FINAL REPORT

Project Title	Development and evaluation of novel, non-toxic solutions for biofouling control and predator exclusion in shellfish aquaculture
Reporting Period	01-Sept-2015 – 31-Aug-2018
Author (Chair)	Sandra E. Shumway
Key Word	Aquaculture, biofouling, prevention, shellfish
Funding Level	Total funds allocated for this project to date. *NOTE: This could be reported by Year. i.e.,* *Year One: FY 2016, $ amount* $91,268 *Year Two: FY 2017, $ amount* $102,314
Participants	**Name(s)/Role(s):** Sandra E. Shumway/PI Institution/Agency/Business: University of Connecticut Address(s): Dept. Marine Sciences, 1080 Shennecossett Road, Groton, CT 06340 Phone(s): 860-405-9282 Email(s): Sandra.shumway@uconn.edu Funded (Yes/No): yes **Name(s)/Role(s):** Stephan Bullard/PI Institution/Agency/Business: University of Hartford Address(s): University of Hartford, 200 Bloomfield Ave, West Hartford, CT 06117 Phone(s): 860-803-6423 Email(s): Bullard@hartford.edu Funded (Yes/No): yes **Name(s)/Role(s):** Tessa Getchis/PI Institution/Agency/Business: Connecticut SeaGrant Address(s): Connecticut SeaGrant, 1080 Shennecossett Road, Groton, CT 06340 Phone(s): 860-405-9104 Email(s): tessa.getchis@uconn.edu Funded (Yes/No): yes **Name(s)/Role(s):** Alex Walsh/Subcontractor Institution/Agency/Business: smartPAINT, Inc. Address(s): 25 Research Road, East Falmouth, MA 02536 Phone(s): 800-258-5998 Email(s): alex@epaint.net Funded (Yes/No): yes
Project Objectives	1) Develop and refine new coatings to prevent the development of biofouling and predation on aquaculture gear; 2) Assess the potential toxicity of the new coatings. The base materials being tested have previously been shown to be non-toxic and are all cleared with FDA and EPA regulations; however, it is important to test any new configurations to confirm non-toxic status; 3) Assess the adhesion of the newly developed formulations on test panels and

	gear; 4) Assess the efficacy of newly developed coatings on test panels and gear at 4 locations (ME, MA, CT, and NH) and other farms as possibilities permit (see letters of support); 5) Assess the ability of coatings to deter predation; 6) Engage aquaculture producers in research and outreach, and disseminate the results to the industrial and scientific communities through presentations at workshops, conferences, outreach publications, web page, and peer-reviewed publications.
Anticipated Benefits	Biofouling is one of the most labor intensive aspects of shellfish aquaculture, and a significant amount of time and economic resources are devoted to removing biofouling from both gear and the cultured shellfish. Biofouling significantly impacts water flow, and therefore, biofouling requires constant attention, especially during warmer months and with gear closer to the top of the water column. The majority of the time, removing biofouling is the primary reason for handling the gear, and therefore results in increased expenses, and therefore reduced profitability. Shellfish farmers often are looking for innovative ways to deal with the issue of biofouling; however, the methods currently available are either toxic to the environmentally sensitive filter feeders, or require large amounts of labor. A new method which does not require periodic dips, environmentally toxic substances or manual cleaning would significantly increase productivity and reduce the amount of time spent tending gear. This will result in higher profit per unit effort, which will result in greater investment, greater expansion of the industry, and a greater chance at economic viability of shellfish farms throughout the Northeast.
Project RESULTS	OBJ. 1, 3 and 4 Develop and refine new coatings and assess the adhesion of the newly developed formulations on test panels and gear; Assess efficacy of newly developed coatings on test panels and gear NOTE: A FULL SET OF PHOTOGRAPHS FROM ALL SITES OVER ALL YEARS WILL BE ARCHIVED AND AVAILABLE UPON REQUEST. **Coating Development** Coatings development efforts in 2017 shifted from using chitosan-based polymers to hyper-branched polyurethane chemistries. The reason for the change is concern regarding the toxicity of residual acrylic monomers used for chitosan polymer synthesis to shellfish. The high costs of these chitosan-based polymers also prohibit practical use in aquaculture. Novel polyurethane coatings were developed with dendritic-like morphology that swell when immersed in water to reveal functional polymer groups useful for biofouling control. Polyurethane resins used to make experimental antifouling treatments differ by being either aliphatic or aromatic. All polyurethanes are supplied in water as

