

magnitude than subsequent major Phanerozoic orogenies. The coupled carbonate carbon isotope record defines a dynamic evolution of seawater $\delta^{13}\text{C}$ throughout the late Early to Late Cambrian, including a large-magnitude negative excursion (4‰) at the Early-Middle Cambrian boundary interval. Superimposed high-frequency, large-magnitude (2 to 4‰) fluctuations in $\delta^{13}\text{C}_{\text{org}}$ and $\Delta^{13}\text{C}$ values record major perturbation in global carbon cycling during this period. Temporal trends in the magnitude and frequency of these C isotope fluctuations, coupled with trends in faunal diversity and distribution, indicate that rapid (10s to 100s ky) and repeated fluctuation in marine primary productivity, seawater CO_2 concentration, and biogeochemical cycling was intimately associated with Early-Middle Cambrian trilobite extinction and radiation.

OS41G-07 1020h INVITED

Chemoautotrophy at oxic-anoxic interfaces: The implications of ocean anoxia in the Phanerozoic

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An enormous amount of interest and studies have focused on the evolution of the biogeochemical sulfur cycle over geologic time. By and large, the emphasis has been on the reductive portion of the oceanic sulfur cycle. This includes studies aimed at determining past sulfate levels, the effect of biota on total sulfate, and the consequences of widespread bottom ocean anoxia. We rely heavily on isotopic analyses (S, C, O) in order to interpret conditions and past Earth events, often based on analogies made with modern marine processes (e.g., S-fractionation associated with biological sulfate reduction). However, it is important to recognize that there is another side to the oceanic biogeochemical sulfur cycle that involves biological processes and has ramifications among multiple isotopic systems: sulfide oxidation. I will discuss sulfide oxidation, the potential effects of sulfide oxidation on various isotopic systems, and the implications these processes may have during past ocean anoxia.

OS41G-08 1035h

Relations Between Long Term Sea-level change, Shelf-Ocean Exchange and Shelf Burial of Organic Material

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Although the present shelf environment represent only 10% of the oceans' area, close to 50% of organic material buried in the marine environment is buried in the shelf and slope sediments. Despite the fact that shelves dominate burial, as much as 80% of the nutrients delivered to the shelf environment to support primary production are supplied from the open ocean. These observations imply that changes in the long term eustatic sea-level and the physical shelf-ocean exchange are of primary importance in modulating the shelf burial and thereby the global carbon and nutrient cycling.

With a simple biogeochemical ocean model and different shelf-ocean exchange parameterizations we explore the relations between long term sea-level changes since the Mesozoic and the global carbon and nutrients cycles. Water column respiration of export production from the surface layer is prescribed as a power function, and burial is taken as proportional to the material reaching the sea-floor. In the simplest shelf parameterization we assume export production is uniform over the ocean and use a global mean hypsographic curve for calculating the carbon and phosphate burial at different depths. The present hypsographic curve is modified above sea-level to be in better accordance with the observed non marine area during the Cretaceous.

With sea-level 200m above present we calculate a ~20% increase in the shelf-slope burial. Because nutrient input have been kept constant, the mean ocean phosphate concentration decrease by ~30% and deep ocean burial decrease by ~40%. The model predicted inverse relation between sea-level and deep ocean phosphate burial is contrary to what is observed. This suggest that changes in shelf-ocean exchange, weathering or other causes probably overwhelmed the influence of sea-level on organic matter burial. Results from a more elaborate shelf-ocean exchange sub-model will be presented and discussed in the context of observations.

OS41G-09 1050h

The Evolution and Radiation of Eucaryotic Phytoplankton Taxa (EREUPT)

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The EREUPT research team, representing a group of 21 scientists from numerous institutions, is focused on understanding the historical origins and environmental conditions that led to selection and radiation of the major eucaryotic phytoplankton taxa, and the ecological processes that contribute to their continued success in the contemporary ocean. The proposed research utilizes a combination of geological, molecular biological, ecological, and models. Our primary goal is to develop the first quantitative models of eucaryotic phytoplankton community structure in the contemporary oceans based on paleoecological and evolutionary inference. The proposed research seeks to test a set of three related hypotheses, from which we will develop a conceptual model for evolution and ecological success (dominance) of key phytoplankton taxa in the contemporary ocean. The central hypotheses are: 1) The three dominant phytoplankton taxa in the contemporary ocean evolved in shallow shelf-seas in the Mesozoic Era in response to changes in the ocean environment, such as anoxia, changes in sea level, or tectonic processes that excluded ecological advantages previously afforded to chlorophytes. 2) Once established, these groups radiated rapidly. The rapid tempo of evolution was a consequence of high mutation frequencies relative to reversion and sexual recombination, resulting in high genetic potential and DNA content relative to genetic expression in the three taxa. The rapid tempo of evolution in the three taxa has permitted rapid radiation and adaptation to changing oceanic conditions throughout the Mesozoic. This rapid tempo continues to the present time. 3) The ecological dominance of the three major eucaryotic phytoplankton taxa is a consequence of pan-division traits that permit individual species within each group to rapidly accommodate large variations in oceanic conditions. These traits include the evolution of cell walls and vacuoles that respectively provide protection from predation while simultaneously optimizing the exploitation of pulsed nutrient supplies. A corollary of this hypothesis is that the structure of marine food webs in the contemporary ocean is primarily a consequence of the tempo of evolution of the three major taxa of eucaryotic phytoplankton, which itself is a consequence of continuous changes in oceanic regimes.

