THE SCIENTIFIC COMMITTEE FOR OCEANOGRAPHIC RESEARCH

WORKING GROUP ON MARINE VIRUSES – WG 126
Virus Ecology in Marine Systems: a workshop on methods
JUNE 1 – 3, 2006, THE UNIVERSITY OF BRITISH COLUMBIA, VANCOUVER, BC

MEETING SCHEDULE

Thursday 1 June
Location: Peter Wall Institute for Advanced Studies, University Centre, 6331 Crescent Rd., UBC

1630 to 1900 ***REGISTRATION AND MIXER***

Friday 2 June
Location: Michael Smith Lab, Auditorium, Rm 102, 2185 East Mall, UBC

INTRODUCTORY REMARKS

0800 **POSTER SET-UP AND LOADING OF TALKS**

0900 *A BRIEF WELCOME AND MEETING LOGISTICS*
CURTIS A. SUTTLE

0910 *OVERVIEW OF WG 126 GOALS- CHALLENGES, ROADBLOCKS AND PITFALLS*
MARKUS G WEINBAUER & STEVEN W. WILHELM

I. ACCURATE ESTIMATES OF VIRUS ABUNDANCE AND PRODUCTION

0925 *COLLECTION, PROCESSING AND PRESERVATION FOR DIRECT COUNTS AND FLOW CYTOMETRY*
CORINA P. D. BRUSSAARD – Royal Netherlands Institute of Sea Research, Netherlands

1000 *WORKING WITH VIRUSES IN MARINE SEDIMENTS*
JANICE E. LAWRENCE – University of New Brunswick, Canada

1035 ***Coffee Break***

1055 *DETERMINING VIRUS-MEDIATE MORTALITY IN MARINE SYSTEMS*
MARKUS G. WEINBAUER- CNRS Oceanographic Laboratory – Villefranche sur Mer, France

1130 *ISOLATION AND CHARACTERIZATION OF NEW VIRUS – HOST SYSTEMS*
KEIZO NAGASAKI – Fisheries Research Agency – Hiroshima, Japan

1205 ***Lunch Break (on your own)***

II. GENE BASED ESTIMATES OF VIRUS DIVERSITY

1400 *COMMUNITY PROFILING WITH DGGE AND SEQUENCING*
STEVEN M. SHORT– University of Denver, USA

1435 *CHARACTERIZATION OF CONSERVED GENES FOR VIRUS GROUP*
FENG CHEN- University of Maryland, USA

1510 **POSTER SESSION**

1730 ***Adjourn for day***

1900 **BANQUET – Peter Wall Institute for Advance Studies, University Centre, 6331 Crescent Rd., UBC**

Saturday 3 June

Location: Michael Smith Lab, Auditorium, Rm 102, 2185 East Mall, UBC

III. GENOMICS, GENOME ANALYSIS and METAGENOMICS

0900 ***Meeting Logistics***
CURTIS A. SUTTLE

0905 *APPLICATION OF MICROARRAYS IN THE CHARACTERIZATION OF VIRUS GENOMES*
WILLIAM H. WILSON – Plymouth Marine Labs, UK

0940 *THE GENOMICS OF PHAGE*
ROGER W. HENDRIX – University of Pittsburgh, USA

1015 ***Coffee Break***

1035 *METAGEMOMICS AND THE MARINE VIRUS COMMUNITY*
K. ERIC WOMMACK– University of Delaware, USA

1110 *THE GLOBAL MARINE VIRIOME*
ROBERT A. EDWARDS– San Diego State University, USA

1145 ***Lunch Break (on your own)***

1400 *METAGENOMICS WITH RNA VIRUSES*
CURTIS A. SUTTLE– University of British Columbia, Canada

IV. GLOBAL IMPLICATIONS FOR MARINE VIRUSES

1435 *THE SYNERGY BETWEEN VIRUSES IN NUTRIENT CYCLES AND SYSTEM GEOCHEMISTRY*
STEVEN W. WILHELM – University of Tennessee, USA

1510 ***Coffee Break***

1530 *THE GLOBAL ROLE OF VIRUSES IN BLOOM TERMINATION, BIODIVERSITY AND MARINE ECOLOGY*
GUNNAR BRATBAK – University of Bergen, Norway

1605 *IMPLICATIONS ON GLOBAL SCALES - THE DEVELOPMENT OF MODELS FOR VIRUS ACTIVITY*
MATHIAS MIDDELBOE - University of Copenhagen, Denmark

1640 **MEETING WRAP-UP – SUMMARY**

1730 ***Meeting Adjourns***

Sunday 4 June

Transportation to Victoria for those attending the ASLO meeting

PAPER

THE GLOBAL ROLE OF VIRUSES IN BLOOM TERMINATION, BIODIVERSITY AND MARINE ECOLOGY

Gunnar Bratbak

University of Bergen, Norway

Algal viruses are important only as far as their hosts play, or can play, qualitative or quantitative significant roles in natural ecosystems. Terminations of both harmful algal blooms and blooms that are important due to size and biomass production have been correlated to viral activity. *Heterosigma akashiwo, Aureococcus anophagefferens, Phaeocystis pouchetii* and *Emiliania huxleyi* may all serve as examples. The actual role of the viruses, being the genuine cause of the bloom termination or just opportunistic parasites attacking when growth have ceased and the hosts are infirm, remains however a question of debate. The possibility that virus prevents bloom formation and keep non-blooming species under constant control has also been considered but convincing case studies are scarce. Virus acts as host specific cause of mortality and their activity will affect community composition and diversity. In ecosystem theory they provide a solution to the fundamental problem of competitive exclusion (Hutchinson's Paradox) but their role can still be understood and analyzed within the framework of a generic ecological model. By lysing host cells and converting particulate biomass into dissolved organic material and nutrients virus may have a profound effect on biogeochemical cycles and food web structure. Their net effect on the pelagic ecosystem as a whole is however constrained by import, export and elemental mass balance in the system.

Gunnar Bratbak
Dept of Biology, P.O.box 7800, N-5020 Bergen, Norway
Gunnar.Bratbak@bio.uib.no

POSTER

MULTIDIMENSIONAL PHYSICAL SEPARATION OF AQUATIC VIRUSES TO ASSESS DIVERSITY

Jennifer R. Brum and Grieg F. Steward

School of Ocean and Earth Science and Technology, University of Hawaii at Manoa

Isolation of aquatic viruses from cultivated microorganisms is currently the only means of directly coupling phenotypic and genotypic data for individual virus types. Metagenomic analyses of virus communities offers large amounts of information about the populations of viruses, but reconstructing viral genomes is often inefficient and results in a disconnection between the phenotype and genotype of viruses. A solution to this may lie in the physical separation of viruses prior to the construction and sequencing of shotgun clone libraries. Viral concentrates were collected near Station ALOHA in the North Pacific Subtropical Gyre and from meromictic, hypersaline Mono Lake. Viruses in the concentrates were then separated based on the following properties: buoyant density (cesium chloride gradients), surface charge (ion-exchange chromatography), and hydrodynamic radius (size-exclusion chromatography). The separation of viruses using these methods was evaluated by determining the number of viral genomes within each separated fraction with pulsed field gel electrophoresis (PFGE). These approaches resulted in a significant reduction of viral diversity in the fractions, with between 1 to 7 dominant viral genome bands shown in the PFGE gel of each fraction. By using three of these separation methods in sequence, the viral diversity of a concentrate was reduced such that one of the resulting fractions had one viral genome band in the PFGE gel and one viral morphotype was observed using TEM. The results demonstrate that physical fractionation can significantly reduce the complexity of natural viral assemblages and suggest that this is a promising approach to more detailed descriptions of uncultivated aquatic viruses.

Jennifer R. Brum
Department of Oceanography, MSB 205, 1000 Pope Rd., Honolulu, HI, 96822
jbrum@hawaii.edu

PAPER

COLLECTION, PROCESSING AND PRESERVATION FOR DIRECT COUNTS AND FLOW CYTOMETRY

Corina P.D. Brussaard

Department of Biological Oceanography, Royal Netherlands Institute of Sea Research, Netherlands

The development of sensitive nucleic acid stains has allowed the detection and enumeration of free viruses in aquatic systems using epifluorescence microscopy and flow cytometry. In addition, flow cytometry allows the discrimination of virus subpopulations with different fluorescence. Epifluorescence microscopy has been used already routinely, whereas flow cytometry becomes more and more in use. For both techniques, however, methodologies have not been consistent to date. The types of fixative, storage conditions, and staining procedures are many. As a result there is a large variation in total virus count. There is an immediate need to have firmly established protocols available that allow accurate estimation of viral abundance in natural samples. Here I will present an overview of those methodologies that came out best after thorough testing. Critical steps will be discussed in more detail.

