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			22%	
212	Pathogens and Nematodes Affecting Plants			7%	
215	Biological Control of Pests Affecting Plants			8%	
311	Animal Diseases			10%	
501	New and Improved Food Processing Technologies			12%	
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			12%	
	Total			100%	

V(C). Planned Program (Inputs)

1. Actual amount of FTE/SYs expended this Program

Year: 2013	Extension		Research	
	1862	1890	1862	1890
Plan	0.0	0.0	2.9	0.0
Actual Paid Professional	0.0	0.0	1.7	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	103848	0
1862 Matching	**1890 Matching**	**1862 Matching**	**1890 Matching**
0	0	116704	0
1862 All Other	**1890 All Other**	**1862 All Other**	**1890 All Other**
0	0	477487	0

V(D). Planned Program (Activity)

1. Brief description of the Activity

NHAES researchers have investigated the several priority issues surrounding human, plant and animal health.

Vibrio bacteria occurs in coastal waters and may grow in oysters; some but not all strains of Vibrio species cause disease in humans. Methods were developed and refined for the detection and enumeration of Vibrio parahaemolyticus and Vibrio vulnificus and their virulence genes in the Great Bay Estuary.

1. "Relay" methods were evaluated by moving newly harvested oysters to locations with low bacterial levels to reduce Vibrio parahaemolyticus from locally grown oysters.
2. Environmental and biological factors associated with reduced concentrations of pathogenic Vibrios were examined in freshly harvested and post-harvest, processed oysters.
3. The multiple genetic changes, which occur in Pseudomonas sp. communities as they adapt as biofilms, were documented.
4. The presence, impacts, and pathways of microcystin toxins exposure in animals and humans were evaluated through a variety of means.
5. The results of various activities were disseminated via scientific, extension, formal, and informal teaching venues .

2. Brief description of the target audience

The target audiences for this work include:

- Vibrio studies: the shellfish industry, public health and shellfish regulatory agencies in New England,
- Microcystins: graduate and undergraduate students, high school students, faculty collaborators, those interested in ALS (Lou Gehrig's Disease), veterinarians, state and regional conservation groups; town planners, decision-makers and conservation commissions, lakes planning commissions, state and federal agencies, lake association members, lake shore residents, public water suppliers, scientists working in related areas, and cooperative extension educators.
- Other research scientists and college students interested in processes related toenvironmental microbiology in relationship to agriculture and water systems.

• Pseudomonas sp. Researchers studying how bacteria evolve diversity and how this contributes to the functioning of surface-associated communities--pathogenic or mutualistic--with the long-range goal of managing biofilms in illness or in health.

3. How was eXtension used?

While eXtension was not used directly for this planned program, two of the research projects in this planned project have an active online presence with resources for the public.

V(E). Planned Program (Outputs)

1. Standard output measures

2013	Direct Contacts Adults	Indirect Contacts Adults	Direct Contacts Youth	Indirect Contacts Youth
Actual	890	1350	50	100

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

Patent Applications Submitted

Year: 2013

Actual: 0

Patents listed

3. Publications (Standard General Output Measure)

Number of Peer Reviewed Publications

2013	Extension	Research	Total
Actual	0	4	0

V(F). State Defined Outputs

Output Target

Output #1

Output Measure

● Number of undergraduate students directly involved in the projects

Year	Actual
2013	23

Output #2

Output Measure

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

Year	Actual
2013	3

Output #3

Output Measure

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

Year	Actual
2013	10

Output #4

Output Measure

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

Year	Actual
2013	10

Output #5

Output Measure

- Number of graduate students directly involved in the research.

Year	Actual
2013	11

Output #6

Output Measure

- Weekly online hits at Feedthedatamonster: a blog including biofilm community evolution research.

Year	Actual
2013	300

Output #7

Output Measure

- Visits to: An-image based key to the Zooplanton of the Northeast, USA Version 5.0.

