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## NRAC ANNUAL PROGRESS REPORT

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**INSTRUCTIONS:** An annual progress report for each funded project must be provided to NRAC by June 30 of each year.

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| <b>Project Title</b>                                      | Oyster Microbiome Changes Resulting From Different Winter Storage Methods  |
| <b>Reporting Period</b>                                   | <u>05/31/2020- 6/30/2021</u>   |
| <b>Author (Chair)</b>                                     | Heidi Yeh  |
| <b>Key Word</b>   | Oyster aquaculture, Overwintering methods  |
| <b>Funding Level</b>                                      | \$14623.63   |
| <b>Participants: Names, Institutions and Contact Info</b> | <p>Project Coordinator: Heidi Yeh, Rutgers U,<br/><a href="mailto:Heidi.yeh@marine.rutgers.edu">Heidi.yeh@marine.rutgers.edu</a></p> <p>Principal Investigator: David Bushek, Rutgers U,<br/><a href="mailto:bushek@hsrl.rutgers.edu">bushek@hsrl.rutgers.edu</a></p> <p>Cooperating/Non-funded: Lisa Calvo, Rutgers U,<br/><a href="mailto:lcalvo@hsrl.rutgers.edu">lcalvo@hsrl.rutgers.edu</a> ; Elizabeth Haskin, Betsy's Cape Shore Salt Oyster Farm, <a href="mailto:betsy_haskin@yahoo.com">betsy_haskin@yahoo.com</a></p> |
| <b>Project Objectives</b>                                 | <ol style="list-style-type: none"><li>1. Test overwintering methods (held on flats, in a marina, and in cold storage) to determine impact on mortality, oyster condition, and Dermo disease status.</li><li>2. Quantify bacteria present on oysters and in adjacent water samples.</li><li>3. Characterize the microbiome composition of the oysters using next-generation DNA sequencing methods.</li></ol>   |

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| <b>Anticipated Benefits</b> | <p>State briefly how the project will benefit the aquaculture industry – directly or indirectly.</p> <p>Providing alternative overwintering methods with which oyster farmers can protect against destruction from winter weather will benefit farmers who experience large annual losses. Another potential trigger for losses are the transfers that are carried out between the overwintering condition and the grow-out condition, so this study examines any mortality effect as well as the impact on the bacterial community to observe any short or longterm changes that may result from this disruption. Insights gained on the microbiome changes that occur in long-term dry storage would also contribute to our understanding of the bacterial dynamics at play when oysters are shipped to market.</p>   |
| <b>Project Progress</b>     | <p>Summarize concisely for each objective the progress toward accomplishment to date. This has an 8,000 character limit.</p> <ol style="list-style-type: none"> <li>1. The fieldwork for this experiment was completed during the winter of 2019-2020. Results for mortality, oyster condition, and Dermo disease status were presented as a poster at the annual conference of the National Shellfisheries Association.</li> <li>2. The original plan to quantify bacteria using plate incubations had to be scrapped due to the onset of the COVID-19 pandemic, but we anticipate being able to recreate this data from the DNA through qPCR analysis, anticipated to start next month.</li> <li>3. We decided to perform the sequencing in-house using Oxford Nanopore Sequencing technology. This is the first-ever application of this novel technology to the oyster microbiome. About 2/3 of oyster and water samples have had DNA extracted, and a successful test run of the Nanopore was performed, the results of which were presented as part of a dissertation proposal. Sequencing should begin in earnest next week, and we anticipate all samples being processed this summer in time to be presented at conferences during the academic year.</li> </ol> |
| <b>Accomplishments:</b>     |   |
| <b>Outreach Overview</b>    | <p>Describe in general how your results have been extended to the intended users. OR, if they haven't yet, explain when &amp; how this will occur.</p> <p>Lisa Calvo will provide assistance and guidance to provide effective outreach to local and regional oyster farmers. We are targeting one of the monthly growers' forums for an informal presentation, as well as the biennial Northeast Aquaculture Conference &amp; Exposition (NACE) in January 2022.</p>   |