Corina P. Brussaard
Dept. Biol. Oceanogr., Royal NIOZ, PO BOX 59, 1790 AB Den Burg, Texel, The Netherlands
corina.brussaard@nioz.nl

PAPER

CHARACTERIZATION OF CONSERVED GENES FOR VIRUS GROUP

Feng Chen

Center of Marine Biotechnology, University of Maryland Biotechnology Institute

Viruses are probably most diverse and abundant biological entities in the ecosphere. Studying the diversity of viruses is a key step towards understanding of virus-host interaction in the natural ecosystem. Although no universal gene markers (e.g. 16S for prokaryotes and 18S for eukaryotes) can be applied for whole viral communities, it is possible to study the genetic diversity of viruses for some specific groups of viruses. For examples, population structure of viruses infecting eukaryotic microalgae has been investigated based on the DNA polymerase gene; diversity of cyanomyoviruses in aquatic environments has been explored based on the conserved viral capsid assembly gene; the photosystem D1 gene was recently found to be conserved among many cyanophages of marine picocyanobacteria. With these gene markers available, it allows us to monitor the spatial and temporal variation of specific viral groups in the natural marine environment. We applied these gene markers to survey the population dynamics of specific viral groups in the Chesapeake Bay, with co-monitoring of marine picocyanobacterial, eubacterial and eukaryotic communities.

Feng Chen
Center of Marine Biotechnology, University of Maryland, 701 E. Pratt St. Baltimore, MD21202
chenf@umbi.umd.edu

POSTER

PHYLOGENETIC DIVERSITY OF GENES ENCODING FOR PHOTOSYNTHETIC PROTEINS IN MARINE CYANOPHAGES AND THEIR HOST

Caroline Chénard[1], Lauren D. McDaniel[2], John H. Paul[2] and Curtis A. Suttle[1,3]

[1]*Department of Earth & Ocean Sciences, University of British Columbia, Vancouver BC, Canada*
[2]*College of Marine Science, University of South Florida, St. Petersburg, FL, USA*
[3]*Departments of Botany and Microbiology & Immunology, UBC, Vancouver BC, Canada*

Cyanophages are widespread in the marine environment and are thought to be a significant player in lateral gene transfer among cyanobacteria of the genera *Synechococcus* and *Prochlorococcus*. For instance, genes (*psbA* and *psbD*) encoding for essential proteins (D1& D2) in photosystem II have been discovered in cyanophages. In order to determine the occurrence of *psbA* and *psbD* in cyanophages, 31 cyanophage isolates (*Myoviridae*) from the Gulf of Mexico were screened with 4 different sets of primers. *PsbA* was found in 28 isolates while *psbD* was detected in 12 isolates. *PsbD* was only amplified from isolates containing *psbA.* Gene-based phylogenies of *psbA* and *psbD* revealed similar tree topologies, with cyanophage sequences clustered together, separate from *Synechococcus* and *Prochlorococcus* sequences. However, the tree topologies for phage genes were not congruent for *psbA* and *psbD*, as sequences from a few cyanophages clustered within different clades, suggesting gene transfer among the phages. These finding confirm that genes encoding the D1 and D2 photosynthetic proteins are common within cyanophages. Furthermore, our data suggest that the evolutionary history of cyanophage *psbA* and *psbD* have evolutionary histories that are distinct from each other and their cyanobacterial hosts

Caroline Chénard
Department of Earth and Ocean Sciences, The University of British Columbia, Vancouver BC V6T 1Z4
cchenard@eos.ubc.ca

POSTER

ASSESSING THE ROLE VIRUSES PLAY IN STRUCTURING LAKE PHYTOPLANKTON COMMUNITY COMPOSITION: A TEMPORAL INVESTIGATION

Jessica L. Clasen[1] and Curtis A. Suttle[1,2]

[1]*Department of Earth & Ocean Sciences, University of British Columbia, Vancouver BC, Canada*
[2]*Departments of Botany and Microbiology & Immunology, UBC, Vancouver BC, Canada*

In lakes, phytoplankton community composition is influenced by physical, chemical and biological variables; these include light, temperature, nutrient concentrations and zooplankton grazing. To asses the role viruses play in structuring phytoplankton communities, the temporal variation in the virioplankton community was determined in four lakes at the Experimental Lakes Area located in northwestern Ontario. In general, total viral abundance (Viruses·mL^{-1}) peaked in the spring/early summer (10^7 Viruses·mL^{-1}) and decreased to minimum values in the fall (10^6 Viruses·mL^{-1}). Interestingly, total viral abundance (Viruses·mL^{-1}) and chlorophyll *a* concentrations (µg·L^{-1}) were tightly coupled during the spring/early summer but not in the fall. To further explore the relationship between viruses and their potential phytoplankton hosts, *Phycodnaviridae* primers (AVS 1 and 2) were used to amplify viruses that specially infect eukaryotic phytoplankton. The resulting DGGE 'fingerprints' indicate that there is substantial temporal variation in the AVS viral community in all four lakes. In all cases, AVS viral richness (measured as OTUs) was significantly higher (mean = 17.5 OTUs) in the spring/early summer than the fall (mean = 9.5 OTUs). These results coupled with the total viral abundance data suggest that viruses play an important role in structuring phytoplankton community composition, particularly during the spring/early summer.

Jessica.L. Clasen
Department of Earth and Ocean Sciences, The University of British Columbia, Vancouver BC V6T 1Z4
jclasen@eos.ubc.ca

PAPER

PHAGES IN THE WORLD'S OCEANS

Robert A. Edwards[1], Ben Felts, Mya Breitbart[1], Peter Salamon[1], Florent Angly[1], Craig Carlson[2], Amy M. Chan[3], Matthew Haynes[1], Scott Kelley[1], Joe Mahaffy[1], Jennifer E. Mueller1, Jim Nulton[1], Robert Olson[1], Rachel Parsons[4], Steven Rayhawk[1], Curtis A. Suttle[3], and Forest Rohwer[1]

[1]San Diego State University; [2] University of California, Santa Barbara,
[3]The University of British Columbia, [4]Bermuda Biological Station for Research

Random community genome sequencing is changing our view on species richness and abundance in marine communities. Until recently these large scale sequencing projects have been limited to select institutions with the resources for massive amounts of high-throughput sequencing. Recent innovations and breakthroughs in sequencing technology have opened a whole new field where all labs can sequence complete environments with little overhead. For a few thousand dollars more than 30 Mbp of sequence can be generated from any environment. The challenges have shifted from the biological sample preparation and sequencing to the bioinformatics analysis of the data generated. Sample viral sequences have been generated from four different oceanic provinces. Complete viral genomes have been sequenced that suggest both temporal and spatial differences in the composition of the environments. This data will be used to highlight some of the bioinformatics approaches that have yielded startling insights into marine microbiology.

Rob Edwards
LS358, Department of Biology, San Diego State University, San Diego, CA
redwards@salmonella.org

POSTER

CHARACTERIZATION OF CHAETOCEROS CF GRACILIS VIRUS ISOLATED FROM CHEASAPEAKE BAY

Yoanna Eissler, D. Wayne Coats

Smithsonian Environmental Research Center

Viruses are widely accepted as playing important roles in the ecology of marine phytoplankton. While many studies have addressed nanophytoplankton-viral systems, only a few have considered viruses of larger phytoplankton like diatoms and dinoflagellates. We recently isolated a nuclear inclusion virus (CgNIV) that infects *Chaetoceros* cf *gracilis* from Chesapeake Bay. To characterize the morphology and lytic cycle of this virus, we conducted a time-course experiment, sampling every four hours over a 72 hours following viral inoculation. In vivo fluorescence began to decline 16 hours after inoculation and was reduced to less than 15% of control cultures by the end of experiment. Transmission electron microscopy confirmed infection within 24 h of inoculation, as indicated by the presence of virus-like particles (VLP) in some cells. Paracrystalline arrays corresponding to the alignment of icosahedral viral particles formed inside host nuclei. Average diameter of viral particles within the paracrystalline arrays was 30 nm. Infection of *Chaeroceros* c.f. *gracilis* with CgNIV caused degeneration of the host nucleolus and non-peripheral heterochromatin concurrent with formation of VLP. Simultaneous production of peripheral heterochromatin may indicate apoptosis as previously suggested (Betteral et al. 2005) or may represent proliferation of material for viral production. Potential burst size of CgNIV based on analysis of TEM images was much larger that previously estimated for diatom viruses using data for free viruses in culture media.

