best way to do the quantification?

If the goal is to detect and identify pathogens such as harmful algal blooms (HABS) or other pathogens such as *Vibrio*, knowing the identity of the organisms in a sample is essential. If the goal is to associate discrete biogeochemical changes with a list of processes, knowledge about the organisms responsible for catalyzing transformations and the presence or absence in a microbial population becomes important. Finally, if new genes or phenotypic characteristics are discovered e.g. discovery of proteorhodopsins, knowledge about the organisms that harbor these genes offers a means to infer their presence in other populations and to understand the evolution of these physiological capabilities.

2) When is it more important to survey diversity and what are the optimal measurement criteria?

Early in a census it is important to obtain an initial measure of diversity because this can guide the ultimate sampling design and measurement technology. As part of this effort, simultaneous analysis of processes can be very informative when evaluating diversity of a population and/or how that diversity relates to functional capabilities of the population. One significant challenge is, “How do we detect/quantify the rare organisms in a survey?” One answer is to use subtractive hybridization techniques that remove the dominant organisms from a population. The group discussed the importance of minor members in microbial populations. These organisms might serve key functions but represent very limited biomass. Alternatively their low numbers might reflect patchiness in space and time.

3) What is the role of genomics and molecular evolution in assessing diversity and how should this be accomplished?

Molecular evolution provides an important tool for identifying microbial diversity in a studied environment. It also provides a qualitative genetic tool for directly comparing microbial populations in different samples. Microbial ecologists commonly use comparisons of Ribosomal RNAs for such analyses but other genes can also be valuable – e.g. comparisons of genes involved in nitrogen fixation, sulfur metabolism, etc. Yet there are limitations to single gene comparisons and much more information about a particular organisms emerges from studies of large DNA inserts that contain homologous genes. By surveying the sequence neighborhood of a functional or structural gene used to select a large DNA insert for analysis, it becomes possible to discover new functional processes and/or understand evolutionary history of a functional or structural gene. In other words the study of large DNA inserts provides genomic contextual information required to understand how individual genes or gene families have evolved. Genome sequences assembled from pure cultures or from properly executed metagenomic investigations opens the door for understanding function and discovery of novel biochemical diversity.

4) Let's assume we have 1 million reads available. What fraction of these reads might we use to look broadly at genetic diversity? What fraction might be just 16S? What fraction might be used for genes responsible for specific metabolic activities and which genes are most important? What fraction should be devoted to metagenomic studies and what should be the format – short insert clones? Long insert clones? Fosmids? Etc.

One million reads is equivalent to shot-gun sequencing 8-10 X coverage of 100 microbial genomes. Single-cell sequencing of uncultivated microbes may soon become tractable but the selection of which single cells to sequence would be challenging. Should we select the 100 most abundant organisms? Should we focus on the 100 organisms that serve the most important biogeochemical functions? From an experimental or technical perspective, how would we make decisions about which single cells should be selected for genome analysis? The most likely method would be to construct single-cell genome libraries and to make decisions according to annotations of a small but preliminary set of sequencing reactions.

5) What physiological measurements can be made to assess diversity?

Physiological measurements can be made on single cells or in batches. However there is a need to develop and carry out experimental manipulation studies.

6) Genome-enabled sensing role via arrays?

Arrays would be a powerful tool for the community. Whole genome expression arrays could be developed for individual organisms but arrays for functional genes would also be of considerable value for assessing diversity of biogeochemical function.

7) How important is culturing? And how can we improve our ability to culture marine microbes?

Culturing capabilities are very important – It makes possible genomics for single organisms and the ability to link genomics to physiological and biogeochemical functions.

The Measurement breakout group considered additional topics that ranged from sampling strategies to methods. There was enthusiasm for sampling along natural gradients including trophic gradients, salinity gradients, depth, and oxic/anoxic. These functional gradients are forcings that will influence the compositions of microbial communities. It is also important to consider fine scaling questions such as the distribution of microbes in organic matter continuums and gels that provide an organic structure to microbial communities. A more general issue for sampling is size-fractionation to separate eukaryotes from prokaryotes.

The group discussed the need for standardization methods and common tools for use by microbial oceanographers. For example Denaturing Gradient Gel Electrophoresis (DGGE) is capable of concurrently detecting single base differences over a few hundred base pairs for many homologous genes but the technology is highly variable in a single laboratory. If the community is to continue use of this technology, there would be merit in establishing common standards.

