December 2009

DEPARTMENT OF MICROBIOLOGY

OBSERVE

WONDER

DISCOVER

RESEARCH AND HIGHER EDUCATION

OSU
Oregon State
UNIVERSITY

FROM THE CHAIR:

Greetings from Nash Hall as 2009 comes to an end. You'll see from these pages that we've all been busy Beavers, even if we don't get the tele-publicity of our football team. We're still teaching 140-odd majors, as well as the many other students from all over campus who take our MB230 Introductory Microbiology, MB302/303 General Microbiology, and MB330 Disease & Society courses. We're training 25-30 graduate students, and publishing apace the results of research conducted by graduate and undergraduate students, postdoctorals, and research associates working alongside professors. Some of our recent research is summarized in this newsletter.

We were unfortunate in being unsuccessful in recruiting an Assistant Professor of Environmental Microbiology this year, after protracted negotiation with our top candidate. With the university now in the midst of an energetic restructuring, we have been forced to delay a follow-up search. Whatever administrative realignments occur during this restructuring, it appears certain that our Microbiology major will remain largely unchanged. We hope to broaden the scope of our graduate program, in the form of an Integrated Microbial Sciences Graduate Program, reaching out to others on campus who are conducting research in microbiology in units such as Pharmacy, Plant Pathology, Oceanography, Bioengineering, and Forestry; VetMed microbiologists are already participating in the Microbiology Graduate Program. We will also likely keep our long-standing affiliations with the College of Science and the College of Agricultural Sciences. CAS is now led by Dean Sonny Ramaswamy, who came last August from Purdue University; Thayne Dutson retired last year after many years as Dean.

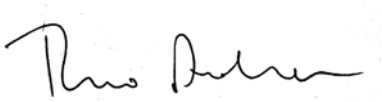
Another aspect of administrative restructuring has already occurred. Before fall term, we implemented the transfer of many of the business functions from the departmental level to a college-level Business Center. Several such Business Centers are being created across campus, with functions transferred upwards from departments and downwards from Kerr Administration Building. We've been fortunate in avoiding major turmoil, because Dick Toliver, our accountant for the last few years is still functioning in that role for us from his new desk in the Business Center. The changes have resulted in a major shrinkage of office staff to only two. This will likely change again as decisions on restructuring are made. These changes are occurring because OSU is facing incredible budget challenges as state support has declined. We are fervently hoping that the January elections do not overturn the legislature's tax increase.

The changes I've described are not the only ones occurring at present. A major utilities renovation is getting underway in Nash Hall, with the entire ventilation system and all windows being replaced, and a sprinkler system being installed. Cindy Fisher, who describes the project in these pages, is coordinating the necessary evacuation of floors one-by-one for three-month periods. We are hoping that the disruption does not slow our research too badly, and are grateful for the long-term benefits the upgrade will bring. This renovation is not the only building activity affecting us. The new Linus Pauling Science Center, which will house the Linus Pauling Institute and part of the Department of Chemistry, is sprouting in the parking lot to the west of Nash Hall. The hole that was dug in September is already filling in with concrete walls and floors; occupation is planned in time for fall term 2011.

This past year we said goodbye to Don Overholser, friend, former Head Advisor and Senior Instructor, and scholarship sponsor to the Department of Microbiology. Don passed away in January, after a long illness. He is remembered everyday when undergrads use the Donald Overholser Microbiology Student Computer Lab (pictured in this newsletter), named in his honor before he died.

As we approach the second decade of the century, we're trying our utmost to keep Microbiology and OSU the great program and university they've been. A great validation of that goal came this year with the awarding of the College of Science Thomas T. Sugihara Young Faculty Research Award to Assistant Professor Martin Schuster.

