Research the Impact of Aquatic Invasive Species on Shellfish Aquaculture in Rhode Island

Project Report
2023

From left: Ryan Reid- student summer intern, Kyle Hess-Owner of Chessawanock Island Oyster Co. Kevin Cute-CRMC Marine Resource Specialist, and PI Skylar Bayer. On right, close up photo of various biofouling organisms found on oyster farming gear. Photos by Susanna Osinski.

By Susanna Osinski[1], Kristen Savastano[1], Sabrina Lyall[1], Caitlin Cleaver[2], Skylar Bayer[1, 3]

[1] Center for Economic and Environmental Development, Roger Williams University, Bristol, RI 02809

[2] Bates-Morse Mountain Conservation Area and Coastal Center, Bates College, Lewiston, ME 02420

[3] Habitat Conservation Division, Alaska Regional Office, NOAA Fisheries, Juneau, AK 99801

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Summary

This report consists of the methods, results and overall conclusions and accompanying appendices for this project. The goal of this research project was to assess the impact of the biofouling community on oyster farms and what portion of the community are aquatic invasive species. We conducted an online survey of farmers on biofouling, assessed percent cover of oyster gear for AIS presence and abundance, and estimated biofouling biomass and oyster mortality through the growing season. We collected monthly data from four farm sites across Rhode Island between April 19 and November 2, 2022, working with three farms: Matunuck Oyster Farm in Ninigret Pond, Chessawanock Island Oyster Company in Eastern Narragansett Bay, and Saltbox Sea Farm which has sites in Eastern and Western Narragansett Bay.

Survey results were from farmers who reported having leases across the state from six different growing areas in Narragansett Bay and the coastal ponds. Respondents were able to identify many invasive tunicate species present on their gear, but pointed to several native species, in particular blue mussels (*Mytilus edulis*) being the most problematic. The peak biofouling season was often during the summer months, but was in April and May at a few locations. Biofouling significantly impacts farmers in terms of cost, particularly because of time and labor. Air drying and flipping floating gear were by far the most effective mitigation methods.

During the 2022 growing season, we observed that our control gear from all four farms had higher percent cover of biofouling than replicates. Matunuck had the lowest percent cover of biofouling and North Kingstown had the highest. On the gear we used, we observed over 46 different species, 18 of which were invasive and most fell into the tunicate taxonomic group. The largest number of invasive species were found in western Narragansett Bay (11) and the coastal ponds (12) (Table 8). We observed seasonal variation of many species, with blue mussels and certain macroalgae species often dominant in the spring (mussels were often reported to set before the start of our study), invasive and native tunicates frequently dominated the middle of the summer, and bushy calcareous bryozoans were common throughout the season.

Environmentally, we saw the biggest changes in salinity and dissolved oxygen in Portsmouth compared to other sites. This site also had the least amount of dried biomass, however biomass did not necessarily indicate percent cover of species at other sites. Dry biomass was also not indicative of how difficult it is to remove a particular biofouling organism nor how heavy it is when wet and handled the most by farmers. Remarkably, mortality and growth rates of oysters were not very different between monthly replicates and season-long controls.

Overall, in this study, current techniques that farmers use are effective at mitigating the growth and impact of biofouling, however these species persist and future research targeted on specific problematic species would be beneficial to both farmers and AIS managers in Rhode Island.

Introduction

Aquatic Invasive Species (AIS) have been anecdotally observed as epiphytes attached to aquaculture field gear, including plastic mesh grow out bags, which contain the oysters from seed to market size, and accommodate the unobstructed flow of plankton rich seawater to feed them. Some AIS such as colonial tunicates will settle on grow out bags and smother them as they spread into solid sheet-like organisms; solitary tunicates and other species will similarly smother grow out bags by settling and growing on them in high densities. Regardless of species, AIS can create a physical barrier that significantly impedes the flow of plankton rich seawater the aquaculturist needs in order to produce an economically viable market product. Further, that AIS directly competes with cultured oysters for the same planktonic food sources further amplifies their impact on the aquaculture industry in Rhode Island.

We hypothesize that cultured oysters occurring in grow out bags with high densities of AIS cover will have lower survival and growth rates than those in grow out bags absent of or with a significantly lower AIS cover density.

Goal: The goal of this research project is to investigate how the presence of aquatic invasive species (AIS) impacts the economic profitability through the production of shellfish aquaculture operations in Rhode Island.

To achieve our goal in this project, we have several specific objectives:

(1) Assess the overall time, effort and cost of managing AIS on a shellfish farm in Rhode Island reported by aquaculture farmers.

(2) Measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear.

(3) Assess the impact that AIS fouling has on production potential of traditional shellfish aquaculture gear.

Methods

Objective 1: Assess the overall time, effort and cost of managing AIS on a shellfish farm in Rhode Island reported by aquaculture farmers.

To assess the overall time, effort, and cost of managing AIS on shellfish farms in Rhode Island, we developed an online survey through Qualtrics that we distributed among aquaculturists in the state via an email list provided by CRMC. The survey questions centered on the issue of biofouling species growing on culture gear and their subsequent cost and time impacts on aquaculture businesses. We included follow up questions on farmers' ability to recognize specific AIS. These questions also focused on location (geography) within the state, the different types of cost which include the loss of product, product growth and requiring more time from hired crew members to clean and handle gear and/or product losses. Finally, the survey asked about the most commonly used mitigation methods and their relative costs, times, and effectiveness. Our protocols, consent form, and the assessment questions are in **Appendix B**.

Objective 2: Measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear.

To measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear, we identified both AIS and native species through photographed sides of bags and organisms collected from replicates of the aquaculture gear used by participant aquaculture operations. Replicate gear will be deployed at each participant aquaculture operation and will match the unique gear types used by each operation. The replicate gear was retrieved and photographed monthly, and replaced by clean replicate gear during the study period. Both sides of all bags within the replicate and control gear were photographed, amounting to more than 24 photos per farm per sampling date. Additionally, at Matunuck Oyster Farm, because they use trays instead of bags, we photographed each of the four sides of the trays in addition to the front and back (6 photos/tray x 4 trays/sampling date = 30 photos). Additional photos were taken up close of the different fouling species seen on the gear to be used to help in later species identification. The photographs were analyzed in ImageJ for (a) total percent cover of the replicate gear by the biofouling community, (b) species composition, (c) classification as invasive, native, or unknown, (d) seasonal variation, and (e) spatial variability with farms as the geographic variable. See **Appendix C** for details on methodology.

Objective 3: Assess the impact that AIS fouling has on production potential of traditional shellfish aquaculture gear.

To assess the impact of AIS biofouling on traditional shellfish aquaculture gear, we partnered with several oyster farmers at multiple locations around Rhode Island. At each farm site through the growing season (April – October), we deployed replicates of traditional oyster culturing gear at the beginning of the growing season (April/May). This gear is the same gear used by the farm in each location (Table 1).

Table 1. Farm details including the site name, Company name, region of Rhode Island (RI) and CRMC Growing Area (GA), coordinates, mean low depth (MLD), and oyster gear type used. Asterisk (*) denotes other gear used on farm site, but not for this project.

Site & Farm	Region of RI	Latitude	Longitude	MLD	Gear Type
Rome Point (North Kingstown) *Saltbox Sea Farms*	Narragansett Bay (GA-7)	N 41°32'30.11"	W 071°25'12.64"	~6 ft	Floating cages, flip bags*
Sakonnet River (Portsmouth) *Saltbox Sea Farms*	Narragansett Bay (GA-3)	N 41°37'50.88"	W 071°13'50.88"	~4 ft	Bottom cages, bottom trays*
Hog Island *Chessawanock Island Oyster Co.*	Narragansett Bay (GA-3)	N 41°38'37.38"	W 071°17'10.28"	~6 ft	Floating cages
Potters Pond *Matunuck Oyster Farm*	Coastal Pond (GA-10)	N 41°22'55.16"	W 071°31'54.34"	~3 ft	AQUA trays, bottom trays* and bags*

At each farm, gear was replicated three times leaving a fourth set as control. Each of the replicates (containing three bags or a single tray were 9 or 12 mm mesh size) were handled as normal without harvesting any oysters and controls without any cleaning. We sampled each set of gear at each farm five times after initial deployments. Every 3-5 weeks (Table 2), gear was pulled out at each farm, photographed, and swapped out with new bags for the replicate treatments after measuring and counting oysters and returning them to the farm. Control bags were also photographed handled to measure and count oysters, but were returned without cleaning or swapping each trip. Biofouling biomass was collected from each bag or tray with a power washer, dried in a drying oven for several days, and weighed for dry biomass.


Images of AQUA trays deployed at Matunuck Oyster Farm (left) and floating cages deployed at Chessawanock Island Oyster Co. (right). Photos by Susanna Osinski.

While we were at each site, we collected environmental data with a YSI, focusing on water temperature, salinity and dissolved oxygen. See **Appendix D** for more details on collection methodologies and data sheets. More data from our field collections can be available upon request (contact: Susanna Osinski). More images from field work are available here.

Table 2. Deployment and sampling dates for 2022 for all farms (Chessawanock = Chessawanock Island Oyster Co., Matunuck = Matunuck Oyster Farm, SSF = Saltbox Sea Farm). In our results text, tables, and figures, we frequently refer to results by collection Months (1-5).

Sampling Dates	**Matunuck**	**Chessawanock**	**Portsmouth - SSF**	**North Kingstown - SSF**
Deployment	April 19	April 22	May 12	May 2
Month 1	May 27	June 2	June 14	June 10
Month 2	June 30	July 8	July 22	July 15
Month 3	August 3	August 12	August 23 & 25	August 24
Month 4	September 9	September 16	September 28	September 26
Month 5	October 13	October 20	November 2	October 26

Results

Objective 1: Assess the overall time, effort and cost of managing AIS on a shellfish farm in Rhode Island reported by aquaculture farmers.

Out of 58 farmers emailed, there were 15 initiations of the survey, eight respondents completed some part of the survey, and seven completed the survey fully. We divided the survey into four sections, (I) Aquaculture Operations, (II) Biofouling & Invasives, (III) Costs and (IV) Further Research. The following results are from the eight farmers that completed any part of the survey.

Section I: Aquaculture Operations

Survey respondents had anywhere between 1 and 4 leases, with an average number of 1.75 leases per farmer. The total number of lease sites provided was 14 that were geographically diverse, spanning across many growing areas of Narragansett Bay, the Coastal Ponds, and Block Island, with the most number of leases in Growing Area 7 (Table 3). These lease sizes ranged from 1 to 9 acres with an average size of 4.2 acres. Farms were in operation between 3-22 years, the average operation time being 11.4 years. All farms operate between May and October, several more operate between March and November, and 37.5% operate year round.