Yoanna Eissler
Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD, 21037,USA
eisslery@si.edu

POSTER

CULTURE INDEPENDENT DETECTION OF ARCHAEAL VIRUSES FROM ACIDIC THERMAL SITES IN YELLOWSTONE NATIONAL PARK

Jennifer Fulton, Josh Spuhler, Blake Wiedenheft, Alice Ortmann, Debbie Willits, Trevor Douglas, Mark Young

Thermal Biology Institute ,Montana State University

To better assess the total Archaeal viral diversity and in an effort to detect and ultimately isolate the first RNA virus infecting an Archaeal host we have adopted a culture independent approach to sampling in Yellowstone National Park. Novel viruses have been observed and we have sequenced the viral metagenome of one pool in Yellowstone. We are currently working to close the genome of a novel virus identified by this method and are concurrently working to develop methods to isolate this virus from the environment. Methods are also being developed to detect very low levels of viral RNA in mixed environmental samples.

Jennifer Fulton
Thermal Biology Institute, Montana State University, Bozeman, MT 59717
jsfulton@montana.edu

POSTER

GENOME ANALYSIS OF A LARGE DOUBLE-STRANDED DNA VIRUS INFECTING A MARINE HETEROTROPHIC NANOFLAGELLATE

Matthias G. Fischer[1] and Curtis A. Suttle[1,2]

[1]*Department of Microbiology & Immunology, University of British Columbia, Vancouver BC, Canada*
[2]*Departments of Botany and Earth & Ocean Sciences, UBC, Vancouver BC, Canada*

Heterotrophic protists are an integral part of the marine food web and factors influencing their mortality have direct effects on grazing rates and community composition. Here we present the 79% complete genome sequence of *Cafeteria sp. virus* (CafV) strain BV-PW1, an unusually large dsDNA virus with a genome size of ~730 kbp, that infects a marine nanoflagellate of the genus *Cafeteria*. This is the first characterized virus found to infect a heterotrophic nanoflagellate. High-throughput sequencing of the viral genome at 454 Life Sciences resulted in 578,774 bp of non-redundant sequence with 22fold coverage, assembled into 46 contigs. The AT-rich genome (77% A+T) contains at least 464 putative protein coding genes and two transfer RNA genes. 32% of the putative open reading frames (ORFs) are significantly similar to genes from *Acanthamoeba polyphaga mimivirus*, the largest virus known to date (Ref. 1). Phylogenetic analyses place CafV closest to Mimivirus in the group of Nucleo-Cytoplasmic Large DNA Viruses (NCLDV), which include the families *Asfar*-, *Irido*-, *Mimi*-, *Phycodna*-, and *Poxviridae*.

Matthias Fischer
Department of Microbiology & Immunology, The University of British Columbia, Vancouver BC V6T 1Z4
mfischer@eos.ubc.ca

POSTER

GET THE PHAGE OUT: ISSUES IN AQUATIC SEDIMENT VIRUS REMOVAL FOR ABUNDANCE AND DIVERSITY ANALYSES

Rebekah. R. Helton[1], K.E. Williamson[1], and K. Eric Wommack[1,2]

(1) Department of Plant and Soil Science, University of Delaware, Newark, DE; (2) College of Marine Studies, University of Delaware, Lewes, DE 19958

Few studies to date have examined viral ecology in aquatic sediments due in part to a lack of reliable methods for efficient extraction of viruses from the sediment matrix. Here, some implications of sample processing for examination of viral ecology in aquatic sediments were addressed. First, efficacy of viral extraction with EDTA/sodium pyrophosphate and vortexing was investigated and confirmed to be a proficient at removing 64%-75% viruses in the first extraction, and ~5% removed by the fourth extraction. Viral extracts also were treated with DNase to determine the validity of epifluorescence enumerations for determining viral abundance. These tests showed no significant difference between the number of viruses in DNase treated and untreated extracts ($P > 0.05$). Furthermore, given that viral morphological diversity is determined via TEM (transmission electron microscopy) analysis, the likelihood exists that viral extraction may significantly alter apparent viral morphologies. Autochthonous viruses having comparatively diminutive tails (e.g. *Myoviridae* and *Podoviridae*) were predominantly found in Chesapeake Bay sediment viral extracts. Experiments utilizing six known phage representing the three tailed phage families were added to sediments and subjected to equivalent extraction procedures. These trials showed that *Siphoviridae* phage exhibited 32%-55% partial or absent tails in contrast to *Myoviridae* (17%-19%) or *Podoviridae* (10%-14%). This indicates that *Myoviridae* and *Podoviridae* frequencies may often be over-reported with TEM analyses. Careful attention to these and other methodological limitations will be essential to pressing forward ecological investigations of viruses within aquatic sediments.

Rebekah R. Helton
University of Delaware, Delaware Biotechnology Institute, 15 Innovation Way, Room 148, Newark, DE 19711
Wommack@dbi.udel.edu

POSTER

VIRAL DIVERSITY ASSESSMENT AT THE GUARAPIRANGA RESERVOIR - SAO PAULO, BRAZIL

Manuela V. Gimenes[1]; Sônia M. Gianesella[2]; Maria Inês Borella[3], Adilson F. Nunes[4]; Patrícia Garrafa[1]; Telma A. Monezi[1] and Dolores U. Mehnert[1]

[1]Laboratory of Human Enteric Viruses, Department of Microbiology, Institute of Biomedical Sciences, University of São Paulo; [2]Oceanographic Institute, University of São Paulo; [3]Department of Cell and Development Biology, Institute of Biomedical Sciences, University of São Paulo; [4]Companhia de Saneamento Básico do Estado de São Paulo - Metropolitan Water Resources Department (SABESP).

In spite of the wideness of the Brazilian water sources there is a lack of information about the viral diversity in such waters. Some studies related to the eventual contamination of those waters by domestic effluent and consequently by human enteric pathogens, have been conducted through the last two decades. Guarapiranga reservoir is one of the largest reservoirs (630 km^2) for public supply of Sâo Paulo city, assisting 3.4 million people. It is located inside the urban area, and, due to unplanned occupation, its surrounding has been undergoing a fast process of degradation and pollution. The purpose of the present study was to establish the first assessment on viral diversity at this environment. Five liters of surface water were collected at three distinct sites in August/2005. Samples were concentrated by filtration through a positively charged membrane (Zeta Plus 60S, AMF Cuno) followed by ultracentrifugation at 100,000 *x g.* The sediment was suspended in 0.1M phosphate buffered saline solution, pH 7.2, effecting an 8,000-fold concentration. Viral concentrates were detoxified by Vertrel-XF and subjected to electron microscopy (EM) and DNA extraction by using Trizol. Virus sequences were detected by PCR, using specific primers for *Phycodnaviridae* and of *Myoviridae* (cyanophages) families. Samples were subjected to reamplification by PCR for DGGE analysis. Physical, chemical and other microbiological parameters were analyzed in the same samples. The concentration method adopted is a good alternative for such studies since both EM and molecular methods detected the two virus families.