With best wishes for a happy and peaceful Holiday Season and 2010,



Theo W. Dreher, Ph.D., Chair and Professor

PETER BOTTOMLEY LAB:

"Ignoring the obvious to focus on the obscure"

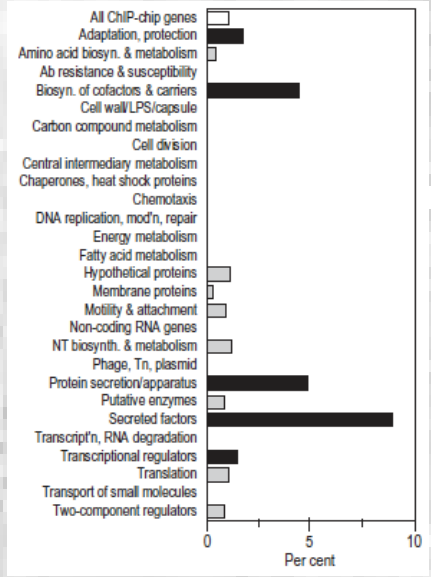
I'll bet that you have never thought once about the composition of the natural gas that heats your house. If you "Google" natural gas you will find that it is made up of about 95-97% methane (C1), 2-3% ethane (C2), and much smaller concentrations of propane (C3) and butane (C4). Given the composition of natural gas you would think that hydrocarbon utilizing bacteria would have evolved to focus on the obvious rather than the obscure i.e., grow on the dominant component of natural gas (methane), and leave the minor components (propane and butane) as an afterthought! In fact, there are many bacteria called methanotrophs that do just that: grow on methane as sole carbon source, and will not grow on ethane, propane, or butane. In contrast, we have studied a β proteobacterium, *Thauera butanivorans* that when cultured on natural gas consumes all of the ethane, propane, and butane, while leaving virtually all of the methane untouched! This, despite the fact that the sequence of the gene that encodes for the monooxygenase enzyme that makes the initial attack on ethane etc. is very similar to the one which oxidizes methane so efficiently in methanotrophic bacteria. This is quite a marvelous accomplishment of "evolutionary engineering", and attests to the exquisite "substrate filtering" ability of the enzyme responsible for the initial oxidation of C2-C4 alkanes. During growth on natural gas, we observed that small amounts of methanol accumulated transiently, and formic acid accumulated permanently in the growth medium of *T. butanivorans*. We went on to show that ATP was derived from the oxidation of methanol, thereby attesting to the metabolic versatility of *T, butanivorans* despite being unable to grow on methane. It is intriguing to wonder how *T. butanivorans* inherited a similar alkane oxidizing enzyme as methanotrophs, yet chose to develop the ability to restrict methane oxidation, detoxify methanol and formaldehyde and use the limited supplies of longer chain alkanes found in natural gas for growth. "Nature can be illogical: go figure!"

For those interested in more:
R.B. Cooley, P.J. Bottomley, and D.J. Arp. 2009.
Growth of a non-methanotroph on natural gas: ignoring the obvious to focus on the obscure.
***Environmental Microbiology Reports* 1: 408-413.**

MARTIN SCHUSTER LAB:

"Two chips are better than one"

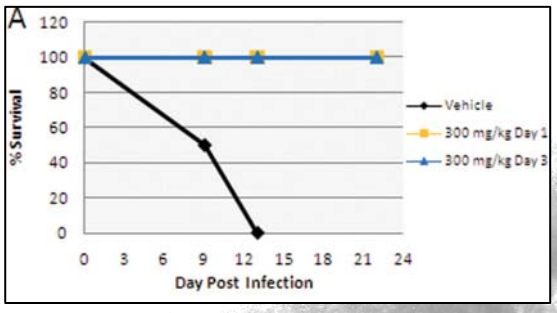
Communication and cooperation are commonplace in the animal world. Although less obvious in microbes, they too share common goods, for example extracellular enzymes that digest food, and coordinate production of these goods by a process called "quorum sensing". Quorum sensing involves the production and perception of chemical signals within a bacterial population. Certain group behaviors are initiated once a threshold level of signal - a "quorum" - has been reached. At the core of the quorum sensing circuitry is a regulatory protein that binds the quorum signal and activates target genes. However, precisely how many such genes in any given bacterium are directly controlled by quorum sensing has not yet been established. We addressed this question in the soil bacterium *Pseudomonas aeruginosa*, which is also an opportunistic pathogen that chronically infects the lungs of cystic fibrosis patients.