The breakout group discussed the potential use of DNA macro arrays as an alternative to more expensive microarrays. It is theoretically possible to produce functional gene macroarrays with standard target genes such as nitrogen geochemical genes. Such arrays could be used to interrogate communities across large spatial scales (polar to tropics). The use of arrays in general could lead to finer temporal/spatial scale resolution). It is possible to produce large numbers of DNA microarrays by using lithographic, printing or spotting technologies. Densities can be greater than 50,000-probes on a chip. When the cost of a well-designed chip is amortized over the research activities of many laboratories, genomic and expression profiling becomes an affordable tool for microbial oceanography laboratories. Through further discussion, the working group could determine which targets (phylogenetic probes versus functional probes versus diversity probes) would be of the greatest value to the microbial oceanography community. Scanners for interpreting the results could be operated in a small number of internationally distributed laboratories. Automated scanners could also be operated and managed as virtual instruments.

The ICoMM community must also consider the archiving of samples from time series measurements and/or the storage of extracted nucleic acids. Resources would be required for maintaining and distributing archived samples and nucleic acids. Culture collections such as the ATCC carry out similar activities for the genome community but given the number of samples that might be generated by the census, the costs will rapidly escalate.

If ICoMM were to focus upon “activity” as a definition of microbial diversity, it will be necessary to develop high throughput assays for productivity. Perhaps specialized laboratories that focus upon standardization of activity measurements could train researchers in particular techniques. In this regard, there was enthusiasm for the development of single-cell activity assays. It would also be desirable to link activity to genotype. This might be accomplished by radio-labeling cells and seeing what genes are

tagged through measurements of radioactive RNA. Non radioactive labeling techniques would also be of considerable interest.

What measurements should be made-time series vs. survey?

Time Series	Survey
JGOFS-type measurements	JGOFS-type measurements
1°Production	1°Production
Bacterial prod	Bacterial prod
Abundance(viruses-to eukaryotic)	Abundance(viruses-to eukaryotic)
N-fix	N-fix
HPLC pigs	
Metabolic indicators (ATP)	
16SrDNA /TRFLP/etc	16SrDNA /TRFLP/etc
FISH samples	
DNA samples (size fractionation)/large insert lib	DNA samples (size fractionation)/ large insert lib
RNA/Transcriptome Analysis	
Organic Matter characterizations.	
Functional gene/array analyses.	
Extinction Culture (occasionally)	

Group 3. Data analysis and training. Molecular sequence data deposited in public archives will serve a key role for molecular studies in all areas of molecular microbial ecology and for ICoMM related activities. The datasets for microbial oceanography are both interdisciplinary and very large. For example the Sorcerer Cruise is generating enormous trace archives, metagenomic assemblies in the form of sequence bins and super scaffolds, and annotations that may change in response to improvements in both data quantity and our ability to more accurately assemble environmental shotgun sequences. These data are collected along with measurements of cell counts, nutrients, temperature, oxygen, GPS coordinates and time. The Data group considered the following questions to guide their discussions.

1) What data elements are most critical to developing a census of marine microbes in the open ocean and coastal systems environments?

In order to compare samples, we need to coordinate measurements in terms of latitude, longitude, time and depth. The molecular databases do not currently support these attributes. The ICoMM community must convince the database community about the importance of these fields. The alternative will be to construct parallel databases that capture both Gene identifiers (GID numbers) and the “big four” parameters – Latitude, Longitude, Depth and Time. As outlined by the Benthic and Technology working groups,

scientific return from ICoMM investments will depend upon the capturing of additional data elements including biogeochemical information and contextual data that describes the studied ecosystem. For example, if the census includes studies of commensal populations associated with metazoans, it will be essential to include taxonomic descriptors for the host.

2) What modes for data analysis are optimal for a census of marine microbes?

The collection of molecular data by the ICoMM community will require many kinds of analysis including annotation of microbial genomes, phylogenetic analysis of genes and genomes, and integration with contextual and biogeochemical databases. Because of the large amounts of information and interdisciplinary nature of the scientific questions, the databases are likely to be specialized yet interdependent. They will contain both archival and dynamic databases. MICROBIS is an example of how federated databases can be integrated across different laboratories. The underlying structure of MICROBIS provides a “turn key” solution for multiple laboratories that gather taxonomic data. It provides the ability to link both in and out to other databases containing molecular, positional, biogeochemical etc. information. MICROBIS forms linkages to other taxonomic databases that contain all of the information in MICROBIS but only serves selected data elements. In return, MICROBIS harvests information from the partner database in order to expand its total information content. This organization of federated databases not only provides for the sharing of software capabilities but also provides for redundancy if the databases are kept in a synchronized state. Data elements within MICROBIS and between its partner databases are linked by a taxonomic naming system served by the uBIO project at the MBL. This allows for alternative taxonomic assignments that reduce to a common numerical identification. The structure can also accommodate other conventions such as the use of GPS, depth and time of sampling coordinates to link together sample information. For such databases to function its data elements and identifiers must be portable to other systems. There was a general consensus that the databases should be distributed and that a “monster” database is not a viable concept.