zero to low VOC solutions. Attempts were made to increase the bioactivity of polyurethane chemistries by adding menthol-like monomers to the polymer backbone. Other attempts to synthesize a low-cost polyurethane were made using soy-based polyols. Test solutions were applied to PVC test panels for adhesion and biofouling resistance testing.

Test Surfaces

Test surfaces (PVC panels) supplied by UCONN were coated with experimental coating formulations. Seven experimental coatings were applied to test surfaces including three commercially available products marketed to the aquaculture industry. Thirty six PVC panels were coated for each of the seven test coatings. Panels were numbered for identification purpose using sheep tags purchased from HASCO TAG CO (Dayton, KY). Panels were then weighed. Experimental coatings were applied to test panels by spray application using a 3M HVLP Accuspray Gun (model HG09). Two coats of test formulas were applied to test panels. Coatings were dried for 8 hours @ 20ºC between coats. Coating thickness (dry film) averaged 120µm. The coatings were dried for >24 hours @ 20ºC and then each panel was reweighed. The total dry film per cm^2 was calculated for each test surface. Coated test surfaces were delivered to UCONN for biofouling resistance testing. Coating adhesion to PVC panels was tested.

Descriptions of each coating are presented in Table 1.

Table 1. Descriptions of Experimental Coatings

Treatment	Experimental Coatings
Group 1	FLEX XI is a copper-based antifouling paint manufactured by Flexdel Corp. for use on salmon net pens.
Group 2	OysterCoat is a zinc-based antifouling paint manufactured by Flexdel Corporation for use on oyster cages.
Group 3	NETMINDER is a photoactive release coating manufactured by Flexdel Corporation for use on oyster cages.
Group 4	Experimental hyper-branched polyurethane release coating developed by smartPAINT, Inc.
Group 5	Experimental bioactive polyurethane release coating based on menthol developed by smartPAINT, Inc.
Group 6	Experimental soy-based polyurethane release coating developed by smartPAINT, Inc.
Group 7	Experimental soy-based polyurethane release coating developed by smartPAINT, Inc.

Adhesion Testing

Adhesion of experimental coatings and controls to PVC panels was measured by following ASTM D-3359-09, *Standard Test Methods for Measuring Adhesion by Tape Test, Method A.* PVC is used in aquaculture as a wire coating. Wire is used for cages and tray fabrication. A multi-tooth cutter blade is used to scribe a grid (6x6 square) in the test coating surface down to the PVC substrate. Permacel™ adhesive tape manufactured by **Nitto Denko Company** of New Brunswick, NJ is specified for the test. Permacel™ is applied to the scribe area and then pulled off the surface in a swift steady motion. Removal of the test coating from the scribed area is rated. Classification of tape adhesion results are described in **Table 2**.

Table 2. ASTM D 3359, Method B - Adhesion Classification Criteria

METHOD B Rating	Post Tape Pull-off Description
5A	No Peeling or removal.
4A	Trace peeling or removal (<5% removal)
3A	Removal of 5-15% of test coating from the grid area
2A	Removal of 15-35% of test coating from the grid area
1A	Removal of 35-65% of test coating from the grid area
0A	Greater than 65% removal from the grid area

Three tests were performed for each test surface. Results from testing adhesion of experimental coatings and controls to PVC are presented in **Table 3.**

Table 3. Adhesion of Experimental Coatings and Controls by ASTM D 3359, Method B

Treatment	Adhesion Rating by ASTM D3359 (Tape Adhesion Test), Method B		
	T1	T2	T3
Group 1	2B	1B	2B
Group 2	5B	5B	5B
Group 3	5B	5B	5B
Group 4	0B	2B	1B

Group 5	5B	4B	4B
Group 6	2B	1B	0B
Group 7	2B	1B	0B

Adhesion of Flexdel XI (Group 1) manufactured by Flexdel Corporation (Lakewood, NJ) to PVC is poor. This copper-based antifouling paint is designed to release copper from a soluble acrylic vehicle. The coating is designed to fall apart over time in water so poor adhesion visible in Figure 1 is not surprising of this sacrificial coating.