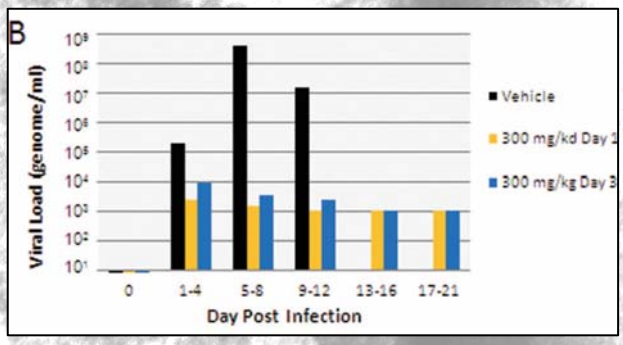
We utilized a novel technique termed "ChIP-chip". We first cross-linked all proteins and DNA in a bacterial culture that had reached a quorum by adding formaldehyde. We then "fished" out our quorum-sensing regulatory protein of interest using a specific antibody. This process is called immunoprecipitation (the first "ChIP"). We then purified the DNA that had been cross-linked to the quorum-sensing regulator and elucidated its identity by microarray analysis (the second "chip"). The microarray we used contains DNA sequences of all the *P. aeruginosa* genes and the regions in between. The purified DNA fragments bind to a complementary sequence on the microarray and are visualized by fluorescence. Using this approach, we identified more than 70 genes under direct control of quorum sensing. While some genes encode unexpected functions, most are involved in the production and secretion of extracellular factors, confirming the notion that the main purpose of quorum sensing in *P. aeruginosa* is to coordinate the production of common goods. This is a clever strategy from the bacterium's point of view. Costly extracellular factors, over which the bacterial cell loses control once secreted, are only produced under favorable conditions such as limited diffusion or high population density.

For more information: Gilbert, K.B., Kim, T.H., Gupta, R., Greenberg, E.P., and Schuster, M. (2009)
Global position analysis of the *Pseudomonas aeruginosa* quorum sensing transcription factor LasR.
***Molecular Microbiology* 73: 1072-1085**

DENNIS HRUBY LAB:



Smallpox, which is caused by variola virus (VAR) infection, is a potentially devastating agent of bioterrorism and a serious threat to human life in the event of its deliberate or accidental release. Likewise, monkeypox virus (MPX) causes similar, though less frequently lethal, disease in humans. Unlike VAR, MPX has not been eradicated. Rather, it is an emerging pathogen in Africa and it has already made an infectious incursion into North America. Infections with either pathogen can be effectively prevented by prophylactic use of a licensed smallpox vaccine; however, potential serious side effects (e.g., eczema vaccinatum, progressive vaccinia, myocarditis, and death) limit its use in the general population in the absence of a verified threat, and the vaccine is contraindicated for use in the treatment of immunocompromised individuals, pregnant women, and individuals with atopic dermatitis. Moreover, the vaccine is only effective when given within a few days after exposure to either VAR or MPX. While safer vaccines based on attenuated vaccinia virus strains are being developed, they are not currently on the market. Given the prolonged prodrome of these human infections, if a sentinel case representing an outbreak of either disease were to be detected, it is probable that a large number of individuals would be harboring infections for which vaccination would provide no benefit. Thus, it has been imperative to develop an antiviral drug for the prevention and/or treatment of VAR or MPX infections and related orthopoxvirus infections to protect individuals who cannot receive the smallpox vaccine and to protect infected nonvaccinated individuals.



Previous work with the antiviral drug ST-246 in a number of different small animal models of orthopoxvirus-induced disease has demonstrated it to be safe and effective. We have extended this work to nonhuman primates challenged with VAR or MPX. The results demonstrated that ST-246, even when administered at a time post infection when disease symptoms were evident, protected the animals from disease or death, supporting the potential use of ST-246 for prophylactic or therapeutic intervention in VAR or MPX disease. This conclusion is further supported by the recent successful use of ST-246 for treatment of several patients suffering from life-threatening vaccine complications. The nonhuman primate model is likely to be predictive of human disease outcome in that VAR- or MPX-induced disease closely resembles the human condition and the characteristics of metabolism of ST-246 in NHP and humans are similar. Thus, this landmark proof-of-concept experiment will lay the foundation for additional experiments to support the licensure and deployment of ST-246 as an anti-poxvirus countermeasure.

