of the free metal ion concentration in the surrounding solution. Also, since the concentrations of all metals at the gel-ligand interface are equal to zero, the sampler also may not reflect the competition amongst metals in complex mixtures for complexation to finite binding sites such as biotic ligands governing metal uptake into biological organisms.

We have developed a sampler based on the equilibration of immobilized ligands held in polyacrylamide gel with the free metal ion concentration in the surrounding solution. The disks, 1cm in diameter x 1mm thick, are impregnated with a metal binding resin (Tosohaas Toyopearl) at a concentration that can equilibrate with the surrounding solution on a time scale of hours, and can collect measurable levels of metals with minimal depletion from the surrounding solution. A procedure based on the complexation of a semi-conservative cation such as Mg can correct for the effects of pH, salinity, or the presence of competing metals on uptake into the gel. Results for copper in artificial seawater solutions of varying salinity, pH, and levels competing metals show agreement between actual and theoretical uptake of copper into the gel based on the free copper ion concentrations in the surrounding solution. Because the uptake of metals into aquatic organisms is a function of competitive interactions of metals, it is hoped that this sampler will also mimic the uptake of metals into biological organisms. Future experiments will examine the correlation between metals taken up by the sampler with metal uptake in fish.

OS22S-09 1550h

Partitioning of Trace Metals Between Particulate, Colloidal and Truly Dissolved Fractions in a Polluted River: the Upper Vistula River (Poland)

Celine Gueguen[1,2] (907-474-2792; celine@iarc.uaf.edu)

[1]University of Alaska fairbanks, International Arctic Research Center 930 Koyukuk Dr., Fairbanks, AK 99775, United States

[2]University of Geneva, Institute F.-A. Forel 10 route de Suisse, CH-1290 Versoix, Geneva, Switzerland

The high industry densities in the Upper Vistula River basin make the river one of the most important polluted river in Europe. The metal partitioning depend on the physical-chemical conditions of system and can be affected by anthropogenic inputs. In this study, we report the results of trace metal partitioning between particulate (>1.2 micron), colloidal (1.2 micron 1kDa) and truly dissolved (< 1 kDa) fractions in the polluted riverine section compared to the non polluted headwaters. It was found that the salt input in the Vistula river induced the decrease of colloid concentration and the increase of SPM. Compared to upstream from the polluted section, the metal concentrations (Co, Cu, Cr, Mn and Zn) in the colloidal fraction were lower. It was mainly due to the rapid colloid coagulation at increased salinity, the competition with ligands and major ions (Ca and Mg) and the weak mobility of metals associated at the pollution sources with particles.

OS22S-10 1605h

Speciation of Hg in the Venezia Lagoon using Ultra-Clean Sampling and Analysis

Nicolas S Bloom[1] (206-622-6960; nicolasb@frontier.wa.com)

Ligia Maria Moretto[2] (39-041-234-8646; moretto@unive.it)

[1]Frontier Geosciences, 414 Pontius North, Seattle, WA 98109, United States

[2]University of Venice, Dept. of Physical Chemistry S. Marta 2137, Venice I-30123, Italy