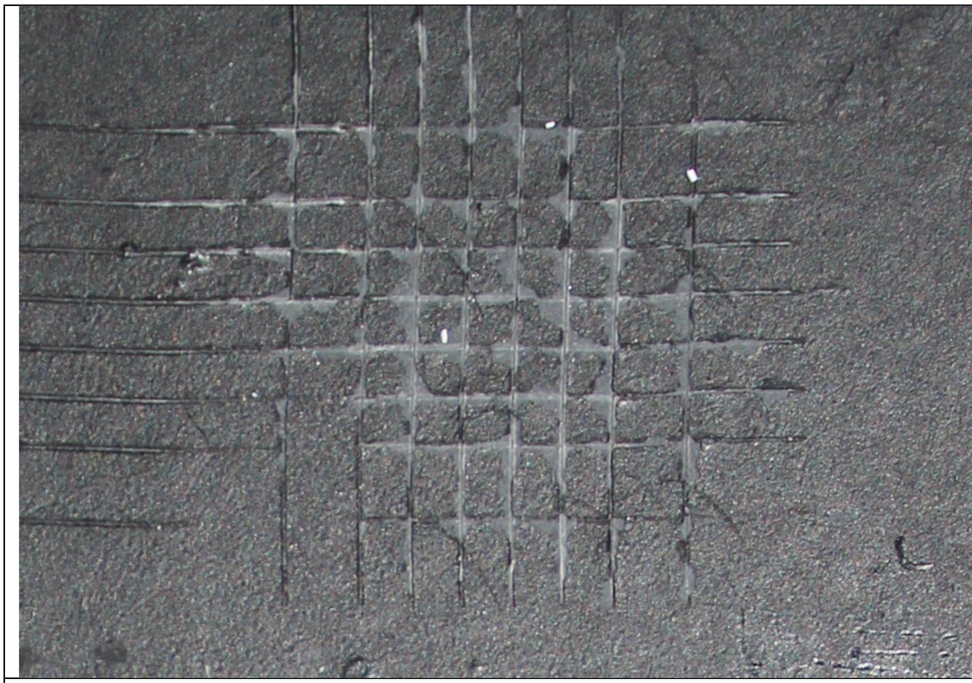

Figure 2. Flexdel Corp FLEX XI (Group 1) adhesion rating 2B by ASTM D3359.

Adhesion of the zinc oxide-based Oyster Coat, also manufactured by Flexdel Corporation, is excellent. Oyster Coat provides a hard acrylic coating that adheres extremely well to PVC.

NETMINDER® (Group 3) also adheres extremely well to PVC. This formula is based on an aliphatic polyurethane vehicle which yields a hard and mar-resistant coating with excellent adhesion to PVC.

The experimental coating used to coat Group 4 panels is based on an experimental hyperbranched polyurethane polymer. This polymer forms a durable film but adhesion to PVC is poor with an average adhesion rating of 1B.

The bioactive experimental polymer was used to coating Group 5 panels adheres well to PVC rating 4-5B. Less than 5% coating damage due to tape removal was observed as visible in Figure 2.

