

# **Microbes and Phosphorus: integrating engineering principles, ecology, and student learning to study eutrophication of freshwater lakes**

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*ABSTRACT: The purpose of the international collaboration was to develop and adopt sensitive methods for microbial population detection and quantification in surface water ecosystems, through the knowledge transfer of two novel molecular techniques, fluorescence in situ hybridization combined with microautoradiography (FISH MAR) and catalyzed reporter deposition fluorescence in situ hybridization (CARD FISH). CARD FISH and FISH MAR are used to identify bacterial population structures and metabolic functions of the populations therein. These techniques will be used to address research aims of Dr. Katherine McMahon's NSF CAREER research project. Graduate student Emily Kara visited the lab of Dr. Stefan Bertilsson at Uppsala University to learn and develop the technique with members of Bertilsson research group. FISH MAR is directly applicable to Kara's graduate research questions in the field of environmental engineering and water quality. During the collaboration, Kara applied CARD FISH and FISH MAR to Lake Vallentunasjon, Sweden, water samples investigating acetate and phosphate uptake by bacterial populations.*

## INTRODUCTION

The research of Dr. Katherine McMahon, Associate Professor of Civil and Environmental Engineering department at the University of Wisconsin, Madison (UW-Madison) has investigated the mechanisms responsible for phosphorus (P) cycling by natural microbial communities in eutrophic lakes since 2007, upon receipt of a NSF CAREER award (CBET-0644949). Though it is generally understood that bacteria and algae control P-cycling in lakes, knowledge of the taxonomic groups responsible for specific P transformations, the biochemical mechanisms involved, and the contributions of different taxonomic groups to P transformations is limited. Studies of eutrophic lakes indicate that freshwater bacterial community composition is variable, and this variation is correlated to differences in nutrient availability. The research aims to increase the understanding of the mechanisms involved in P cycling, particularly with respect to bacterial community composition. Ultimately, this knowledge will improve the ability of lake managers to better predict the impacts of management practices on water quality. Methods to study P cycling employed by McMahon's CAREER research project include traditional limnologic and water chemistry methods, and several modern molecular microbial ecology techniques.

International cooperation was carried out under IREE and the Global Lake Ecological Observatory Network (GLEON) Student Site Exchange Fellowship, as a conduit of expertise in modern microbial ecology methods between the labs of McMahon and Dr. Stefan Bertilsson, of Uppsala University, Sweden. In order to maximize the sensitivity and number of techniques available to address the research aims of McMahon's CAREER research project, graduate student Emily Kara was chosen to visit Bertilsson's lab group. Kara was chosen based on her research interests in microbial mechanisms of P cycling and water quality. Kara was best qualified for this opportunity as a 2<sup>nd</sup> year graduate student because of her oral and written communication skills, ability to work independently, problem-solving skills for practical and theoretical problems, motivation to expand her laboratory skill set, interest in international collaboration, and capacity for cultural exchange. Kara traveled to Uppsala, Sweden from September 15, 2008 through November 25, 2008.

Bertilsson's research at Uppsala University focuses on microbial influences on geochemical cycling of nutrients and how the function and composition of microbial communities is controlled by the environment. Bertilsson's research group is part of the Limnology group within the Department of Ecology and Evolution, and currently includes five post doctoral researchers and three PhD students ([http://www.ebc.uu.se/limno/staff/Stefan\\_Bertilsson/home.html](http://www.ebc.uu.se/limno/staff/Stefan_Bertilsson/home.html)). The research activities of this host laboratory are vibrant and diverse; it is an ideal environment for education and research.

Kara was successfully trained in the CARD FISH and FISH MAR methods by post doctoral researcher Laura Alonso-Saez in Bertilsson's lab. Following training, Kara used CARD FISH and FISH MAR to investigate P uptake rates and identity of bacterial taxa actively taking up P under different nutrient regimes. Results of the experiment are forthcoming. In the following months, Kara will establish and continue to develop at the home institution, and over the following years, Kara will apply the molecular methods to address the questions outlined by McMahon's CAREER research project.