Venezia and its lagoon are, simultaneously, one of the cultural wonders of the world and a sensitive marine estuary under stress due to centuries of anthropogenic pollution. Although many studies have dealt with problems related to environmental pollution, practically no comprehensive research has been undertaken to explore the biogeochemistry of Hg within this fragile ecosystem. This is particularly surprising, given that the western side of the lagoon is bordered by the Marghera Industrial zone, which houses many industries that have discharged wastes directly to the lagoon since before world war II. Although many wastes contain Hg, of particular interest is the presence of a mercury cell chlor-alkali plant, which operated with little or no pollution control from 1953 to 1985. Studies that were conducted in the 80s and 90s have shown high levels of total mercury (1-20 mg/kg) in the sediments off shore of Marghera, with levels decreasing to approximately 0.2 mg/kg in the more isolated northern lagoon. Hg is transported on fine pariculate matter into the Adriatic Sea, where elevated levels of up to 0.5 mg/kg have been reported in pellitic sediments outside the barrier island of Lido di Venezia. Based on deep core sections collected in these these earlier studies, background Hg concentrations in these sediments are expected to be approximately 0.05 mg/kg. No results for methyl Hg have ever been reported for any compartment of the Venice Lagoon ecosystem. Since the lagoon is shallow and poorly flushed, contains high sulfate, and high nutrient loadings from agricultural runoff and urban sewage, it is expected to act as a vigorous microbiological incubator for Hg methylation. In this paper, we will report the first findings of a synoptic survey of Hg and methyl Hg in water column, suspended matter, sediment cores, and select marine biota samples collected using ultra-clean sampling techniques during November, 2001. Samples were taken in areas ranging from the relatively unimpacted northern lagoon, through the urban waters surrounding Venezia and Murano, and up into the Marghera Industrial zone itself. Samples were also collected in the Adriatic Sea, just outside the barrier islands (during incoming tide) to provide a contemporary regional background for comparison. Because the lagoon is very shallow and vertically well mixed, the use of suspended matter tracks the local surface sediment concentrations, while also allowing the calculation of very accurate sediment/water distribution coefficients for Hg and methyl Hg. This paper also reports the results of studies which look at the mercury speciation and methylation potential for chemical waste pond solids (primarily alkaline mineral material from an historic alumina extraction facility) as they erode into the lagoon.

OS22S-11 1620h

Effects of Habitat Type and Size on Species Composition, Nursery Function, and Refuge Quality for an Estuarine Fish and Macroinvertebrate Community

Jana LD Davis[1] (janalddavis@yahoo.com)

Margaret A Kramer[1] (kramer@serc.si.edu)

Alicia C Young-Williams[1] (young@serc.si.edu)

Anson H Hines[1] (hines@serc.si.edu)

[1]Smithsonian Environmental Research Center, 647 Contees Wharf Rd, Edgewater, MD 21037, United States

In the Chesapeake Bay, as in other estuaries, human activity and natural forces have altered shallow-water benthic habitats, fragmenting them and replacing them with other habitat types. Some suggest that the new habitats assume the ecological roles fulfilled by the original habitat types. The purpose of this study was to experimentally test the effects of habitat fragment size and type on species composition, nursery function, and refuge quality for shallow-water macrofauna. We compared the density of fish and macroinvertebrates among five habitat types that have been impacted by human activity in the Bay. Oyster reef, submerged vegetation (SAV), and woody debris have been declining in percent cover, while bare sediment and riprap (artificial shoreline armor) have been increasing. Most of the dominant species (8 of 11) preferred (occupied in highest densities) either SAV or oyster reef. Wood and bare sediment were occupied by some species, but never preferred. For most species, density increased with patch size in their preferred habitat. Two, however, were most dense in smallest patches. New recruits of most species were most abundant in SAV, suggesting a larger nursery role than the other habitat types. Oyster reef and SAV offered the highest degree of protection when tested for one prey species (grass shrimp). Neither nursery nor refuge function depended on fragment size. Although fragment size affects community structure, habitat type appears to be more important at the scale of our study. Results also indicate that the value of the five habitat types is species- and age-specific. Habitats that are increasing in abundance due to human activity (riprap and bare sediment) are therefore unlikely to adequately assume the roles of those habitats in decline.