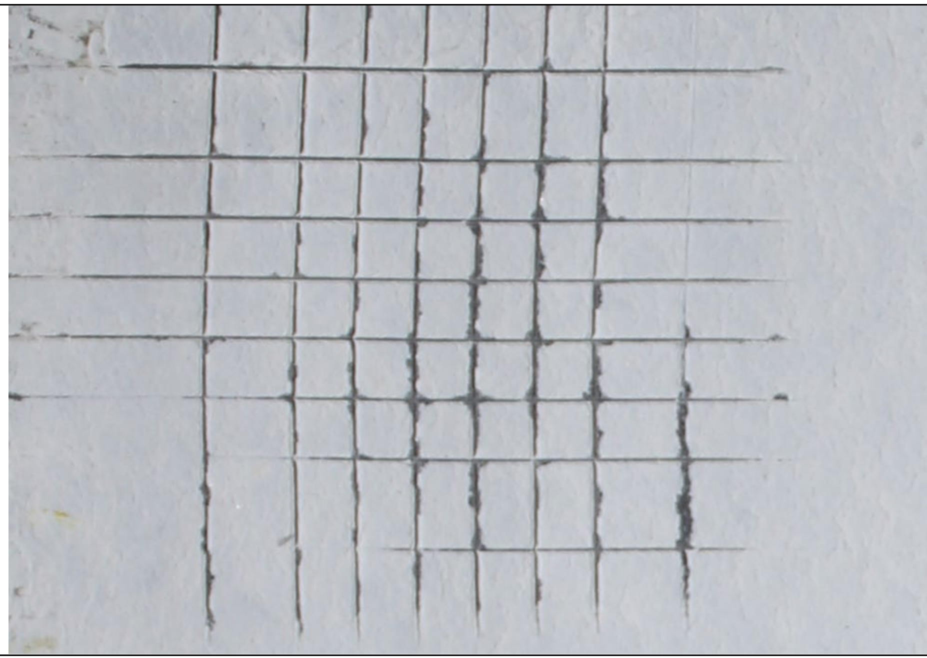

Figure 2. smartPaint test coating 259-45 (Group 5) adhesion rating 4B by ASTM D3359.

The Group 6 coating is based on an experimental soy-based polymer. This soy-based polymer does not adhere to PVC rating 0B.

Group 7 coating is also based on an experimental soy-based polymer which does not bond well to PVC.

Biofouling Testing

PVC coated panels treated with experimental coatings were deployed from the UConn dock (Groton, CT) mid-August, 2017. After three months exposure, experimental coatings developed by smartPAINT, Inc. that are based on non-toxic polyurethane release coating chemistries are resisting biofouling as well as the copper-based pesticide FLEX XI manufactured by Flexdel Corporation. Percent biofouling coverage on test surfaces (average of three panels per treatment) after three months exposure in Connecticut waters are presented in Figure 3.

