FINAL PROGRESS REPORT

Project Title	Safe feedstocks for bivalve aquaculture
Reporting Period	9/1/15 – 8/31/18
Author (Project Coordinator)	Dr. David Rowley, University of Rhode Island
Key Word	Probiotic, vibrio, shellfish, hatchery
Funding Level	$194,000
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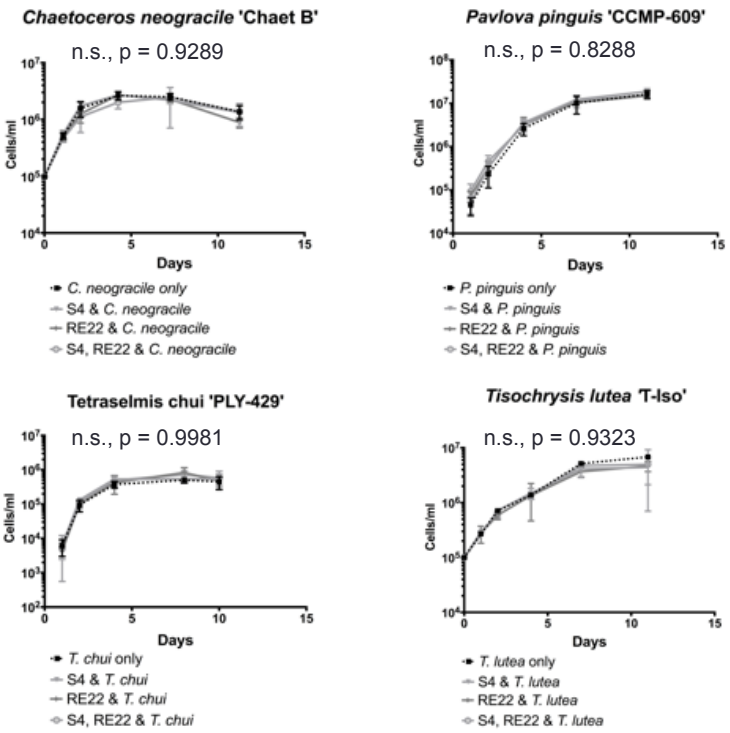
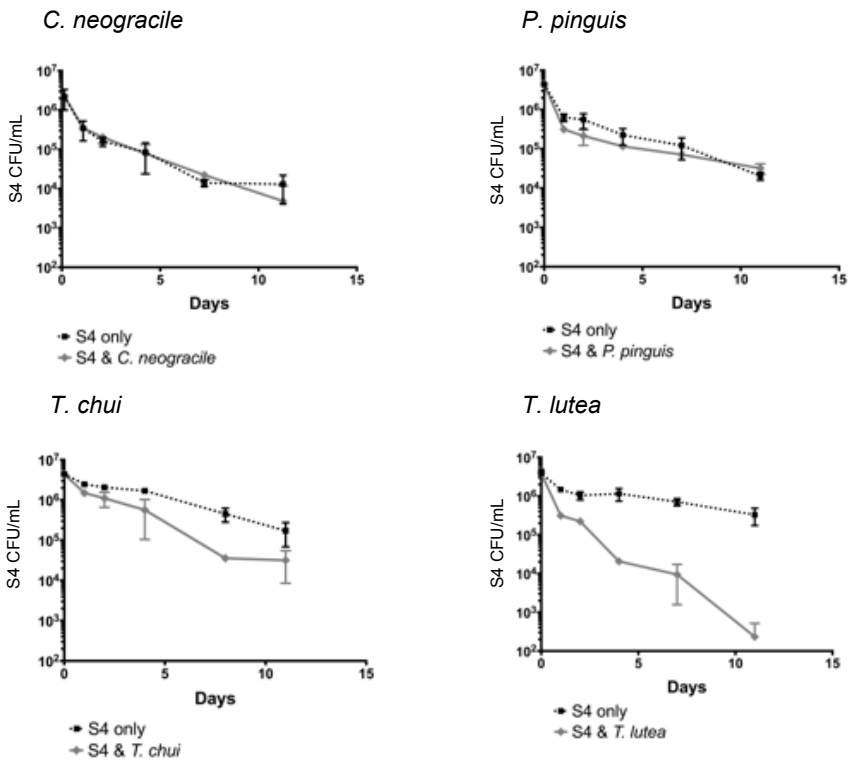
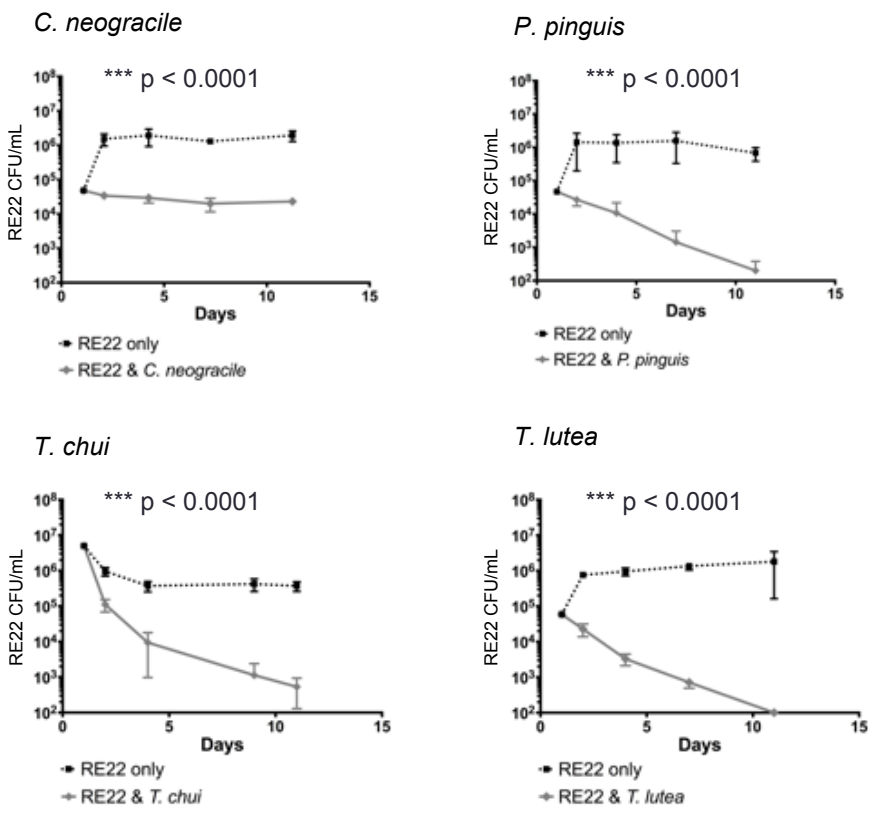
Project Objectives	Objective 1 : Measure the reduction of vibrios and other potentially pathogenic bacteria in algal cultures following the addition of the two beneficial bacterial probiotic strains. Objective 2: Measure the growth and survival of algae in response to probiotic treatment Objective 3: Measure the survival of bivalve larvae that are fed probiotic-treated algae prior to being challenged with bacterial pathogens. Objective 4: Outreach and Extension: The results of this research will reach potential end users through direct collaboration, workshops, and meetings with commercial hatchery managers.
Anticipated Benefits	State briefly how the project will benefit the aquaculture industry – directly or indirectly. Hatchery production of shellfish seed is required to supplement natural recruitment and meet rising industry demand for oysters, scallops, and hard clams. Despite considerable measures aimed at the elimination of bacterial pathogens from aquaculture systems, disease continues to be a major impediment to reliable larviculture in hatcheries. Microalgal feedstocks can be a persistent source of bivalve pathogens. Since shellfish ingest microalgae, attached pathogens can be delivered directly to the larvae where they can initiate disease outbreaks. The addition of probiotic microorganisms is a promising strategy to eliminate pathogenic bacteria from feedstocks, promote larval growth, and improve disease resistance. However, no probiotic additives have yet been commercially developed specifically for bivalve aquaculture in the Northeast United States. Our research has uncovered promising probiotic bacteria to help prevent disease outbreaks in hatcheries. This research will investigate the use of probiotics to prevent/eliminate pathogens in algal feedstocks.