Year	Actual
2013	57353

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	Number of citizens engaged in educational presentations and workshops related to toxic algal blooms and the potential health impact of microcystins
6	Development of a laboratory infection model to identify virulence factors of the harmful bacteria Vibrio parahamolyticus.

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
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Gastroenteritis infections caused by V. parahaemolyticus (Vp), and death in certain health-compromised individuals caused by V. vulnificus (Vv), are typically associated with shellfish from warmer areas like the Gulf of Mexico. Recent Vp outbreaks in more-northern Pacific and Atlantic waters are evidence of widening infections and have heightened nationwide public health concerns.

What has been done
Not all strains of these bacteria can cause disease. Researchers therefore tested water, shellfish, and sediments for the presence of genes associated with clinical (disease-causing) strains to determine their incidence and where/when/why they are present. This knowledge will help manage shellfish harvesting and processing to reduce public health infection and disease threats.

Results
Recent fieldwork has demonstrated that Vp and Vv in oysters, sediments, and water are present in more areas and for longer periods of time compared with those observed in the 1990's. Elevated temperatures in the Gulf of Maine Region are a likely cause. Those considered to be pathogenic strains were not detected from Great Bay estuary oysters, water or sediments until this year. Pathogenic strains were detected in oysters from MA and CT, collected from areas closed to harvesting, in 2013 because of Vp infections. Detection of these putatively pathogenic strains is significant because they are rarely detected in environmental samples from New England waters.

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

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 Action Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Little is known about where and when it is safe to harvest shellfish in New England coastal waters during warmer months when pathogenic Vibrio species may be present. To reduce economic hardships associated with limiting shellfish harvesting during warm summer months, there is a need for the development of post-harvest treatments of shellfish that reduce bacteria to safe levels and maintain high quality shellfish that can be eaten raw.

What has been done
Long-term (2007-13) surveillance of the occurrence and levels of Vibrio parahaemolyticus, V. vulnificus, and V. cholerae in oysters, sediment, and overlying waters in the Great Bay estuary of New Hampshire and in Maine show they are present longer, in more areas, and at higher levels compared to the 1990?s. Oyster relaying?moving newly harvested oysters to locations with low bacterial levels for several weeks?has been introduced to reduce pathogenic loads of Vibrio in locally harvested oysters.

Results
State shellfish industries in New England can use the results of this work to design Vibrio surveillance programs to track conditions where shellfish can be safely harvested without undue risk of disease from pathogenic Vibrio species. Postharvest relaying provides shellfish harvesters with a strategy for continuing the harvesting of shellfish even during those times during the year when pathogenic Vibrio species may otherwise limit harvesting.

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

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
2013	10

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

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
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Biofilms are likely the predominant mode of life for bacteria, yet the ecology, diversity, and physiology of these structured communities remains mostly unknown. Understanding how diversity contributes to the functioning of surface-associated communities?pathogenic or mutualistic?is an essential next step toward the long-range goal of managing biofilms in illness or in health.

What has been done
An experimental evolution method was used that allowed diverse biofilm communities of Pseudomonas species to evolve under defined conditions. New mutations that arose in this system were characterized by next generation DNA sequencing and bioinformatic analysis.

Results
1. Adapting Pseudomonas populations are dynamic, genetically and phenotypically, due to strong biotic interactions between members.

2. Experimentally evolved populations converge on several traits commonly observed during chronic lung infections, including modification to Type IV pili and RNA polymerase.

3. Adapting Pseudomonas biofilms repeatedly evolve an elevated mutation rate, or hypermutation, more than expected by chance alone.

4. A molecular mechanism of adaptation, which enabled the fixation of hypermutation in one replicate population, also evolved a mutation in the ppk gene that alters phosphate storage.

4. Associated Knowledge Areas

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

Outcome #5

1. **Outcome Measures**

   Number of citizens engaged in educational presentations and workshops related to toxic algal blooms and the potential health impact of microcystins

2. **Associated Institution Types**

   - 1862 Research

3a. Outcome Type:

Change in Condition Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	246

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)

What has been done

Results

4. Associated Knowledge Areas

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

Outcome #6

1. **Outcome Measures**

   Development of a laboratory infection model to identify virulence factors of the harmful bacteria Vibrio parahamolyticus.