URL: http://marine.rutgers.edu/ebme/html_docs/project_Biocomplex.html

OS41G-10 1105h

Dynamical Analysis of a Network Model of Phosphorus for the Phanerozoic Earth

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We present a dynamical model of the global biogeochemical cycling of phosphorus. The primary parameters of the model are seafloor spreading rate, eustasy, efficiency of thermohaline circulation, and the ratio of primary biological productivity between the oceans and land. The first three can be measured or estimated from the geologic record of Phanerozoic time. The last depends on biological innovation and climate limitations. The water column is modeled as euphotic zone and deep water components that exchange at a rate given by the THC parameter. High-temperature hydrothermal systems also strip P from the deep ocean at a rate proportional to the rate of seafloor spreading. Phosphorus is removed from the euphotic zone by sinking in organic particles at a rate that is proportional to primary productivity. Remineralization of the organic particulate matter and release of P in the deep ocean is a sensitive function of the oxygen content of the water column. We assume that the amount of particulate organic matter buried in ocean sediments is stoichiometrically related to the amount of net respiration of organics and oxidative weathering of minerals on land. Remaining organic matter reaches benthic sediments, where it can be slowly released into pore water upon diagenesis. The sediments are subducted at the rate of sea-floor spreading. Phosphorus is also sequestered on continental shelves: Marine regressions result in weathering and release of this P. Surface waters receive P both from riverine input and from aerial transport of soil-derived dust particles. These fluxes depend on the rate of weathering. A major difference between this model and previous efforts is that an explicit temperature-dependence to weathering is

not included: Although laboratory experiments support an Arrhenius-like temperature dependence of mineral weathering rates, this has not been supported by basin-scale measurements and terrestrial biological activity has probably been a more important factor on the Phanerozoic Earth. We explore the dynamics of this model and examine the system's response to slow changes in parameters as well as sudden perturbations (e.g., impacts or eruption of large igneous provinces). We also determine the long-term trend of total biologically available phosphorus, a potential determinant of global biodiversity.

OS41G-11 1120h

Simulation of Coastal Upwelling Circulation and Nitrogen Isotopic Fractionation With a Coupled Physical Biogeochemical Model

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A coupled physical-biogeochemical model is used to simulate the evolution of the sedimentary nitrogen isotopic signal during the last glacial-interglacial transition in coastal upwelling systems. The biological model is a simple nitrogen-based trophic chain model, which also computes the nitrogen isotopes fractionation. Photosynthesis and zooplankton excretion fractionations influence the detrital nitrogen isotopic signal, which is compared to the sedimentary data. The 2D physical primitive equation model simulates the coastal upwelling circulation.

This coupled model is applied for different sea level situations to reproduce the sedimentary $\delta^{15}\text{N}$ and organic nitrogen flux signals, and we reconstruct some paleoceanographic scenarios. Offshore Cap Blanc, Mauritania, the effect of the sea-level rise, inducing the shelf immersion, is the main factor to explain the organic nitrogen flux and isotopic signal variations along the last deglaciation. Between 15 and 5.5 kyrs, 60 % of the sedimentary isotopic signal could be explained by this local shelf immersion effect. Any sedimentary diagenetic process or any nitrogen loss/gain budget occurring in other ocean areas and affecting the nutrient isotopic signature would account only for 40 % on these local sedimentary isotopic signal variations. We thus conclude that the global oceanic fixed nitrogen budget during the last glacial-interglacial transition may have been more balanced and stable than previously assumed. This effect is modulated by an upwelling seasonality that may have been much longer at the Last Glacial Maximum, around 10 months instead of 5-6 months at present. This model has been also successfully applied to the upwelling system of Benguela (South West Africa) to study some recycling processes.

OS41H HC: 318 B Thursday 0830h

Molecular Ecology of Carbon and Nitrogen Cycles in Ocean Margins I

Presiding: F Wilkerson, San Francisco State University; J Paul, University of South Florida

OS41H-01 0830h

High Density Sampling in the Coastal Ocean.

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The oceanographic community is gaining a better understanding about the population dynamics of bacterioplankton communities on different spatial and temporal scales. The use of molecular techniques targeted

to specific microorganisms has greatly enhanced our knowledge base. Now, the current generation of automated, fluorescent sequencers provides a means to accomplish high-density environmental sampling to place these observations in a more meaningful context. As an example, monthly fingerprints of microbial communities ($n=280$) will be presented from a 34 km transect off the New Jersey coast near the Long-Term Ecosystem Observatory (LEO-15) from 1994 to 1997. Microbial populations at this site have been characterized using 16S ribosomal genes and terminal restriction fragment length Polymorphism (TRFLP) analysis. Preliminary data demonstrates near-shore to offshore differences in average TRFLP peak numbers (48 ± 16 , 51 ± 20 , and 60 ± 18), suggesting a diversity gradient in this coastal ocean setting. Furthermore, frequency plots of TRFLP peaks reveal 13 SSU genes are present in greater than 65% of all samples, indicating a specific suite of dominant microbes at LEO-15. In addition, specific TRFLP peaks have been identified which appear associated with seasonal change, oxygen availability or spatial variability. As a second example of high-density fingerprinting, data from the Black Sea (the largest anoxic basin in the world) will also be presented. Microbial populations from these samples have been characterized by TRFLP and library construction for: i) Eubacterial and Archaeal ribosomal rRNA genes and ii) functional genes, including: particulate methane monooxygenase (*pmoA*), dissimilatory sulfite reductase (*dsrA*), dissimilatory nitrous oxide reductase (*nosZ*) and nitrogenase (*nifH*). Distinct members of both the Eubacterial and Archaeal communities along the oxic/anoxic interface have been discerned for a variety of microbial groups involved in biogeochemical transformation. This detailed information on the microbial populations will prove useful in understanding background variability in microbial communities and identifying specific mechanisms that might affect microbial diversity in marine environments and on much smaller scales.