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Dolores Ursula Mehnert
Departamento de Microbiologia, Instituto de Ciências Biomédicas, Universidade de São Paulo
dumehner@icb.usp.br

PAPER

GENOMICS OF THE TAILED BACTERIOPHAGES

Roger W. Hendrix, Graham F. Hatfull, Jeffrey G. Lawrence

Pittsburgh Bacteriophage Institute & Department of Biological Sciences, University of Pittsburgh

The tailed dsDNA bacteriophages are thought to constitute an absolute majority of organisms on our home planet, and they are important forces in the evolution of their bacterial and archaeal hosts. We have sequenced and analysed genomes of these phages from a variety of bacterial hosts and habitats as an entrée into their mechanisms of evolution and the structure and dynamics of their population. The phages evolve in the first instance through horizontal swapping of sequences through non-homologous recombination. The novel sequence joints produced in this way can move through the population by homologous recombination. Less than half the genes in a newly sequenced genome from an unexplored corner of phage space typically match anything in the databases, and this has changed surprisingly little as we have gone from having tens of sequences to hundreds. The genetic diversity in this population is evidently large. An emerging question is how many fundamentally different ways there are to construct a phage genome and how those different strategies are distributed over the host populations. Within the constraints of sparse and biased selection, it appears that there are a countable number of phage genome strategies for phages of a given host but with tremendous variation in specific sequences within each type. It is not yet clear whether there are sharp boundaries between types or a more continuous gradation.

Roger Hendrix
Dept of Biological Sciences, A340 Langley Hall, University of Pittsburgh, Pittsburgh, PA 15260
rhx@pitt.edu

POSTER

CO-MONITORING VIRIOPLANKTON AND BACTERIOPLANKTON IN CHESAPEAKE BAY

Jinjun. Kan[1], Erla Örnólfsdóttir[1], Kui Wang[1], Kate O'Mara[1], K. Eric. Wommack[2], and Feng Chen[1]

1. Center of Marine Biotechnology, University of Maryland Biotechnology Institute, 2. Delaware Biotechnology Institute, University of Delaware

Viruses could affect the population structure and genomic diversification of their hosts via killing the winner populations and horizontal gene transfer. To understand ecological impacts of marine viruses on microbial consortia, we monitored the abundance and community structure of both virioplankton and bacterioplankton in the Chesapeake Bay between September 2002-October 2004. Both viral and bacterial counts were relatively high in the summer and low in the winter. Composition of virioplankton (revealed by PFGE) and bacterioplankton (revealed by DGGE) communities showed stronger seasonal than spatial variations. Both viral and bacterial communities exhibited repeatable annual patterns. Nonmetric multidimensional scaling (MDS) and the cluster analysis revealed that seasonal succession of virioplankton and bacterioplankton communities followed a similar pattern and the winter communities were distinct from the summer-fall ones. Canonical discriminant analysis (CDA) based on the environmental variables agreed with MDS results and determined that temporal variations in bacterial and viral communities were stimulated by changes in water temperature and Chl *a*. Furthermore, dissolved oxygen, ammonia, nitrite and nitrate also contributed significantly to the bacterial seasonal variations. The results imply that repeated annual succession of bacterial communities in the temperate estuary is likely structured by virioplankton, phytoplankton and water temperatures as well.

Jinjun Kan
Center of Marine Biotechnology, University of Maryland, 701 E. Pratt St. Baltimore, MD21202
Kan@umbi.umd.edu

POSTER

THE IMPORTANCE OF PHAGE-HOST DENSITY ASSESSMENT FOR VIRAL ECOLOGY STUDIES IN THE GULF OF TRIESTE (NORTHERN ADRIATIC SEA)

Ana Karuza and Paola Del Negro

Department of Biological Oceanography, National Institute for Oceanography and Experimental Geophysics

Although it has been known for years that phage-host density plays a crucial role in determining viral life strategy, there is still a lack of in field studies that concern this aspect of viral ecology. During six-years study of virioplankton dynamics in the Northern Adriatic Sea we verified the prevalence of the lytic life strategy through the product of viral and bacterial abundances, when it exceeded the threshold value of approximately 10^{12} ml^{-2}. The study was carried out on multiscale basis: by 6-years temporal study with bimonthly sampling strategy in a coastal station of the Gulf of Trieste, by three seasonal spatial distribution surveys of microbial components in the Northern Adriatic Sea (typically displaying strong trophic gradient) conducted during 2004, by experimentally induced increase of trophic state of the system and by serial dilution technique to assess virus-mediated mortality of bacterioplankton. By observing dynamics within microbial loop our results evidenced that phage-host density is tightly related to viral life strategy and therefore we propose Virus-to-Bacterium Product (VBP) to be more frequently used. The uncoupling between Virus-to-Bacterium Ratio (VBR) and VBP evidenced the anomalous functioning of microbial loop during mucilage formation occurred in the Northern Adriatic Sea in June 2000.

Ana Karuza
Department of Biological Oceanography, OGS, Via A. Piccard 54, 34010 Trieste, Italy
akaruza@ogs.trieste.it

PAPER

WORKING WITH VIRUSES IN MARINE SEDIMENTS

Janice Lawrence

Department of Biology, University of New Brunswick

Marine sediments may be important environmental reservoirs for virioplankton, provide historical accounts of the temporal and spatial dynamics of viruses and their hosts, and function as a persistent source for isolating new viruses when their hosts may be seasonally absent from the plankton. I will provide an overview of sediment sample collection, processing and storage, and examples of techniques for examining sediment samples, outlining their inherent strengths and weaknesses. Examples of bioassays will be shown to illustrate their use for examining the abundance and distribution of specific viruses in sediments, and to permit the isolation of viruses infecting specific hosts. Molecular approaches, such as PCR, pulsed-field gel electrophoresis and denaturing gradient gel electrophoresis will be presented to demonstrate their utility for examining for the presence, abundance, diversity and distribution of viruses in sediments.

Janice Lawrence
Biology Department, University of New Brunswick, PO Bag Service 45111, Fredericton, NB, E3B 6E1
jlawrenc@unb.ca

PAPER

THE USE OF MODELS FOR SIMULATING VIRUS-HOST DYNAMICS

Mathias Middelboe

Marine Biological Laboratory, University of Copenhagen, Denmark

Viruses influence bacterial community composition and population dynamics by infection and lysis of specific bacterial subpopulations, potentially causing temporal fluctuations in individual virus and bacteria populations, as described in the "Killing the Winner"-model. The development of virus-resistant clones following lysis of a particular bacterial host may further complicate these interactions, as it introduces a clonal dynamics of susceptible and resistant clones a given bacterial host, on top of the species dynamics. Consequently, the dynamics of bacterial and viral populations (clones, strains, species) in natural environments are very complex and difficult to predict, or even to describe. Relatively simple virus-host dynamics have been demonstrated in both single- and multi-species chemostat experiments under controlled conditions in the lab, and subsequently analysed by mathematical models (Stella and LabView software). With these models, we were able to simulate the observed dynamics of bacterial and viral populations in culture studies at variable growth conditions. Central process parameters such as rates of infection, predation, and nutrient uptake, and organism parameters such as size, elemental composition, growth efficiency and burst size can each be specified for individual bacterial populations in the models. The models therefore provide a valuable framework for exploring the regulation of virus-host interactions and the potential effects of viruses on the dynamics of individual populations. Moreover, such models serve as powerful tools in attempts to explain the diversity and dynamics of microbial populations in marine systems, and to predict possible scenarios of microbial community dynamics, in response to changing environmental conditions.

Mathias Middelboe
Marine Biological Laboratory, University of Copenhagen, Strandpromenaden 5, DK-3000 Helsingør, Denmark
MMiddelboe@bi.ku.dk

PAPER

ISOLATION AND CHARACTERIZATION OF NEW VIRUS–HOST SYSTEMS

Keizo Nagasaki

National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Japan

Establishment of new virus-host systems is essential for accelerating the progress of algal virology. So far, more than 17 viruses infecting marine eukaryotic phytoplankton have been isolated and their physiological, ecological, and genetic characteristics are illustrated at different levels of resolution. The flow of traditional virus isolation procedure is very simple: screening, cloning, and maintenance. In screening viruses infectious to a certain algal species, it is strongly recommended to use multiple clones (belonging to the algal species) as hosts, because virus sensitivity spectra can differ at the strain levels. In many cases, exponentially growing cultures tend to be more sensitive to viral attacks than stationary phase cultures are. Microalgal viruses are included not only in seawaters but also in marine sediments; thus, sediments are also a promising resource for new virus isolation. When a viral infection is successfully detected, the pathogen suspension should be cloned by two cycles of extinction dilution procedure, made free from bacterial contamination by filtration, and appropriately stored. Conditions required for stable preservation differ among viruses; hence, it is necessary to maintain the virus by repetitious transfer to fresh host cultures before establishing a suitable storage method. However, it should be noted that it might cause a loss of infectivity, which is presumably due to the increase of defective interfering particles. Other than this traditional method, a new virus isolation technique using particle bombardment is now under way.