The most commonly grown species were eastern oysters (*Crassostrea virginica*), with two respondents indicating they also grow mussels (*Mytilus edulis*). One of those respondents grows a variety of species, with eastern oysters being the majority of their crop (65%), soft-shelled clams or *Mya arenaria*, (20%) being their second, and mussels (15%) being their third most abundant crop. A few respondents indicate they also grow kelp, one respondent indicated they also grow bay scallops (*Argopecten irradians*), and several selected “other.” However, these species did not come up in anyone’s top three species production list.

Respondents primarily use floating and bottom gear (Table 3). However, suspended longline, direct bottom planting, rack and bags, and nursery or FLUPSY systems are also used across the state, with a majority of FLUPSY systems in the West Passage (Growing Area 7). A few farms use suspended longline gear in the Narragansett Bay growing areas, but no respondents with leases in the coastal ponds or Block Island reported use of suspended line gear.

Table 3. Regions and growing areas for Narragansett Bay (NB), the coastal ponds, and Block Island with growing area codes in parentheses. Regions and growing areas determined by CRMC. For more details on gear types, see Appendix B.

Region (Growing Area)	Gear Type(s)	Species	Leases
NB: East Middle Bay (GA-3)	Floating, Suspended Bottom	*Crassostrea virginica* *Crassostrea virginica*	1 1
NB: East Passage (GA-6)	Floating, Bottom, Suspended, Racks Floating, Bottom, Suspended	*Crassostrea virginica* *Mytilus edulis*	1
NB: West Passage (GA-7)	Floating, Bottom, FLUPSY, Direct Bottom Direct Bottom Floating, Bottom, Direct Bottom Floating, Bottom, FLUPSY Floating, Bottom, Suspended	*Crassostrea virginica* *Mya arenaria* *Mytilus edulis* *Crassostrea virginica* *Crassostrea virginica*	1 5 1
Point Judith & Potters Ponds (GA-10)	Floating, Bottom	*Crassostrea virginica*	2
Ninigret & Green Ponds (GA-11NG)	Floating, Bottom, FLUPSY	*Crassostrea virginica*	1
Block Island (GA-13)	Racks, Bottom	*Crassostrea virginica*	1

Section II: Biofouling & Invasive Species

Survey participants indicated that biofouling species are a major concern for their operations, averaging 88.7 on a sliding scale (0-100), ranging from 74-100. Five respondents indicated that they could not tell the difference between invasive and native biofouling organisms, and two responded "maybe/sometimes". Given that most respondents could not identify invasive species reliably, it is not surprising then that five respondents then said they hadn't noticed an increase in invasive species since they began their operation. Two responded that maybe there was an increase in invasive species.

In response to our question about common biofouling species that we provided, a majority were commonly encountered by respondents, especially acorn barnacles, boring sponges, slipper snails, blue mussels, and most tunicates (Table 4). The invasive tunicate species *Botryllus schlosseri*, blister worms and seaweeds were still reported to be problematic by more than 50% of respondents. When asked what species were most problematic, there was a diversity in responses with blue mussels being listed the most frequently (Table 5). Tunicates were listed as a problematic species in the West Passage area and East Middle Bay. Biofouling was commonly reported to increase between April and November, with peak months being June through August in the East Middle Bay and usually in April in May in the Western part of the state (Table 5).

While the cleaning season often spanned between May and October, the greatest intensity of cleaning was often reported as a particular set of months within that time frame (Table 5).

Table 4. Biofouling organisms listed in survey and percentage of respondents (n=7) who selected these organism groups that they have encountered on their aquaculture gear.

Organism	%	Organism	%	Organism	%
Acorn barnacle	71.4	*Tunicate - Didemnum*	71.4	*Red seaweed*	0
Boring sponge	71.4	*Club tunicate*	85.7	*Seaweeds*	57.1
Slipper snails	85.7	*Tunicate - Botryllus schlosseri*	57.1	*Predators*	28.6
Blue mussels	85.7	*Tunicate - Botrylloides violaceus*	85.7	*Other: starfish*	14.3
Mud/blister worm	57.1	*Tunicate - Sea squirts*	71.4	-	-

Table 5. Respondents' top problematic biofouling organisms cross referenced by growing area, and responses about biofouling and cleaning seasons. Respondents (n=7) were allowed to write in multiple species (n = number of times mentioned).

Region (Growing Area)	Most Problematic Species	Biofouling Season (Worst months)	Cleaning Season (Worst month)
NB: East Middle Bay (GA-3)	Sea squirts/tunicates (1) Boring sponge (1)	May- September (June - August) May - October	April - November (June - August) May - October (May)
NB: East Passage (GA-6)	Mussels (1)	Year round	June - October
NB: West Passage (GA-7)	Mussels (5) Tunicates (1) Barnacles (1) Seaweed (1) Sponges (1)	Year round March - December (April - May) April - October (April - May) April - November (May - September)	June - October March - November (March - June) March - October (March, October) April - October (May - August)
Point Judith & Potters Ponds (GA-10)	Barnacles (1) Boring Sponge (1) Mud/Blister Worm (1)	May - November (June - September)	April - November (June - September)
Ninigret & Green Hill Ponds (GA-11NG)	Mussels (1)	April - October (April - May)	March - October (April - September)

Respondents overwhelmingly reported that oysters are the most affected species, with reduced flow through, growth rates, increased mortality, increased labor (time), and increased cost of operation being the top impacts of biofouling (Table 6). Increased labor (time) was the only category that 100% of respondents selected. When asked to estimate what percentage of their work time their staff spent cleaning biofouling, the average was 44.3% with a range of 10-80%.

Table 6. Impacts on aquaculture farm operations from biofouling listed in survey and percentage of respondents (n=7) that could select more than one option.

Direct Impacts on Animals	%	Operation & Gear Impacts	%
Reduced Growth	85.7	Increased Labor (Time)	100
Increased Size Distribution & Crowding	57.1	Increased Costs	85.7
Increased Disease	14.3	Reduced Water Flow	71.4
Increased Predation	14.3	-	-
Increased Mortality	85.7	-	-

When asked about cleaning methods for biofouling, at least one respondent selected each of the available choices (Table 7) and all respondents used air drying techniques or flipping of gear so that the gear could dry in the air. One respondent selected the "other" category writing in, "Bottom cages are brought in to dry starting in March and cycled through all gear with oysters through May," which is another air drying technique that's seasonally dependent. When asked what was most effective, most respondents selected air drying, with one respondent selecting "all three" (air drying, brine/hot water dips, power/pressure washing). It's worth noting that this respondent has been using these techniques together for 20 years.

Table 7. Biofouling cleaning techniques and percentage used by respondents (n=7) as well as percentage that said it was most effective. Respondents could select more than one option.

Method of Biofouling Reduction	Selected (%)	Selected as most effective (%)	Years using most effective technique
Gear type	28.5	0.0	-
Method of grow out	42.9	0.0	-
Air drying/flipping	100	85.7	3, 3, 8, 10, 11, 20
Brine/hot water dips	42.9	28.6	1, 20
Power/Pressure washing	57.1	28.6	8, 20
Other:	14.3	0.0	-

Section III: Costs

All respondents (100%) agreed that biofouling increases the costs of their operation. Labor was the most costly with all respondents reporting that labor costs increased from biofouling, with repair and maintenance coming in second (57.1%), new gear (42.9%) and anti-fouling equipment (42.9%) came in third, marketing/packaging coming in last (14.3%).

The estimated cost spent on biofouling annually ranged from $500-100,000 with an average of $36,500. The range of percent of estimated costs of annual biofouling maintenance ranged from 10 to 60% with an average of 23.6% from participants.

Three of the seven respondents stated that the percent of their operating costs have increased in the last five to ten years, two selected that it was the same and two selected that it was less. We noted that the two respondents who selected costs are less than they were five or ten years ago have been in operation for over 20 years and are both located in the western Narragansett Bay.

Three out of seven respondents reported that they currently do pay more for biofouling resistant gear while the majority reported that they did not. Only one respondent stated they would pay more for biofouling resistant gear regardless of cost, four stated they would but it would depend on the price and one selected maybe, but that they'd need to think more about it.

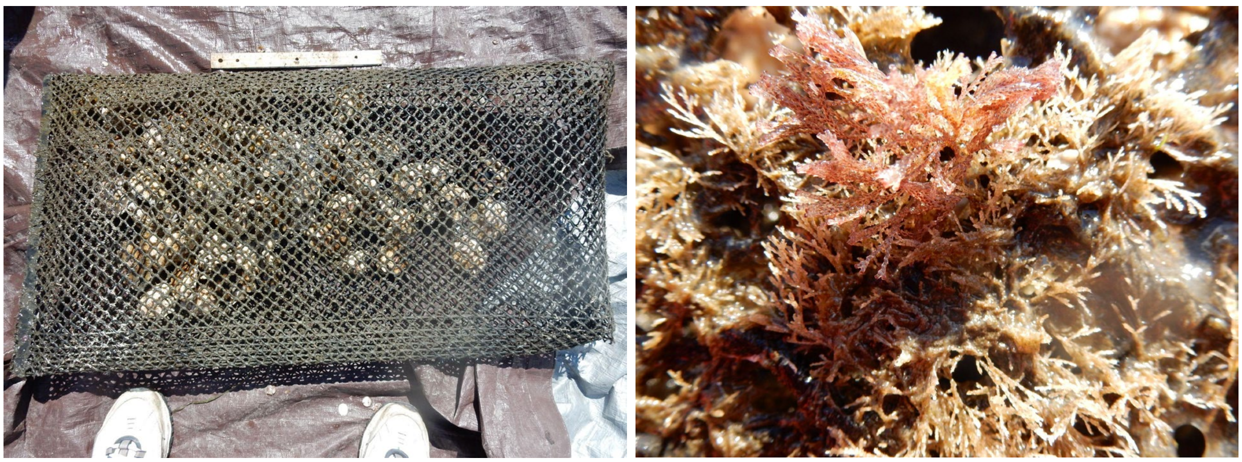
Section IV: Further Research & Other Comments

In the last section of the survey, we polled the respondents for what they think causes a majority of biofouling in their area. 71.4% selected "equipment" as being the major cause for biofouling, 57.1% selected "grow-out method" and 42.9% selected "location" as the primary cause. When asked if they wished there was more information available about biofouling, five responded "Yes" and the remaining two selected "I don't know." When asked if they wished there was more equipment available to resist biofouling, six responded "Yes" and one selected "I don't know." Three of seven respondents stated they did not have any specific biofouling problems they'd like to see addressed with better research and tools for control. Three respondents stated they'd like more research or tools that address the timing of mussel and/or barnacle sets, and one respondent stated "floating gear that works best to combat biofouling for oyster culture."

Finally, respondents were asked if they had anything else important to share. One respondent stated that air drying is the most cost effective way there is to handle biofouling and being able to flip gear at the site instead of bringing it elsewhere is also very important to keeping costs and time down. One respondent did also comment that the wealthy in Rhode Island should consider floating aquaculture gear to be the same as mooring balls for yachts and pleasure boating. Additionally, it was suggested that aquaculturists who do not take their businesses seriously should not be allowed to continue their operations. One final comment that was buried within the survey was that one farmer started growing and selling mussels as a way to make the best of the mussel biofouling problem.