The GMOD (Genome Model Organism Database) consortium offers another example of federated databases that have the potential to share functional capabilities and information. The combination of databases such as MICROBIS (image rich microbial diversity descriptions), GMOD (graphical displays of genome annotations) Micro-Mar (graphical displays of global distributions of rRNA sequences in marine environments) and ARB (multiple sequence alignments and phylogenetic representations) could provide a basis for a powerful network of federated databases for molecular genomic data linked to archival information from GenBank, cruise data and other sources of contextual information. Currently MICROBIS is designed to function as a “switching” or “integrating” system for linking together databases that share common data descriptors e.g. taxon ids, gene ID’s or sampling coordinates. Analytical platforms such as GMOD can be readily integrated into MICROBIS but current phylogenetic content of ARB will require significant changes to its database design. To maintain and operate this federation of databases it will be necessary to establish common procedures of synchronization and data sharing, to train researchers in the use of these databases and associated tools, and to establish a biology-driven community of software developers for implementation of new analytical tools.

3) How should census data be integrated with contextual databases?

The ICoMM community must encourage NCBI to either host appropriate molecular databases and/or provide fields for integration into other data repositories that collect relevant non-molecular data. The simplest mode for achieving this goal would be to require the submission of GPS coordinates, depth and time elements when environmental molecular data is submitted. For all of the databases, information must be released within time frames agreed to by the community of microbial oceanographers.

4) How important is quantitative data for building ecological models? Not discussed in detail but modeling is an important outcome if ICoMM and microbial oceanography are to have significant impacts on interpretations of Ocean Observing systems and management activities.

5) Can we assume there will be a succession of bacterial groups under specific environmental conditions, for example a phytoplankton bloom? This issue was not discussed.

6) How can we integrate new information with what is already known and is worth assembling into a database? The capturing of existing information into on-line databases was discussed but represents an open-ended challenge that the entire biological community currently faces. There are efforts to digitize journals that appeared before the age of digital publishing. We anticipate that web-crawlers, and other agents will play important roles in extracting valuable information from these future resources.

7) Training issues: How can we move forward with integrating the next generation of marine microbiologists with the challenge of bioinformatics? – What are the optimal training environments? Should they be oriented around genome centers or around marine microbiology laboratories?

For ICoMM related activities, the key to education will be “cross-training”. There are a variety of options that range from post-doctoral rotations across fields to special graduate and post-doctoral summer courses that include an equal number of faculty and students. The emphasis should be bioinformatics but should also include laboratory or ship time in order to maintain connections for the enormous scope of field and biochemical work required for ICoMM related efforts in microbial oceanography. There was a strong suggestion to develop a workshop on molecular ecology – or a mixed community workshop. The group discussed other training models including long-term rotations (3-6 months) for post doctoral students at labs with significant bioinformatics activities. The idea is to train them to go beyond the mere use of menu-driven data bases. They need to learn how to use powerful software tools in concert with scripting skills. To facilitate training, a valuable community resource that could be established today would be a database of Powerpoint and PDF slides for lectures and talks in microbial oceanography.

With respect to the databases, there will be a meeting in Woods Hole on September 25th and 26th, 2005 sponsored by ICoMM to discuss databases in much greater detail.

**International Census of Marine Microbes: Open Ocean and Coastal Systems
Workshop**

May 10th and 11th, 2005

**Imin International Conference Center, Jefferson Hall,
University of Hawai'i at Manoa, East West Road, Honolulu, HI 96822**

This agenda is only a guide for discussions by the Open Ocean and Coastal Systems workshop participants on May 10th and 11th. The times for discussion topics will shift in response to specific interests, questions, etc. At the end of this meeting we would like to outline a consensus "Roadmap" for ICoMM's future directions.

Tuesday, May 10th, 2005

07:45-08:00: Hotel pick up (We will furnish van transportation to the East West Center.)

0815: Coffee and pastries at the Imin International Conference Center

0830: Welcome to the University of Hawaii

0900: **Dave Karl** - Brief Introductions by workshop participants

0920: **Mitchell L. Sogin** - The Ocean's hidden majority and ICoMM

0950: **Dave Karl** - Microbial Oceanography: A time for action

1020: **Coffee Break**

1045: **John Heidelberg** - Shotgun genome sequencing of marine microbial communities

1100: **Forest Rohwer** - Molecular diversity of uncultured viral communities

1115: **Heidi Sosik** - Continuous flow cytometry of picophytoplankton.

