

**Annual Report for Period:**02/2003 - 02/2004**Submitted on:** 12/07/2003**Principal Investigator:** Boomer, Sarah M.**Award ID:** 0237167**Organization:** Western Oregon University**Title:**

RUI-Microbial Observatories: A Longitudinal Molecular Diversity and Chemical Survey of Red Layer Microbial Communities in Yellowstone National Park

**Project Participants****Senior Personnel****Name:** Boomer, Sarah**Worked for more than 160 Hours:** Yes**Contribution to Project:**

In addition to fulfilling previous MO grant obligations, I have facilitated the following new projects based on specific goals in the renewed grant (to be described in more detail in other sections): (1) implemented new research-driven lab curriculum modules in Microbiology (Biology 331); (2) implemented new research-driven lab curriculum modules in Molecular Biology (Biology 475); (3) Facilitated management of new monthly Upward Bound outreach project using Microbiology-inspired curriculum (primary instructor and assessor is Kelly Shipley); (4) Designed, advertised and recruited for two teacher workshops based on RLMO curriculum, filling available spots for January and March offerings, 2004; (5) Designed, advertised, and recruited for new summer undergraduate research experience, filling more than available spots for summer, 2004 offering; (6) Along with two research students (Will Drury and Melissa Boschee, described participants in the last MO grant) and collaborators (Bryan Dutton and Danny Lodge), I completed a poster and proceedings/chapter submission on the RLMO Database for the Thermal Biology Institute's Yellowstone meeting (October, 2003).

**Name:** Shipley, Kelly**Worked for more than 160 Hours:** No**Contribution to Project:**

Kelly began designing pre-college outreach projects, as proposed in this grant to begin year two, over the summer of 2003 when an early opportunity to run an Upward Bound cohort arose. Kelly is completing this special Upward Bound project (including regular assessment) part-time while working her masters in teaching on campus; she will begin her full-time position with me in June, 2003. Kelly began teaching a monthly Upward Bound course in October based on new microbiology curriculum (water chemistry using new portable chemistry test kits, culture-based approaches to identifying local river microbes, and DNA-based methods to identifying new isolates from RLMO sites). Kelly's course provides 6 local high school students (including 5 Hispanic females) the opportunity to earn 0.5 elective credit for high school graduation (40 hours hands-on science using this project on campus).

**Name:** Dutton, Bryan**Worked for more than 160 Hours:** No**Contribution to Project:**

Bryan, as described in the Final Report for our last MO grant, has been an outstanding colleague and collaborator, assisting with curriculum assessment and revision, phylogenetics, and more importantly database design (I daresay the database was his conception more than it was mine even though I now embrace it the most). Bryan co-advises Will and as been integral in the moving forward and presentation of the database. Bryan has organized a regular series of pre-college outreach science and math courses (modeled after the Saturday Academy program) for all secondary science students (i.e. not just Upward Bound) to begin in January, 2003. Currently, I will offer 2 weekend activities under this general program between March and June, 2004.

**Post-doc****Graduate Student****Undergraduate Student****Name:** Students, Molecular Biolo**Worked for more than 160 Hours:** No**Contribution to Project:**

Ten undergraduates participated in the revised Molecular Biology curriculum this spring, 2003. As proposed and outlined in this grant, students mastered new methods in 'macroarray' (blotting and hybridization) and DGGE-based population genetics. They used lab-dedicated computers to assemble web pages about their projects, incorporating all new digital technology in the lab (digital cameras, Photodyne system, and upgraded DNA sequencer).

**Name:** Students, Microbiology

**Worked for more than 160 Hours:** No

**Contribution to Project:**

Thirteen undergraduates participated in the revised Microbiology curriculum this fall, 2003. As proposed and outlined in this grant, students mastered five weeks of traditional culture-based environmental enrichment and phenotype-based identification testing followed by five weeks of culture-independent molecular methods using the RLMO project. Specifically, students performed new methods in plasmid isolation, DNA fingerprinting, DNA sequence analysis, and computational assessment of retrieved sequences using NCBI/BLAST. During the environmental portion of the new curriculum, students also utilized new portable water chemistry test kits to analyze local rivers from which coliforms and Pseudomonads were isolated. The latter is part of a collaborative project between several faculty in the division to assess impacted stream drainages. As with Molecular Biology, students used lab dedicated computers to assemble web pages about their projects, incorporating all new digital technology in the lab.