Objective 2: Measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear.

To complete this objective, a large amount of data was collected through image analysis and hundreds of hours from staff were required to assess these images in a standardized consistent manner. A working species identification guide was made to assist in ImageJ species identification accumulating many of the various organisms and potential alternatives found throughout the four farms.



An example image analyzed in ImageJ (left), and example of an additional photo used for close-up species identification (right). Photos by Susanna Osinski.

From our biofouling images, we identified over 46 different species or species groups (spp.) (Table 8), with the most number of species falling into the tunicate (nine species or species groups) and arthropod (nine species) taxonomic groups. Some species were difficult to identify through photography and available guides and were therefore identified only to a genus or higher taxonomic group level. We identified 18 invasive species, most of which were tunicates, and five species groups that were a mix of possible invasive and native species.

Species that were found at all four sites included a mix of native and invasive species (Table 8). Of those found at all four farms, four were bryozoans (a mix of invasive and native), one was a mollusc (native, blue mussels), one was a crustacean (native, acorn barnacles), four were tunicates (three invasive, one native), one was a red algae (native) and one was a brown algae (native).

Saltbox Sea Farm's North Kingstown's site in GA-7 (Rome Point) had the most species observed (33), with 11 being invasive and three species groups that were native/invasive species. Matunuck Oyster Farm located in GA-10 (Potters Pond) had 12 invasive species and four native/invasive species groups. Saltbox Sea Farm's Portsmouth site in GA-3 (Sakonnet River) had the least number of species observed (20), nine of which were invasive. Chessawanock Island Oyster Co.'s site in GA-3 (Hog Island) had 23 species observed, three native/invasive species groups and nine invasive species.

Table 8. Major taxonomic groups, species groups (spp.), and individual species present (✔) at farms Matunuck (MT), Chessawanock (CH), Saltbox Sea Farm's Portsmouth site (PM) and North Kingstown site (NK) during the growing season. Species are indicated as being native (N) or invasive (I). Asterisk (*) denotes possible species presence. Gray cells indicate species presence across all four farms with invasive species bolded.

Group	*Species*	N/I	MT	CH	PM	NK
Sponges/Porifera	*Clathria prolifera*	N	✔*			
	Halichondria spp. *(Halichondria panicea)*	N/ I	✔			✔
Bryozoans	*Bugula neritina*	I	✔		✔	✔
	Bugulina stolonifera	N	✔	✔	✔	✔
	Electra pilosa	N	✔	✔	✔	✔
	Schizoporella unicornis	**I**	✔	✔	✔	✔
	Tricellaria inopinata	**I**	✔	✔	✔	✔
Molluscs	*Anomia simplex*	N				✔
	Crepidula fornicata	N	✔		✔	✔
	Crepidula plana	N		✔		
	Littorina littorea	I		✔		
	Mytilus edulis	N	✔	✔	✔	✔
Arthropods	*Callinectes sapidus*	N			✔	
	Caprella mutica	I				✔
	Dyspanopeus sayi	N				✔
	Hemigrapsus sanguineus	I	✔		✔	
	Uniciola irrorata	I				✔
	Libinia emarginata	N	✔			
	Palaemon elegans	I	✔	✔		✔
	Panopeus herbstii	N		✔		✔
	Semibalanus balanoides	N	✔	✔	✔	✔
Annelids	*Alitta virens (Nereis virens)*	N				✔

	Spirobranchus triqueter *(Pomatoceros triqueter)*	N	✔		✔	✔
Tunicates	*Aplidium* spp.	N/I				✔
	Ascidiella aspersa	I			✔	
	Botrylloides violaceus	**I**	✔	✔	✔	✔
	Botryllus schlosseri	**I**	✔	✔	✔	✔
	Molgula manhattensis	N	✔	✔	✔	✔
	Didemnum albidum	**I**	✔	✔	✔	✔
	Didemnum vexillum	I	✔		✔	✔
	Diplosoma listerianum	I	✔	✔		✔
	Styela clava	I	✔			✔
Green Macroalgae	*Chaetomorpha* spp.	N/I	✔	✔		✔
	Codium fragile	I	✔			
	Ulva spp. *(including Ulva lactuca, Ulva flexuosa)*	N/I	✔	✔	✔	
Red Macroalgae	*Ahnfeltia plicata*	N	✔			✔
	Gracilaria tikvahiae	N	✔	✔	✔	✔
	Gracilaria spp.	N/I	✔	✔		
	Grinnellia americana	N				✔
Brown Macroalgae	*Chordaria flagelliformis*	I		✔		✔
	Dictyosiphon foeniculaceus	I	✔			
	Ectocarpus siliculosus	N	✔	✔	✔	✔
	Fucus spp.	N		✔		
	Punctaria tenuissima	N		✔		✔
Sea Grasses	*Zosteraceae/Zostera marina*	N	✔			✔
Vertebrates	*Tautoga onitis*	N	✔			

Control gear consistently had the same or higher percent cover of biofouling than the replicates (Figure 1). Surprisingly, controls did not consistently increase in percent cover over the growing season suggesting that some biofouling fell off naturally or through Monthly handling through the season. North Kingstown controls had the highest percent biofouling observed with means ~60% in Month 3 (August 24) and Month 5 (October 26, Figure 1d). Overall, Matunuck appeared to have the least amount of biofouling percent cover compared to all sites (maximum of ~10%). Chessawanock had the second highest percent cover overall during the last month of collection (Month 5, October 20) at 40% for controls. Chessawanock also frequently had the same amount of biofouling cover for controls and replicates in a sampling month (Figure 1b).

For sampling dates with no data, ImageJ analysis was not used due to poor quality of data. At Matunuck, during the Month 1 collection (May 27, Table 2) we were still developing our methodology with Kevin Cute, and didn't have comparable pictures to the rest of our image collection, although most trays were covered with green macroalgae. However, Matunuck did have a high amount of biofouling biomass collected during that first sampling (see Figure 6a). At Saltbox Sea Farms' North Kingstown site, photos were taken with the wrong setting on Month 1 (June 10, Table 2) and were overexposed and biofouling could not be distinguished from the bags in the photos. However, North Kingstown did experience a large amount of biofouling biomass during Month 1 (see Figure 6d).

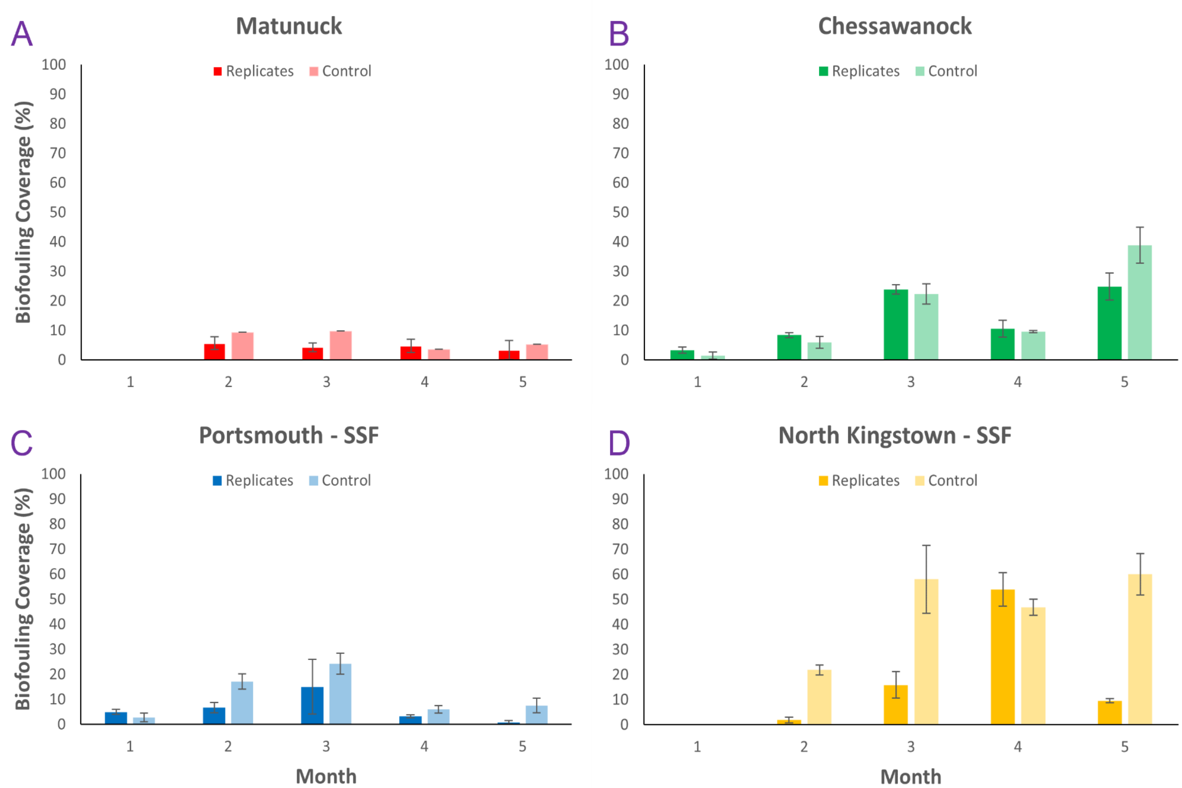


Figure 1. Mean ± standard deviation (SD) for total percent of biofouling cover from ImageJ analysis for replicates and control from each time point collection from a) Matunuck, b) Chessawanock, c) Portsmouth and d) North Kingstown.

When we divided biofouling cover by invasive, native or unknown (native/unknown mix within the same genus or taxonomic group), we saw a mix of natives, invasives or unknowns, with a majority of species falling into the unknown/mix category. Different farms experienced very different seasonal trends of these three categories, which could be a reflection of both geography, gear types and operation protocols.

Matunuck had ~100% of native species in the summer months, Months 3 (August 3) and 4 (September 9), which was the highest across all farms for the entire growing season (Figure 2 & 3). Matunuck replicates (Figure 2a) and controls (Figure 3a) had similar seasonal patterns in native and unknown groups, with the **replicates** having noticeably more invasive species than controls in Month 2 and 5.

Chessawanock **controls** had the most consistently high proportions (90-100%) of native species across all the farms through the growing season with the exception of Month 5 (October 20) (Figure 3b). This farm experienced the largest percentage of invasive species during Month 3 (August 12) and the largest percentage (almost 100%) of mixed/unknown species in Month 5 (October 20, Figure 2b & 3b). The **replicates** had a larger proportion of invasive species (Figure 2b) for the same date than controls (Figure 3b). In Month 2 (July 8), there was also a noticeably larger percentage of mixed/unknown species than native for **replicates** than controls (Figure 2 & 3).