OS41H-02 0845h

Denitrification in Inshore and Continental Margin Sediments of Washington

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Arguably, the largest sink for combined nitrogen in the global marine system is denitrification in continental margin sediments. To help understand the controls on sedimentary denitrification we have taken a number of cores from the Washington State continental margin and from several sites within Puget Sound. Pore water profiles of showed that both dissolved oxygen and nitrate was depleted within the upper few cm of the sediment column at all locations. Denitrification potential at various depths in the cores was determined by the acetylene inhibition technique after addition of nitrate. Community structure was evaluated by T-RFLP of the nitrite reductase gene (*nirS*). Denitrification potential was detected at almost all depths in all samples and in the Puget Sound samples there was very little lag time observed within the upper 40 cm. Only in very deep sediments, 70 - 140 cm down core, was denitrification potential undetectable over the course of the incubation. Given a sedimentation rate of less than a cm per year, this indicated that even in sediments that had been depleted of nitrate for many years, denitrifiers were able to resume active denitrification almost immediately. Previous workers have determined that these sediments are actively bioturbated within approximately the upper 20-30 cm. Thus we suspect active bioturbation mixes the sediments on a time scale that allows denitrifiers to remain viable at least down to 40 cm. The lack of denitrification activity below 40 cm is probably because these sediments were below the bioturbation zone. Community structure as determined by T-RFLP of *nirS* was very similar in the upper 40 cm of all Puget Sound sediments, which is also consistent with bioturbation.

OS41H-03 0900h

Nitrification rates and Diversity of Ammonia Oxidizing Bacteria in Monterey Bay, California

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Temporal variability in nitrogen cycling at a central station in Monterey Bay, California was investigated by measuring rates of nitrogen assimilation, nitrification and the diversity and composition of the ammonia oxidizing community on bimonthly cruises over two years. The site was influenced by the episodic and seasonal upwelling of the northern bay, such that nitrate concentrations in the surface waters were usually high and the water column was relatively well mixed. Nevertheless, depth profiles often showed a nitrification rate maximum near the 1 % light depth. Nitrification rates integrated over the euphotic zone (0.1 % light depth) varied by a factor of 2.5 over the two year period. We investigated the extent to which the temporal and vertical structure in the rate distributions was reflected in the diversity and composition of the nitrifier community using sequence analysis of the 16S rRNA and ammonia monooxygenase (*amoA*) genes of beta- and gamma-subdivision ammonia oxidizing bacteria. Most of the beta-subdivision 16S rRNA sequences retrieved were most similar to cultivated *Nitrosospira* strains, rather than *Nitrosomonas*. We found relatively little diversity among the 16S rRNA sequences of the Monterey Bay clones, but distinct clusters appeared to be associated with specific depths and sampling dates. *AmoA* sequences displayed greater diversity than did the 16S rRNA sequences, but also formed distinct clusters according to depth and sampling date. Many of the cloned *amoA* sequences do not appear to represent cultivated strains. The gamma-subdivision 16S rRNA sequences retrieved from Monterey Bay were more similar to each other than to the cultivated strains of *Nitrosococcus oceanii*. Thus, even over a depth range of less than 100 m in a relatively well mixed water column, the nitrifier assemblage exhibited detectable temporal and vertical structure.

OS41H-04 0915h

Impact of DOM on Bacterial Community Structure in the Delaware Estuary

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Dissolved organic material (DOM) is a large component of material and energy cycles in aquatic systems, including estuaries such as the Delaware River and Bay, and is thought to be an important, if not a limiting factor affecting the standing stocks and growth of heterotrophic bacteria in these systems. Less is known about the role of DOM in shaping bacterial community structure. We used a series of DOM enrichment experiments to investigate the impact of DOM on bacterial community structure in the Delaware Estuary, with some work also in the Chesapeake Bay. Biomass production of the entire heterotrophic bacterial assemblage appeared to be limited by organic carbon, since addition of glucose or protein stimulated thymidine and leucine incorporation, although bacterial biomass (cell numbers and biovolume) did not vary substantially when grazers were present. These additions also led to changes in bacterial community structure as revealed by fluorescence in situ hybridization (FISH). Addition of glucose to whole water caused an increase in the abundance of gamma proteobacteria, and there was a correlation between relative gamma proteobacterial abundance and the stimulation of total biomass production (leucine incorporation). Addition of protein to grazer-free incubations had a different impact and seemed to be more complex. The abundance of Cytophaga-Flavobacteria often increased in these experiments, but sometimes the increase was observed in the control as well. The addition of protein also affected subdivisions of proteobacteria depending on the location of the experiment. The stimulation of Cytophaga-Flavobacterial abundance is consistent with Mico-FISH experiments suggesting that Cytophaga-Flavobacteria dominate protein use whereas alpha-proteobacteria use free amino acids. The protein additions also led to dramatic changes in bacterial fatty acids with odd chain and branched chain acids increasing over four-fold compared to the control. These experiments shed some light on what controls bacterial community structure and may allow us to link rRNA-based bacterial phylogeny with organic markers of bacterial communities and thus the contribution of bacterial carbon to preserved organic matter in aquatic systems.