OS31A HC: Hall III Wednesday 0830h

Bridging the Gap: From Molecular Biology to Marine Ecology II

Presiding: **G F Steward,** University of California, Santa Cruz

OS31A-01 0830h POSTER

Phylogenetic Analysis of Metabolically Active Heterotrophs in the Oregon Upwelling System

Krista Longnecker[1] (541 737 2359; klongnecker@coas.oregonstate.edu)

Barry Sherr[1] (sherrb@coas.oregonstate.edu)

Evelyn Sherr[1] (sherre@coas.oregonstate.edu)

[1]College of Oceanic and Atmospheric Sciences, COAS/OSU 104 Ocean Admin Bldg, Corvallis, OR 97331-5503, United States

The ability to link cell-specific metabolic activity with phylogenetic diversity is an important step towards identifying the role of microorganisms within marine ecosystems. The amount of cell-specific metabolic activity has been shown to vary more than the abundance of cells. Whether the variation in cell-specific activity is accompanied by a shift in the diversity of cells present or is a shift in the metabolic activity of each group within the community remains unknown. To address these questions, samples were collected during four sampling periods from May 2001 to October 2001 at a site 10 nm east of Newport, Oregon. Temperature, salinity, density, and fluorescence were measured concurrently with the water sample collection. Incubating the water samples with the fluorogenic redox compound 5-cyano-2,3-ditolyl tetrazolium chloride (CTC) indicated that 5% of the cells had an active electron transport system (ETS). Flow cytometry using the nucleic acid stain SYBR Green I indicated that small cells with high nucleic acid contents (SHNA) were the majority of the microbial community at depths below 40 m. Cells with low nucleic acid contents (LNA) were more prevalent immediately below the thermocline, while the large, high nucleic acid cells (LHNA) were less than 10% of the total heterotrophic microbial assemblage. Denaturing gradient gel electrophoresis (DGGE) of the V3 variable region of the small subunit ribosomal RNA gene was used to compare the diversity of whole seawater to cells with an active ETS (CTC+ cells). Furthermore, since other research has indicated that cells with high nucleic acid contents are responsible for the majority of bacterial production, whole seawater samples were compared to cells with different concentrations of nucleic acids (LHNA, SHNA and LNA cells). These data will examine the temporal variability in the diversity of metabolically active cells and examine whether all cells in marine systems are metabolically active or if only a subset of those cells are active at any one point.

OS31A-02 0830h POSTER

Molecular Probes of Sediment Stress in the Reef Building Coral Montastrea Faveolata

Sara E Brogdon[1] (678-556-0295; sbrogdon36@hotmail.com)

Terry W Snell[1] (404-894-8906; terry.snell@biology.gatech.edu)

[1]Georgia Institute of Technology, School of Biology, Atlanta, Ga 30332-0230, United States

A variety of stressors including high water temperature, sedimentation, changes in salinity, and exposure to UV radiation have contributed to a rapid decline of corals worldwide. Our work has focused on developing molecular biomarkers of sedimentation stress for the reef building coral Montastrea faveolata. Stressors cause a remodeling of gene expression by regulating specific genes. We used subtractive hybridization, differential screening, and sequencing, to identify coral genes up regulated in response to sedimentation stress. Techniques for extracting RNA from coral produced between 1000 to 4000 ug of total RNA, and approximately 0.4 to 1 percent of message RNA per 40 centimeters squared of coral tissue. A cDNA library of genes was created and 96 colonies were screened, with 15 to 20 percent showing differential hybridization. Further screenings demonstrated that 1 to 3 percent of the original colonies exhibited differential expression. These genes were further processed for probe development. By identifying and monitoring these genes in the field, we can estimate stressor impact, and rank stressors according to their effects. The probes could also be used to compare regions of high sediment stress to low stress areas, and to compare sediment stress to other stressors impacting corals.