Portsmouth had the lowest percentages of unknown/mixed species groups in biofouling overall (Figure 2 & 3). The bags from this site had the highest proportion of invasive species of all the farms, with **controls** (Figure 3c) having the highest percentages in Month 3 (August 23 & 25, ~80%) and Month 5 (November 2, ~80%). In contrast, replicates had 30% and 50% of biofouling cover identified as invasive species for Months 3 and 5 respectively (Figure 3c).

North Kingstown had very high percentages of unknown/mixed species groups for both controls and replicates (Figure 2 & 3). Month 4 (September 26). **Replicates** had 100% unknown/mixed (Figure 2d) and controls had ~90% unknown/mixed with the remainder identified as invasive species (Figure 3d). Very little of the biofouling was identified as native species, with controls having a peak in identifiable native species (~40%) during Month 2 (July 15, Figure 3d).

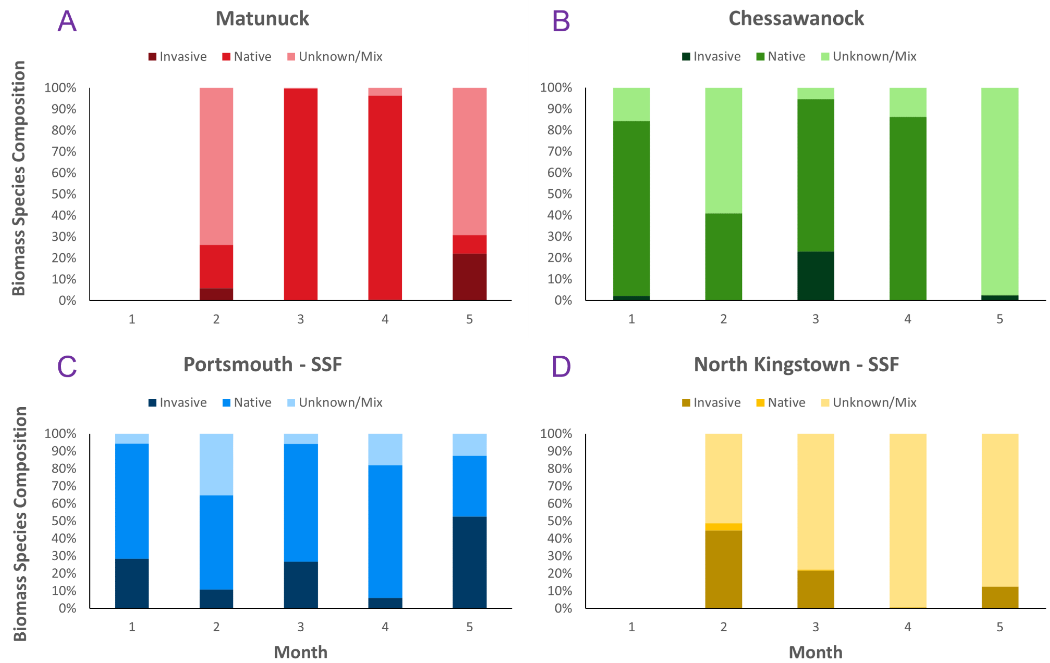


Figure 2. <u>Replicates</u>: Percentage of total biofouling area identified as invasive, native and unknown/mix categories for bags or trays. Unknown/mix are groups are a mix of native and invasive species or species where level categorization was not reliable.

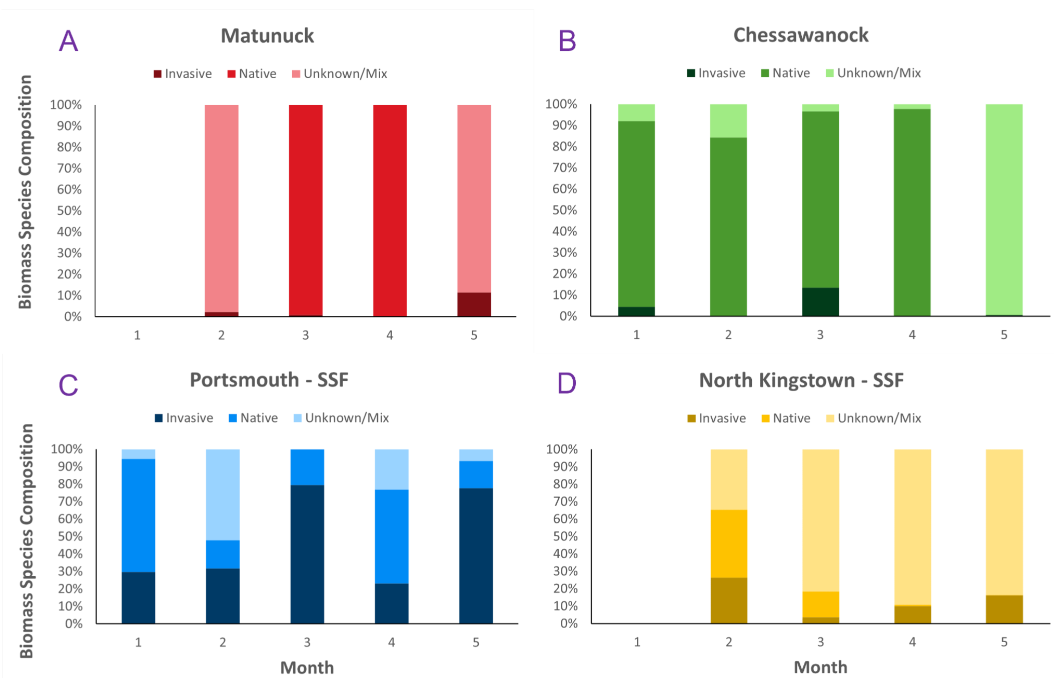


Figure 3. <u>Controls</u>: Percentage of total biofouling area identified as invasive, native and unknown/mix categories for bags or trays. Unknown/mix are groups are a mix of native and invasive species or species where level categorization was not reliable.

In all months for all farms and all samples (replicates and controls), there was at least one invasive (**bolded**) or possible invasive species/species groups (**bolded**) listed in the top three by percent cover. Out of the 18 sampling Months (4-5 per farm x 4 farms), 13 had one species or species group that composed more than 50% of the biofouling area (Table 9). Of these 13, three had an invasive species identified as the number one, and four were possibly invasive species (Table 9).

For Matunuck's Month 1 (May 27) top three species (Table 9), no ImageJ analysis was done due to the lack of available photos. However, in person identification was conducted by Kevin Cute who noted that there were many invasive ***D. vexillum**, **B. schlosseri**,* and a few ***P. elegans*** among all trays. No species or species groups were seen in the top three list all five sampling months, however, ***Gracilaria* spp.**/*G. tikvaihiae* and *Semibalanus balanoides* were seen in all Months 2-5 samples, with ***Gracilaria* spp.** in the top three species for all those months (Table 9).

At Chessawanock, there was a shift in the season from a brown algae species (*E. siliculosus*) and blue mussels (*M. edulis*) dominating the top three biofouling species (Table 9) in the first few months to bryozoans and tunicates during Month 2 and 3 respectively. In Month 4, red seaweeds dominated samples, however bushy calcified bryozoans (***T. inopinata/****B. stolonifera*) were present in the top three biofouling species in three of the five sampling months (Table 9). This species group, while comprising a large percent of the biofouling, was not one that many farmers reported during the field work or in the Qualtrics survey. This species group (it is unclear with ImageJ analysis if it is the native or invasive variety) was present (although not always in the top three species list) for all five months, along with blue mussels and *Ulva* spp.

In Portsmouth, like at Chessawanock, the brown algae species *E. siliculosus* was the most prevalent species during the first sampling Month (Table 9). This shifted to bushy calcified bryozoans dominating in Month 2, then the invasive tunicate ***B. violaceus*** during Month 3 and Month 5, with a shift to red seaweed in Month 4. During Months 2-5, *G. tikvahiae* was consistently present in samples, making it into the top 3 Months 2-4 (Table 9). Species that were present all five months (including those not listed in the top three) were ***B. violaceus***, ***T. inopinata/****B. stolonifera*, ***Didemnum* spp.** (likely ***D. albidum***), and ***Halichondria* spp**.

Biofouling in North Kingstown was dominated by tunicates, calcareous bryozoans and skeleton shrimp, groups that were likely a mix of invasive and native species. Several species were seen every month: ***D. vexillum***, ***B. violaceus***, ***C. mutica***, *C. fornicata*, ***D. listerianum***, *S. balanoides*, ***S. clava***, and ***T. inopinata***/*B. Stolonifera*. This last bushy calcareous bryozoan was observed in the top three list Months 3-5 (Table 9), comprising more than 50% of the biofouling area for those three months, however the native *G. tikvahiae* did make it into the top three during Months 2-3.

Table 9. Dominant three biofouling species at each site at each farm by percent cover of the biofouling area for all samples (replicates and controls) for each sampling Month (M). Invasive species are bolded. Asterisk (*) denotes additional information provided. Genus is abbreviated, refer to Table 7 for full genus spelling and taxonomic grouping.

M	Matunuck	Chessawanock	Portsmouth - SSF	North Kingstown - SSF
1	N/A*	1. *E. siliculosus* - - - - - 57% 2. *M. edulis* - - - - - - - 25% 3. ***T. inopinata***/ *B. stolonifera* - - - - - - 13%	1. *E. siliculosus* - - - - 63% 2. ***D. vexillum*** - - - - - 18% 3. ***B. violaceus*** **- - - - -** 7%	N/A
2	1. ***Gracilaria* spp.** - - - - 53% 2. ***Ulva* spp.** - - - - - - - - 32% 3. ***B. violaceus*** - - - - - - 5%	1. *E. siliculosus* - - - - - 51% 2. *E. pilosa*/ ***S. unicornis*** - - - - - - - 47% 3. *M. edulis* - - - - - - - 2%	1. ***T. inopinata***/ *B. stolonifera* - - - - - - 43% 2. *H. panicea* - - - - - - 29% 3. *G. tikvahiae* - - - - - 8%	1. ***D. listerianum***/ *E. siliculosus* - - - - 28% 2. ***C. mutica*** - - - - - 25% 3. *G. tikvahiae* - - - 22%
3	1. ***Gracilaria* spp**. - - - - 98% 2. ***D. albidum*** - - - - - - - <1% 3. ***Ulva spp.*** - - - - - - - - <1%	1. *M. manhattensis* - - - 50% 2. *G. tikvahiae* - - - - - 24% 3. ***B. schlosseri*** - - - - - 21%	1. ***B. violaceus*** - - - - - 40% 2. *G. tikvahiae* - - - - - 22% 3. *M. manhattensis* - - 17%	1. ***T. inopinata***/ *B. stolonifera* - - - - 73% 2. ***C. mutica*** - - - - - 10% 3. *G. tikvahiae* - - - 7%
4	1. *S. triqueter* - - - - - - - 93% 2. **Chaetomorpha spp.** - 3% 3. ***Gracilaria* spp.** - - - - - 1%	1. *G. tikvahiae* - - - - - - 89% 2. ***T. inopinata***/ *B. stolonifera* - - - - - - 11% 3. ***B. violaceus*** - - - - - <1%	1. *G. tikvahiae* - - - - - 45% 2. *H. panicea* - - - - - - 21% 3. ***B. violaceus*** - - - - - - 9%	1. ***T. inopinata***/ *B. stolonifera* - - - - 82% 2. ***D. listerianum***/ *E. siliculosus* - - - - 13% 3. ***D. albitum*** - - - - 1%
5	1. ***Gracilaria* spp.** - - - - 44% 2. ***T. inopinata***/ *B. stolonifera* - - - - - - - 30% 3. ***D. albitum*** - - - - - - - 12%	1. ***T. inopinata***/ *B. stolonifera* - - - - - - 98% 2. ***B. violaceus*** - - - - - 1% 3. ***D. listerianum***/ *E. siliculosus* - - - - - - <1%	1. ***B. violaceus*** - - - - - 69% 2. *H. panacea* - - - - - - 12% 3. ***T. inopinata***/ *B. stolonifera* - - - - - - 8%	1. ***T. inopinata***/ *B. stolonifera* - - - - 68% 2. ***C. mutica*** - - - - 9% 3. ***D. albitum*** - - - - 6%

Objective 3: Assess the impact that AIS fouling has on production potential of traditional shellfish aquaculture gear.