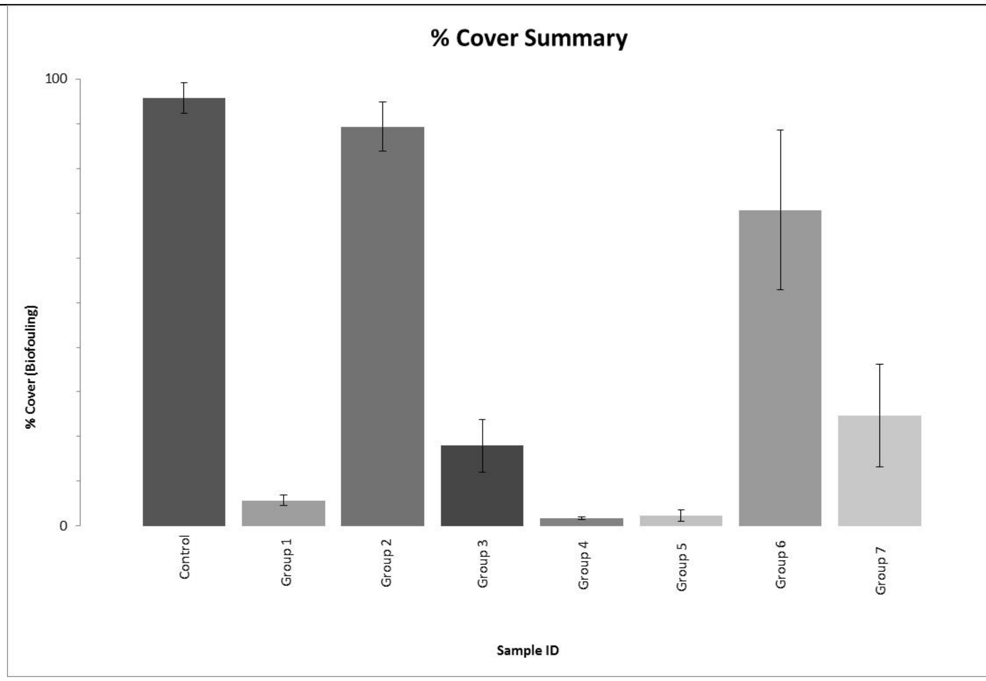

Figure 3. Average % coverage by biofouling on test surfaces after 3 months exposure at UConn Avery Point (Groton, CT)

Untreated test panels (control) are completely covered after three months exposure whereas the copper-based antifouling paint FLEX XI is fouled by a thin film of algae. The zinc-based OysterCoat does not resist biofouling after three months exposure. The photactive biofouling release coating NETMINDER® is active, but not as effective as the experimental polyurethane test coatings from Groups 4 & 5 that were developed by smartPAINT, Inc. Average percent biofouling coverage of Group 4 treated test surfaces is 1.7±0.3%, and 2.3±1.3% for Group 5 treated test surfaces. Biofouling resistance of Groups 4 & 5 treated test surfaces is significantly better than the copper-based antifouling paint FLEX XI. This is a significant achievement considering the non-toxic nature of Groups 4 & 5 experimental coatings.

OBJECTIVE 5

Predator Assays

A primary goal of Phase One is to determine whether test compounds deter predators. We conducted several preliminary predator assays designed to see whether a model predator, the oyster drill (*Urosalpinx cinerea*), would be willing to move across surfaces coated with test compounds. Assays were conducted in 3.8 L aquaria. Aquaria were filled with ~0.5 L of seawater and placed into water tables with flowing seawater to maintain ambient temperature (Fig 1A).

In the first preliminary assay, we assessed whether oyster drills would be willing/able to cross a strip of PVC (5 cm wide, ~2 mm high) to reach cracked oysters. Two aquaria were prepared, both with a strip of PVC dividing the aquaria into two sides. One aquaria was empty, the other had 3 cracked juvenile oysters on one side of the PVC strip. Ten oyster drills that had been starved for 72 hours were placed into one side of each aquaria - the side without the oysters in the second aquaria (Fig 1B). Oyster drills were observed every 15 min for 1.5 hours and then again at 24 hours, and the number of oyster drills on the opposite side of the aquaria from their start position were recorded. At the end of the assay, similar numbers of oyster drills were found on both sides of both aquaria, suggesting that oyster drills would cross the PVC strip. We were concerned, however, that oyster drills could move around the PVC on the side of the tanks, and that securing additional strips to the aquaria sides to prevent this would be challenging. We therefore decided to assess a different experimental design.

In the second assay, we placed 3 cracked juvenile oysters into two aquaria. In the first aquaria, the cracked oysters were simply placed on one side of the tank. In the second aquaria, the cracked oysters were placed on a platform formed by five parallel PVC strips (Fig 1C). Ten oyster drills that had been starved for 72 hours were placed into the two aquaria on the opposite side from the oysters. The number of oyster drills feeding on the oysters was recorded after 24 hours. At the end of the assay, a total of 6 oyster drills were feeding, 4 in the aquaria with PVC, 2 in the aquaria without PVC. These results indicated that oyster drills would cross a PVC platform to reach cracked oysters, but that the overall rate of oyster drill movement was very low.

