V(A). Planned Program (Summary)

Program # 3

1. Name of the Planned Program

Food Safety

☑ Reporting on this Program

V(B). Program Knowledge Area(s)

1. Program Knowledge Areas and Percentage

KA Code	Knowledge Area	%1862 Extension	%1890 Extension	%1862 Research	%1890 Research
133	Pollution Prevention and Mitigation			4%	
135	Aquatic and Terrestrial Wildlife			21%	
212	Diseases and Nematodes Affecting Plants			8%	
215	Biological Control of Pests Affecting Plants			7%	
311	Animal Diseases			10%	
501	New and Improved Food Processing Technologies			7%	
711	Ensure Food Products Free of Harmful Chemicals, Including Residues from Agricultural and Other Sources			25%	
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins			10%	
723	Hazards to Human Health and Safety			8%	
	Total			100%	

V(C). Planned Program (Inputs)

1. Actual amount of FTE/SYs expended this Program

Year: 2014	Extension		Research	
	1862	1890	1862	1890
Plan	0.0	0.0	1.5	0.0
Actual Paid	0.0	0.0	3.4	0.0
Actual Volunteer	0.0	0.0	0.0	0.0

2. Actual dollars expended in this Program (includes Carryover Funds from previous years)

Extension		Research	
Smith-Lever 3b & 3c	**1890 Extension**	**Hatch**	**Evans-Allen**
0	0	127743	0
1862 Matching	**1890 Matching**	**1862 Matching**	**1890 Matching**
0	0	203867	0
1862 All Other	**1890 All Other**	**1862 All Other**	**1890 All Other**
0	0	0	0

V(D). Planned Program (Activity)

1. Brief description of the Activity

Several different research projects are conducted under this program, and activities include:

- Using experimental evolution to identify the suites of adaptations that occur as Pseudomonas sp. to form symbiotic or pathogenic biofilms;
- Developing, refining, and applying methods for the detection and enumeration of Vibrio parahaemolyticus and Vibrio vulnificus;
- Developing new methods to distinguish between benign and virulent strains of Vibrio parahaemolyticus and Vibrio vulnificus;
- Evaluating, through a variety of means, how microcystins are spread across landscapes to animal and human food sources;
- Disseminating research outcomes via scientific, extension, formal and informal venues, and to stakeholder groups and natural resource managers.

2. Brief description of the target audience

The target audiences for these research activities include both discrete and overlapping audiences.

- For bacterial biofilms, the audience targeted is peer researchers, students, and ultimately agricultural producers concerned with the role of Pseudomonas in crop or animal productivity and disease.
- For Vibrio pathogens in shellfish, the target audience includes the shellfish industry, shellfish regulatory agencies, graduate and undergraduate students, high school students, faculty collaborators, and other scientists.
- For microcystins from cyanobacterial bloom, the target audience includes students (college and pre-college), scientists, lake shore residents, lake association members, local and regional decision makers, source water protection and watershed managers, surface drinking water suppliers, and public health and environmental agencies.

3. How was eXtension used?

eXtension was not used in this program

V(E). Planned Program (Outputs)

1. Standard output measures

2014	Direct Contacts Adults	Indirect Contacts Adults	Direct Contacts Youth	Indirect Contacts Youth
Actual	1390	11250	112	25

2. Number of Patent Applications Submitted (Standard Research Output)

Patent Applications Submitted

Year: 2014

Actual: 0

Patents listed

3. Publications (Standard General Output Measure)

Number of Peer Reviewed Publications

2014	Extension	Research	Total
Actual	0	3	0

V(F). State Defined Outputs

Output Target

Output #1

Output Measure

- Number of undergraduate students directly involved in the projects

Year	Actual
2014	22

Output #2

Output Measure

- Number of university courses in which project results have been incorporated

Year	Actual
2014	7

Output #3

Output Measure

- Number of presentations at regional, national, or international scientific meetings

Year	Actual
2014	14

Output #4

Output Measure

- Number of workshops, training sessions, and presentations to non-scientific and regulatory stakeholders

Year	Actual
2014	12

Output #5

Output Measure

- Number of graduate students directly involved in the research.