Environmental Variables - Water Temperature, Salinity, Dissolved Oxygen

The four farms had similar temperature increases and decreases throughout the growing season with Portsmouth experiencing the highest temperatures of 25 °C during our sampling dates (Figure 4a). Matunuck Oyster Farm experienced the coldest temperatures during our sampling early in the season in mid-April (< 10 °C). Salinity was high and consistent in Potters Pond, while other farms had greater changes through the season, likely due to rain events. Portsmouth experienced the lowest salinities at 24 ppt in mid-June (Figure 4b). Dissolved oxygen varied considerably for the Portsmouth site, varying from 80-170% over the course of two months; the other three sites ranged from 90-110% through most of the season (Figure 4c). However, Chessawanock saw a dip in dissolved oxygen during early August down to 70%, the lowest recorded in the field.

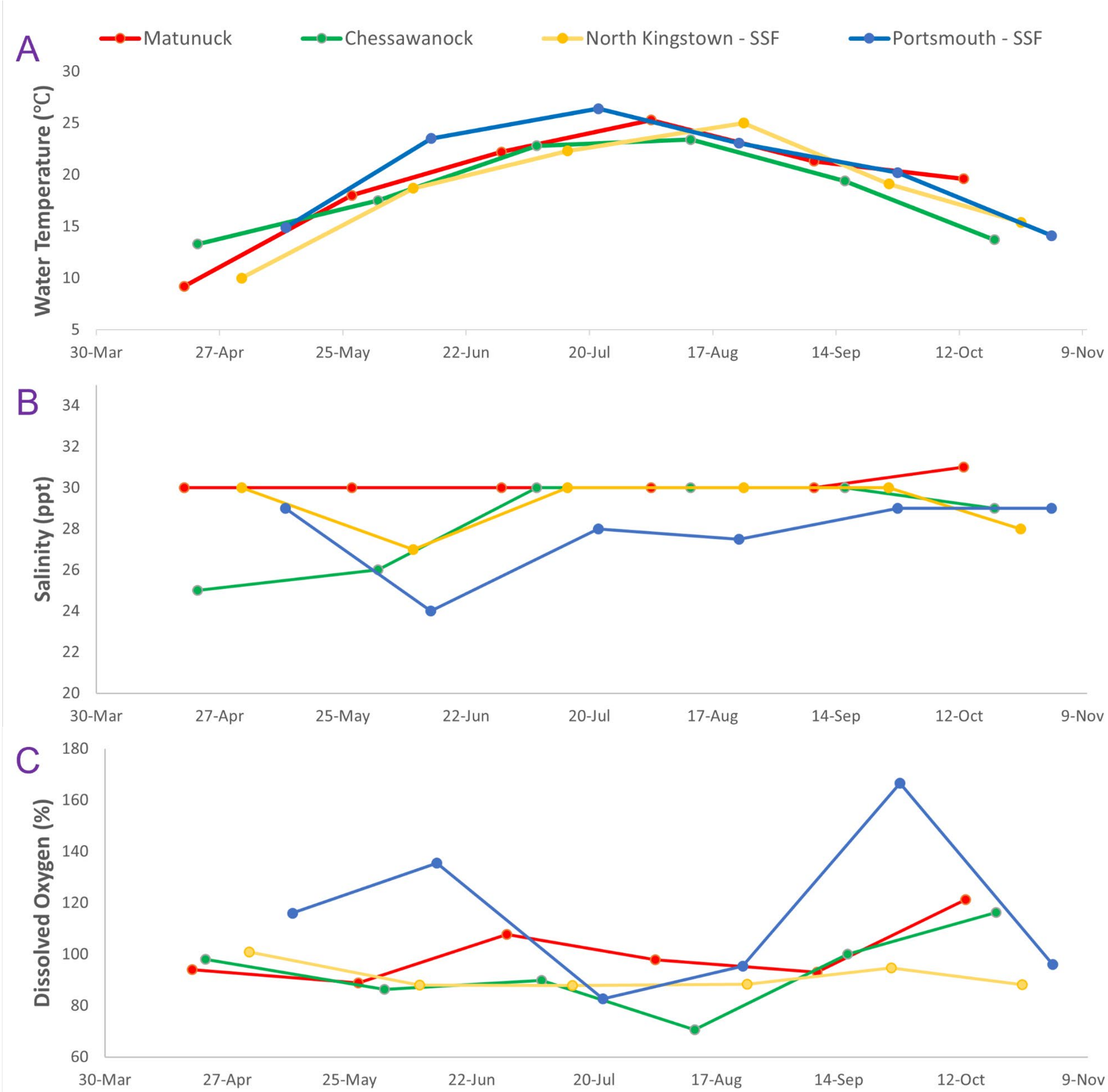


Figure 4. Environmental parameters a) water temperature, b) salinity and c) dissolved oxygen for all farms throughout the growing season. Note that environmental data was collected on different days at different farms that correspond to monthly collections (Table 2).

Oysters - Seasonal Mortality

Oysters at each farm were counted out for each bag or tray for each replicate of gear at the beginning of the experiment, at each monthly time point, and at the end of the season. Surprisingly, many controls showed higher survival rates than replicates with the exception of the Portsmouth site (Table 10). This may be due to the stress of flipping cages and/or air drying.

Table 10. Mean ± SD starting and ending counts of live oysters per bag/tray at each of the four farms. Note that Matunuck has a smaller number of replicates (n= 3) and only one control but has more oysters/tray. All other sites have three bags per each replicate (n=12) or control (n=3).

Farm	**Start**		**End**	
	Control	*Replicates*	*Control*	*Replicates*
Matunuck Oyster Farm	268 ± 0.00	268 ± 0.00	174.00 ± 0.00	164 ± 64.53
Chessawanock Island Oyster Co.	138.67 ± 0.58	138.56 ± 0.53	112.00 ± 10.54	97.00 ± 12.39
Portsmouth Saltbox Sea Farm	125 ± 0.00	125 ± 0.00	103.33 ± 6.51	104.78 ± 7.92
North Kingstown Saltbox Sea Farm	125 ± 0.00	125 ± 0.00	89.00 ± 2.00	86.00 ± 9.37

Mortality over the entire growing season for the four farms showed had means between 20-35%, expanding to 10-45% when including standard deviations (Figure 5). Oyster mortality rates for controls (no biofouling community removed) was actually lower than the replicates for all sites except North Kingstown. Matunuck Oyster Farm did see a slight loss from one of their replicate trays before the month five collection due to a storm. The farm did try to recover many of the oysters, but not all were retrieved.

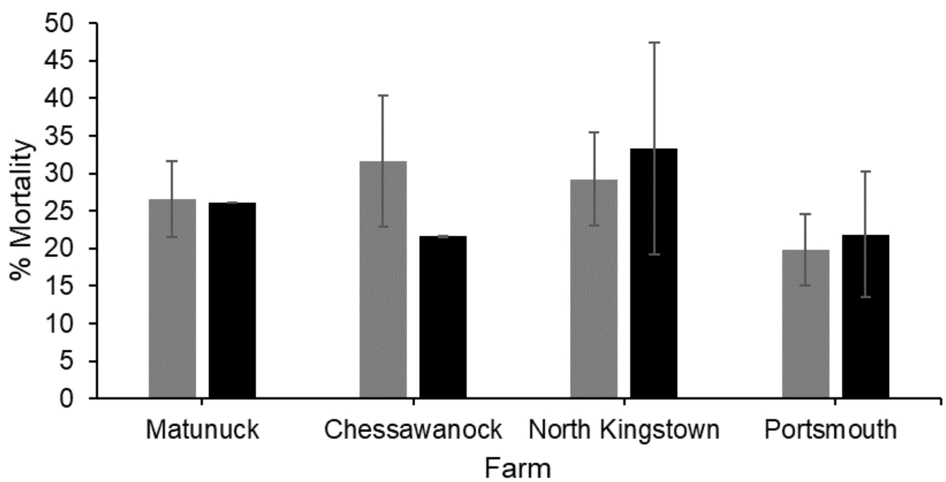


Figure 5. Mean ± SD mortality (%) for farm sites during the growing season. For each farm, there was one replicate for controls (black) over the five sampling periods (n = 5). For replicates (gray), three replicates were measured each month (n = 15) at each farm. Replicates and controls had three bags of oysters except for Matunuck which only had four trays. See Table 10 for details about bag or tray population sizes.

Oysters - Seasonal Growth

Starting sizes of oysters were ~ 60 mm in length for Matunuck, Portsmouth, and North Kingstown farms (Figure 6). By the end of the growing season, Matunuck oysters grew ~10 mm in length (Figure 6a), Portsmouth oysters grew ~20 mm (Figure 6c), and North Kingstown oysters grew ~20 mm (Figure 6d). Chessawanock had starting sizes closer to ~70 mm in length and grew ~20 mm during the season (Figure 6b). Controls and replicates often had similar growth trajectories, although replicates in Portsmouth and North Kingstown appeared to be a little smaller in size than controls, but this wasn't statistically significant. Matunuck, Portsmouth, and North Kingstown sites all appeared to have growth slow at the end of the season (Month 5 collections), while Chessawanock has a growth curve that suggests there could be continued growth into November.