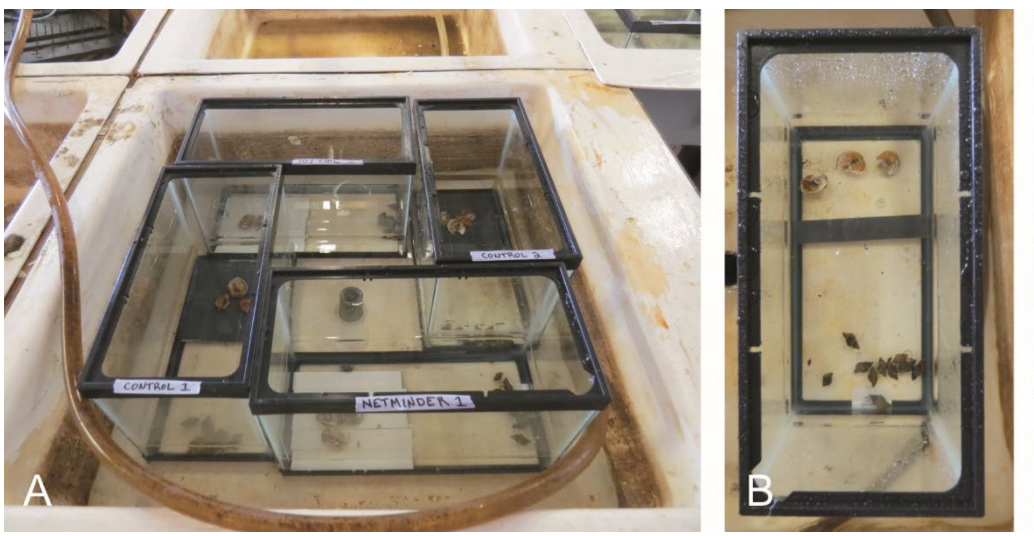

Figure 1. Predator assays. (A) 3.8 L aquaria in water table. (B) Preliminary assay 1, snails separated from oysters by a strip of PVC. (C) Preliminary assay 2, oysters placed on platform of PVC strips.

For the coating trials, test compounds were applied to PVC surfaces to see if the compounds would keep oyster drills from moving across the coated surfaces to reach oysters. For each assay (n = 4 replicates per treatment), 10 snails were placed on one side of a 3.8 L aquaria and 3 cracked oysters were placed onto flat PVC strips on the other side. The only way snails could reach the oysters was if they crawled across the PVC strips. For controls, no

	coatings were applied to the PVC strips. For treatments, the PVC strips were coated with one of several test compounds: Netminder, Zinc, Birch extract, Menthol, or Capsaicin. Snails were placed into the aquaria and left undisturbed for 24 hours. After 24 hours, the number of snails feeding on oysters was recorded. Due to space limitations, it was not possible to test all of the compounds simultaneously. Instead, Netminder and Zinc were tested in one assay, Birch extract and Menthol tested in a second assay, and Capsaicin tested in a third assay. None of the coatings significantly deterred snails from reaching the oysters. The number of oyster drills feeding on oysters was highly variable in all treatments, including the controls (Table 1). Some snails fed on oysters in all treatments. Table 1. Mean number of oyster drills feeding (± 1 SE) on oysters after 24 h. Assay 1 — # Snails Control — 2.5 ± 1.0 Zinc — 0.7 ± 0.9 Netminder — 1.5 ± 1.3 Assay 2 Control — 2.3 ± 1.9 Birch extract — 2.5 ± 3.1 Menthol — 2.7 ± 3.4 Assay 3 Control Capsaicin Though not significant, the initial results are promising. Numerically, Netminder and Zinc both had lower mean numbers of oyster drills feeding on oysters at the end of the assays. A larger, more detailed study, is needed to more fully elucidate the potential of using aquaculture coatings to deter benthic predators. *OBJECTIVE 6 – see below.*
Outreach Overview	**Objective 4. Engage aquaculture producers in research and outreach, and disseminate the results to the industrial and scientific communities through presentations at workshops, conferences, outreach publications, web page and web conference, and peer-reviewed publications.** **Overview** The primary goal of the outreach effort was to work towards improved aquaculture producer knowledge on various aspects of marine biofouling. The

Table 1. Mean number of oyster drills feeding (± 1 SE) on oysters after 24 h.