## RESEARCH ACTIVITIES AND ACCOMPLISHMENTS OF THE INTERNATIONAL COOPERATION

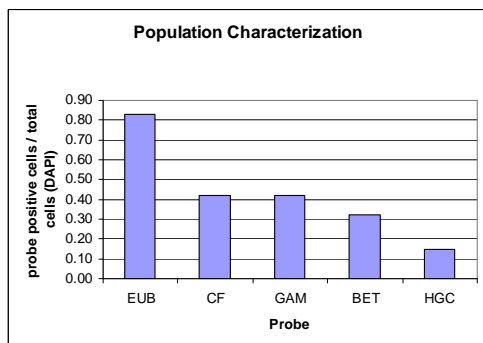
Goals of the GLEON and IREE travel supplements included training of Kara by Bertilsson lab members in FISH MAR and CARD FISH techniques, application of techniques on water from a local lake, and establishment of the technique at the home institution following Kara's return from Sweden. Progress of the goals is well underway. Final sample analysis and method set-up at UW-Madison are incomplete as of completion of this report, but are expected to be finished by May 2009 (Table 1).

Over 2.5 months at Uppsala University, Kara worked in the Bertilsson research group, interacting on a daily basis with Bertilsson and members of the Bertilsson research group. In addition to immersion into the Bertilsson research group, Kara planned experiments, carried out laboratory and field work, participated in research seminars and in three international workshops.

For the first six weeks of the collaboration, Kara was primarily engaged in mastering the CARD FISH and MAR FISH techniques under the supervision of Alonso-Saez. During these weeks Kara also attended three international workshops: the Global Lake Ecological Observatory Network (GLEON) 7<sup>th</sup> meeting (Norrtalje, Sweden 30 Sept 2008-1 Oct 2008), International Aquatic Microbial Dynamics Workshop (Norrtalje, Sweden 29 Sept 2008-4 Oct 2008), and the Lake Ecosystem Response to Environmental Change (LEREC) Workshop IV: Ecological Stoichiometry (Umea University, Sweden 23 -24 Oct 2008).

In the final four weeks of the collaboration, Kara put into practice CARD FISH and MAR FISH methods by investigating differential P uptake by bacterial populations from a eutrophic lake under differing nutrient regimes. The study site, Lake Vallengnasjon, is located in southeastern Sweden, 20 km north of Stockholm. Surface area of Vallengnasjon is 6.2 km<sup>2</sup>, mean depth is 2.7m, and max depth is 5m. The lake received domestic wastewater until the 1970s, and as a result it is subject to internal loading of P from the sediments. The trophic status of the lake and the extent of internal loading make Vallengnasjon an ideal study system for the investigation of P transport and transformation between the water column and sediment, in Sweden. Current trophic status and history of P loading to Lake Vallengnasjon are similar to that of Lake Mendota, WI, a McMahan CAREER research project study site.

For application of techniques during the visit, Lake Vallengnasjon surface water was collected during Nov 2008. Sample water was stored in the dark at 4°C and immediately transported to the laboratory. Nutrient addition incubations began within two hours of sample collection. Four treatments were applied to sample water: ~ 20mL water was incubated for two hours with four different concentrations of unlabeled (PO<sub>4</sub><sup>3-</sup>) and radiolabeled (<sup>33</sup>PO<sub>4</sub><sup>3-</sup>) substrate concentrations; treatments ranged from 0.5 mg/L to 0.0005 mg/L total phosphate. Bulk activity was measured using a liquid scintillation counter, and filters were prepared for quantification of metabolically active and probe-positive cells. CARD



**Figure 1. Lake Vallengnasjon, Sweden bacterial population characterization by CARD FISH.**

FISH using ribosomal ribonucleic acid (rRNA) oligonucleotide probes for hybridizations included general bacterial probes (EUB I,II,III), Betaproteobacteria (BET), Gammaproteobacteria (GAM), Actinobacteria (HGC), and Cytophaga-Flavobacteria (CF) probes. DAPI was used as a counterstain, and to quantify total bacterial cells. The sample was dominated by taxa Cytophaga-Flavobacteria and Betaproteobacteria. Uptake results are forthcoming.