**Name:** Drury, Will

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Will Drury, as described in the Final Report for our last MO grant, was supported to upgrade the RLMO Database over the summer of 2003 (including the addition of a query system). Will has continued to assist with database modification, presentations, and proceedings chapter authorship (TBI Yellowstone Meeting, October 2003) and will be supported by computer service funding, as needed, throughout this year. As stated in the original grant, we will perform major database upgrades each summer; Will has also enthusiastically agreed to assist with the database teaching curriculum for the first offering of the new summer research experience.

**Name:** Manning, Terry

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Terry was one of two RLMO-supported summer stipend recipients (June 2002-3) and went on to earn one of 25 national ASM summer research fellowship for a continuation of this work (June 2003-4). Terry performed water chemistry at RLMO sites, used site-specific chemical information to develop designer media, and compared retrieval rates using designer media and standard GNS media (developed by S. Hanada). In terms of impact on this first year of renewed funding, Terry trouble-shot DGGE methodology, using these approaches to compare enrichment outcomes for his ASM-supported culture project this summer. Terry co-authored a Yellowstone TBI poster (October, 2003) is completing an Honors Thesis about this work, and will develop an ASM poster and manuscript in spring, 2004. Terry will graduate in June, 2005 and hopes to go to medical school.

## Technician, Programmer

**Name:** Lodge, Danny

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Danny was a full-time technician (70% research and 30% teaching) supported on salary by our last MO/RUI grant (which overlapped with this grant through September 1, 2003). Danny has performed data entry during the ongoing maintenance of the RLMO database. Most importantly, Danny was the key individual who facilitated the acquisition and set-up of all new equipment (DGGE, hybridization, PCR, DNA sequencer upgrade, portable water chemistry test kits) and lab computers (six lab-dedicated and networked computers, linked to existing digital equipment). Danny troubleshooted all new equipment and developed teaching protocol during February and March such that we were able to use all new equipment in the spring, 2003 offering of Molecular Biology. Danny directly assisted in teaching this lab, running new sessions on DGGE and 'macroarrays.' Danny is pursuing an advanced degree in engineering at OSU.

**Name:** Kernan, William

**Worked for more than 160 Hours:** Yes

**Contribution to Project:**

Bill, as described in the Final Report for our last MO grant, has been integral in the development and support of the RLMO Database. Bill served as a co-advisor for Will Drury over the summer of 2003 and co-authored the TBI/Yellowstone poster and

carried out by students like Will (who receive the only direct support) as this aspect of the grant continues.

## **Other Participant**

### **Research Experience for Undergraduates**

#### **Organizational Partners**

##### **Upward Bound, Western Oregon University**

After two years of collaborative, short-term projects, Upward Bound provided 6 students (all first generation college-bound, minority) for a year-long program featuring RLMO-based curriculum in Microbial Diversity and Biotechnology. Kelly Shipley is currently leading and assessing this program with assistance from Upward Bound Director Sandra Hastings.

##### **American Society For Microbiology**

ASM provided Terry Manning (research student June 2002-present) with one of 25 national undergraduate summer research fellowships. Terry is completing this work at this time, described more in the participant section.

##### **Thermal Biology Institute, MSU**

As described in the Final Report for our last MO grant, TBI - specifically Dr. Tim McDermott - provided one week of on-site training in DGGE methods for my research assistant (August, 2002). TBI recently hosted and supported 2 undergraduates and I during the TBI Yellowstone meeting (October, 2003), at which we presented a poster about the RLMO database.

#### **Other Collaborators or Contacts**

Bev Pierson (U. Puget Sound, Tacoma, WA), Dick Castenholz (U. Oregon, Eugene, OR), and Tim McDermott (Thermal Biology Institute, Bozeman, MT) have all provided mentoring, reagents, and advice.