Assay 1	# Snails
Control	2.5 ± 1.0
Zinc	0.7 ± 0.9
Netminder	1.5 ± 1.3
Assay 2	
Control	2.3 ± 1.9
Birch extract	2.5 ± 3.1
Menthol	2.7 ± 3.4
Assay 3	
Control	
Capsaicin	

project investigators developed a PowerPoint presentation, “Biofouling 101” and accompanying script containing science-based information on the following topics:

- Identification of common marine biofouling species
- Biology and ecology of common marine biofouling organisms
- Effects on biofouling on various gear types and species
- Available management (prevention and control) strategies
- How to report new or unusual biofouling organisms

The “Biofouling 101” presentation was piloted at the Northeast Aquaculture Conference & Exposition held in January 2017 in Providence, Rhode Island. The purpose of the workshop was to: (1) assess attendee knowledge on the topic, (2) provide basic information on the aforementioned topics, and finally, (3) evaluate whether or not attendee’s knowledge on these topics improved following the presentation.

The workshop commenced with distribution of a pre-test on biofouling topics. After attendees completed the pre-test, it was collected and the project investigators began the presentation. The workshop began with an overview on the five biofouling topics given by researchers and outreach staff. This was followed by presentations given by aquaculture producer which highlighted the results of field trials of biofouling coatings (the focus of this research project) on their individual farms. Workshop participants had the opportunity to view coated and uncoated gear pieces, and ask cooperative farmers about their experiences with the different antifouling coatings. Researchers were on hand to listen to suggestions with respect to product development and future directions for biofouling research and to answer questions.

There was a total of 57 attendees and 7 speakers involved in this workshop. Of the total number of attendees, 30 pre- and post-tests were submitted and 24 of those surveys were considered complete. The response rate was 42%. In all cases, participants scores were higher on the post-test than on the pre-test. Workshop attendees also provided a considerable amount of input regarding their own experiences with anti-fouling strategies, and suggested future directions for anti-fouling research.

In the short-term (within the project period), we are making a concerted effort to outreach the results (indicating benefits and tradeoffs of the new antifouling coating) to growers and grower associations.

Additionally, the project investigators will assemble a final project report, and at least one peer-reviewed journal article that describes the research effort, results, and implications for shellfish aquaculture producers.

Future work

In the longer term (within the first 2-5 years following the grant period), the PI

	will be tracking the use and acceptance of the new coating through product sales and consumer comments. These results will be reported back to NRAC in periodic impact reports. **<u>Accomplishments</u>** Getchis, T.L., Shumway, S.E., Walsh, A., Bullard, S. 2017. Farmer to Farmer: What Works and Doesn't When It Comes to Biofouling Control (Workshop). Northeast Aquaculture Conference & Expo, Providence, Rhode Island. **<u>Anticipated Outcome(s)/Impact(s)</u>** The overall focus of this project was to develop and make available an alternative hazard management strategy (prevention as opposed to removal) for marine biofouling in aquaculture. In the short-term, we were able to expand aquaculture producer understanding about various aspects of biofouling, including promising strategies to help prevent its occurrence, and ultimately resulting in a significant cost-savings to the producer. In the longer term, adoption of this management strategy should result in a reduction in effort necessary to address biofouling; and in some cases, product will be of better quality and more valuable in the marketplace.
Targeted Audiences	Results obtained from this research will reduce the costs and effort associated with biofouling on aquaculture farms.
Outputs:	In the short-term (within the project period), we are making a concerted effort to outreach the results (indicating benefits and tradeoffs of the new antifouling coating) to growers and grower associations. Ultimately, the project investigators will assemble a final project report, and at least one peer-reviewed journal article that describes the research effort, results, and implications for shellfish aquaculture producers. Future work In the longer term (within the first 2-5 years following the grant period), the PI will be tracking the use and acceptance of the new coating through product sales and consumer comments. These results will be reported back to NRAC in periodic impact reports. **Accomplishments** Anticipated Outcome(s)/Impact(s) The overall focus of this project was to develop and make available an alternative hazard management strategy (prevention as opposed to removal) for marine biofouling in aquaculture. In the short-term, we were able to expand aquaculture producer understanding about various aspects of biofouling,