Year	Actual
2014	6

Output #6

Output Measure

- Number of reviewed, bulletin, popular and other publications

Year	Actual
2014	1

Output #7

Output Measure

- Number of websites in which project results have been incorporated

Year	Actual
2014	5

Output #8

Output Measure

- Number of surveys or other means of gathering information and data from participants

Year	Actual
2014	30

Output #9

Output Measure

- Postdoc and other scientists trained in cutting edge research methods

Year	Actual
2014	14

V(G). State Defined Outcomes

V. State Defined Outcomes Table of Content

O. No.	OUTCOME NAME
1	Increased knowledge about the incidence and detection of vibrio in oysters.
2	Knowledge of environmental and biological factors associated with reduced concentrations of vibrios in harvested and processed oysters.
3	Number of agencies and stakeholder groups involved in research outreach related to vibrios in shellfish.
4	Increased knowledge about mechanisms of biofilm adaptation and diversification in pathogens and symbionts.
5	Knowledge about the changes in Vibrio genomes, which cause transitions to virulence;
6	Understanding of how microcystin toxins spread from lakes to the terrestrial food chain
7	Implement an evolution-in-action curriculum that allows high school students to observe bacterial evolution associated with biofilms in a week-long study.

Outcome #1

1. Outcome Measures

Increased knowledge about the incidence and detection of vibrio in oysters.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Shellfish harvesting and public health are being threatened by the emerging incidence of Vibirosis in consumers of shellfish from the Northeastern United States. Changes in pathogenic Vibrio populations in the Northeast US are likely consequences of changing environmental conditions and their detection is complicated because not all strains are virulent. A simple method for screening pathogenic Vibrio species is essential for industry and regulators to shape locale-specific harvest management plans.

What has been done
A tiered conceptual approach for detection and enumeration of pathogenic Vibrio species in coastal ecosystems has been further optimized based on new findings. Over the past year we have expanded our routine surveillance at a new site and included plankton as an added ecosystem matrix for tracking Vibrio population dynamics. PhD and undergraduate students have worked with plankton ecologists to develop novel ways of sampling for and separating zooplankton from phytoplankton in estuaries, and have teamed with students and a professor from a local technical college to determine photosynthetic pigments present in the phytoplankton and water samples. Efforts were further modified to include more frequent (biweekly) sampling to determine environmental variability in Vibrio and plankton levels during the warmer summer months

Results
The research to date has continued to result in significant changes in knowledge about the dynamics of Vibrios in shellfish in Northeast estuarine ecosystems. Conditions, as in sea surface temperatures, in regional larger coastal ecosystems like the Gulf of Maine and Long Island Sound also appear to influence Vibrio species populations in estuarine ecosystems. Recent surveillance of Vibrio concentrations in water, shellfish and sediments now suggests that association with

plankton is a significant ecosystem factor that affects Vibrio populations, and that remote sensing may aid the need to track and predict risk conditions. Much needed knowledge about the emergence of pathogenic strains of Vibrio parahaemolyticus in the Northeast as a result of collaborations with state public health and resource agencies.

4. Associated Knowledge Areas

KA Code	Knowledge Area
501	New and Improved Food Processing Technologies
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins
723	Hazards to Human Health and Safety

Outcome #2

1. Outcome Measures

Knowledge of environmental and biological factors associated with reduced concentrations of vibrios in harvested and processed oysters.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Shellfish harvesting and public health are being threatened by the emerging incidence of vibirosis in consumers of shellfish from the Northeastern U.S. Where more northerly states with colder coastal ecosystems had rare disease occurrence in the past, recent increases in incidence has triggered costly harvest management and monitoring needs for industry and public health agencies alike.

What has been done
The understanding of conditions that allow for reducing pathogenic Vibrio levels in harvested shellfish has been enhanced. In 2014 was the fourth year of monthly oyster relay experiments, where oysters were relayed from a harvest area with elevated levels of Vibrio species to one where Vibrio levels were minimal.

Results
Overall results suggest that relay is a viable strategy for post-harvest treatment to reduce Vibrio concentrations in oysters within 14 days. The underlying mechanisms for why this happens continue to be the focus of our research. Trends in Vibrio and non-Vibrio bacterial taxa in oysters during the relay process have been tracked using metagenomic analysis through the Illumina platform. The bacterial sequence data were analyzed using high throughput sequence analysis software to determine which bacteria increase when pathogenic Vibrio species decrease, and thus may be involved in competitive exclusion of pathogenic Vibrio species during the relay process. Taxa identified from this approach and those cultured from oyster and estuarine water samples collected during relay will be used in in vitro competition experiments against local Vibrio parahaemolyticus strains to select the best candidates to test for experimentally excluding pathogenic Vibrio species from harvested shellfish.