1130: **William Li** - Geographic variations in microbial cytometric diversity

1145: **Carlos Pedrós-Alió** - Marine microbiology from space

1200: **Lunch Buffet** (East West Center Dining Room)

1330: **Oswaldo Ulloa** - Microbial biogeochemistry of oxygen minimum zones off Chile

1345: **Gordon Taylor** - Microbial processes in the Cariaco Basin
1400: **Dave Caron** - The SPOTS Microbial Observatory
1415: **Mike Zubkov**: Atlantic Meridional Transect (AMT)
1430: **Matt Church** - Hawaii Ocean Time-series: 17 years and counting
1445: **Craig Carlson** - BATS/ Bermuda Microbial Observatory
1500: **Open Mic Session** - Part 1

1530: **Break**: Cookie/fruit, juice/water

1550: **Open Mic Session** - Part 2, Open discussion

1630: **Linda Amaral-Zettler** - Formation of Break-out Groups

1730: End of Day 1--- Dinner at the Willows, an "old Hawai'ian style" restaurant in the residential section of Moiliili, 817 Hausten Street (roughly half way between the University and Waikiki)

May 11th, 2005:

0800: Hotel pick up

0815: Coffee and pastries at the Imin International Conference Center

0840: **Dave Karl:** Progress summaries, General discussion

0900: Break-out group meetings

1100: **Dave Karl:** Plenary summary of initial group discussions:

1200: **Lunch Buffet** (East West Center Dining Room)

1330: Break-out group meetings

1530: Cookie/fruit break, juice/water

1600: **Mitch Sogin:** Plenary summaries and next steps

1700: END

Questions relevant for all ICoMM working groups

Diversity:

- 1) What metric can be used to describe microbial diversity?
- 2) For molecular measures, what are the strengths and weaknesses of single-gene, genomic, and populations-level perspectives? Which of these will have the greatest long-term benefits?
- 3) How should the dynamics of diversity be handled, and should the approach be based on species (phylotypes) or populations?
- 4) How will approaches to microbial diversity differ from those used in the Census of Marine Life, which focuses mainly on metazoans?

Integration:

- 1) What level of biodiversity is necessary to interpret ecological, physiological and process-related observations?
- 2) How can process and ecological data inform us about diversity and what levels of information are required?
- 3) What are the key scientific questions that a census can address?
- 4) Where are the gaps in the investigative framework?
- 5) How is diversity related to process stability? Can predictive frameworks be defined?

Sampling, prioritization and coordination with other programs:

- 1) Schedules, locations and priorities.
- 2) Relationship to sampling program in the Census of Marine Life and related subprojects.
- 3) Are there mileposts that will logically define phases of the project?
- 4) What observations are needed at each sampling site?
- 5) How should we address temporal variations?
- 6) How should we address spatial heterogeneity, particularly with regard to commensal populations and chemosynthetic environments (e.g. seeps, whale falls, wood falls)?

Databases:

- 1) What is the structure of the information that will be produced?
- 2) What are the preferred techniques for carrying out a census?
- 3) How can databases be structured to facilitate communication?

Relationships with other programs:

- 1) RIDGE, ODP, Genomes to Life, Microbial Observatories, existing CoML field projects
- 2) Biodiversity Organization issues: Centralized, Coordinated, Distributed, Combinational Models

How to Proceed:

1) Funding Strategies

- i. What are the highest priorities?
- ii. Where could ICoMM seed monies be most effective towards initiating programs and collaborations in benthic, open ocean and coastal systems?
- iii. What are the key programs for benthic, open ocean and coastal studies? How can benthic, open ocean and coastal diversity research be better advocated within these or other programs for multi-institutional and international programs?

2) White Papers

- i. What audiences should be targeted?
- ii. What are the most important messages?

Sampling questions:

Group 1

William Li - Group Leader
David Kirchman- Rapporteur
Matthew Church
David Caron
Gordon Taylor
John Waterbury
Osvaldo Ulloa
Daniel Vaultot
Karin Björkman
James Cowen
David Karl
Lucas Stal

Measurement questions:

Group 2

Jonathan Zehr- Group Leader
John Paul-Rapporteur
Linda Amaral-Zettler
Heidi Sosik
Claire Mahaffey
Michael Zubkov
Grieg Steward
Michael Rappé
Robert Bidigare
Valerie Franck
Karen Selph
Craig Carlson

Data questions:

Group 3

Anthony Michaels- Group Leader
Forest Rohwer-Rapporteur
Mitchell L. Sogin
Lita Proctor
Oscar Schofield
Carlos Pedrós-Alió
Chris Winn
Alexandra Worden
Zachary Johnson
Ricardo Letelier
John Heidelberg

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*ICoMM Open Ocean and Coastal Systems Working Group Members (Bess Ward and Peter Burkhill were unable to attend)