	including promising strategies to help prevent its occurrence, and ultimately resulting in a significant cost-savings to the producer. In the longer term, adoption of this management strategy should result in a reduction in effort necessary to address biofouling; and in some cases, product will be of better quality and more valuable in the marketplace. We will have a presence at the upcoming Aquaculture 2019 Triennial Conference in March of 2019 and will present final results and hope to convince the shellfish aquaculture community that our results hold great promise for their industry and use of developed coatings will significantly reduce the costs and labor associated with biofouling on their farms.
Outcomes/Impacts	The following outcomes/impacts are planned, industry members are currently engaged in testing in several areas (see Project Progress) and routine interaction is integral to the project. **Short term** • An alternative hazard management strategy (prevention as opposed to removal) is developed and made available to address biofouling and predation • Producers are more aware of ways to manage biofouling and predation **Medium term** • Adoption of this management strategy (product use) results in a reduction in effort (hours spent) necessary to address biofouling and predation; in some cases, product is of better quality and more valuable in the marketplace • Producers are more easily able to identify biofouling species, when and how they can affect aquaculture operations, recognize new species and know who to notify (e.g. natural resource managers and extension professionals) **Long term:** • A significant cost savings to the producer • Biofouling is no longer considered one of the most costly problems in aquaculture
Impacts Summary	1. **Relevance:** Biofouling in shellfish aquaculture 2. **Response:** Coatings are being developed and field-tested to mitigate fouling 3. **Results:** The work is in the first season and results will not be available until the end of the fouling season in October/November *4.* **Recap:** Coatings are being developed and field tested to mitigate biofouling in shellfish aquaculture.
Publications and Presentations	**PUBLICATIONS** No formal publications have yet been submitted as the work has been developmental and the coatings are proprietary. That being said, we have freely made information available to our industry colleagues and will continue to do so through lectures and presentations.

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A Book Chapter is currently in preparation: Biofouling in Shellfish Aquaculture (Shumway, Bullard, and Walsh). NRAC will be clearly identified in the final publication as a partial sponsor of that effort and a copy provided to NRAC.
Some materials have only just been retrieved from experimental locations. A separate paper will be produced for publication in an appropriate scientific journal as soon as all results are examined.
PRESENTATIONS
Numerous presentations were made at formal conferences, seminars, and industry gatherings. Poster presentations were made at the US Aquaculture Society meeting in Las Vegas, NV, January 2018; San Antonio, Texas, in February, 2017, and the National Shellfisheries Association annual conference in Seattle, WA, in March, 2018. PI Shumway had the opportunity to make presentations at many national and international venues as part of other invited activities, i.e. at no cost to the project. These included: National Taiwan Ocean University (October, 2017), Tokyo University of Marine Science and Technology (January 2018), University of the Algarve, Portugal (March 2018); Institute of Oceanology, Chinese Academy of Sciences; First Institute, Qingdao, an aquaculture farm China (May, 2018), and the University of Washington. an informal seminar at Roger Williams University, and will be presented during an upcoming visit to the Ocean University of Shanghai – all highly productive centers of shellfish aquaculture.
The NRAC grant was clearly identified as a funding source at each presentation.
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<tr><td>Students/ Participants:</td><td>
Provide the following information for every student that worked with you during the reporting period:

Name: Elisabeth Eudy, University of Connecticut undergraduate
Whether Degree was completed during the reporting period (name, yes/no): no
New or Continuing Student: continuing
Capstone/Thesis Title (actual or anticipated): not applicable
Date of Graduation: 2018
Provide link to thesis/dissertation document: not applicable
Name: Maria Rosa, University of Connecticut graduate student; summer participant 2016, not related to her thesis research; PhD. Awarded 2016

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<tr><td>Partnerships</td><td>
List any partners that you worked with on your project. Provide the following information for each Partner:
<table>
<tr><th>Partner</th><th>Specific Type</th><th>Level</th><th>Nature of</th></tr>
<tr><td>Dan Ward
Leslie Sturmer</td><td>Cooperating testing</td><td></td><td></td></tr>
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