Keizo Nagasaki
2-17-5 Maruishi, Hatsukaichi, Hiroshima 739-0452, JAPAN
nagasaki@affrc.go.jp

POSTER

FOUR DIATOM-INFECTING VIRUSES

Keizo Nagasaki, Yuji Tomaru, Yoko Shirai, Hiroyuki Mizumoto, Yoshitake Takao

National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Japan

Authors are now examining four diatom-infecting viruses. RsRNAV is a ssRNA virus infecting *Rhizosolenia setigera*, the genome of which has been fully sequenced (DDBJ accession number: AB243297). The genome is 8,877 nucleotides (nt), polyadenylated, lacking a cap structure, and has two large open reading frames (ORFs): ORF-1 (4,818 nt), a polyprotein gene coding for replicases, ex. RNA-dependent RNA polymerase [RdRp], RNA helicase; and ORF-2 (2,883 nt), a polyprotein gene coding for structural proteins. Phylogenetic analysis of the RdRp domain indicated RsRNAV is a new previously unknown virus. CsNIV is a DNA virus infecting *Chaetoceros salsugineum*; its genome consists of a single molecule of covalently closed circular ssDNA (6,000 nt) and a segment of linear ssDNA (997 nt) and the latter is complementary to a portion of the closed circle creating a partially double-stranded genome. It was re-sequenced and now six potential ORFs are identified in the genome; only one of which shows a low similarity to replicase of circoviruses (DDBJ accession number: AB193315). Recently, a ssDNA virus infectious to *C. debile* (CdDNAV) and a ssRNA virus infectious to *C. neogracilis* (CnRNAV) were successfully isolated. The propagation pattern of CnRNAV is distinctive from the other viruses; 4-5 replication cycles occurred before algal lysis even when virus inoculation was conducted at a m.o.i. of 7.9. It may be that a diverse array of ssRNA viruses and ssDNA viruses exists in marine environments, and they may also affect the dynamics of diatoms.

Keizo Nagasaki
2-17-5 Maruishi, Hatsukaichi, Hiroshima 739-0452, JAPAN
nagasaki@affrc.go.jp

POSTER

MARINE VIRUSES ON THE CANADIAN ARCTIC SHELF

Jérôme Payet [1] and Curtis Suttle [1,2,3]

Departments of Earth and Ocean Sciences[1], Microbiology and Immunology[2], and Botany[3], University of British Columbia

Spatial and temporal distribution and production of viruses were assessed across the Beaufort Shelf between September 2003 and August 2004 as a part of the international Canadian Arctic Shelf Exchange Study. Viral abundances were estimated by epifluorescence microscopy (EFM) and flow cytometry (FCM). A good correlation was found between EFM and FCM (R=0.81) and viral concentrations ranged from 1.7×10^5 to 1.3×10^7 mL^{-1}. In terms of depth profile, generally one or two mid-water peaks were observed in the upper 50 m, below which viral abundances decreased markedly. Viruses increased in subsurface following the spring bloom and were generally high during summer. There was a low fluorescence population that averaged ~70% of the total, and which was highest near the surface during the summer, and a high fluorescence population that occurred at lower concentrations but which increased during and after the spring bloom. Viral production rates estimated from dilution approaches ranged from 1.0 to 5.9×10^5 mL^{-1} d^{-1}, and were higher during the summer. Our results indicate that viruses are important and active components of the microbial food web in the Arctic Ocean, and may consequently significantly influence the nutrient cycling and the food-web structure.

Jérôme Payet
6270 University Blvd, Vancouver, BC, Canada V6T 1Z4
jpayet@eos.ubc.ca

POSTER

DETECTION AND ENUMERATION OF MARINE VIRUSES BY FLOW CYTOMETRY

Qiwei Qin[1], Karina Yew-Hoong Gin[2] and Alice Ilaya Gedaria[3]

[1] *State Key Laboratory of Bioncontrol, School of Life Sciences, Sun Yat-sen University, 135 West Xingang Road, Guangzhou 510275, P. R. China*
[2] *School of Civil and Environmental Engineering, Nanyang Technological University, Blk N1, 50 Nanyang Avenue, Singapore 639789, Singapore*
[3]*Tropical Marine Science Institute, National University of Singapore, 14 Kent Ridge Road, Singapore 119223, Singapore*

Ballast water exchange from ships at port has the potential to introduce foreign organisms, some of which are capable of causing disease. In diagnosing disease, the speed and accuracy by which these microbes can be identified is of paramount importance for protecting human, aquaculture and ecosystem health. In this study, we use flow cytometry to detect and enumerate marine viruses which attack aquaculture stock. Using SYBR Green I, a highly fluorescent nucleic acid dye, flow cytometry was used to detect and enumerate the marine fish virus, Singapore grouper iridovirus (SGIV) and the shrimp white spot syndrome virus (WSSV). Individual particles of viruses could be resolved from background noise levels, after only 10 minutes of staining with SYBR Green I. In addition, a panel of 6 monoclonal antibodies (mAbs) against the marine fish virus, SGIV, was developed by immunization of Balb/c mice with purified virus preparations. Using the monoclonal antibodies as probes, a sensitive and accurate flow cytometry-based method was developed to detect and quantify SGIV in cell cultures. The flow cytometry procedure enables large numbers of cells to be screened rapidly for infectivity, and can detect low levels of virus infection. As early as 8 h after inoculation with the virus, 0.34% of infected cells were detected in cell culture. The maximum level of infection was obtained at 72 h. The efficiency of the flow cytometry method was also compared with that of the standard method of immunofluorescence microscopy.

Qiwei Qin
State Key Laboratory of Bioncontrol, School of Life Sciences, Sun Yat-sen University, 135 West Xingang Road, Guangzhou 510275, P. R. China
lssqinqw@mail.sysu.edu.cn

POSTER

THE EFFECT OF VIRUS SIZE CLASS ENRICHMENT ON BACTERIAL PRODUCTION

Johanna M. Rinta-Kanto and Steven W. Wilhelm

Department of Microbiology, The University of Tennessee

The “virus enrichment” assay is now a commonly used approach to study the effects of viruses on bacterial communities. In this approach the abundance of viruses in a water sample is increased by addition of “virus size-class” (typically 30 kDa – 0.2 µm) concentrate. Along with this high density of viruses, viral concentrates contain a considerable amount of non-viral dissolved organic matter (DOM) as well as non-heat-labile components which have been shown to have effects on bacterial communities. Thus, it is possible that the effects seen upon virus-size-class enrichment result from the combined effects of alterations in viral impact and additional DOM. We have examined the effect of addition of viruses and the non-viral carbon on bacterial carbon production during these experiments. Bacterial community biomass production was determined through ^{3}H-leucine incorporation and direct bacterial counts. We show here that enrichments with virus concentrates leads to increased bacterial production and cell proliferation in marine samples from the Sargasso Sea and Pacific Ocean, and that neutralization of viruses in this samples enhances this process further. Differences in the rates of bacterial production, relative to the level of virus enrichment, will be used to provide some insight into the direct impacts of increased virus / host contact rates at the community level.