OS41H-05 0930h

Development and Use of Molecular Techniques to Identify Urea-degrading Microorganisms

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Important linkages between the carbon and nitrogen cycles are likely to be found in the fluxes and fates of dissolved organic nitrogenous compounds (DON). Urea is often the single most abundant component of the large and complex DON pool, and many microorganisms are capable of using urea as a source of ammonium and/or carbon. Most organisms use the enzyme urease, a nickel-requiring metalloenzyme, to catalyze the hydrolysis of urea into two molecules of ammonia and one molecule of carbonic acid. As part of the Biocomplexity project examining relationships between ecosystem level fluxes of nitrogenous compounds (such as urea) and the organisms possessing genes encoding the enzymes (such as urease) responsible for driving those fluxes in Chesapeake Bay, we set out to develop a universal molecular method to detect and identify urea-degrading organisms. Thanks to the well-conserved nature of urease sequences, we were able to use the CoDeHOP strategy to design PCR primers which are able to amplify a wide array of both known and novel urease sequences. Using our universal urease primers, we have PCR-amplified, cloned, and sequenced a 560bp region of the ureC gene from several pure cultures of prokaryotes and eukaryotes, from one non-axenic algal culture and from four samples from the Choptank River (a major tributary of Chesapeake Bay). Phylogenetic reconstructions from our sequence data appear to provide a reasonable level of taxonomic resolution. There are many distinct sequence clusters with strong bootstrap support, including two different cyanobacterial clusters, a cluster formed around *Tetraselmis* sp., and several clusters of sequences of unknown phylogenetic affinity obtained directly from the Choptank River samples. An extremely high level of diversity of ureases in the environment is indicated both by TRFLP analyses and by the fact that of the 100 sequences collected to date from the four Choptank samples, none are identical (or even differ only by the *Taq* polymerase error rate). Strategies for defining and analyzing the highly diverse urealytic organisms like to be found throughout the Chesapeake are currently being developed.

URL: <http://geoweb.princeton.edu/research/biocomplexity/>

OS41H-06 0945h

Uptake and utilization of dissolved organic nitrogen by the brown tide alga *A. anophagefferens* in Long Islands Coastal Waters

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Intense blooms of *Aureococcus anophagefferens*, or Brown Tides (BT), were first observed in 1985 at three separate locations along the Northeast Coast of the United States. The simultaneous occurrence of BT in geographically isolated regions suggested that regional-scale, climatological forcing may play a role in triggering the blooms. Yet, attempts to correlate bloom initiation with rainfall or wind stress have failed to provide a consistent explanation for bloom. In a recent paper we have hypothesized that BT blooms are controlled by interannual variability in the supply of dissolved organic nitrogen (DON).

Dissolved organic nitrogen is potentially available to phytoplankton through bacterial degradation to inorganic nitrogen or as simple organic nutrients such as amino acids and urea. Whether more complex forms of DON are also directly available to phytoplankton is not known. The availability of DON may depend on its chemical composition and on the enzymatic hydrolytic capabilities of the phytoplankton. To determine if eukaryotic picoplankton can grow directly on HMW DON as a source of nitrogen without the intervention of bacteria, we investigated growth of *A. anophagefferens* on HMW DON in axenic culture. HMW DON was isolated by ultrafiltration from sediment pore waters and

surface sea waters, processed to remove salts, and analyzed for N content. HMWDON was characterized by NMR and molecular level techniques, and aliquots were dissolved in artificial seawater and used as the sole N source in *A. anophagefferens* cultures. We find that *A. anophagefferens* grows well on HMW DON, and that the utilization of HMW DON is matched by the increase in PON. We demonstrate the presence of a 26 kD membrane protein exclusively induced in cells grown on HMW DON and not in cells grown on low molecular weight DON or inorganic nitrogen. We further explored the use of DON by *A. anophagefferens* by testing four different classes of dissolved organic compounds, including urea, amines, peptides and chitin. Our choice of substrates was determined by current information on DON composition. We found that *A. anophagefferens* possesses several biochemical pathways that enable it to utilize multiple components of DON. For example, *A. anophagefferens* can hydrolyze peptides extracellularly, suggesting that at least one component of HMW DON is bioavailable to marine phytoplankton. However, peptides comprise only 1-2% of HMW DON and our experiments demonstrate that *A. anophagefferens* may deplete 12-30% of the nitrogen available in HMW DON. Either current estimates of proteins in DON are too low, or additional components of DON must play a role in the nitrogenous nutrition of picoeukaryotes.