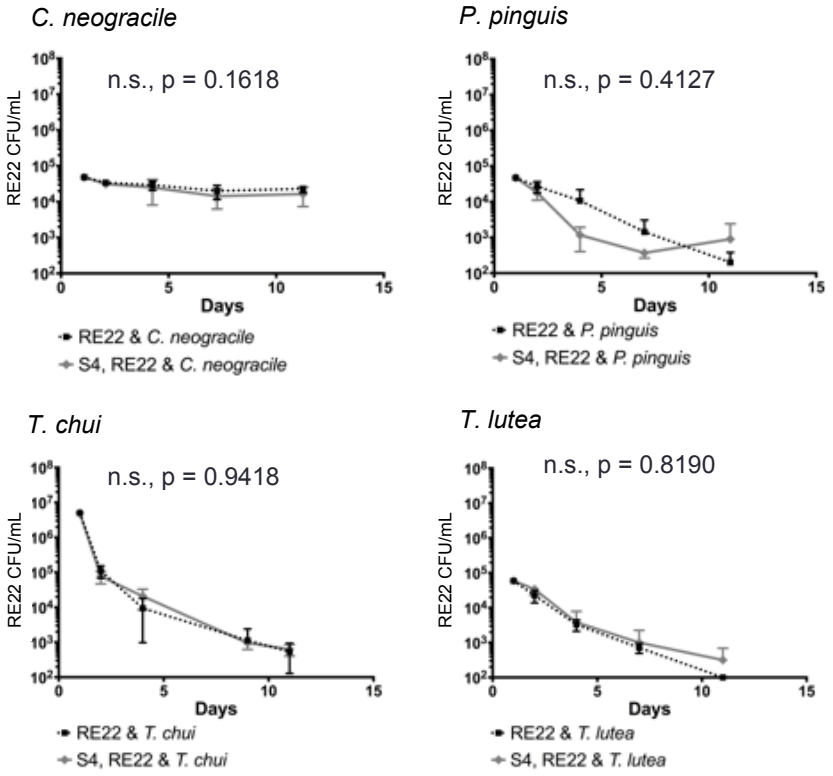
Project Progress	Major findings: 1. Probiotic bacteria do not adversely impact the growth and survival of microalgal feedstocks. 2. Microalgae used as feedstocks decrease the abundance of both probiotic and pathogenic bacteria under co-culture conditions. 3. Microalgae reduce the numbers of probiotic bacteria to below the threshold necessary for achieving a beneficial impact when added to shellfish larvae. Therefore, this method does not appear to be the most advantageous method for providing probiotics to the larvae. 4. Adding probiotic bacteria did not reduce the abundance of vibrios in microalgal cultures beyond the observed reductions due to interactions with the microalgae alone. Specific data supporting the above findings is presented below. Four different microalgal feedstocks were cultured in the presence of the probiont *Phaeobacter inhibens* S4 and/or the shellfish pathogen *Vibrio coralliilyticus* RE22. Neither bacterium had an effect on algal growth or survival (Objective 2; see figure below).  Probiont S4 cell abundance in culture water was negatively impacted by certain microalgal cells (see figure below).