Two OSU Microbiology students participated in this study. Chelsea Byrd received her B.S. in Microbiology and Ph.D. in Molecular & Cellular Biology, working in the Hruby lab. Tove' Bolken was a Microbiology major.

For more information:

J. Huggins, A. Goff, L. Hensley, E. Mucker, J. Shamblin, C. Wlazlowski, W. Johnson, J. Chapman, T. Larsen, N. Twenhafel, K. Karem, I.K. Damon, C. M. Byrd, T. C. Bolken, R. Jordan, and D. E. Hruby. 2009. Non-human primates are protected from smallpox virus or monkeypox virus challenges by the antiviral drug ST-246. ***Antimicrobial Agents and Chemotherapy*** **53:2620-2625**

MICROBIOLOGY STUDENT ASSOCIATION

The Microbiology Student Association (MSA) is a student club at OSU that strives to promote the field of microbiology, through education and extension activities. In addition, MSA enhances opportunities and relationships for microbiology majors, by providing social activities and educational field trips.

MSA Field Trip to Firesteed Winery

Twice a year MSA participates in Discovery Days, an outreach program sponsored by the Colleges of Science and Engineering, where hundreds of K-5 children engage in interactive exhibits. In the past year MSA has expanded its booth offerings to include a prize wheel with questions on microbiology topics and a "create a science bracelet" activity. In January MSA will participate in the Linn County cub scout "Lock-in" at the fairgrounds, where 800 cub scouts will get the chance to design their own microbe and have it made into a bracelet or necklace.

Each term MSA students vote on their choice for field trip locations. This term MSA visited Firesteed Winery in Rickreall and learned about wine production. Next term MSA is planning to visit research labs at OHSU in Portland. In spring term the American Society for Microbiology will have its 110th general meeting in San Diego, CA, providing a wonderful opportunity for many MSA members to travel to a national meeting to attend talks, poster sessions, and make connections for future employment. Thanks to generous alumni support, the club is hoping to send at least 10 MSA members to California in May!

MSA Service Project at Discovery Days

CONSTRUCTION, CONSTRUCTION AND MORE CONSTRUCTION

The reprieve from the noise and disruption of the previous seismic construction project at Nash Hall was short lived as construction in the area has engulfed us once again. After nine months of design meetings, work began in Nash early this fall on a $14,000,000 project to control the temperature fluctuations in the research laboratories and reduce the building's energy consumption. The members of OSU project team remain the same for this second phase of construction with Cindy Fisher continuing as the building liaison for the current project. Anderson Construction, GLaS Architectural firm and KPFF Engineering were chosen for the project.

The preliminary work has been concentrated in the mechanical spaces of the building but beginning in January, the construction will invade the labs and offices on each floor for three months at a time. The fifth floor residents will be the first group to enjoy all the new amenities that will come from this project but they were also the first to purge, pack and move their entire labs and offices to new locations in the Nash. When the project is finished, the temperature in the research labs will remain a steady 72F, twenty four hours a day, year in and year out! Auxiliary rooms and offices will be on motion and heat sensors and will provide heating or cooling as needed when the rooms are occupied. In addition to the new HVAC system, Nash will have new chemical fume hoods in the main laboratories; new interior lighting and energy efficient windows when the construction is completed.

The new Linus Pauling building is being built in the NW section of the Nash parking lot. Anderson Construction was selected for both projects and the plan is to have the construction on Nash Hall and Linus Pauling completed by fall term 2011. A web cam has been set up to view the progress on the new building at http://webcam.oregonstate.edu/lpsc/ .