#### **Activities and Findings**

##### **Research and Education Activities:**

**Research Activities:** This first year of renewal significantly overlapped with our last MO grant and many major research findings, in my opinion, are more appropriate to the Final Report for that Grant (submitted November, 2003). The key reason we asked to overlap grants was because this first year of renewed funding was designed to acquire and troubleshoot new equipment for new research-driven educational programs, including database development for long-term data entry. I will highlight our preliminary research findings using new equipment with the following:

**Geochemical Testing Methodology:** We have successfully acquired and implemented geochemical testing methodology in the field using new portable Hach kits (troubleshooted by undergraduates and I in the field); two years of preliminary water and mat chemistry data for four RLMO sites (those proposed for long-term monitoring studies in the renewed grant) have been entered into the RLMO database (available on-line at <http://wilbur.wou.edu/pls/wou/nsboomer.public3.main>). These data repeatedly demonstrate that RLMO sites, despite superficially similar appearances (temperature, pH, and community structure), show wide variation in 15 common salts and metals selected for testing. We have also noted some fluctuations over two years and, after hearing recent data presented at the TBI/Yellowstone meeting (October, 2003), decided to conduct site analysis twice each summer and modify target compounds we will continue to assess. Chemical testing kits have also been successfully used for collaborative river-based water testing projects used in Microbiology (Biology 331), Upward Bound activities, and pending teacher workshops.

**DGGE Methodology:** We have successfully implemented DGGE methodology into Molecular Biology (Biology 475). Over the summer, Terry Manning (supported by previous funding and ASM) used the new DGGE equipment to monitor enrichments in designer media he developed using site-specific chemistry data. Although Terry was not able to ultimately isolate red GNS using these novel approaches, he isolated a novel green GNS and described a variety of new Proteobacteria and Gram Positives. Terry upgraded our collective lab DGGE skills by successfully cloning and sequencing specific DGGE bands, a method I hope to bring to Molecular Biology during its next offering. Terry's project, supported by an ASM Undergraduate Research Fellowship, will be presented at the forthcoming General Meeting in May, 2004.

Education Activities: Thus far, this grant has served 13 undergraduates in Microbiology (Biology 331), 10 undergraduates in Molecular Biology (Biology 475), and 6 pre-college Upward Bound participants. I have actively recruited 16 teachers for pending teacher workshops (these activities supported by release time) and 8 undergraduates for the pending new summer research program (these will be pared to 6 via competitive applications for reasons of support and impact). Equipment and computer service support from this grant have facilitated undergraduate projects being completed by Terry Manning and Will Drury (both of whom received stipends from the last grant). In addition to the above equipment, several other key pieces of equipment have served the expansion of research-driven curriculum using the RLMO Project, including:

The Digital Molecular Microbiology Lab: Using new dedicated lab computers, projection systems, and digital cameras, students in Microbiology and Molecular Biology now make mandatory web-based presentations of their term-long projects (in addition to hard-copy notebooks that are graded as part of a new 'writing intensive' course status). Research-driven curriculum and student projects are maintained on my long-term RLMO Website (<http://www.wou.edu/~boomers/boomer.html>). Software and hardware upgrades to our existing DNA sequencing system have finally permitted multiple user access to what used to be a stand-alone terminal for DNA editing and sequence management. These methods are, at this time, trickling down to outreach workshops (although at this time, Kelly is writing the websites û not the high school students) and will be actively employed by teachers during pending workshops. I am VERY PLEASED with these new systems because they have greatly improved teaching concepts and methods to larger groups of students. Although initially intimidated, students have responded extremely well to web-page projects because they are visual and visible beyond the scope of the classroom and students understand the value of being able to cite them in portfolios or resumes. It should be noted that the use of Word-based templates (that I designed) has served to emphasize data presentation and interpretation, not the mechanics of how to make a web site. I look forward to presenting these new curricula at the ASM General Meeting in May, 2004.

### **Findings:**

Again, I am only presenting limited and preliminary findings based on specific new equipment acquired:

- (1) Despite superficial appearances, RLMO sites (water and mats) are chemically distinct (based on 15 salts and metals, archived in the RLMO Database). These studies are ongoing components of our long-term monitoring project.
- (2) Site-defined media based on the above information yielded different enrichment outcomes than published anoxygenic phototrophs media used to propagate traditional GNS. However, simple sterilized spring water from two RLMO sites has supported the best enrichment for Red GNS (although we have not yet fully isolated target organisms). Assessment of cultures was made using side-by-side comparisons of DGGE results with bacterial 16S libraries.

### **Training and Development:**

As described in the 'major research and education' section, this project has provided training (field, independent research, or class-based research) for 25 undergraduates in the first year. Outreach activities will, starting in January, provide research-based training in molecular techniques and microbial diversity for an additional 8 local secondary science teachers completing masters degrees or continuing education.

