

Adrian Tinsley Program Semester Grant

Fall 2009

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Mentor: Dr. Jenna Mendell

Title: Microbial Thiaminase Activity in Alewife (*Alosa Pseudohaerngus*) and its Impact on Salmonid Early Mortality Syndrome

Research Question: The recent decline of the salmonid population in the Great Lakes region has been attributed to Early Mortality Syndrome (EMS) in which swim-up fry die between hatching and first feeding. Previous research has shown a strong correlation between EMS and low maternal thiamine levels. In an effort to establish the cause of this deficiency, it has been hypothesized that the salmonid diet of alewife may be a contributing factor, as alewife is rich in thiaminase, an enzyme that degrades thiamine.

The overall goal of the proposed research is to determine if the source of thiaminase in alewife is bacterially derived by focusing on two specific questions.

1. What is the microbiology community structure of the alewife gastrointestinal (GI) tract, and does it include *Paenibacillus thiaminolyticus*, a species with known thiaminase activity?
2. Do the experimentally determined bacterial species of the alewife GI tract possess thiaminase gene sequences?

Methodology: DNA will be extracted from fixed alewife gastrointestinal contents, pooled and analyzed using gel electrophoresis. This will facilitate assessment of the DNA concentration and efficiency of extraction. The extracted DNA will be amplified using polymerase chain reaction (PCR), targeting the 16s rRNA gene sequence with 8F forward and 1492R reverse primers. These 16s rRNA gene products will be ligated into a plasmid vector and then *Escherichia coli* cells will be transformed with these plasmids. The success of this transformation will be determined by blue/white colony screens using LB/ampicillin/X-gal plates. These colony screens will then be used by a fellow researcher for DNA sequencing and bioinformatics analysis to generate operational taxonomic units and phylogenetic trees of the alewife GI microbial structure. Any hazardous material will be handled according to safety protocol.

General Significance: As a top predator within the Great Lakes ecosystem, a decline in salmonid population has broad repercussions within the entire ecosystem, as well as considerable economic impact for the region as a whole. Analysis of the alewife GI community is the first step towards understanding how this population contributes to EMS in these fish.

Proposed Budget:

Books:

At the Bench: A Laboratory Navigator by Kathy Barker \$41.31

Supplies:

Proteinase K, Acros Organics \$99.20

Acetic Acid \$39.84

X-Gal \$61.82

Filters, Syringes \$57.83

Total \$300.00

Budget Justification:

At the Bench: A Laboratory Navigator contains lab protocols essential for this project (reference manual).

Proteinase K, acetic acid and the syringe filters will be used in DNA extraction to purify the nucleic product and prepare it for PCR.

X-Gal will be used in the blue/white colony screen to select for *E. coli* cells that have transformed the ligated plasmid vector.