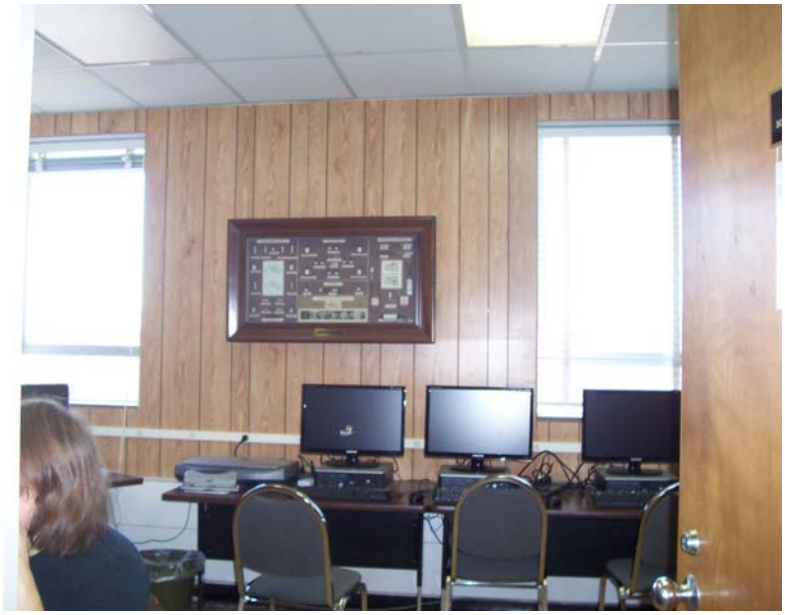
We all will breathe a sigh of relief when the construction on both projects is completed.

Installing the variable speed fans to replace single speed fans for the building. 12,000 pounds worth of metal!

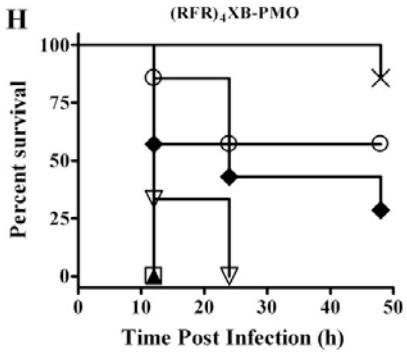
Linus Pauling Science Center construction in Nash Hall parking lot

DONALD OVERHOLSER MICROBIOLOGY STUDENT COMPUTER LAB

Artist: Tamsen Polley, MB undergrad



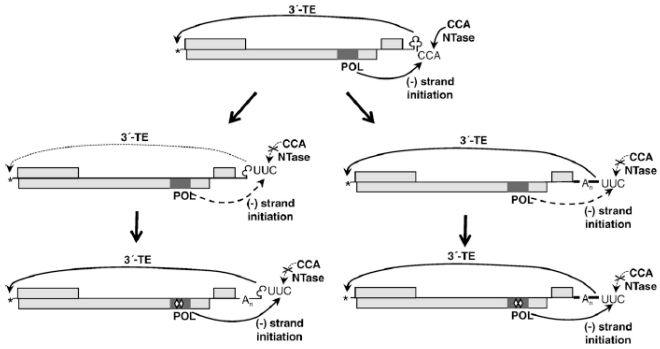
BRUCE GELLER LAB:



Jesse Deere, Brett Mellbye, Susan Puckett and Luke Tilley are all recent graduates of our Microbiology program, and they all are now graduate students in top-tier programs across the United States. Susan worked on this study for her Honors thesis, while the other three were employed as lab technicians after graduation. The thread that links all four, besides their undergraduate major, is their participation in an on-going research project to develop a revolutionary new antibiotic. They contributed to the development of a new antisense antibiotic, a DNA mimic that is unlike any antibiotic that is currently on the market. Antisense antibiotics kill bacteria by base-pairing to specifically targeted sites on the bacteria's messenger RNA, inhibiting gene expression. Traditional antibiotics target enzymes or the membranes that surround bacterial cells. Jesse made the initial discovery that we could kill bacteria with antisense compounds, and showed that mice infected with bacteria could be cured by treating them with an antisense antibiotic. Luke greatly improved the potency of the antisense antibiotic by devising a chemical modification that increases uptake into bacterial cells. Susan found that bacteria could become resistant to the antisense antibiotic, and that the resistance was linked to the chemical modification that Luke discovered. Fortunately, she also showed that different kinds of modifications to the antisense antibiotic could overcome the resistance. Brett optimized the potency of a number of different antisense antibiotics and showed that they were effective against many pathogens, including those that cause serious hospital-acquired infections and are resistant to many available antibiotics.