OS31A-03 0830h POSTER

Widespread N-acetyl-D-glucosamine Uptake Among Marine Bacteria: Implications for Particle Colonization in the sea

Lasse Riemann[1] (45-48267600; lriemann@vip.cybercity.dk)

Farooq Azam[1] (fazam@ucsd.edu)

[1]Scripps Institution of Oceanography, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0202, United States

N-acetyl-D-glucosamine (NAG) is among the largest pools of amino sugars in the ocean. NAG is a main structural component in chitin and a constituent of bacterial peptidoglycan and lipopolysaccharides. While enzymatic degradation and recycling of chitin has received considerable attention, uptake of NAG by marine bacteria has not been well examined. We combined experiments with isolated bacteria and in situ measurements of NAG uptake in order to elucidate the distribution and kinetics of the Phosphoenolpyruvate:NAG phosphotransferase Systems (PTS) in marine bacteria. Of the analyzed 79 bacterial isolates, 41 took up high amounts, 19 took up low amounts, and 19 were unable to take up 3H-labeled NAG. Uptake rates were highly variable among the isolates. No systematic pattern in NAG uptake ability relative to phylogenetic affiliation were found, except that all isolates within Vibrionaceae took up high amounts of NAG. Turnover time and the upper limit for the ambient concentration of NAG in samples from off Scripps Pier (La Jolla, California) was 5.9 days and 5.2 nM, respectively. Competition experiments indicated that glucose, glucosamine, mannose, and fructose were taken up by the same system as NAG. The fraction of a natural bacterial assemblage taking up NAG was estimated by use of the antibiotic and structural NAG analog Streptozotocin (STZ). STZ had no effect on isolates incapable of taking up NAG, but completely inhibited cells with a high NAG uptake. In seawater samples, STZ caused a 28.4 % reduction in thymidine incorporation and a 43 % reduction in bromodeoxyuridine (BrdU) incorporation as detected with a novel single-cell BrdU-antibody staining technique. Growth of the isolates on pH indicator plates showed that isolates capable of taking up NAG (thus, possessing a PTS), were predominantly facultative anaerobes. The combined laboratory and field studies suggest that roughly one third of the active bacteria off Scripps Pier take up NAG by PTS and, hence, may be predominantly facultative anaerobes. The adaptational value of fermentative metabolism in the pelagic environment is potentially significant and might be important for bacteria colonizing microenvironments such as marine snow, which may experience O2-limitation.

OS31A-04 0830h POSTER

Calibration of Bromodeoxyuridine Incorporation to Growth and Thymidine Incorporation for Diverse Marine Bacteria

Ena Urbach[1] (865-974-3800; eurbach@utk.edu)

Patricia A. Jegier[1] (865-947-8327; pjegier@utk.edu)

[1]University of Tennessee, Department of Microbiology M409 Walters Life Sciences Bldg., Knoxville, TN 37996, United States

Bacterioplankton taxonomic composition varies as a function of depth and location, suggesting that different microorganisms occupy different ecological niches. Population densities reflect relative rates of growth and loss, which vary with environmental conditions. ^{3}H-thymidine incorporation is used to estimate bacterioplankton community growth in situ but gives no information about taxon-specific rates. We are developing tools using bromodeoxyuridine (BrdU), an immunogenic thymidine analog, to estimate taxon-specific, in situ growth rates. Pulse-labeling of microbial populations with BrdU incorporates the antigen into newly synthesized community DNA. PCR amplification of SSU rRNA genes from immunochemically-purified BrdU-DNA extracted from these populations is used to identify actively growing taxa. Calibration is required to estimate growth rates for these active taxa. We are examining relationships among BrdU incorporation (immunochemical dot-blot assay), growth rate (turbidity measurements) and ^{3}H-thymidine assimilation (liquid scintillation counting) for phylogenetically diverse, cultured marine bacteria. Direct calibration is possible for marine populations of Roseobacter, which can be grown in culture, and approximate calibrations for uncultivable marine microbes. Comparison of growth, BrdU and thymidine incorporation rates helps us to evaluate ^{3}H-thymidine-based estimates of community growth and, eventually, understand the ecological roles of marine microbial taxa. This technology will enable future experiments to delineate the ecological niches of marine microbes important for global biogeochemical cycling.

