



CMOP Undergraduate Intern Mentoring Opportunity

Deadline: **March 28, 2011**

Selections Announced: **April 1, 2011**

Name/Title/Institution(s) of senior mentor(s): Peter Zuber, Professor, OHSU

Name/Title/Institution(s) of frontline mentor(s): Pete Kahn, Graduate Student OHSU

Project Title: Development of molecular probes for heterotrophic protists found in the Columbia River and implementation via quantitative PCR.

Context for Project:

Microbial eukaryotes, or protists, including phytoplankton and heterotrophs, play important roles in energy transfer and nutrient transformations in the complex estuarine food web. The Columbia River estuary, which divides Oregon and Washington in the United States, is typically classified as a detritus-driven system due to high water flushing times and light-limited conditions that hinder phytoplankton growth. Much of the microbial activity is therefore heterotrophic and associated with suspended particles. While the particle-associated bacterial community has been examined in the Columbia River estuary, major consumers of bacteria (bacterivores) in the estuary are not well characterized. Molecular analysis of protists assemblages in the Columbia River estuary indicates that during the spring, the heterotrophic flagellate, *Katablepharis*, dominates the estuary, while sequences of an uncultured alveolate resembling the heterotrophic ciliate *Frontonia* are numerous in the summer months. Both organisms could play an important and previously unseen role in phagotrophy and the fate of organic matter in the estuary. However, specific molecular probes must be developed and implemented through qPCR in order to quantify the presence of these organisms in the estuary.

Brief Description.

The student's research will focus on developing specific molecular probes for *Katablepharis* and the uncultured alveolate. For *Katablepharis*, we currently have a full sequence of the 18S, internal transcribed spacer (ITS) 1, 5.8S, and ITS2 regions of rDNA as well as a partial 28S region of rDNA. The partial 28S sequence of *Katablepharis* CRE (the strain of *Katablepharis* found in the estuary) contains a 332 bp variable region that has not been found in 28S sequences of katablepharids or any other protist in the NCBI database. The student will use this region to design a specific probe for *Katablepharis* CRE. For the uncultured alveolate, we currently have only an 18S sequence, so the student will work to develop a full rDNA sequence of the organism and design specific probes from this. They will then use these probes to conduct a quantitative analysis of the presence of these organisms through qPCR. The student will conduct qPCR on samples collected during 2009 and 2010 as well as samples they collect in the estuary on a monthly/ bi-monthly scale throughout their internship.

This project fits in with CMOP framing hypothesis 3, as *Katablepharis* and the uncultured alveolate both display a repeatable pattern of occurrence that could potentially be used to track environmental shifts. Due to their high abundance, these organisms could play an important, previously unseen role in the estuarine food web.

Proposed Outcomes/Broader Impact:

This project will give us a finer scale, more quantitative assessment of the presence of *Katablepharis* and the uncultured alveolate than we currently have. The probes designed in this project can then be implemented via the environmental sample processor (ESP) that will be deployed in the estuary.

Proposed timeline (within a 10 week span):

First Half:

Week 1-5: Familiarize student with protists (specifically *Katablepharis* and ciliates), the Columbia River, and rDNA; Work to develop specific primers to target the ITS1-5.8S-ITS2 region of rDNA for the uncultured alveolate, followed by primers for the 28S region. Travel to estuary to collect samples. Learn lab techniques (DNA extraction, PCR, cloning, miniprep). Use specific probes for *Katablepharis* to track presence first through PCR, and begin qPCR experiments.

Weeks 6-10: Build full rDNA sequence and design specific probes for the uncultured alveolate. Analyze genetic variability of sequences 28S and ITS sequences. Travel to estuary to collect samples. Implement probes via qPCR.

Intern academic experience and skill set should include:

Science majors with some background with molecular biology or lab work is preferred, which would probably lead to a more experienced candidate.