4. Associated Knowledge Areas

KA Code	Knowledge Area
501	New and Improved Food Processing Technologies
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins
723	Hazards to Human Health and Safety

Outcome #3

1. Outcome Measures

Number of agencies and stakeholder groups involved in research outreach related to vibrios in shellfish.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Condition Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	9

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)

What has been done

Results

4. Associated Knowledge Areas

KA Code	Knowledge Area
501	New and Improved Food Processing Technologies
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins
723	Hazards to Human Health and Safety

Outcome #4

1. Outcome Measures

Increased knowledge about mechanisms of biofilm adaptation and diversification in pathogens and symbionts.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Defining adaptive mutations in bacterial populations growing on surfaces presents a huge challenge for understanding this important process in host adaptation.

What has been done
A method was developed for identifying haplotypes from mutations with shared trajectories in evolving populations and also statistical methods for assigning predicted functions to these mutations.

Results
Major lineages define mutants that are broadly adaptive, which continually give rise to low-frequency specialists, which as a group are highly diverse. Thus the specialists with unique functions for adherence and biofilm assembly are derived from many lineages.

4. Associated Knowledge Areas

KA Code	Knowledge Area
212	Diseases and Nematodes Affecting Plants
215	Biological Control of Pests Affecting Plants
311	Animal Diseases

Outcome #5

1. Outcome Measures

Knowledge about the changes in Vibrio genomes, which cause transitions to virulence;

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
There is a rising threat of seafood-borne illness from Vibiro parahaemolyticus due to complex factors, including changing harvest practices, increased shellfish production, increased summer seasonal demand and climate changes.Even with these complex factors, there is ample evidence that changes in bacterial populations have also contributed to disease, and that in recent years, both endemic pathogens have emerged and non-native strains have invaded the region and are causing illness. It is currently difficult to detect rare pathogenic strains.

What has been done
Several alternative host models have been developed to assay for virulence. Of these Arabidopsis thaliana, C. elegans were studied in more detail

Results
There has been success with the C. elegans model in discriminating between clinical strains of varying virulence, and the assay can distinguish between harmless environmental and clinical strains, although variability continues to be an issue and must be addressed before large scale application. Virulence was quantified for more than 50 clinical and environmental strains using the assay. The traits that influence virulence (animal death and intestinal colonization) in the model including capsule, siderophore, and hemolysin of the bacteria. It has been demonstrated that human body temperature primes this virulence, as only pathogenic strains have enhanced

nematode killing at 37C compared to 20C. The stress hormone norepinephrine enhances virulence and even acts as a cue for virulence gene expression in the absence of an important global regulator.

4. Associated Knowledge Areas

KA Code	Knowledge Area
311	Animal Diseases
712	Protect Food from Contamination by Pathogenic Microorganisms, Parasites, and Naturally Occurring Toxins

Outcome #6

1. Outcome Measures

Understanding of how microcystin toxins spread from lakes to the terrestrial food chain

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Reported incidences of harmful cyanobacteria blooms in freshwaters (HCBs) have increased worldwide. There are also frequent reports of deaths of domestic animals such as dogs and cattle caused by drinking water contaminated with toxic cyanobacteria. Recent evidence indicates toxins produced by aquatic cyanobacteria are linked to a wide array of human diseases including liver tumors and amyotrophic lateral sclerosis (ALS) or Lou Gehrig's disease. Water bodies with frequent HCBs may have higher incidences of diseases such as ALS, although the means of transmission of the toxins are not known.

What has been done
Experiments evaluated the exposure of leafy crops to lake and pond water containing microcystin-producing cyanobacteria.