OS41H-07 1020h INVITED

The Role of it ntcA in Nitrogen Acquisition and its Use as a Molecular Diagnostic Tool for Assessing Nitrogen Stress in Marine Cyanobacteria

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Nitrogen metabolism in cyanobacteria is regulated by the transcriptional activator NtcA. Inactivation of the it ntcA gene in marine *it* *Synechococcus* sp. strain WH 7803 leads to the inability of the cell to utilize or grow on nitrate or nitrite. Expression analyses of the gene encoding the nitrate transporter (*it napA*) shows that the expression of this gene is under the control of NtcA. Therefore for these cyanobacteria to utilize new nitrogen sources such as nitrate, NtcA must be expressed. In contrast, growth on ammonium was not impaired in the NtcA-deficient mutant indicating that NtcA expression is not required for ammonium utilization. NtcA also plays a role in the adaptation of cyanobacteria to prolonged nitrogen deprivation.

it ntcA is differentially expressed by cells grown under various nitrogen regimes. In the presence of ammonium expression of this gene is basal whereas transcript levels are greatly enhanced under conditions of nitrogen deprivation in both *it* *Synechococcus* sp. strain WH 7803 and *it* *Prochlorococcus* strain PCC 9511. Expression is intermediate between basal and maximal when *it* *Synechococcus* is grown on nitrate. Thus *it ntcA* transcript levels, relative to basal and maximal expression, can be used to assess the nitrogen status of natural populations of these unicellular, non-nitrogen fixing cyanobacteria. Analysis of RNA extracted from bulk seawater samples during the years 1999 and 2000 in the Gulf of Aqaba, Red Sea, show that *it* *Synechococcus* populations were not N deprived even when inorganic nitrogen concentrations were low. In most cases *it ntcA* expression was basal indicating that the cells were utilizing ammonium. This suggests that the supply of nitrogen through rapid ammonium regeneration was sufficient to meet the needs of the *it* *Synechococcus* populations. However during a spring bloom of massive proportions, *it ntcA* expression was intermediate indicating that ammonium was insufficient and that an alternative nitrogen source such as nitrate, was being utilized. Having *it* *Synechococcus* populations report on their nitrogen status through *it ntcA* gene expression has revealed that these cyanobacteria were not deprived of nitrogen and that they contributed to both new and regenerated production.

OS41H-08 1035h INVITED

ntcA mRNA levels as an indicator of picocyanobacterial nitrogen status in the Gulf of Mexico.

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Coastal plumes are highly productive features known to support large populations of phytoplankton, including picoplankton such as *Synechococcus*. Plumes originate as high nutrient coastal water and are believed to have nutrients exhausted as they reach the oligotrophic ocean. Yet, photosynthetic carbon fixation in such plumes exceeds that of adjacent, non-plume oceanic water by at least an order of magnitude. To determine the nitrogen nutritional status of picocyanobacterial populations in the Mississippi River plume, we have measured mRNA levels of the nitrogen controlling gene *ntcA* along the plume axis. We have adapted the method of Lindell and Post (2001, Appl. Environ. Microbiol. 67:3340-3349) to estimate nitrogen nutritional status of picocyanobacteria in this plume. By this method, *ntcA* mRNA levels are measured in natural populations under ambient conditions, in the presence of high levels of exogenous ammonium, and during methionine sulfoximine-induced N-starvation. A nitrogen deficiency index (NDI) was calculated from such measures, and was found to be lowest in coastal and non-plume stations (ave. 37 +/- 16.8, n=4) and highest in offshore plume stations (ave. 76.7 +/- 14.4, n = 3, p = 0.0206). These results indicate that the ambient *Synechococcus* population in coastal plumes is subject to nitrogen stress but not complete nitrogen deprivation. Such results are consistent with concept of efficient utilization of low levels of recycled nitrogen in picocyanobacteria in offshore plume environments.

OS41H-09 1050h

Diversity of Cyanobacterial Nitrate Reductase and Development of Molecular Probes for Nitrogen Assimilation Genes

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Phytoplankton in coastal upwelling zones experience dynamic shifts in light and nutrient availability that results in changes in the composition of phytoplankton community members themselves. To understand nitrogen uptake dynamics of cyanobacteria in ocean margins at the molecular level, we developed probes to a key enzyme in the nitrogen assimilation pathway, assimilatory nitrate reductase (*narB*). Full-length cyanobacterial *narB* amino acid sequences were aligned and analyzed by the CODEHOP algorithm that assigns degenerate PCR primers to blocks of highly conserved amino acid homology. PCR primers were selected based on homology blocks most conserved in cyanobacteria, but less conserved in closely related bacterial *nasA* sequences. Two pairs of *narB* CODEHOP primers were used in two sequential rounds of PCR to amplify partial 900 bp *narB* sequences from culture collection isolates, coastal and open ocean water samples, and microbial mats. Samples were selected to determine both the diversity of cyanobacterial *narB* in aquatic environments and to assess the utility of the primers. All sequences obtained to date clustered with *narB* rather than *nasA* genes, suggesting that the primers are specific for cyanobacteria. With the exception of sequences from the equatorial Pacific that are 99% identical to *Trichodesmium*, there were no other sequences with over 80% similarity to *narB* sequences in GenBank and from culture isolates. Within most environments, individual sequences are almost identical on the amino acid level. However, sequences are no more than 75% similar when compared between different environments, suggesting that cyanobacterial *narB* diversity might high, but limited within an ecosystem. Once more *narB* genes in coastal cyanobacteria are identified, we will use this sequence information to monitor the contribution of cyanobacteria to nitrate uptake in coastal upwelling zones