Johanna Rinta-Kanto
Department of Microbiology, The University of Tennessee, Knoxville, TN 37996, USA
jrintaka@utk.edu

POSTER

ISOLATION AND CHARACTERIZATION OF BACTERIAL AND VIRAL AGENTS INFECTIOUS TO *AUREOCOCCUS ANOPHAGEFFERENS*

Janet M. Rowe[1], John R. Dunlap[2], Ashley D. Frazier[1], Christopher J. Gobler[3], O. Roger Anderson[4], Mary D. Gastrich[4,5], and Steven W. Wilhelm[1]

1. Department of Microbiology, The University of Tennessee, 2. Division of Biological Sciences, Program in Microscopy, The University of Tennessee, 3. Marine Science Research Consortium, Stony Brook University, 4. Lamont-Doherty Earth Observatory of Columbia University, 5. New Jersey Department of Environmental Protectio,

To better understand the natural pressures on populations of *Aureococcus anophagefferens* and examine the agents which cause mortality, field samples collected during Brown Tide events (2002-2003) were tested for the presence of lytic agents. Through selective filtration and repetitive controlled infections, both viral and bacterial agents were isolated that were capable of lysing cultures of *Aureococcus anophagefferens* CCMP 1784. Five unique bacterial isolates, which resulted in lytic activity similar to viruses, were identified and characterized by sequencing and subsequent blast analysis of their 16S rDNA. Lytic virus lysates were examined under transmission electron microscopy and scanning electron microscopy to reveal virus-like particles consistent with the morphological characteristics of previously described phycodnaviridae. Fixation of samples at the beginning of infections and subsequent examination demonstrated that these particles rapidly associated with cells of *A. anophagefferens*, countering previous observations of small phage-like particles and in agreement with thin section micrographs of infected cells. The results of this study will be presented in the context of screening assays for virus activity.

Janet M. Rowe
Department of Microbiology, University of Tennessee, Knoxville, TN, 37996
jrowe2@utk.edu

PAPER

CONFOUNDING ERRORS ASSOCIATED WITH DGGE ANALYSIS OF ALGAL VIRUS COMMUNITIES

Steven M. Short and Katherine I. Hall

Department of Biological Sciences, The University of Denver

Size-independent DNA separation with denaturing gradient gels was first described in the early 1980s, and a decade later the method was used to analyze the diversity of PCR-amplified small subunit ribosomal genes from complex bacterial communities. Since then, the technique has been used extensively to study the diversity of numerous genes amplified from wide range of aquatic microbial communities. However, as many researchers have noted, DGGE has limitations that must be recognized in order to accurately interpret experimental results and avoid erroneous conclusions. Because it has the potential to confound sample fingerprints and similarity comparisons, the production of multiple bands from the amplification of a single target sequence may be the most profound error associated DGGE analysis. Nonetheless, DGGE has a considerable advantage when compared to other fingerprinting methods, i.e. it permits the recovery of sequence information from individual, resolved DNA fragments. Therefore, despite its limitations, the method is still widely used, and in the near future it will likely remain an important method in molecular ecology. Recently, refinements have been suggested to help minimize multiple-band artifacts. Generally, multiple-band artifacts are produced during PCR amplification, and consequently, most of these refinements involve PCR protocol modification. In this paper, I will compare the efficacy of two modified PCR protocols designed to minimize DGGE artifacts produced during the degenerately primed amplification of algal virus DNA polymerase genes.

Steven M. Short
Department of Biological Sciences, The University of Denver, 2190 E. Iliff Avenue, Denver, CO 80208
sshort@du.edu

PAPER

METAGENOMIC ANALYSIS INDICATES THE SEA IS A RESERVOIR OF PREVIOUSLY UNKNOWN RNA VIRUSES

Alexander I. Culley[1], Andrew S. Lang[2] and Curtis A. Suttle[1,3]

[1]*Department of Botany, University of British Columbia, Vancouver BC, Canada*
[2]*Institute of Marine Sciences, University of Alaska, Fairbanks AK, USA*
[3]*Departments of Earth & Ocean Sciences and Microbiology & Immunology, UBC, Vancouver BC, Canada*

RNA viruses infect a wide variety of marine organisms ranging from bacteria to whales; yet, RNA virus communities in the sea are largely unexplored. Reverse-transcribed whole-genome shotgun sequencing is an approach that can be used to explore the composition of uncultivated marine RNA virus communities. Using this approach we found that most sequence fragments showed no significant similarity (tblastx e value < 0.001) to sequences in the NCBI database. However, sequence fragments with significant homology to those in databases demonstrated the existence of a diverse assemblage of RNA viruses, including a broad group of marine picorna-like viruses, and distant relatives of viruses infecting arthropods and higher plants that occur in BC coastal waters. The composition of the RNA virus communities was very uneven, and was heavily dominated by distinct genotypes with small genome sizes. This allowed us to completely assemble the genomes of several hitherto undiscovered viruses. The results indicated that there is a broad group of distinctly marine picorna-like viruses, as well as numerous other previously unknown RNA viruses. The oceans are clearly a reservoir of uncharacterized RNA viruses that are likely major players in marine ecosystems.

Curtis A. Suttle
Department of Earth and Ocean Sciences, The University of British Columbia, Vancouver BC V6T 1Z4
csuttle@eos.ubc.ca

POSTER

THE DYNAMICS OF VIRUSES INFECTING MARINE EUKARYOTIC DECOMPOSER THRAUSTOCHYTRIDS

Yoshitake Takao[1], Yuji Tomaru[1], Yukari Sasakura[2], Keisuke Yamane[2], Keizo Nagasaki[1], Daiske Honda[2]

[1] National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Japan [2]Konan University, Kobe, Japan

Thraustochytrids are widely distributed osmo-heterotrophic protists. They are an important eukaryotic decomposer in coastal ecosystems. Nevertheless, their natural dynamics and ecological roles *in situ* are not well understood. We isolated and established 152 viral strains infecting thraustochytrids from a coastal sea area in Japan. Based on their characteristics, the isolated viral strains were divided into two groups: a small icosahedral single-stranded RNA virus (*Schizochytrium* single-stranded RNA virus: SssRNAV [ø 25nm]), and a large roundish (but not icosahedral) double-stranded DNA virus (Thraustochytrids DNA virus: ThDNAV [ø 140nm]). These two virus groups exhibited significantly different dynamics throughout the field survey conducted in Hiroshima Bay, Japan in 2004-2005: SssRNAV temporarily increased in abundance following *H. akashiwo* blooms; in contrast, ThDNAV remained at a relatively low concentration with no drastic changes in abundance. The dynamics of each virus group likely reflect changes in the abundance of its host. There are at least two thraustochytrid groups coexisting in Hiroshima Bay that are ecologically different with dissimilar fluctuation patterns; one that utilizes dying and dead algal cells and the other mainly functioning as a decomposer of organic matter of land origin. It might be that the two host groups are dominant in the coastal sea area in Japan, and each of them is affected by a distinct type of virus.

Yoshitake Takao
2-17-5 Maruishi, Hatsukaichi, Hiroshima 739-0452, JAPAN
takaoyo@affrc.go.jp

POSTER

VISUALIZATION AND ENUMERATION OF GTA PARTICLES USING SYBR GOLD STAIN

Kui Wang[1], Robert Belas[1], Mary Ann Moran[2], Feng Chen[1]

1. *Center of Marine Biotechnology, University of Maryland Biotechnology Institute*

2.Department of Marine Sciences, University of Georgia

The Gene Transfer Agent (GTA) is a type of “defective” bacteriophage whose capsids contain small randomly acquired fragments of host DNA (~4.5 kb in Rhodobacter). GTA particles resemble phage morphology, and could mediate the genetic exchange between strains of the same species via horizontal gene transfer. Although GTA was discovered more than three decades ago, a direct counting method specific to GTA particles has not been developed. In the past, the relative abundance of GTA particles has been estimated from the transduction rate of specific gene markers (an indirect method). SYBR Gold has been widely used to count viral-like particles in seawater, and in this study, we applied SYBR Gold staining to enumerate the GTA particles produced by two species of marine *Roseobacter* (*Silicibacter pomeroyi* and *Silicibacter* sp. TM1040). SYBR Gold-stained GTA particles fluoresced brightly indicating the method is sufficiently sensitive in detecting a small DNA fragment packaged in GTAs. A significant proportion of GTA particles were found attached to either the cell surface or filament-like structures extending beyond the surface, implying that the transduction frequency when used with cell-free GTA particles could be underestimated. The concentration of GTA particles obtained from the marine roseobacter species was low ($<10^3$ particles/ml) at the beginning of growth curve and gradually increased with time, e.g., GTA particle density reached a maximum of ca. 10^6 to 10^7 particles/ml at stationary phase. These results demonstrate the usefulness of SYBR Gold staining as a rapid tool to enumerate GTA particles produced by roseobacters.

