CURE: Metagenomics Bioblitz: Discovering the Microbial Diversity of the Lumber River

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INTRODUCTION

During the fall semesters of 2017 and 2018, students in Conner Sandefur's **Microbiology Lab (MB)** collaborated with students in Lisa Kelly's **Conservation Biology (CB)** course in a **Course-based Undergraduate Research Experience (CURE**). The goal was to engage students in an investigation of the microbial diversity of the *National Wild and Scenic* Lumber River. Field studies connect people with nature and can tie them to place, and the Lumber River is integral to the region.

Conservation biology seeks to protect Earth's biodiversity, and microbes are among the most important organisms on Earth, critical for nutrient cycling, decomposition, photosynthesis, disease processes, and innumerable plant-animal interactions, etc.

Microbes have been overlooked in biodiversity surveys. New genomics technologies allow detection of microbes that otherwise are "invisible."

LABORATORY ACTIVITIES

Groups of students (2 MB + 2 CB) participated in hands-on field and laboratory experiences, involving ~ 40 students per semester.

MB students tutored their CB partners in microbial culturing techniques, while CB students tutored their MB partners in conservation values and goals.

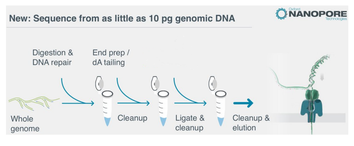
Students could identify and microscopically examine only a small number of microbial taxa using conventional culturing techniques in the laboratory.

Oxford Nanopore Technologies' MinION device uses cutting-edge genomics technology to identify microbes based on their DNA sequences. From two water samples analyzed by the MinION, a large number of microbes were identified. A much larger number of DNA sequences could not be matched to known microbes.

Phylogenetic tree results:
147 taxa

While only a few taxa were identified using conventional techniques

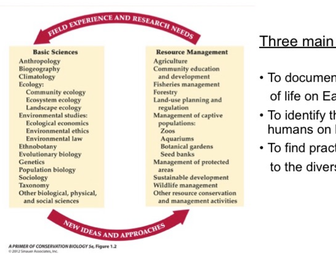
http://phylot.biobyte.de/



As illustrated by the cladogram above (left), the MinION device methodology above (right) identified 147 taxa of bacteria, archaea, and fungi from just two water samples. Conventional culturing techniques identified far fewer microbes.

INTERDISCIPLINARY COLLABORATION

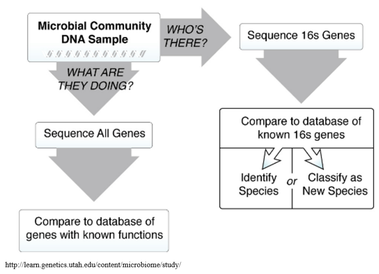
Conservation biology, a multidiscipline science, seeks to protect Earth's biodiversity



Three main goals:

- To document the diversity of life on Earth
- To identify the impacts of humans on life
- To find practical solutions to the diversity crisis

Metagenomics allows analysis of 'invisible' organisms



Microbiology Lab students and Conservation Biology students addressed the goals of conservation biology (above, left) while engaging in an authentic research experience. New genomics technology (above, right) was used to identify microbial taxa of the Lumber River.

Course-specific and collaborative activities were planned for students in the CURE

Activities	Outputs	Outcomes
preliminary list of taxa via literature review	Dynamic and publically available database	Explain threats to biodiversity and consequences of biodiversity loss.
list of taxa identified during initial field excursion to collect samples		
CB field: intensive taxa search; MB lab: intensive culture based testing		Discuss how metagenomics can be a tool to study microbes
DNA extraction and sequencing		
Data analysis		
Meetings for collaborative data and methodology sharing	Presentation slides	Use genomic tools to assess the microbial diversity of an environmental sample
CB-MB teams: share data and include an issue of concern for Lumber River related to observed/predicted microbial life/taxa		

DISCUSSION

OUTPUTS:

A preliminary biodiversity database has been created based on metagenomics. Students used these data, plus data from conventional culturing techniques, to create end-of-semester group (PowerPoint) presentations that addressed one or more of the three major goals of conservation biology.

OUTCOMES:

2017: The CURE generated much excitement among students by creating a unique learning environment, one that included opportunities to hone laboratory skills, to visualize microbes microscopically, and to create science by way of new genomics technology.

The instructors in this new CURE, however, did not adequately prepare students for their group presentations. Laboratory schedules for the two courses did not overlap entirely, which meant that some students were coming in early or leaving late from lab. The instructors were also caught off guard by the limited shelf life of the MinION reagents and by the big investment in time of processing and analyzing water samples on the MinION.

2018: By addressing problems encountered during the first CURE, the 2018 CURE ran more smoothly, students were more comfortable with their CURE partners, and they were better prepared for their end-of-semester group presentations. Unfortunately, laboratory time lost as a result of Hurricane Florence, plus other issues, meant that water samples collected during 2018 were not analyzed via the MinION. This disappointed some students. These samples were cultured and examined using conventional culturing techniques only.

FUTURE COURSE PLANS

Conner Sandefur and his undergraduate researchers continue to study the microbial diversity of the Lumber River. He is now collaborating with Maria Pereira in a CURE in their Principles of Genetics Laboratory sections.

Lisa Kelly has plans to partner Conservation Biology with the Lumbee Tribal Cultural Center in a Service-Learning project to document the center's biodiversity.

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