OS41H-10 1105h

Cloning and Characterization of Assimilatory Nitrate Reductase Gene (*nar*) in Marine Green Alga *Dunaliella tertiolecta*

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Nitrate is used as a nutrient for growth by many organisms through the nitrate assimilation pathway. The key enzyme involved in nitrate assimilation is nitrate reductase (*nar*). Biochemical and genetic properties of assimilatory nitrate reductase (*nar*) have been well elucidated in bacteria, fungi, fresh water green algae and higher plants. There is very little information available, however, for *nar* genes in marine eukaryotic phytoplankton, although the biochemical characteristics of the enzymes are well studied. Therefore, we attempted to clone and characterize *nar* genes in marine eukaryotic phytoplankton. Degenerate primers for the *nar* gene were designed from a highly conserved region in molybdenum-pterin and iron-heme domains by comparing amino acid sequences of *nar* genes from higher plants, fungi and unicellular algae. These primers were used to amplify *nar* genes from genomic DNAs from the marine green alga *Dunaliella tertiolecta* and marine diatoms *Skeletonema costatum* and *Thalassiosira weissflogii*. A *nar* gene was successfully amplified only from *D. tertiolecta*. Rapid Amplification of cDNA Ends (RACE) technique was performed to obtain a complete *nar* gene from a cDNA of *D. tertiolecta*. The complete *nar* gene of *D. tertiolecta* was 2.6Kb size and showed strong similarity at the amino acids level to *nar* genes from freshwater green algae *Chlorella vulgaris* (50.6%), *Chlamydomonas reinhardtii* (58.4%) and *Volvox carter* (59.4%). The induction and repression of *nar* mRNA transcript were measured with the RNase Protection Assay (RPA) technique with an internal *nar* gene probe. The induction of *nar* mRNA transcript in *D. tertiolecta* was maximized within one hour after nitrate was added in nitrogen depleted cells. However, no significant differences were observed between the in light and dark periods. The level of *nar* mRNA transcript was significantly reduced by the addition of ammonium and urea, as well as by nitrogen depletion. Interestingly, *nar* mRNA transcript was induced a quarter fold in cells growing with nitrite relative to cells growing with nitrate. Thus, we report here the first *nar* gene from marine eukaryotic phytoplankton and regulation of *nar* mRNA transcript with different nitrogen sources.

OS41H-11 1120h INVITED

Diatom Population Dynamics: Molecular Identification of Genetically and Physiologically Distinct Subpopulations of the Centric Diatom, *Ditylum brightwellii*

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Diatoms are one of the most abundant groups of aquatic eukaryotic microorganisms found in nature, with a wide distribution throughout marine habitats. Because of their dominance under high nitrate conditions and their enhanced sinking rates, diatoms play a critical role in the biological pump that mediates carbon and nitrogen fluxes out of euphotic zones and into deeper waters. We have begun developing molecular markers, based on highly repetitive regions of the genome known as microsatellites, to examine how the genetic and physiological structure of individual species of diatoms are shaped by their environment. The overriding theme of our work thus far is that remarkably high levels of genetic and physiological diversity characterize diatom populations. Recently, we have used our high throughput DNA fingerprinting techniques to genetically define estuarine- and ocean-derived subpopulations of the centric diatom *Ditylum brightwellii*, a common component of coastal ecosystems. Furthermore, we have found that, in general, estuarine-derived isolates display a slower maximum growth rate than the ocean-derived isolates. The fact that subpopulations with distinct attributes can exist within a single species from an environment such as Puget Sound, WA where all the waters eventually mix, implies the action of strong environmental selection that presumably operates on relatively short time scales. It now appears that we can use these techniques to examine in detail the link between environmental forcing and biological response at the level of individual species. Moreover, these results suggest that species characteristics may be somewhat ephemeral, with a strong dependence on sampling locale.

OS41H-12 1135h

A Proton Buffering Role for Silica in DiatomsAllen J. Milligan¹ ((609) 258-4674; allenm@princeton.edu)Francois M.M. Morel¹¹Princeton University, Guyot Hall Geosciences Department Princeton University, Princeton, NJ 08544, United States

Diatoms concentrate carbon in order to overcome the carbon oxidation reaction of RubisCO encourage the carboxylase reaction. Diatoms possess a carbon concentrating mechanism which utilizes an extracellular carbonic anhydrase (CA) and catalyses the equilibrium reaction between bicarbonate and CO₂. Forms of CA which have high catalytic rates require a pH buffer to either provide or receive a proton in the dehydration / hydration reaction. The proton transfer reaction between the pH buffer and the active site has been shown to be the rate limiting step in catalysis. We have found evidence that the siliceous cell wall of diatoms is an effective pH buffer and that it enables the enzymatic conversion of bicarbonate to CO₂, an important step in the acquisition of inorganic carbon by these organisms. We find that diatom silica has the appropriate buffering capacity and pKa to provide pH buffering to CA. Using membrane inlet mass spectrometry we demonstrate that both bovine and diatom CA's can be catalytically active in a system buffered with cleaned diatom silica. In situ experiments show that live cells of *T. weissflogii* do not require a pH buffer to exhibit catalytically active CA but that the green algae *Chlamydomonas* requires pH buffering. We hypothesize that diatoms possess the capacity to utilize the pH buffering ability of the silica cell wall to aid in uptake of bicarbonate, the major inorganic carbon source in seawater.