Kui Wang
Center of Marine Biotechnology, University of Maryland, 701 E. Pratt St., Baltimore, MD21202.
wangk@umbi.umd.edu

PAPER

DETERMINATION OF VIRUS-INDUCED MORTALITY IN MARINE SYSTEMS

Markus G. Weinbauer

Microbial Ecology and Biogeochemistry Group, Laboratoire d'Océanographie de Villefranche, Villefranche-sur-mer, france

Several methods have been developed to assess viral production and virus-induced mortality of heterotrophic prokaryotes, however, none of these methods has a level of acceptance as methods used to estimate primary and bacterial production. Nevertheless, results using different methods show often the same trends, although absolute values can differ. Assessing virus-induced mortality for primary producers is even more difficult. Most of the methods used to estimate virus-induced can also be applied in combination with an inducing agent to determine lysogeny in prokaryotes. Clearly, more intercalibration studies of different methods and development of new or refined methods are needed to obtain harder data on virus-induced mortality. Molecular tools are one avenue on this way.

Markus G. Weinbauer
Laboratoire d'Océanographie de Villefranche, BP 28, 06234 Villefranche-sur-mer, France
wein@obs-vlfr.fr

PAPER

THE SYNERGIES BETWEEN MARINE VIRUS DYNAMICS, NUTRIENT CYCLES AND SYSTEM GEOCHEMISTRY

Steven W. Wilhelm

Department of Microbiology, The University of Tennessee, Knoxville, T N 37996 USA

The relationship between virus activity and system biogeochemistry in marine environments is one which is accepted to exist, but poorly describe. By inducing microbial mortality, viruses drive the transformation of nutrients from the particulate pool to the dissolved pool, often releasing these nutrients in chemical forms that have highly altered bioavailabilities relative to other nutrient input / regeneration processes in marine systems. At the same time the input of nutrients from autochthonous or allochthonous processes influences both rate processes and diversity associated with primary and secondary producers. Subsequent changes in virus activity, brought about by these changes in host dynamics, further influence system geochemistry. Using Fe and P as model elements, the above relationships will be considered in a survey of our recent data from an ecosystem scale manipulation (SEEDS II) as well as mesocosm experiments where the biological availability of elements regenerated by virus-mediated cell lysis has been examined. The goal of this presentation will be to try and capture the "big picture" of synergies between virus activity and biogeochemical cycles, and to provide some direction for future model efforts in ecosystem level geochemistry.

Steven W. Wilhelm
Department of Microbiology, The University of Tennessee, Knoxville, TN 37996 USA
wilhelm@utk.edu

PAPER

FROM PCR TO MICROARRAYS: WHAT CAN A MOLECULAR TOOLBOX TELL US ABOUT THE ECOLOGY OF COCCOLITHOVIRUSES

William. H. Wilson

Plymouth Marine Laboratory, Plymouth, UK, whw@pml.ac.uk

The virus genus Coccolithovirus is a group of large, double stranded DNA viruses that infect the globally important marine coccolithophorid Emiliania huxleyi. Coccolithoviruses are now known to be one of the causative agents of E. huxleyi bloom demise. We have developed diagnostic molecular tools to analyse the dynamics of coccolithoviruses and their hosts during natural blooms. We have recently sequenced the 407,339 bp genome of one coccolithovirus and revealed that only 14% of the predicted genes confer any significant database homology. The genome encodes a range of unexpected genes never previously observed in a virus, some perhaps providing insights into the ecology of coccolithoviruses. Most notably are those involved in biosynthesis of ceramide, a sphingolipid better known for it's role in apoptosis induction. Microarray analysis of genes on the virus genome is starting to provide clues to the propagation mechanism of this unusual group of viruses. A lot to fit in, it's sure to be a roller coaster ride.

W.H. Wilson
Plymouth Marine Laboratory, Plymouth, UK
whw@pml.ac.uk

PAPER

METAGENOMICS AND THE MARINE VIRUS COMMUNITY

K. Eric Wommack

Delaware Biotechnology Institute, Dept. of Plant and Soil Sciences, and Graduate College of Marine Studies, University of Delaware, USA

Application of high-throughput DNA sequencing technologies to the analysis of whole microbial communities, i.e., microbial metagenomics, has truly begun to reveal the detailed inner workings of the proverbial microbial black box and ushered in the era of gene ecology. By and large, microbial metagenome sequencing efforts have focused on bacterioplankton; however, as compared to other classes of microorganisms, metagenomics may be best suited to examination of viral communities. Arguably, laboratory cultivation-bias is greatest for viruses; thus, known viruses likely represent less than 1/100th of total viral diversity. Although a number of marker genes have been important to constraining the diversity of viral groups, there is no universal phylogenetic marker for viruses. Because of the small, coding dense genomes of viruses, viral community metagenome sampling should reveal genes which are active within the environment. The handful of characterized viral metagenomes examined to date reveal that viral communities contain an extraordinarily diverse number of genotypes and are the largest reservoir of unknown genes on Earth. As viral metagenomics is a nascent field and a number of new and cheaper technologies for high-throughput DNA sequencing will soon be widely available, methodological approaches to the metagenomic characterization of viral diversity are in flux. This paper will summarize the current state of viral metagenomics; outline the methodologies and bioinformatic analyses used in these studies; and highlight the future promise of metagenomic approaches to viral ecology.

K. Eric Wommack
Delaware Biotechnology Institute, Dept. of Plant and Soil Sciences, and Graduate College of Marine Studies, University of Delaware, USA
wommack@dbi.udel.edu

POSTER

DETECTION OF CO-OCCURRENCE OF ALGAL VIRUSES AND EUKARYOTES USING CULTURE-INDEPENDENT METHODS

Leanne Yeung[1], Jessica L. Clasen[2], Curtis A. Suttle[3]

[1]*Integrated Sciences Program, University of British Columbia*
[2]*Department of Earth and Ocean Sciences, University of British Columbia*
[3]*Departments of Earth and Ocean Sciences, Microbiology and Immunology, and Botany, University of British Columbia*

Detection of co-occurrence among algal viruses and eukaryotes in water samples was done using denaturing gradient gel electrophoresis (DGGE) gels generated from the 2003 study by Short and Suttle. Community composition fingerprints of algal virus and eukaryotes were compared using band matching. The presence or absence of the bands was converted into binary data for the use of statistical analyses. A similarity matrix was generated between the detected bands and was used to carry out NMDS and single-linkage cluster analysis. Co-occurrence of algal viruses and eukaryotes was detected in the 3D-NMDS map and single-linkage cluster analysis. At a cut-off of 65% similarity of occurrence, algal virus and eukaryotic DGGE bands clustered together in the NMDS analysis. The results showed that this method could determine possible groups of specific algal viruses and their particular hosts. This use of culture-independent methods to examine ecological interactions between a specific virus and eukaryote could aid in the continuing search of how viruses affect marine ecology.

Leanne Yeung
6270 University Blvd, Vancouver, BC, Canada V6T 1Z4
leanney@interchange.ubc.ca

POSTER

SPATIAL DISTRIBUTION OF VIRUSES AND DECAY RATES IN NORTHERN SOUTH CHINA SEA

Kedong Yin[1,2], Xiangcheng Yuan[1,2], Lei He[1,2], Paul J. Harrison[2]

[1]*LED, South China Sea Institute of Oceanology, Chinese Academy of Sciences, Guangzhou, China*
[2]*AMCE/Biology Dept, Hong Kong University of Science and Technology, Clear Water Bay, Kowloon, Hong Kong SAR, China*

The South China Sea is one of the largest inland seas in the world and is an oligotrophic ocean. This is the first study to examine spatial distribution of viruses in the entire northern South China Sea. Surface concentrations of viruses ranged from 39.4×10^6 to 0.18×10^6 VLP ml^{-1} with a mean of 13×10^6 VLP ml^{-1}. These concentrations were basically comparable with those reported in other areas. The ratio of mean viral count to mean bacterial count for surface water was calculated to characterize the relationship between bacterial and viral communities. Viral abundance decreased from coastal waters to open waters and appeared to be related to bacterial and chl a concentrations. Viral abundance was high at the surface and decreased with depth. At some station, there was a subsurface maximum of viral abundance coinciding with the subsurface chl a maximum and also the subsurface maximum of bacterial abundance. Virus decay rates (VD) ranged from 0.8-10% and UVR can increase VD significantly ($P<0.05$). Normalized by UVR energy, viral decay under UVR in open oceans was higher than that in coastal waters, which indicated more importance of solar radiation to viral decay in open oceans.