OS31A-05 0830h POSTER

Bioinformatics and DNA Arrays for Investigating the Molecular Ecology of Nitrogen Fixation

Grieg F Steward[1] (gsteward@cats.ucsc.edu)

Andi Stephens[1]

Bethany D Jenkins[1]

Jonathan P Zehr[1] (zehrj@cats.ucsc.edu)

[1]UCSC, Ocean Sciences Dept, Santa Cruz, CA 95062, United States

Rapidly expanding molecular databases provide new opportunities for exploring how diversity and expression patterns of functional genes relate to ecosystem structure and function. Among genes of particular ecological and biogeochemical importance are those coding for nitrogenase, an enzyme used by prokaryotes to fix nitrogen (reduction of dinitrogen gas to ammonia). Nitrogen fixation can have a significant influence on ecosystem productivity, which is often limited by the rate of nitrogen supply. Many sequences are now available for nifH, which encodes the iron protein of nitrogenase. We have compiled a database of over 1000 aligned nifH sequences. We are using this database to investigate the diversity and bigeography of nitrogenases and to develop DNA micro- and macroarrays as tools to rapidly assess spatial and temporal variations in nifH diversity and expression.

A database of aligned DNA and protein sequences was created in Arb using the alignment program HMMER 2.2 (http://hmmer.wustl.edu/) and custom PERL scripts. Phylogenetic trees built in Arb allow one to rapidly query and view phylogenetic relationships of nifH genes from different environments. We also used the database in a tree-building strategy for choosing optimal regions of the gene to target on oligonucleotide microarrays (currently under construction and testing). We have also created macroarrays on nylon membranes by spotting 350 bp nifH gene fragments from plasmid clones derived from bacterial isolates and environmental samples. Tests of arrays with single target sequences revealed negligible cross-hybridization between sequences sharing less than 85% sequence identity. This is sufficient discrimination to resolve clusters within major bacterial groups (e.g., sub-groups of the cyanobacteria). Differences in signal intensity were observed for sequences sharing 86 to 99% identity suggesting that finer scale discrimination is also possible. Achieving this higher resolution in mixed environmental samples will require deconvoluting multiple levels of non-specific cross-hybridization. To facilitate this we are currently testing a strategy that uses parallel arrays hybridized at different stringencies. A comparison of clone libraries, TRFLP analysis, and DNA array data for determining diazotroph diversity in natural samples will be presented.

OS31A-06 0830h POSTER

Phylogenetic Characterization and Enzymatic Activity of Marine Bacteria Isolated on Different Solid Media From the Northern Adriatic Sea

Bruno Cataletto[1] (39-0-40-224400; catale@univ.trieste.it)

Paola Del Negro[1] (39-0-40-224400; delnegro@univ.trieste.it)

Francesca Malfatti[1] (39-0-40-224400; fmalfatti@hotmail.com)

Farooq Azam[2] (858-534-3196; fazam@ucsd.edu)

Serena Fonda Umani[1] (39-0-40-224400; labbioma@univ.trieste.it)

[1]Marine Biology Laboratory, Via Auguste Piccard 54, Trieste 34010, Italy

[2]Scripps Institution of Oceanography, University California, La Jolla 92093, San Diego, CA 0202, United States

The phylogenetic diversity of bacteria isolated on two different solid media from the Gulf of Trieste (Northern Adriatic Sea) was investigated by means of 16S rRNA gene sequence analysis. Surface seawater samples were monthly collected from October 2000 to April 2001 in one station in the Gulf of Trieste. Of the 22 sequenced isolates, 13 were obtained by spreading samples of surface seawater onto ZoBell agar plates and 9 spreanding the same samples onto MPG agar plates. A majority of the isolates were assigned to */gamma* subdivision of the class Proteobacteria while the other isolated bacteria were distributed among the */alpha* and */beta* subdivisions and the Cytophaga-Flavobacterium group. The ZoBell-isolated bacteria occur mainly in marine ecosystems while the MPG-isolated bacteria occur mainly in sewage and flowing water. Ectoenzymatic activity (leucine-aminopeptidase,*/beta*-D-glucosidase and alkaline phosphatase enzymes) measurements with fluorogenic-substrate of isolates on solid media showed distinct differences in the expression of certain enzymes. The enzymatic activities of bacteria showed the importance of substrate induction. All MPG-medium isolates showed a high */beta*-D-glucosidase activity. These results provided support for studies of bacterial diversity in the Gulf of Trieste and to understand the role of different marine bacteria to degrade different fractions of organic matter in the sea.