Brett L. Mellbye, Susan E. Puckett, Luke D. Tilley, Patrick L. Iversen and Bruce L. Geller, 2009, Variations in Amino Acid Composition of Antisense Peptide-Phosphorodiamidate Morpholino Oligomer Affect Potency against *Escherichia coli* In Vitro and In Vivo. *Antimicrobial Agents and Chemotherapy*, 53: 525–530.

THEO DREHER LAB:



Tymoviruses are positive strand RNA plant viruses. The 30 nm non-enveloped spherical virions contain RNA genomes that are ready to serve as mRNAs as soon as they are released into a newly infected cytoplasm. Because the genomes also act directly in both replication and protein synthesis (unlike DNA, for example), they must carry signals to regulate and coordinate these processes. We have studied this issue for some years, focusing much attention on the right hand (3´) end of the genome. For eukaryotic cellular mRNAs, this end is a poly(A) tail, but for Turnip yellow mosaic tymovirus (TYMV) and most of its relatives, the 3´-end closely mimics transfer RNA, the molecular adaptor that reads the codons in mRNA and brings amino acids to the ribosome for protein synthesis. Over the years, we found that the tRNA-like structure of TYMV is important in maintaining an intact 3´-end and in promoting the translation of the RNA in a regulated way. However, genome replication also begins in this feature, and the final –CCA nucleotides are important in this step. In our recent paper, we studied an atypical tymovirus, dulcamara mottle virus (DuMV) whose genome has no tRNA-like structure or –CCA 3´-end, but is a fit virus all the same. The key experiment was to construct a chimeric genome that is mostly TYMV but has the 3´-end of DuMV. We observed that this chimera was infectious (albeit weakly so). We think of the chimera as a snapshot in evolution, with which we have caught a typical tymovirus like TYMV in the act of evolving into a virus with a remarkably different 3´-end. To survive, the chimera would have to acquire further genetic changes that improve its fitness. Our experiments show that virus evolution can be remarkably flexible, leading to changes that could alter host range or pathogenicity.

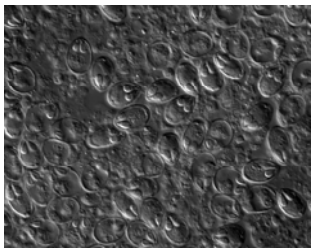
Ioannis E. Tzanetakis, Ching-Hsiu Tsai, Robert R. Martin and Theo W. Dreher. 2009. A tymovirus with an atypical 3'-UTR illuminates the possibilities for 3'-UTR evolution. *Virology* 392:238-245.

JERRI BARTHOLOMEW LAB:

We recently completed an interstate collaborative study that was led by **Daniel Horner**, a current undergraduate Microbiology major. For the first time in 26 years of surveillance, emerald shiners with hemorrhagic skin lesions and swollen abdomens were observed by personnel of the Wisconsin Department of Natural Resources in 2007 and 2008 in Lake Superior. Infected fish were sent to the Bartholomew lab for analysis. Based on appearance of the spore, site of infection and gross pathology, the pathogen was identified as a myxozoan parasite, *Myxobolus notropis*, which has been recorded in other species of shiner. The parasite was only detected in samples collected later in the summer and we postulate that changes in local environmental conditions may have contributed to the recent emergence of severe infections: 'While rising water temperature and decreasing water levels typically occur over the course of the summer, water levels in 2007 in Lake Superior were at the lowest recorded average level since the 1930s. These environmental changes may have caused emerald shiners to occupy different habitats than in previous years, exposing them to myxozoans not encountered previously and putting them in closer proximity to the putative invertebrate host of *M. notropis*.' Dan was awarded a Howard Hughes Medical Institute scholarship during the summer 2009 to support his research interests in the Bartholomew lab; he continues to work in the lab for research credit.

D Horner[1], S D Atkinson[1,2], D M Pratt[3], S Marcquenski[4] and J L Bartholomew[1]
***Myxobolus notropis* from emerald shiner, *Notropis atherinoides* Rafinesque, in Lake Superior**
Journal of Fish Diseases, accepted 2009

Left: Infected emerald shiners from Lake Superior.