OS41I HC: 317 A Thursday 0830h**Biogeochemical Processes in Anoxic and Suboxic Environments II****Presiding: M Scranton, State**University of New York; **J Murray, University of Washington**

OS41I-01 0835h

Ventilation of the Black Sea: New Hydrographic and Nutrient Data From the 2001 R/V Knorr CruiseJames W. Murray¹ ((206) 543-4730; jmurray@u.washington.edu)Sergey K. Kononov² (sergey@alpha.mhi.iuf.net)Temel Oguz³ (oguz@ims.metu.edu.tr)Sukru Besiktepe³ (sukru@ims.metu.edu.tr)Suley Tugrul³ (tugrul@ims.metu.edu.tr)¹University of Washington, School of Oceanography Box 355351, Seattle, WA 98195-5351²Marine Hydrophysical Institute, Kapitanskaya St., 2a, Sevastopol 99000, Ukraine³Middle East Technical University, Institute of Marine Sciences P.O. Box 28, Erdemli-Icel 33731, Turkey

The sampling plan of the 2001 Knorr research cruise was designed to examine variability associated with the Bosphorus inflow along the SW coast near Turkey and the NW shelf area near Ukraine. The cruise was divided into two legs of approximately 10 days each. There were 48 scientists from five countries participating. A web site describing the cruise is available at www.ocean.washington.edu/cruises/Knorr2001.

The only source of salinity to the Black Sea is the Bosphorus inflow. The only source of cold water is the surface. Thus, distributions of hydrographic data can be used to evaluate variability in the Black Sea due to variable inputs from the Bosphorus and variable intrusions from the surface. Variability in oxygen from both sources influences the depth and intensity of the suboxic layer. The cold intermediate layer in 2001 had lower oxygen than in previous years suggesting lower rates of ventilation than in the past. The density of the first appearance of sulfide was the same, however.

Oxygen input with the Bosphorus inflow is an important sink for the upward flux of sulfide and the stations in the SW corner of the Black Sea were well sorted to observe these intrusions.

OS41I-02 0850h

The Lateral Flux of Oxygen From the Bosphorus Into the Black Sea: Controls on the Distributions of Oxygen, Sulfide and Redox ChemistrySergey K. Kononov¹ (sergey@alpha.mhi.iuf.net);James W. Murray² ((206)543-4730;jmurray@u.washington.edu); George W. Luther³ ((302) 645-4208; luther@udel.edu); Ken O.Buesseler⁴ ((508) 289-2309; kbuesseler@whoi.edu);Gernot Friederich⁵ ((831)775-1713;frge@mbari.org); Brad Tebo⁶ ((619) 534-5470;btebo@ucsd.edu); Leonid I. Ivanov¹(leonid@alpha.mhi.iuf.net); Anatoly Samodurov¹ (ass@alpha.mhi.iuf.net)¹Marine Hydrophysical Institute National Academy of Sciences of Ukraine, Kapitanskaya St. 2a, Sevastopol 99000, Ukraine²University of Washington, School of Oceanography Box 355351, Seattle, WA 98195-5351³University of Delaware, College of Marine Studies, Lewes, DE 19958⁴WHOI, Dept. of Marine Chemistry and Geochemistry, Woods Hole, MA 02543⁵MBARI, 7700 Sandholdt Road, Moss Landing, CA 95039⁶Scripps Institution of Oceanography, 9500 Gilman Drive, La Jolla, CA 92093-0202

The lateral flux of oxygen input from the Bosphorus plays an important control on the vertical oxic/anoxic structure and redox budget of the Black Sea. We use the results of numerical modeling calibrated and verified against historical data from 1960 to 1995 and the most recent data from the 2001 Knorr cruise to support the following points: 1. Injection of Mediterranean water into the Black Sea results in intrusions of oxygen in the suboxic and anoxic layers. 2. The volume of these intrusions and the lateral flux of oxygen decreases exponentially towards the deeper layers of the main pycnocline. 3. The lateral influx of oxygen results in oxidation of sulfide inside the anoxic zone. As much as 70% of the total vertical flux of sulfide is oxidized by this oxygen, mainly through redox Mn(II) - Mn(III,IV) cycling of manganese. This appears to be the main reason for the stable density of the onset of sulfide in the main pycnocline, even though the depth of both the onset of sulfide and pycnocline vary by more than 20 meters on the time scale of decades. 4. The lateral flux of oxygen appears to be the main reason for the presence of the suboxic layer, as the downward flux of oxygen is exhausted at the depth of the upper boundary of the suboxic layer and the position of the lower boundary is mainly controlled by the lateral flux of oxygen. The suboxic zone can exist when the flux of organic matter is enough to deplete the downward flux of oxygen and the lateral flux of oxygen is sufficient to compensate the upward flux of sulfide and other reduced substances. 5. Both climate- and human-induced changes in the flux of nutrients and oxygen are essential for the temporal evolution of the suboxic zone and the entire biogeochemical structure of the Black Sea water column.