### **Outreach Activities:**

This project has directly supported the development and implementation of research-driven curriculum for both secondary students and teachers. In the context of outreach activities (a new year-long Upward Bound Program), 6 local students are using RLMO materials to analyze 16S libraries, perform DNA sequence analysis, and/or learn how to use BLAST. Newly recruited-for teacher training workshops and general Saturday Academy-like workshops will serve at least 40 teachers and pre-college in the coming four months.

### Journal Publications

#### Books or Other One-time Publications

W.M. Drury, B.E. Dutton, D.P. Lodge, M.S. Boschee, W. Kernan, and S.M. Boomer, "The RLMO Database: A Model for the Integration and Dissemination of Biological and Geological Data via the World Wide Web", (2003). Book, Submitted  
 Editor(s): Thermal Biology Institute, Montana State University  
 Collection: Proceedings from the First Annual Geothermal Biology and Geochemistry in Yellowstone Meeting  
 Bibliography: Meetings Proceedings Chapter

### Web/Internet Site

**URL(s):**

<http://www.wou.edu/~boomers/research/allresearch.html>

**Description:**

This is our comprehensive RLMO website. It contains RLMO-generated educational course curriculum, outreach projects and assessment, project methodology, and links to our Oracle-based RLMO Database.

### Other Specific Products

**Product Type:** Data or databases

**Product Description:**

Although the RLMO Database was proposed for this MO renewal, response to this project has been so great that we are ahead of schedule in terms of this aspect of the project. Consequently, we are adding important database entry training and proficiency to the summer research experience curriculum. As described in our Final Report, the RLMO Database Project is a web-accessible Oracle database that integrates physical, chemical, and molecular data from RLMO sites. This application, in its second year of development, was written using PL/SQL, HTML, and Javascript. Each research site in this study is assigned a unique identifier that is linked to the following tables: Geochemical Data (pH, 15 common salts and metals); DNA Sequence Data (16S clone name, GenBank-linked accession number, BLAST-inferred identity); Macroscopic and Microscopic Image Data; and Student Collection Team Information. Using the administrative URL, all data can be entered and edited through restricted web-access. Using the public URL, users can view and query all data. Site Query results can be formatted to display any combination of geochemical parameters across one or more years and/or sites. Sequence Query results can be formatted to display inferred identity and GenBank-linked accession numbers across one or more sites. The RLMO Database, in its current form, is designed to accommodate physical, chemical, and molecular information as part of a five-year longitudinal study.

- Excerpted from TBI/Yellowstone meeting abstract (October, 2003)

**Sharing Information:**

The RLMO database is provided on-line, as described above. Users can browse the public portion of this database in a non-restricted manner.

### Contributions

**Contributions within Discipline:**

In terms of molecular microbial systematics, the RLMO project continues to improve our understanding of GNS diversity with an emphasis on novel red anoxygenic phototrophs. Ongoing studies suggest that geochemical mechanisms drive site-specific selection provide models for bacterial evolution and selection. Current geochemical data are also providing novel models for media design approaches. The RLMO database (similar to Dr. Mary Ann Moran's SIMO Database) is a model database for other PIs involved in long-term environmental monitoring studies to archive and analyze environmental and molecular data.

In terms of undergraduate education, RLMO-based molecular biology curricula has provided exemplary models for comparable undergraduate Courses and we look forward to presenting novel curricula for teaching General Microbiology (with an emphasis on culture-dependent and culture-independent approaches) and Computational Biology at ASM's General Meeting in May, 2004.

**Contributions to Other Disciplines:**

These topics have been covered and summarized extensively in this report under participants and activities/findings sections.

**Contributions to Human Resource Development:**

Improved outreach and workshop development for secondary science teachers is improving exposure to culture-independent approaches to identifying novel microbial diversity.

**Contributions to Resources for Research and Education:**

These topics have been covered and summarized extensively in this report under participants and activities/findings sections.

**Contributions Beyond Science and Engineering:**

### Special Requirements

**Special reporting requirements:** None

**Change in Objectives or Scope:** None

**Unobligated funds:** less than 20 percent of current funds

**Animal, Human Subjects, Biohazards:** None

**Categories for which nothing is reported:**

Any Journal

Contributions: To Any Beyond Science and Engineering