Right: The pathogen – a myxozoan parasite.

[1] Department of Microbiology, Oregon State University, Corvallis, OR, USA
[2] School of Chemistry and Molecular Biosciences, The University of Queensland, St. Lucia, QLD, Australia
[3] Wisconsin Department of Natural Resources, Superior, WI, USA
[4] Wisconsin Department of Natural Resources, Madison, WI, USA

Microbiology Ph.D. student Sarah Bjork's research has focused on the myxozoan parasite, *Ceratomyxa shasta*, which infects the intestine of salmon and trout, often killing the host. The infective stage is an actinospore, which proliferates in the fish host and transforms into a myxospore. This then infects an invertebrate worm, in which the life cycle is completed with the formation of new actinospores. Determination of the infective dose for fish is critical to interpreting and predicting disease effects on valued stocks. Sarah's research determined that just a single actinospore was sufficient to cause lethal infection in susceptible rainbow trout, while more resistant fish, Chinook salmon, did not become infected even when challenged with up to 5,000 actinospores. One fatal infection occurred in a coho salmon challenged with 1,000 actinospores. Sarah discovered that prevalence of infection is influenced by parasite concentration but not by the size of the fish host. She developed an infection model for *C. shasta* in rainbow trout that should facilitate the investigation of other host/parasite interactions. Funding for this work was provided by the California Energy Commission and Oregon Sea Grant. Sarah will be completing her Ph.D. in the coming months.

Sarah J. Bjork, Jerri L. Bartholomew Department of Microbiology, Oregon State University, Corvallis, OR,
Effects of *Ceratomyxa shasta* dose on a susceptible strain of rainbow trout and comparatively resistant Chinook and coho salmon
***Diseases of Aquatic Organisms*, Vol. 86: 29–37, 2009**

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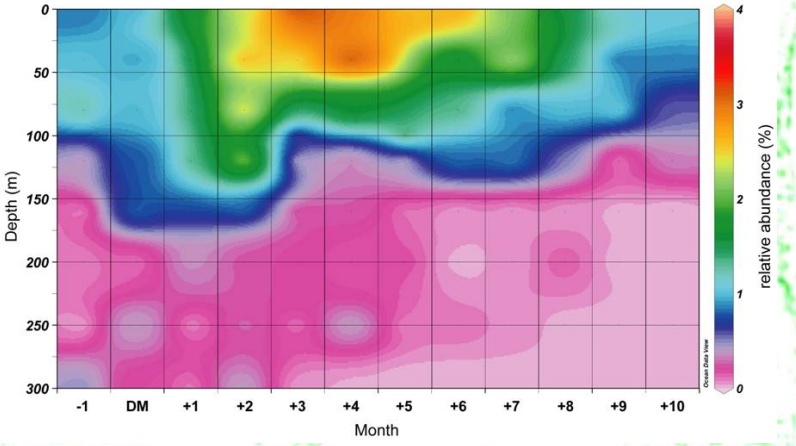
STEPHEN GIOVANNONI LAB:

The illustration shows a summertime bloom of the SAR86 clade of bacteria in the Sargasso Sea (months are numbered on the X-axis, depth on the Y-axis and population density is reflected by color). Warming of the atmosphere may stimulate blooms like this. The SAR86 clade was discovered by students in the Giovannoni laboratory in the early 1990's. The work shown in the figure is from a long-term collaboration with the Bermuda Institute of Ocean Sciences. This work is funded by the National Science Foundation. The primary author of the paper, Alexander Treusch is a German citizen who was a postdoctoral fellow in the Giovannoni laboratory and has recently taken a faculty position at the University of Southern Denmark in Odense. While working on this project, Dr. Treusch was supported by a fellowship from the Alexander von Humboldt Foundation.

Alexander Treusch, Ph.D.



Treusch A.H., K.L. Vergin, L.A. Finlay, M.G. Donatz, R.M. Burton, C.A. Carlson and S.J. Giovannoni. 2009. Seasonality and Vertical Structure of Microbial Communities in an Ocean Gyre. *ISME J.* 3:1148-63.