Results
The transfers of MCs within lakes that may result in biomagnification at higher levels in the lake food chain, such as in fish, thereby representing a potential source of toxins for humans and animals. Additionally, MCs may be transferred to crops; for example, MCs were detected in lettuce that was irrigated with water from a lake that frequently experiences blooms of cyanobacteria. Similarly, moderate to high levels of MCs were found in blueberries grown near a lake with persistent cyanobacteria. Soil samples taken at varying distances from the shore of a cyanobacteria-dominated lake tested positive for MCs as well as for living cyanobacteria. Tests to determine whether cyanobacteria and their toxins are transported from lake water as aerosols show evidence of airborne movement of both the toxins and cells of cyanobacteria. Aerosol testing was carried out on eight lakes in New Hampshire as well as Lake Attitash, MA. Cyanobacteria cells as well as MCs were detected from each of the lakes. Work with aerosols suggests a mechanism to disperse cyanotoxins directly to crops and humans that reside near lakes with HCBs.

4. Associated Knowledge Areas

KA Code	Knowledge Area
133	Pollution Prevention and Mitigation
135	Aquatic and Terrestrial Wildlife

Outcome #7

1. Outcome Measures

Implement an evolution-in-action curriculum that allows high school students to observe bacterial evolution associated with biofilms in a week-long study.

2. Associated Institution Types

- 1862 Research

3a. Outcome Type:

Change in Condition Outcome Measure

3b. Quantitative Outcome

Year	Actual
2014	80

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Teaching evolution is challenging because it is typically not viewed as an empirical science.

What has been done
NHAES researchers (#226672) developed an innovative evolution-in-action curriculum that allows high school students to observe bacterial evolution associated with biofilms in a week. This also teaches biotechnology, ecology, microbiology, and genetics. During 45 minutes each day, students ranging from grades 9-12 organize themselves in working groups, define responsibilities, document their procedures, count and record their observations, and statistically analyze their results. They articulate new hypotheses and predictions and even test some of them.

Results
This curriculum not only demonstrates that evolution is a tangible process that happens in real time, but also provides practical laboratory experience and knowledge of fundamental concepts in microbiology, ecology, genetics, and physiology. Classes committed to this curriculum see the genomes of their bacterial mutants completely sequenced, putting these 14-year-olds at the cutting edge of biotechnology and introducing them to the 21st century language of genomics. Learning with bacterial evolution curriculum, as judged by common assessments, improved by 50 per cent which was strongly significant, relative to the standard evolution curriculum.

4. Associated Knowledge Areas

KA Code	Knowledge Area
212	Diseases and Nematodes Affecting Plants
215	Biological Control of Pests Affecting Plants
311	Animal Diseases

V(H). Planned Program (External Factors)

External factors which affected outcomes
- Other (none)

Brief Explanation

V(I). Planned Program (Evaluation Studies)

Evaluation Results

The basic research projects covered in this planned program are most often evaluated by publication in peer-reviewed journals and successfully defended masters or Ph.D. theses and include:

Haney, J.F. and W. Lampert. 2013. Spatial avoidance of Microcystis aeruginosa by Daphnia: fitness consequences and evolutionary implications. Limnol. Oceanogr. 58 (6): 2122-2132.
Cooper V.S. The Origins of Specialization: Insights from Bacteria Held 25 Years in Captivity. 2014. PLoS Biol. 12(2):e1001790. doi: 10.1371/journal.pbio.1001790.
Cooper, V.S., R.K. Staples, C.C. Traverse, and C. N. Ellis. 2014. Parallel evolution of small colony variants in Burkholderia biofilms. Genomics. pii: S0888-7543(14)00184-0. doi:

10.1016/j.ygeno.2014.09.007.

Key Items of Evaluation

- The Cooper project (#226672) extended a decade of investment in building a curriculum that engages young biology students to study evolution-in-action. This curriculum demonstrates that evolution is a tangible process that happens in real time, but also provides practical laboratory experience and knowledge of fundamental concepts in microbiology, ecology, genetics, and physiology. Students ranging from grades 9-12 articulate new hypotheses and predictions and even test some of them. Classes committed to this curriculum will see the genomes of their bacterial mutants completely sequenced, putting these 14-year-olds at the cutting edge of biotechnology and introducing them to the 21st Century language of genomics.
- Research lead by Haney (#227004) demonstrated that microcystins toxins produced by cyanobacteria in lakes are transferred by irrigation and aerosols to food crops. Microcystins are hepatotoxins, and are also suspected as factors contributing the onset of Amyotrophic Lateral Sclerosis.