OS31A-07 0830h POSTER

Diversity of Nitrite Reductase Genes from Chesapeake Bay Sediments

Chris A Francis[1] (609-258-2612; francis@princeton.edu)

Bess B Ward[1] (609-258-5150; bbw@princeton.edu)

[1]Princeton University, Department of Geosciences, Guyot Hall, Princeton, NJ 08544, United States

Denitrification accounts for the main loss term from the fixed nitrogen budget, yet the diversity of organisms responsible for this process in most aquatic environments is not known. The functional genes (*nirS* and *nirK*) encoding nitrite reductase, the metalloenzyme which catalyzes the first committed step in the denitrification pathway, are useful targets for PCR detection of denitrifiers. Our objective is to relate the distribution, diversity, and expression of *nirS* genes to ecosystem function (i.e., N transformations) in the Chesapeake Bay. The study site encompasses gradients of nutrient loading and trophic status along the hydrographic gradient from the upper river to the sea. We have PCR-amplified, cloned, and sequenced *nirS* genes from DNA extracted from sediment cores collected at stations throughout the bay, including upper and lower bay stations as well as the Choptank River. Sequence analysis of over100 *nirS* clones from Choptank River sediments revealed both extensive diversity and low redundancy among clones. Relative to *nirS* sequences of known denitrifying strains, our sequences shared nucleotide identities ranging from <50% to >80%. Phylogenetic analysis revealed that most of the *nirS* sequences fell into coherent clusters distinct from sequences of known denitrifiers, suggesting that cultivated denitrifiers may not be representative of natural microbial assemblages. Comparative analysis of *nir* sequences (from DNA and RNA extracts) between stations within the Chesapeake Bay should help reveal the extent to which denitrifier diversity is influenced by environmental gradients and whether functional diversity or community composition is reflected in the measured denitrification rates under different environmental conditions.

OS31A-08 0830h POSTER

Bacterioplankton DOM Interactions: A Bacterial Community Fractionation Study Using Capillary Electrophoresis

Jesus M. Arrieta[1] (31-222-369-380; txetxu@nioz.nl)

Erwin Schoonhoven[1] (31-222-369-380; urfs@bio.vu.nl)

Gerhard J. Herndl[1] (31-222-369-507; herndl@nioz.nl)

[1]Dept. of Biological Oceanography, Netherlands Inst. of Sea Research (NIOZ), P.O. Box 59, Den Burg 1790 AB, Netherlands

It has been shown that the bulk low molecular weight DOM is more refractory than high molecular weight DOM. However, the influence of the different molecular weight fractions of the DOM on bacterioplankton diversity remains unknown and it is likely that different molecular weight fractions of DOM are used by different bacteria. In order to address this issue, bacterial assemblages were separated by capillary electrophoresis and inoculated in seawater containing the total DOC or the DOC fraction <1000 Da. The purpose of these manipulations was to create a series of what-if scenarios where part of the members of the bacterial community and/or part of the available substrates are removed. The development of these cultures was followed by measuring the bacterial abundance and production and by DNA fingerprinting of the microbial communities. The results of these experiments indicate that a different community developed in the 2 different molecular weight DOM fractions. Furthermore, the low molecular weight DOM supported a higher richness of bacterial species than the unfractionated DOM regardless of the starting community used.