OS41I-03 0905h INVITED

Mediterranean Inflow Through the Bosphorus Injects Massive Amounts of O₂ into the Suboxic Zone of the Southwest Black SeaGeorge W. Luther¹ (302-645-4208; luther@udel.edu);Donald B. Nuzzio² (908-788-7022); Brian Glazer¹;Sergey K. Kononov³ (sergey@alpha.mhi.iuf.net);Gernot Friederich⁴ (frge@mbari.org); Robert E.Trouwborst¹; Alexander Romanov³; Brad M.Tebo⁵¹Univ. of Delaware, College of Marine Studies, Lewes, DE 19958, United States²Analytical Instrument Systems, Inc. Road, 1059C Old York, Ringoes, NJ 08851, United States³Marine Hydrophysical Institute, 2 Kapitanskaya St., Sevastopol 99011, Ukraine⁴MBARI, 7700 Sandholdt Rd., Moss Landing 95039, United States⁵Scripps Institution of Oceanography, University of California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0202, United States

An in situ voltammetry analyzer from Analytical Instrument Systems, Inc. coupled to the MBARI CTD-pump profiler system was deployed to determine the redox chemistry of the upper 200 m (from the oxic to suboxic to anoxic zones) of the western Black Sea. Solid-state gold-amalgam electrodes were used to measure

O₂, H₂S and polysulfides (S_n²⁻) at < 1 meter resolution. In the Southwest, massive intrusion of O₂ (from 10 to 100 micromolar concentrations) into the suboxic zone was measured over intervals of 4 to 5 meters and correlated with increases of temperature of only 0.2 to 0.3 °C. Up to 3 submaxima of O₂ could be detected in the suboxic zone and 2 subminima of H₂S in the H₂S zone could be detected. O₂ and H₂S were not observed to co-exist at any depth. This "fingering" of O₂ was not observed in the West or Northwest section of the Black Sea and leads to significant consequences on the oxidation of manganese and sulfide in the southwestern section of the Black Sea. We present data from the in situ work as well as from a voltammetric flow cell and from discrete samples. High resolution data are essential to determine the chemical characteristics of the water column.

OS41I-04 0920h

Spatial and Temporal Variations in the Hydro-chemical Properties of the Black Sea Upper Layer Water ColumnIlkay Salihoglu¹ (+90 324 521 2150; ilkay@metu.edu.tr)Suleyman Tugrul¹ (+90 324 521 2406; tugrul@ims.metu.edu.tr)¹Ilkay SALIHOGLU, METU Institute of Marine Sciences, P.O.BOX 28, Erdemli/ICEL 33731, Turkey

In this report we utilized Knorr-2001 Black Sea cruise nutrient data to evaluate spatial variability in the hydro-chemical properties (dissolved oxygen (DO), nitrate, phosphate, silicate, dissolved iron and manganese) of the upper layer water column of the Black Sea, including the upper depths of the anoxic layer. Nutrient profiles reveal that the photic zone waters contained very low concentrations of nitrate-nitrite (0.05-0.1 μM) and phosphate (0.02-0.04 μM) ions. The cold intermediate layer (CIL) in the coastal waters became poor in nitrate as compared to the late 1980-1995 period data, during which the open sea contained higher nitrate values at the depth of the nitrate maximum. Interestingly, in May-June 2001, the oxycline of the deep basin commenced at a shallower density surfaces as compared to the onset of nitrate. This finding indicates that denitrification processes became effective within the steep oxycline and caused nitrate lost from the water column. Comparison of long-term nitrate profiles in the studied sites confirms our idea. Combined profiles of the nitrate and DO data also indicate that the upper nitricline sharpened and deepened whilst the oxycline was modified by sinking particulate organic matter (POM). In the open sea the suboxic boundary (DO < 10 μM) has moved upward slightly and the DO deficiency (<5.0 μM below the 15.5 density surface) appeared to have increased in the suboxic zone, supporting the increasing nitrate loss from the transition zone. In the deep basin, the dissolved manganese concentrations become detectable consistently at about 15.85-15.90 σ density surfaces where the both DO and nitrate dropped to very low levels. Though the slopes of nitricline and oxycline were modified the sulphide bearing anoxic waters have remained almost at the same density surface in recent years, suggesting the existence of the other oxidizing agents (e.g. manganese) within the interface. In coastal margin off the Bosphorus Strait, the intrusion of the Mediterranean inflow to the Black Sea intermediate depths could be traced by its high nitrate values. In the shelf break; the excess oxygen in the Mediterranean inflow oxidize all sulfides in the receiving water masses whilst the nitrate and ammonia co-existed at elevated concentrations, indicating lower oxidation rate of ammonia. Such similar features were also observed in the coastal waters off Sakarya, in the southern Black Sea. In the suboxic interface between 16.1-16.35 σ density surfaces of this site, the concentrations of nitrite, intermediate product of redox reactions of dissolved inorganic nitrogen compounds, increased to levels of 0.2-0.35 μM in a water column of about 30 m thick, where the DO and nitrate both ranged between 30-45 μM and between 2-2.8 mM, respectively. This suboxic water also contained measurable concentrations of both dissolved manganese ions and ammonia (determined by colorimetric method) at 1.0-3.0 μM levels.

OS41I-05 0935h INVITED

High Resolution Measurements of Carbon Dioxide Distributions in the Black Sea: Indication of Horizontal Mixing Processes in the Suboxic Zone and Unexpected Surface Layer Carbon DeficitGernot E Friederich¹ (831-775-1713; frge@mbari.org)William T Hiscock² (wthiscoc@rsmas.miami.edu)George W Luther³ (luther@udel.edu)