2. **Associated Institution Types**

- 1862 Research

3a. Outcome Type:

Change in Knowledge Outcome Measure

3b. Quantitative Outcome

Year	Actual
2013	0

3c. Qualitative Outcome or Impact Statement

Issue (Who cares and Why)
Currently researchers are unable to definitively determine whether shellfish harbor harmful Vibrio parahaemolyticus because of a lack of understanding of the characteristics of virulent strains. Identification of virulence characteristics that define infectivity or transmissibility could inform specific diagnostic detection protocols, which?when implemented as part of a management plan--could aid in ensuring shellfish is safe for consumption. Virulence assessment requires laboratory infection models, which are currently lacking.

What has been done
Alternative laboratory models (fruit flies, mice, nematodes, Arabidopsis) were evaluated for laboratory infection systems to quantify virulence of different Vibrio isolates. Two promising model systems?nematodes and the plant Arabidopsis thaliana?were further optimized for the assay system.

Results
Nematodes proved to be a highly reproducible laboratory model for virulence in Vibrio parahaemolyticus. This model was used for the evaluation of natural isolates from both clinical and environmental sources, and for screening mutant bacteria for reduced or attenuated virulence.
Mutations that reduce virulence are potential diagnostics for virulence factors. The laboratory model was used to confirm a prior hypothesis that normal human body temperature is an important cue in triggering virulence by infective strains?but not in harmless environmental strains?, further indicating that virulence traits may be identified by comparison of gene expression at normal human body temperature.
UNH investigators are collaborating with a researcher at the FDA lab in Dauphine Island to transfer the nematode protocols, so that the laboratory model will be more widely used and potentially applied in assaying oyster samples for pathogens as a biological surveillance mechanism.

4. Associated Knowledge Areas

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

V(H). Planned Program (External Factors)

External factors which affected outcomes

- Appropriations changes

Brief Explanation

The NHAES suffered a ~23.9% cut in state funds in the budget that ran through FY13. This has necessitated cuts in investigator support budgets and funding for students/postdocs.

V(I). Planned Program (Evaluation Studies)

Evaluation Results

The success of research projects in this planned program is measured in several ways:

- For new projects, is there evidence of progress toward objectives?
- Research projects in this planned program all demonstrated significant progress toward their objectives.
- For more mature projects, have results been published in peer reviewed journals? Do project directors participate in national and international conferences?
- Researchers for all projects have actively participated in regional, national, and international conferences.
- Where appropriate are outcomes/protocols, etc. disseminated to stakeholders or adopted by the public safety agencies.
- The new laboratory model, developed by NHAES researchers, for evaluating the virulence of natural Vibrio isolates has been transferred to Food and Drug Administration labs in order to improve monitoring for the presence of pathogenic Vibrio in oysters.
- Results have been broadly disseminated on a local, regional, and international level.
- The testing protocols of the microcystin toxin study are being widely disseminated to regulatory agencies throughout New England.
- Collaborations with NHAES researchers have been initiated with public safety agencies across New England, related to monitoring for and reducing potential infections loads of Vibrios in oysters.

The NHAES believes research conducted under the auspices of this program have been very effective by the measures listed above.

Key Items of Evaluation

- New methods have been developed for the detection of virulent strains of pathogenic

Vibrios that may be associated with oysters and other mollusks, and cause serious human disease.

- A new laboratory model system has been developed for the analysis of virulence factors in Vibrios, naturally occurring bacteria in marine environments that are associated with shellfish-borne diseases.
- The NHAES is working with the NH Department of Environmental Services' Source Water Protection program and town water supply utilities to discuss whether to set state standards for microcystins in fresh water lakes. Sampling protocols developed by NHAES researchers will be used in monitoring.