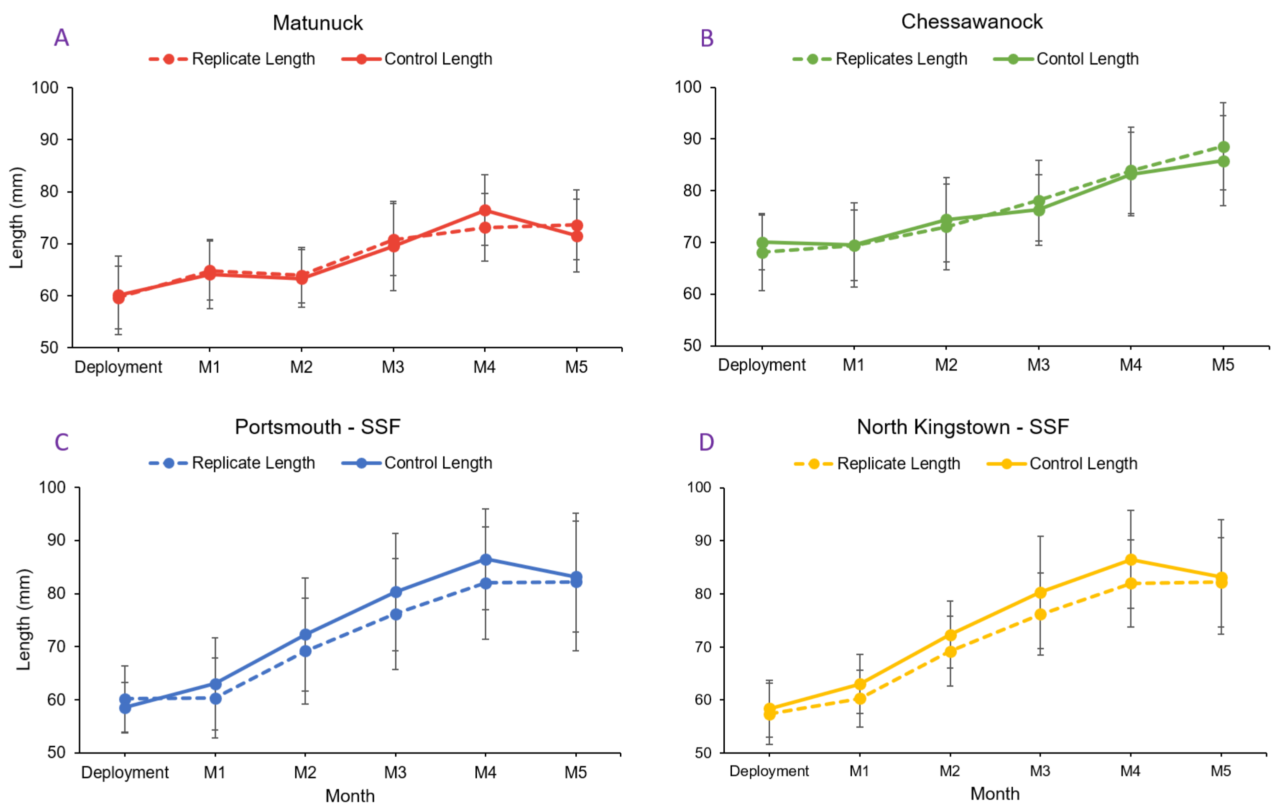


Figure 6. Mean ± SD length (mm) for oysters during the growing season for a) Matunuck, b) Chessawanock, c) Portsmouth and d) North Kingstown. For each month, 25 oysters were measured for each bag (bags = 9, total = 225 oysters), and control series (3 bags = 75 oysters). Matunuck only had three trays for replicates (total = 75 oysters) and one control tray (25 oysters).

Biofouling - Biomass

Dried biofouling biomass was the highest for Matunuck, Chessawanock, and North Kingstown during the first month of collection (Figure 7). Overall, Matunuck had the highest mean dried biomass during Month 1 at ~160 g (Figure 7a), Chessawanock and North Kingstown experienced the second highest means of ~120 g during Month 1, however Chessawanock had very large standard deviations implying a lot of variation in biomass across replicates in Month 1 (Figure 7b). This is likely due to the placement of bags within the floating cages. The Portsmouth samples had the least amount of dried biomass, never exceeding much beyond ~20 g (Figure 7c), which may be due to the species composition and their biomass when dry (Table 9). Overall, all sites had a decrease of dry biomass through the season. Matunuck did have an increase in dry biomass at the end of the season, implying that the species observed during this time had a larger dry biomass than in previous Months. Portsmouth's Month 5 collection was not completed due to weather conditions at the time, and one of the nine replicate bags from Chessawanock Month 4 was missing, reducing the number of replicates.

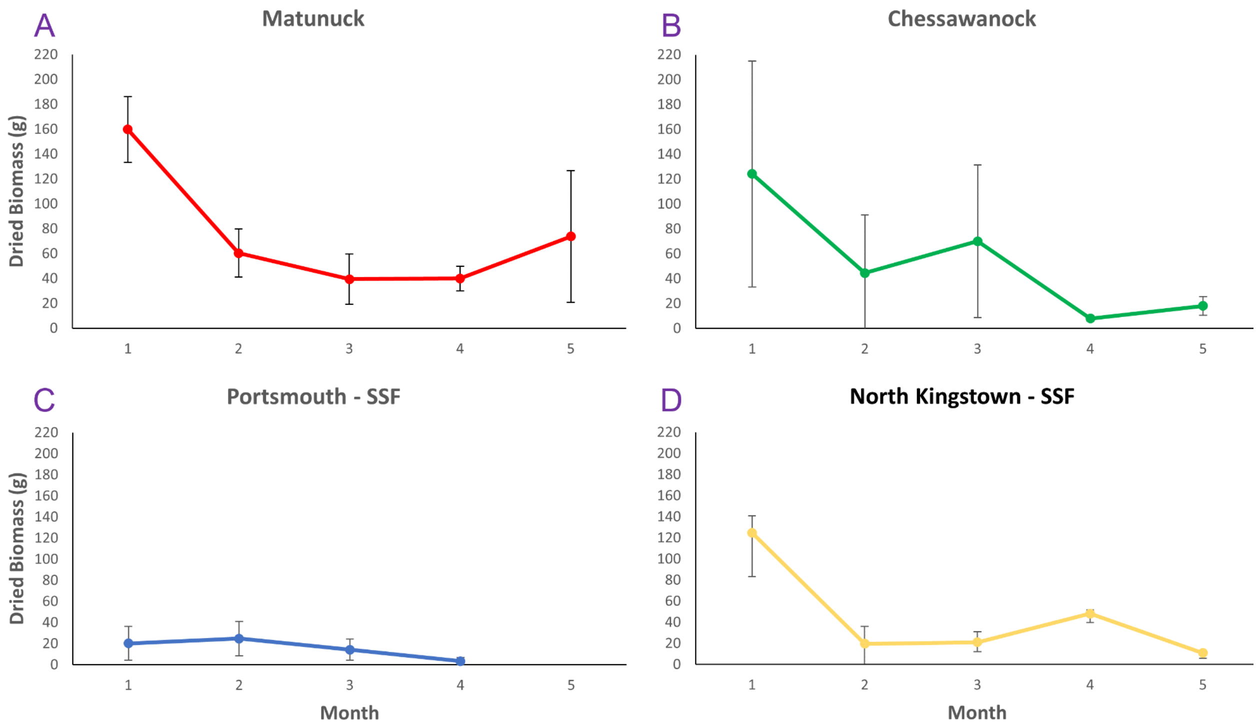


Figure 6. Mean ± SD dried biomass weight (grams) collected monthly from replicates at farms a) Matunuck (n=3), b) Chessawanock (n=9), c) Portsmouth-Saltbox Sea Farm (n=9) and d) North Kingstown-Saltbox Sea Farm (n=9). During Month 4, Chessawanock only had 8 replicate bags.

Conclusions

The results from our Qualtrics survey indicated that overall, air drying was by far one of the most effective forms of dealing with biofouling organisms – this matched a lot of our observations during the fieldwork portion of this project. Respondents in the survey did not necessarily know how to distinguish between native and invasive species, but had observed many invasive species commonly reported in Rhode Island on their gear (Table 4). Most respondents expressed a lot of interest in understanding and mitigating the impact of barnacle and mussel sets at farms across the state, which are native species. However, there was some input from farmers in Growing Area 3 that tunicates, which comprise many invasive species, are a problematic biofouling group in that region. As our Portsmouth and Chessawanock field sites are both located in Growing Area 3 and we observed many tunicate species there (Table 8 & 9), the survey and the field data match well in this regard.

Species that are deemed the most problematic by farmers are usually dependent on how hard they are to remove from the gear or how likely they are to negatively impact growth and survival of their oysters, even if for a short period of time. For example, Chessawanock Island Oyster Company finds that sea squirts are difficult to remove from their gear. Sea squirts can be problematic for their juvenile oysters because they can reduce flow and smother the smaller oysters. Chessawanock Island Oyster Company also noted that they have very large sets of blue mussels, especially in early spring, before we started our study. Blue mussels have also been noted by Saltbox Sea Farms as a problematic biofouling species in their North Kingstown site. Matunuck Oyster Farm noted that they get an abundance of macroalgae on their oyster trays, a group of species that seems to thrive predominantly in the coastal ponds.

One very interesting observation in our data is that oyster survival in our control samples (no cage cleaning through the season) had similar growth rates to replicates at every site and in some cases exceeded the growth rate of the replicate bags which experienced cleaning and fresh bags every 3-5 weeks (Figure 6). Mortality rates were not statistically significant between replicates and controls either (Figure 5) suggesting that perhaps biofouling, if left unattended wasn't causing major problems. However, all controls were removed from the water for several hours for counting and measuring, and in some cases, oysters from replicate cages may have experienced extra stress from air drying and tumbling. In future experiments, it may be helpful to have oysters in gear that are not counted or measured for the entire season to see if reduced handling results in higher mortality rates.

It was clear from our data collection that mussels often appeared in the beginning of the season (Table 9) and before our study started, and tunicates often increased at sites in the summer, with variation in species composition between sites. Bushy calcareous bryozoans were extremely abundant at multiple sites, often appearing in our top three biofouling species for each sampling (Table 9). However, likely because these species dry rapidly and are easy to remove, they are not often reported as problematic by farmers.

Dry biomass measurements were not necessarily indicative of percent cover or indicate how problematic species were for removal (Figure 6). Macroalgae, colonial tunicates, and bryozoan species were initially heavy when removed from the water, but as stated before, they are extremely light once dry. This is true for many species observed to have large percent covers. Organisms like mussels, however, often do not change much from wet to dry weight (relative to other species), and therefore will weigh more. While wet, they can cause issues of flow and the ability to move gear around, causing increased handling time and cleaning effectiveness. Mitigation and cleaning of gear, while helpful in removing biofouling, does not affect the presence of the species in the area. Future research directions should include considering wet weights of gear and species present, especially problematic species like mussels, barnacles, tunicates, bryozoans, and macroalgae.

Our field samples and ImageJ analysis indicate that invasive species are present and prevalent throughout the different areas of Narragansett Bay and the coastal ponds (Figure 2, Figure 3 Table 9), however North Kingstown had the highest number of invasive species in Growing Area 7, western Narragansett Bay. Many species appear seasonally, with variation over time possibly dependent on environmental factors. Portsmouth exhibited high percentages of known invasive species and had the lowest salinities of all the sites. However, it also had relatively low biofouling percent cover (Figure 1) although not as low as Matunuck, which had very consistent oxygen and salinity profiles at their site (Figure 4).