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| <b>Targeted Audiences</b> | <p>Provide information on the <b>target audience</b> for efforts designed to <b>cause a change in knowledge, actions, or conditions.</b></p> <p>Growers in the northeast and mid-atlantic region who wish to avoid damage to their crops can consider cold storage as a viable alternative to intertidal or tidal storage of oysters.</p>  |
| <b>Outputs:</b>           | <p>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.</p> <p>A graduate student was trained in conducting research on an oyster farm and in molecular protocol development.</p>   |
| <b>Outcomes/Impacts:</b>  | <p>Describe how findings, results, techniques, or other products that were developed or extended from the project generated or contributed to an outcome/impact. <b>Outcomes/impacts are defined as changes in Knowledge, Action, or Condition.</b></p> <p>To be determined after we perform outreach activities.</p>  |
| <b>Impacts Summary</b>    | <p>Provide short statements (2-3 sentences) about each of the following: (pre-established fields for Researchers to complete short statement answers)</p> <ol style="list-style-type: none"> <li>1. <b>Relevance:</b> Issue – what was the problem?<br/>Variable winter weather poses a threat to oyster crops exposed to the elements. There is anecdotal evidence that long-term storage in a cooler may be an effective way to circumvent damage, but the resulting mortality and impact on the product had not been rigorously tested. Introducing more transfers into the production life cycle of oysters also carries the risk of long-term disruption to the oysters and the bacteria that live on them.</li> <li>2. <b>Response:</b> What was done?<br/>Oysters were stored in three different conditions (intertidal, subtidal, and in temperature-controlled dry storage) and assessed for differences in mortality, condition, and disease status. Samples were collected to assess changes to the bacteria living in the oysters using next-generation sequencing methods.</li> <li>3. <b>Results:</b> How did your work make a difference (<b>change in knowledge, actions, or conditions</b>) to the target audiences?<br/>The trial of different overwintering methods confirms that there is no significant difference in survival, condition, or disease-status. The microbiome analysis at the core of this particular study has yet to be completed.</li> <li>4. <b>Recap:</b> One- sentence summary<br/>Different methods for overwintering oyster crops (intertidal, subtidal, and cooler storage) were compared with a specific focus on short- and long-term impact on the bacteria that live with oysters.</li> </ol> |
| <b>Publications</b>       | <p>Follow the format to list publications in the following categories:</p> <ul style="list-style-type: none"> <li>• Presentations: <ul style="list-style-type: none"> <li>○ Oral</li> </ul> </li> </ul>  |

|                               | <ul style="list-style-type: none"> <li>○ Posters: Overwintering alternatives for Eastern oysters (<i>Crassostrea virginica</i>) farmed in Delaware Bay; Aislinn Mohyla, Elizabeth Haskin, Mitch Tarnowski, and David Bushek; the National Shellfisheries Association 2021 annual meeting.</li> <li>● Peer-reviewed: <ul style="list-style-type: none"> <li>○ Print (journal, etc.)</li> <li>○ Digital (websites, videos, etc.)</li> </ul> </li> <li>● Non-Peer-reviewed: <ul style="list-style-type: none"> <li>○ Extension factsheets</li> <li>○ Popular articles</li> </ul> </li> </ul>  |                |  |              |                              |                  |        |       |  |
|-------------------------------|--|----------------|--|--------------|------------------------------|------------------|--------|-------|--|
| <b>Students/Participants:</b> | <p>Provide the following information for <b>every</b> student that worked with you during the reporting period:</p> <ul style="list-style-type: none"> <li>● Name: Heidi Yeh</li> <li>● Whether Degree was completed during the reporting period (name, yes/no): Heidi Yeh, no</li> <li>● New or Continuing Student: Continuing</li> <li>● Capstone/Thesis Title (actual or anticipated): 2022</li> <li>● Date of Graduation: 2022</li> <li>● Provide link to thesis/dissertation document:</li> </ul>   |                |  |              |                              |                  |        |       |  |
| <b>Partnerships</b>           | <p>List any partners that you worked with on your project. Provide the following information for each Partner:</p> <table border="1" data-bbox="550 1150 1430 1346"> <thead> <tr> <th data-bbox="550 1150 745 1188"><b>Partner</b></th> <th data-bbox="745 1150 972 1188"><b>Specific Type</b></th> <th data-bbox="972 1150 1200 1188"><b>Level</b></th> <th data-bbox="1200 1150 1430 1226"><b>Nature of Partnership</b></th> </tr> </thead> <tbody> <tr> <td data-bbox="550 1188 745 1346">Elizabeth Haskin</td> <td data-bbox="745 1188 972 1346">Farmer</td> <td data-bbox="972 1188 1200 1346">Level</td> <td data-bbox="1200 1226 1430 1346">Helped to design the study and collect samples</td> </tr> </tbody> </table> | <b>Partner</b> | <b>Specific Type</b>                           | <b>Level</b> | <b>Nature of Partnership</b> | Elizabeth Haskin | Farmer | Level | Helped to design the study and collect samples |
| <b>Partner</b>                | <b>Specific Type</b>   | <b>Level</b>   | <b>Nature of Partnership</b>                   |              |                              |                  |        |       |  |
| Elizabeth Haskin              | Farmer   | Level          | Helped to design the study and collect samples |              |                              |                  |        |       |  |