**Table 1. Progress of IREE Travel**

| IREE Travel Award Goals                | % Completed | Date (expected) |
|--|-------------|-----------------|
| <b>Training</b>                        |             |                 |
| Learn CARD FISH                        | 100         | Nov-12          |
| Learn MAR FISH                         | 100         | Nov-12          |
| <b>Application</b>                     |             |                 |
| CARD FISH population characterization  | 100         | Nov-12          |
| MAR FISH time course                   | 75          | (Feb-09)        |
| Sample Quantification                  | 60          | (Mar-09)        |
| <b>Post-travel</b>                     |             |                 |
| Independent application in McMahon Lab | 0           | (May-09)        |

## **BROADER IMPACTS OF THE INTERNATIONAL COOPERATION**

Activities carried out under the supplement award promoted diversity, enhanced international collaboration and future interaction between UW-Madison and Uppsala University directly expands the scope of McMahon's CAREER award, and increased Kara's experience with Swedish and European languages, culture, and academic institutions.

The representation of women in science, technology, engineering and mathematics (STEM) fields is and has been traditionally low. The international cooperation funded by the IREE supplement provided graduate student Kara with training and research opportunities that will allow her to succeed in the field of environmental science and engineering. Kara's research will be enhanced by this opportunity by increasing lab experience and tools available to answer relevant questions regarding microbial ecology and environmental engineering.

As described above, the research methods developed and carried out during the collaboration will be applied directly to questions proposed in McMahon's CAREER award project. The tools available to simultaneously identify the structure and function of microbial communities are limited; CARD FISH and MAR FISH are two techniques that make this connection possible.

McMahon and Bertilsson are planning to submit two collaborative proposals over the next six months, with research topics related directly to the methodological skills acquired by Kara during her visit. They are also co-authoring two review manuscripts and co-chairing a session at an international conference. Kara submitted an NSF Graduate Research Fellowship Proposal to support continued work with these tools.

While in Sweden, Kara interacted with a variety of Swedish scientists, and scientists from Germany, Austria, Spain, Argentina, Switzerland, Ireland, Romania, and Poland, among other countries. In addition to great diversity within Bertilsson's group, Kara attended and participated in three international workshops. One workshop resulted in the creation of a manuscript, of which Kara is an author. The length of stay allowed these opportunities to be fulfilled, in addition to allowing more immersion into Swedish culture.

## **DISCUSSION AND SUMMARY**

University of Wisconsin Environmental Engineering graduate student Emily Kara traveled from the lab of Dr. Katherine McMahon to the lab of Dr. Stefan Bertilsson, at Uppsala University, Sweden. During the exchange, graduate student Emily Kara learned and developed modern molecular techniques directly applicable to McMahon's CAREER Award. While in Sweden, Kara applied the methods to environmental samples in an independent research experiment. Kara interacted daily with an international group of scientists in a vibrant research environment, within the Limnology group of the Ecology and Evolution Department at the host institution.

The GLEON and IREE travel supplements allowed the sharing and development of technical knowledge across international and cultural boundaries. A traditionally underrepresented gender benefitted from the supplement in many ways, including international collaboration, gaining technical expertise, increasing cultural familiarity and expertise, attendance of international workshops, and increasing communication skills with individuals who speak English as a second language.

## **ACKNOWLEDGEMENTS**

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### **BRIEF BIOGRAPHIES OF RESEARCHERS**

**Katherine McMahon** received B.S. and M.S. degrees in Civil and Environmental Engineering from the University of Illinois at Urbana-Champaign in 1995 and 1997, respectively. She received her Ph.D. degree in Civil and Environmental Engineering from the University of California at Berkeley in 2002. She was an assistant professor at the University of Wisconsin from 2002 until 2008 in the Department of Civil and Environmental Engineering and is now an associate professor with joint appointments in Civil and Environmental Engineering, and Bacteriology. Her interests include microbial ecology, environmental biotechnology, biochemical engineering, environmental engineering, water quality, metabolic engineering, and freshwater microbiology.

**Stefan Bertilsson** received a Ph.D. degree in Water and Environmental Studies from Linköping University, Sweden. He has been an associate professor at Uppsala University, Sweden, since 2005. His interests include microbial ecology and biogeochemical cycling.

**Emily Kara** received a B.S. degree in Environmental Science from the University of Arizona, Tucson, in 2004, and an M.S. degree in Environmental Science in 2007 from Indiana University, Bloomington. Kara is currently a Ph.D. student in the Department of Civil and Environmental Engineering at the University of Wisconsin-Madison. Her interests include water quality, eutrophication, and microbial ecology.