We think that investigating the occurrence of biofouling organism growth on gear, especially invasive species, should be further investigated on a shorter and more in depth time scale. It may be valuable to observe how quickly certain invasive species accumulate on a farm during the growth season. Future research into environmental variables should include flow rates which were not included in this study. This could be combined with research directions mentioned above regarding gear type, mesh size, and the prevalence of certain invasive species. Invasive species size and shape should also be studied more carefully in relation to gear type and flow rates. For example, some sheet-like organisms may be more problematic than those with bushy structures (i.e. bryozoans). This could be combined with further research on how certain cleaning methodologies or mitigation strategies could be applied for specific invasive or problematic species.

Regardless of whether species are invasive or native, biofouling causes extra time and labor for aquaculture farmers. Farmers are also uniquely positioned to observe the biofouling community frequently throughout the year and are therefore important and valuable partners in further biofouling and invasive species studies.

RWU staff members (from left) Liam Brosnahan, Susanna Osinski, and Kristen Savastano measuring oysters during a collection day. On right, RWU Summer student interns Ryan Reid and Thomas Desnoyers sorting oysters on collection day. Photos by Susanna Osinski.

Statement of Work & Acknowledgements

The initial proposal for this project was developed by Dale Leavitt, Matt Griffin, and Kevin Cute. Skylar Bayer was the lead on writing the statement of work within the Cooperative Agreement, making initial contacts with farmers, and finalizing the budget with the help of Dale, Matt, and Kevin, and writing the final report. Kevin developed the research goals, priorities, and methodologies with the Roger Williams University research team. Susanna Osinski was the lead for the data collection of this project, developing collection methods and communication routines with the farmers, developing the standard operating procedures for all aspects of the field, lab, and ImageJ analysis work, and overseeing multiple interns and staff who worked on this project. Bradford Bourque and Susanna both drove the boat for deployments and retrievals at multiple farm sites. Kristen Savastano oversaw and completed the ImageJ analysis and species identification with help from Sabrina Lyall. Kristen also assisted in much of the data entry and analysis, field collections, and training summer interns. Sabrina also helped assemble the species identification guide used in this report. Caitlin Cleaver aided Skylar and Susanna with the development of the Qualtrics survey. Ryan Reid and Thomas Desnoyers were the two summer interns hired for this project in 2022, who helped with collections and data entry. We thank Erin Tooley for advice and guidance through the HSRB process at Roger Williams University. We thank RWU staff Liam Brosnahan, and students Brian Mejia, Jackie Griffith, Cassidy Pilate, and Marissa Michaud for their help with field collections, measurements and data entry during the growing season. Thank you to students Emily Leonard, Maraynah Vasconcelos, and Jill Haudenshield for helping with data entry and organization. Many thanks to Brian Wysor for his help in identifying algae species in biofouling samples. Additionally, thanks to Kelly Meyer with purchasing and management of this project, as well as Tim Scott, Bob McCarthy, and Polla Mearns for financial, accounting, management, and reporting requirements of the grant.

Appendix A: Cooperative Agreement

Cooperative Agreement
Between RI Coastal Resources Management Council
And the Roger Williams University
For a Project to
Research the Impact of Aquatic Invasive Species on Shellfish Aquaculture in Rhode Island

The Coastal Resources Management Council (CRMC) and Roger Williams University hereby agree as follows:

The Rhode Island Coastal Resources Management Council agrees to pay for the services of RWU in an amount not to exceed $32,000.

1. CRMC will pay RWU, as set forth in the attached scope of work (SOW) after RWU submits on or before November 30, 2022 supporting documentation for the services rendered to CRMC Business Affairs.
2. All items in this agreement may be modified by mutual written consent.

These agreements made and entered by:

STATE OF RHODE ISLAND, RI COASTAL RESOURCES MANAGEMENT COUNCIL

_______________________________________ __________________
Jeffrey M. Willis Date
Executive Director, CRMC

Roger Williams University

_______________________________________ __________________
Nicole Turner
Assoc. VP for Accounting & Treasury Management, RWU Date

Statement of Work
The CRMC will execute a sub-recipient award agreement with a Roger Williams University Principal Researcher to conduct the following:

Introduction: Aquatic Invasive Species (AIS) have been anecdotally observed as epiphytes attached to aquaculture field gear, including plastic mesh grow out bags, which contain the oysters from seed to market size, and accommodate the unobstructed flow of plankton rich seawater to feed them. Some AIS such as colonial tunicates will settle on grow out bags and smother them as they spread into solid sheet-like organisms; solitary tunicates and other species will similarly smother grow out bags by settling and growing on them in high densities. Regardless of species, AIS can create a physical barrier that significantly impedes the flow of plankton rich seawater the aquaculturist needs in order to produce an economically viable market product. Further, that AIS directly compete with cultured oysters for the same planktonic food sources further amplifies their impact on the aquaculture industry in Rhode Island.

We hypothesize that cultured oysters occurring in grow out bags with high densities of AIS cover will have lower survival and growth rates than those in grow out bags absent of or with a significantly lower AIS cover density.

Goal: The goal of this research project is to investigate how the presence of aquatic invasive species (AIS) impacts the economic profitability through the production of shellfish aquaculture operations in Rhode Island.

To achieve our goal in this project, we have several specific objectives:

(1) Assess the overall time, effort and cost of managing AIS on a shellfish farm in Rhode Island reported by aquaculture farmers.
(2) Measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear.
(3) Assess the impact that AIS fouling has on production potential of traditional shellfish aquaculture gear.

Methods: For each objective, we will employ different assessment techniques and tools.

Objective 1: Assess the overall time, effort and cost of managing AIS on a shellfish farm in Rhode Island reported by aquaculture farmers.

To assess the overall time, effort and cost of managing AIS on shellfish farms in Rhode Island, we will develop an online and/or phone interview-based survey that we will distribute among aquaculturists in the state. The survey questions developed will be centered around the issue of AIS growing on culture gear and their subsequent cost and time impacts on aquaculture businesses. These questions will also consider location (geography) within the state, the different types of cost which include the loss of product, product growth and requiring more time from hired crew members to clean and handle gear and/or product losses. Finally, the survey will ask about the most commonly used mitigation methods and their relatively costs, times, and effectiveness.

Objective 2: Measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear.

To measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear, we will identify both AIS and native species as photographed and collected from replicates of the aquaculture gear used by participant aquaculture operations. Replicate gear will be deployed at each participant aquaculture operation, and will match the unique gear types used by each operation. The replicate gear will be periodically photographed, retrieved, and replaced by clean replicate gear during the study period. The photographs will be analyzed for total percent cover by the biofouling community, and further differentiated by percent cover of invasive versus native species.

The photographs will be analyzed in Image J or similar software for (a) total percent cover of the replicate gear by the biofouling community, (b) species composition and classification as invasive or native, (c) seasonal variation, (d) annual variation, and (e) spatial variability (northern and southern Narragansett Bay and coastal ponds as applicable.

In addition, the entire biofouling community will be removed from the replicate gear and be classified by species as invasive or native, and measured by weight to determine its total biomass, and percent composition of invasive and native species.

Objective 3: Assess the impact that AIS fouling has on production potential of traditional shellfish aquaculture gear.

To assess the impact of AIS biofouling on traditional shellfish aquaculture gear, we will partner with several oyster farmers Rhode Island at multiple locations around Rhode Island.

At each farm site through the growing season (April – October), we will deploy replicates of traditional oyster culturing gear at the beginning of the growing season (April/May). This gear will be the same as the gear used by the farmer in each location (see table below).

Site	**Region of RI**	**Mean Low Water**	**Gear Type**
Rome Point	Narragansett Bay	15 ft	Floating and bottom cages
Sakonnet River	Narragansett Bay	3 ft	Bottom cages
Ninigret Pond	Coastal Pond	3 ft	Bottom cages
Potters Pond	Coastal Pond	3 ft	Floating and Bottom cages

At each site, gear will be replicated three times at each site. Each piece of gear will be handled as for a normal operation without harvesting oysters. All gear types will have two replicate oyster bags (9 or 12 mm mesh size). The gear will be cleaned monthly with the fouling community collected and dried to measure total biomass, and percent biomass by invasive and native species for each month of the growing season. Each replicate will be sampled for the biomass of fouling seven times (7 x 3 = 21 measurements per site). We will rotate clean pieces of gear out as we collect them for fouling community harvest. Oyster mortality will be measured in each bag at these intervals as well (7 x 3 x 2 = 42 data points per site). These bags will be rinsed gently to remove sediment and oyster waste. Oysters will be transferred to clean bags for re-deployment with the new gear. An additional piece of gear will be left untouched from the beginning of the season until the end of the season when the total biomass of biofouling growth will be dried and weighed as well as overall mortality of the two oyster bags.

Final Assessment: Combining the data from objectives 2&3, we will be able to describe the fouling community over a seasonal cycle and estimate the relative proportions of AIS and native fouling species. We will also measure the biomass of fouling community on replicate gear and be able to assess differences of impact on gear typically used in geographically specific regions of Rhode Island. These field data combined with survey data will provide an assessment on production impact of AIS on shellfish aquaculture in Rhode Island.

Reporting and Timeline: In regards to Objective 1, the survey to be distributed to aquaculturists will be developed and approved through the Human Subject Research Board of Roger Williams University during the fall of 2021. Survey data will be collected during the Winter and Spring of 2021-2022.

In regards to Objectives 2 & 3, analysis of biofouling images will take place Spring and Summer 2022 and data collection of biofouling accumulation on oyster gear and weight analysis will be performed in late Spring through Summer (April – October) 2022. Data will be finalized and reported to CRMC by November 30, 2022.

The Impact of Aquatic Invasive Species on Shellfish Aquaculture

PRINCIPAL INVESTIGATORS: S. Bayer & D. Leavitt			**2021-2022** **18 months**
A. SALARIES AND WAGES:	**person-months**		
1. Senior Personnel	**No. of people**	**Amount of Effort**	**Requested Funds**
a. (Co) Principal Investigator:	1	0.67	$5,367
b. Associate (Faculty or Staff):			
Sub Total:	0	0	$5,367
2. Other Personnel			
a. Professionals:			
b. Research Associates:			
c. Res. Asst. / Grad Students:			
d. Prof. School Students:			
e. Pre-Bachelor Students:	2	6.0	$14,040
f. Secretarial - Clerical:			
g. Technicians:			
h. Other:			
Total Salaries and Wages:	3	6.67	$19,407
B. FRINGE BENEFITS (FICA only @ 9.18%):			$1,782
Total Personnel (A and B):			$21,189
C. PERMANENT EQUIPMENT:			
D. EXPENDABLE SUPPLIES AND EQUIPMENT:			$2,552
E. TRAVEL:			
1. Domestic			$806
F. PUBLICATION AND DOCUMENTATION COSTS:			
G. OTHER COSTS:			
Total Other Costs:			$0
TOTAL DIRECT COST (A through G):			$24,547
INDIRECT COST (On campus 38.4% of salary):			$7,452
TOTAL COSTS:			$32,000

Appendix B: HSRB Documents for the Objective 1 Social Science Survey

Research Protocol Form for New Individual Research Project

Project Description

The overall goal of the broader research project is to investigate how the presence of aquatic invasive species (AIS) impacts the economic profitability through the production of shellfish aquaculture operations in Rhode Island. It is a subcontract to Roger Williams University through the Coastal Resources Management Council (CRMC) of Rhode Island.