Kedong Yin
AMCE, Hong Kong University of Science and Technology, Hong Kong
kyin@ust.hk

Workshop Participants

BRATBAK, Gunnar (Gunnar.Bratbak@bio.uib.no); University of Bergen, Department of Biology, P.O. Box 7800, N-5020 Bergen, Norway

BRUM, Jennifer R. (jbrum@hawaii.edu); University of Hawaii at Manoa, Department of Oceanography, MSB 205, 1000 Pope Rd., Honolulu, HI 96822 USA

BRUSSAARD, Corina (corina.brussaard@nioz.nl); Royal Netherlands Institute for Sea Research, Department of Biological Oceanography, P.O.Box 59, NL-1790 AB Den Burg (Texel), The Netherlands

CHAN, Amy M. (achan@eos.ubc.ca); University of British Columbia, Department of Earth & Ocean Sciences, 1461-6270 University Blvd., Vancouver, BC, V6T 1Z4, Canada

CHEN, Feng (chenf@umbi.umd.edu); University of Maryland Biotechnology Institute, Center of Marine Biotechnology, 701 E. Pratt St. Baltimore, MD 21202 USA

CHÉNARD, Caroline (cchenard@eos.ubc.ca); University of British Columbia, Department of Earth & Ocean Sciences, 1461-6270 University Blvd., Vancouver, BC, V6T 1Z4, Canada

CLASEN, Jessica L. (jclasen@eos.ubc.ca); University of British Columbia, Department of Earth & Ocean Sciences, 1461-6270 University Blvd., Vancouver, BC, V6T 1Z4, Canada

EDWARDS, Rob A. (redwards@salmonella.org); Department of Biology, San Diego State University, LS358, San Diego, CA, USA

EISSLER, Yoanna (eisslery@si.edu); Smithsonian Environmental Research Center, 647 Contees Wharf Road, Edgewater, MD 21037 USA

FISCHER, Matthias G. (mfischer@eos.ubc.ca); University of British Columbia, Department of Microbiology & Immunology, 1461-6270 University Blvd, Vancouver, BC, V6T 1Z4, Canada

FUHRMAN, Jed A. (fuhrman@usc.edu); University of Southern California, Department of Biological Sciences, 825, Bloom Walk, ACB 308, Los Angeles, CA 90089 USA

FULTON, Jennifer (jsfulton@mymail.msu.montana.edu); Montana State University, Thermal Biology Institute, Bozeman, MT 59717 USA

HELTON, Rebekah (rrhelton@udel.edu); University of Delaware, Delaware Biotechnology Institute, 15 Innovation Way, Newark, DE 19711 USA

HENDRIX, Roger W. (rhx@pitt.edu); University of Pittsburgh,Department of Biological Sciences, A340 Langley Hall, Pittsburgh, PA 15260 USA

KAN, Jinjun (kan@umbi.umd.edu); University of Maryland Biotechnology Institute, Center of Marine Biotechnology, 701 E. Pratt St. Baltimore, MD 21202 USA

KARUZA, Ana (akaruza@ogs.trieste.it); National Institute for Oceanography & Experimental Geophysics, Department of Biological Oceanography, Via A. Piccard 54, 34010 Trieste, Italy

Workshop Participants

LABONTÉ, Jessica (jess_labonte@hotmail.com); University of British Columbia, Department of Microbiology & Immunology, 1461-6270 University Blvd, Vancouver, BC, V6T 1Z4, Canada

LAWRENCE, Janice (jlawrenc@unb.ca); University of New Brunswick, Biology Department, PO Bag Service 45111, Fredericton, NB, E3B 6E1, Canada

LOVEJOY, Connie (connie.lovejoy@bio.ulaval.ca); Université Laval, Dépt de Biologie, Pavillon Vachon, Québec City, QC, G1K 7P4, Canada

MARTINEZ, Joaquin Martinez (joaquin.martinez@nioz.nl); Royal Netherlands Institute for Sea Research, Department of Biological Oceanography, P.O.Box 59, NL-1790 AB Den Burg (Texel), The Netherlands

MEHNERT, Dolores Ursula (dumehner@icb.usp.br); Universidade de São Paulo, Departamento de Microbiologia, Instituto de Ciências Biomédicas, Av. Prof. Lineu Prestes 1374, São Paulo, 05508-900, Brasil

MIDDELBOE, Mathias (mmiddelboe@bi.ku.dk); University of Copenhagen, Marine Biological Laboratory, Strandpromenaden 5, DK-3000 Helsingør, Denmark

NAGASAKI, Keizo (nagasaki@affrc.go.jp); National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, 2-17-5 Maruishi, Hatsukaichi, Hiroshima 739-0452, Japan

ÖRNÓLFSDÓTTIR, Erla (ornolfsd@umbi.umd.edu); University of Maryland Biotechnology Institute, Center of Marine Biotechnology, 701 E. Pratt St. Baltimore, MD 21202 USA

ORTMANN, Alice (aortmann@montana.edu); Montana State University, Thermal Biology Institute, Bozeman, MT 59717, USA

PAUSZ, Clemens (cpausz@eos.ubc.ca); University of British Columbia, Department of Earth & Ocean Sciences, 1461-6270 University Blvd., Vancouver, BC, V6T 1Z4, Canada

PAYET, Jérôme (jpayet@eos.ubc.ca); University of British Columbia, Department of Earth & Ocean Sciences, 1461-6270 University Blvd., Vancouver, BC, V6T 1Z4, Canada

PERSONNIC, Sébastien (sebastien.personnic@thonon.inra.fr); Institut National de la Recherche Agronomique, 75 avenue de Corzent, Thonon les Bains, 74200, France

QIN, Qiwei (lssqinqw@mail.syse.edu.cn); Sun Yat-sen University, School of Life Sciences, State Key Lab of Biocontrol, 135 West Xingang Road, Guangzhou 510275, P. R. China

RINTA-KANTO, Johanna (jrintaka@utk.edu); University of Tennessee, Department of Microbiology, 1414 W. Cumberland Ave., Knoxville, TN 37996 USA

ROWE, Janet (jrowe2@utk.edu); University of Tennessee, Department of Microbiology, 1414 W. Cumberland Ave., Knoxville, TN 37996 USA

Workshop Participants

SHORT, Steven M. (sshort@du.edu); University of Denver, Department of Biological Sciences, 2190 E. Iliff Avenue, Denver, CO 80208 USA

SUTTLE, Curtis A. (csuttle@eos.ubc.ca); University of British Columbia, Departments of Earth & Ocean Sciences, Microbiology & Immunology, and Botany, 1461-6270 University Blvd, Vancouver, BC, V6T 1Z4, Canada

TAKAO, Yoshitake (takaoyo@affrc.go.jp); National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, 2-17-5 Maruishi, Hatsukaichi, Hiroshima 739-0452, Japan

WANG, Kui **(**wangk@umbi.umb.edu); University of Maryland Biotechnology Institute, Center of Marine Biotechnology, 701 E. Pratt St. Baltimore, MD 21202 USA

WEINBAUER, Markus **(**wein@obs-vlfr.fr); Laboratoire d'Océanolographie de Villefranche, BP28, Villefranche-sur-mer, 06234 France

WILHELM, Steven W. (wilhelm@utk.edu); University of Tennessee, Department of Microbiology,
1414 W. Cumberland Ave., Knoxville, TN 37996 USA

WILSON, Willie H. (whw@pml.ac.uk); Plymouth Marine Laboratory, Plymouth, UK

WOMMACK, Eric K. (wommack@dbi.udel.edu); University of Delaware, Delaware Biotechnology Institute, 15 Innovation Way, Newark, DE 19711 USA

YEUNG, Leanne **(**leanney@interchange.ubc.ca); University of British Columbia, Integrated Sciences Program, Department of Earth & Ocean Sciences, 1461-6270 University Blvd, Vancouver, BC, V6T 1Z4, Canada

YIN, Kedong (kyin@ust.hk); South China Sea Institute of Oceanology, Academy of Sciences, Guangzhou, and Hong Kong University of Science and Technology, AMCE, Clear Water Bay, Kowloon, Hong Kong SAR, China