Vibrio abundance was also negatively impacted by microalgal cells (see figure below).



Probiotic additions to microalgal cultures do not further decrease vibrio abundance (Objective 1). See figure below.



Accomplishments:

Outreach Overview	The primary benefactors to this study are commercial shellfish hatcheries throughout the NRAC region and nationally. We have communicated one-on-one with participating hatcheries in the region to test and optimize the methods of delivery of probiotics. Three participants in this project (Leavitt, Smolowitz and Gomez-Chiarri) are in regular communication with shellfish growers and hatcheries throughout the NRAC region. In a more generic outreach effort, presentations were made at aquaculture meetings. We will also ensure that the NRAC Aquaculture Extension community is aware of the details of the probiotic application and can assist us in outreaching the program to hatcheries or other interested parties within their states.
Targeted Audiences	Provide information on the **target audience** for efforts designed to **cause a change in knowledge, actions, or conditions**. The main target audience for this project is **research and commercial shellfish hatchery managers**. The goal of our research and outreach efforts is to provide hatchery managers with environmentally friendly, economic, and effective tools to mitigate and prevent the impacts of infectious diseases in shellfish hatcheries. Our research and outreach efforts also target **aquatic pathologists and microbiologists**, by

	providing knowledge about potential mechanisms of action of these probiotics, knowledge that is fundamental for the rational use of effective and safe probiotics.
Outputs:	Outputs are tangible, measurable products (website, events, workshops, products [AV, curricula, models, software, technology, methods, websites, patents, etc.], trainees, etc.). Do NOT include publications as they're listed separately. Outputs from this research included presentations at scientific conferences, and training of 4 graduate students and 2 undergraduate students. This research will help lead to 3 Ph.D. dissertations and 1 MS. thesis. In addition, we have established a Materials Transfer Agreement with two companies that are interested in licensing our probiotic bacteria.
Outcomes/Impacts:	The general outcome of this research is the development of an environmentally friendly, economic, and effective method for the management of infectious diseases in bivalve shellfish hatcheries. This method will serve as an alternative to antibiotic treatments (not allowed by FDA regulations) and the need for expensive water treatment systems. We anticipate that our probiotic bacteria will result in two commercial products for the prevention of bacteria diseases in shellfish hatcheries. We are currently negotiation with two interested companies regarding licensing.
Impacts Summary	1. **Relevance:** Issue – what was the problem? The proposed project is aimed at the development of highly promising marine microorganisms as probiotic additives to reduce and/or eliminate the presence of pathogens in microalgal feedstocks used for shellfish larviculture. *No probiotic agents are currently commercially available specifically for shellfish aquaculture in Northeast US.* 2. **Response:** What was done? We tested the addition of probiotic bacteria to microalgal feedstocks to determine if there was (1) an adverse impact on microalgal growth and (2) if pathogenic bacteria could be eliminated from the microalgae. 3. **Results:** How did your work make a difference **(change in knowledge, actions, or conditions)** to the target audiences? The probiotic bacteria are safe to the algal cultures. However, the abundance of the probiotic bacteria did not remain high enough to use microalgal treatment as a means to treat bivalve

	larvae. Nevertheless, the direct probiotic treatments of the larvae have resulted in licensing discussion with two companies. We are optimistic that our probiotic bacteria will result in a new commercial tool for hatcheries in the near future. 4. **Recap:** One- sentence summary *This project aims to eliminate shellfish pathogens that can persist in microalgal feedstocks.*
Publications	Hughes, Samuel; Chesler, Amanda; Rowley, David; Nelson, David; Gomez-Chiarri, Marta. "Coculture of probiotic bacteria in algal feedstocks for disease management in bivalve hatcheries". National Shellfisheries Association Annual Meeting, 18-22 March 2018, Renaissance Hotel, Seattle, WA. Poster presentation. Hughes, Samuel; Brooks, Kathryn; Chesler, Amanda; Rowley, David; Nelson, David; Gomez-Chiarri, Marta. "Coculture of probiotic bacteria in algal feedstocks for disease management in bivalve hatcheries". Northeast Aquaculture Conference and Exposition, 11 January 2019, Boston Park Plaza Hotel, Boston, MA. Oral presentation. Upcoming article is now in press in Fish Farming News (http://fish-news.com/ffn/).
Students/Participants:	• Name: Tejashree Modak • Whether Degree was completed during the reporting period: PhD, yes • New or Continuing Student: continuing • Date of Graduation: Dec 2018 • Name: Samuel Hughes • Whether Degree was completed during the reporting period (name, yes/no): MS, no • New or Continuing Student: new • Date of Graduation: May 2019 • Name: Katheryn Brooks • Whether Degree was completed during the reporting period: BS Marine Biology, no • New or Continuing Student: new • Date of Graduation: May 2019 • Name: Christopher Schuttert • Whether Degree was completed during the reporting period: PhD Cell and Molecular Biology, no • New or Continuing Student: continuing • Date of Graduation: May 2020 • Name: Eisen Villaluna

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Whether Degree was completed during the reporting period: BS, 2018
New or Continuing Student: continuing
Date of Graduation: May 2018

Name: Rebecca Stevick
Whether Degree was completed during the reporting period: PhD Oceanography, no
New or Continuing Student: continuing
Date of Graduation: Dec 2019

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<tr><td rowspan="2">Partnerships</td><td colspan="4">List any partners that you worked with on your project. Provide the following information for each Partner:</td></tr>
<tr><td>Partner</td><td>Specific Type
Type</td><td>Level
Level</td><td>Nature of Partnership</td></tr>
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