To achieve our goal in this project, we have several specific objectives:

(1) Assess the overall time, effort and cost of managing AIS on a shellfish farm in Rhode Island reported by aquaculture farmers.
(2) Measure the relative contribution of AIS to the total fouling community on traditional aquaculture gear.
(3) Assess the impact that AIS fouling has on production potential of traditional shellfish aquaculture gear.

To assess **objective 1**, the overall time, effort and cost of managing AIS on shellfish farms in Rhode Island, we developed an online interview-based survey that we will distribute among aquaculturists in the state. This online interview also provides the option to follow up with participants via phone calls (this would be a second study). The survey questions developed are centered around the issue of biofouling organisms growing on culture gear and their subsequent cost and time impacts on aquaculture businesses. To whatever extent we can determine from farmers, we will assess (1) the relative economic impact of biofouling on their businesses and (3) what amount of biofouling is caused by AIS. These questions also consider location (geography) within the state, the different types of cost which include the loss of product, product growth and requiring more time from hired crew members to clean and handle gear and/or product losses. Finally, the survey asks about the most commonly used mitigation methods and their relative costs, times, and effectiveness.

Participants

Participants will be recruited from a database of aquaculturists who have lease permits through the CRMC. This list is maintained by CRMC staff, and these leases are publicly known as the public is made aware of all lease-holdings in the state of Rhode Island. We will contact participants through email. We will include a statement in our email that indicates that participating in the study is voluntary and will not impact their lease-holding status nor future applications or reapplications.

Procedures and Methodology

Research Setting. Participants will be sent a link via email to the survey, which is hosted through Qualtrics, an online survey website. Participants will complete the survey on their own computers at a time of their choosing prior to the close of the study. It is estimated that providing responses to the survey will take between 5 and 15 minutes.

Procedures. After following the link, participants will be brought to an electronic Informed Consent Document on Qualtrics, where they will click a button to indicate agreement with the consent statement. After leaving the Informed Consent page, participants will be taken to the actual study measures. Participants will be informed that the survey consists of several measures (See Measures Attachment) assessing their experiences as aquaculture farm owners, specifically in regards to biofouling. Aquaculturists will be required to answer all questions (except one question about specific dollar amounts) to complete the survey. The last question will ask if we can follow up with them with more questions through a semi-structured phone interview (we will submit this as a second study proposal). After completing the study, participants will be thanked for their participation, and provided with contact information for the researchers. There will be no deception utilized for this study.

Data Collection. Responses will be collected via Qualtrics. Information about the participants' aquaculture farms and experience with biofouling will be collected. Those that wish for us to follow up with further questions will provide contact information for follow up phone calls as part of a second study.

Data Analysis. The proposed survey-based study will be analyzed using correlational analyses, ANOVA analyses to examine differences between tutors and the comparison group, and descriptive statistics in SPSS.

Consent Procedures and Data Confidentiality and Anonymity

This study will adhere to guidelines established by the American Psychological Association. Participants will be informed that they can withdraw from the study at any time without penalty. Each participant will be asked to read the electronic consent form and click a button to indicate agreement with the consent statement. Only the researchers conducting the study will have access to this data. Once the information has been downloaded from Qualtrics, names will be deleted from the data set and the data will be stored on the Principal Investigator's password-protected computer.

Risks/Discomfort and Benefits to the Participants

It is believed that participants should experience no risks or discomforts.

Informed Consent

Title of Project:	Impact of Biofouling on Aquaculture in Rhode Island
Principal Investigators:	Skylar R. Bayer, Roger Williams University Susanna Osinski, Roger Williams University Caitlin Cleaver, Bates College
PI Contact Information:	401-254-3091 sbayer@rwu.edu

You are being asked to participate in a research study. The section below highlights key information about this research for you to consider when deciding whether or not you want to participate. Carefully consider this information and be advised that you have the right to ask questions about any aspect of the study you do not understand before you decide whether to participate.

KEY INFORMATION REGARDING THIS STUDY

- **Consent**. You are being asked to participate in a research study. It is your decision whether or not you choose to participate. This consent form will give you the information you will need to understand why this study is being done and why you are being invited to participate. There will be no penalty if you wish to discontinue participation. Consent to participate is voluntary.

- **What is the purpose of this study?** The purpose of this study is to evaluate the impact of biofouling, including aquatic invasive species, on the aquaculture industry in Rhode Island.

- **Where will the study take place and how long will it last?** This is an online study, hosted on Qualtrics. The study will be completed on your own computer at a time you choose before the study's closing time. This study will take approximately 5-10 minutes of your time on one day.

- **What will I be asked to do?** If you agree to be in this study, you will be asked to complete a survey by answering questions about your background as an aquaculturists, the impact of biofouling on your time and financial resources, and if you would be willing to speak with us further about biofouling impacts via a phone interview. Questions on background and biofouling impacts are not optional, however there is an optional section at the end to provide further information or insight. We recommend that you complete this in one sitting as it is short, however, there is nothing that will prevent you from answering the questions during multiple sessions.

- **What are the risks if I participate in this research?** We believe there are no known risks associated with this research; however, a possible inconvenience may be the time it takes to complete the study.

- **What are the benefits associated with participation in this research?** There is no direct benefit from participation in the study; however, the data collected in this study will help marine resource managers in Rhode Island understand the impacts of biofouling and aquatic invasive species on the aquaculture industry. Answering this survey will have no bearing on your aquaculture lease status or future applications with CRMC.

- **How will my privacy and data confidentiality be protected?** Your participation in this research is confidential. Only the primary investigator will have access to the study records. When the data is downloaded onto the primary investigator's computer, that information will be deleted and the data deidentified. Data will be kept in a password protected document on the principal investigator's computer. This data may be stored for up to seven years after it is collected. At the conclusion of this study, the researchers may publish their findings. Information will be presented in summary format such that you will not be identified in any publications or presentations. Additionally, Rhode Island state agencies including Coastal Resource Management Council (CRMC) will not be aware of who chooses to participate in this survey.

- **Will I receive payment for taking part in this study?** No

- **What if I want to stop participating in this research?** You are not required to take part in this study. Participating in this research is your personal decision; even after you consent, you may stop at any time. You have the right to choose not to participate in any study activity or completely withdraw from continued participation at any point in this study without penalty or loss of benefits to which you are otherwise entitled. Your decision whether or not to participate will not affect your relationship with the researchers or any other key individuals, including those who work at CRMC.

- **Who can answer my questions about this research?** If you have questions or concerns about this study, contact the research team at:

Dr. Skylar R.Bayer
(401) 254-3091
sbayer@rwu.edu

The Roger Williams University Human Subjects Review Board ("HSRB") is overseeing this research. An HSRB is a group of individuals who perform independent review of research studies to ensure that the rights and welfare of participants are protected. If you have questions about your rights or wish to speak with someone other than the research team, you may contact:

Becky L. Spritz, Ph. D.
Director, Human Subjects Review Board
Roger Williams University
Bristol, RI 02809
401 254-5738
hsrb@rwu.edu

STATEMENT OF CONSENT

I have had the opportunity to read and consider the information in this form**.** By clicking "I agree" below I am indicating that I am at least 18 years old, have read and understood this consent form, and agree to participate in this research study voluntarily. I understand that I can withdraw from the study at any time, without any penalty or consequences.

If I have any questions, or would like a copy of this consent letter, I can contact the Principal Investigator at

Dr. Skylar R.Bayer
(401) 254-3091
sbayer@rwu.edu

I Agree

I Do Not Agree

Assessment from Qualtrics

We also included images of invasive species for Question 9 of the survey.

OF CONSENT I have had the opportunity to read and consider the information in this form. By clicking "I agree" below I am indicating that I am at least 18 years old, have read and understood this consent form, and agree to participate in this research study voluntarily. I understand that I can withdraw from the study at any time, without any penalty or consequences.

If I have any questions, or would like a copy of this consent letter, I can contact the Principal Investigator at Dr. Skylar R. Bayer (401) 254-3091 sbayer@rwu.edu

o I agree (1)

o I do not agree (2)

I *In this section (eight questions), we will ask about your aquaculture operation.*

Q1 In which town do you LIVE? Fill in the blank.

____

Q2 How many leases do you have? Fill in the blank.

o # leases (1) ____

Q3 Where is your FIRST lease located? Fill in all blanks.

o Total acres leased (1) ____

o Total acres in current production (2) ____

o Water body (3) ____

o Town (4) ____

o Growing area (5) ____

Q3a Where is your SECOND lease located? Fill in all blanks.

o Total acres leased (1) ____

o Total acres in current production (2) ____

o Water body (3) ____

o Town (4) ____

o Growing area (5) ____

Q3b Where is your THIRD lease located? Fill in all blanks.

o Total acres leased (1) ____

o Total acres in current production (2) ____

o Water body (3) ____

o Town (4) ____

o Growing area (5) ____

Q3c Where is your FOURTH lease located? Fill in all blanks.

o Total acres leased (1) ____

o Total acres in current production (2) ____

o Water body (3) ____

o Town (4) ____

o Growing area (5) ____

Q4 How many YEARS has your farm been in operation? Fill in the blank.

o Years (1) ____

Q5 Which months of the year are you consistently (at least 20 hours/week) on your farm? Check all that apply.

▢ January (1)

▢ February (2)

▢ March (3)

▢ April (4)

▢ May (5)

▢ June (6)

▢ July (7)

▢ August (8)

▢ September (9)

▢ October (10)

▢ November (11)

▢ December (12)

Q6 Which species do you culture? Check all that apply.

▢ Eastern oysters (1)

▢ Quahogs (2)

▢ Razor clams (3)

- ▢ Soft shell clams (4)
- ▢ Blood Arc clams (5)
- ▢ Bay scallops (6)
- ▢ Kelp/Algae (7)
- ▢ Other (8) ____

Q7 How much does each species grown contribute to (%) total production?

		Species	Total Production
		Name (1)	% (1)
1st species (1)			
2nd species (4)			

3rd species (5)			

Q8 What kind(s) of gear do you use for Species 1? Check all that apply.

- ▢ Floating cages/bags (e.g. OysterGro, ADPI bags, Flow N Gro) (1)
- ▢ Bottom cages/bags (e.g. stacked cages, shelf cages, ADPI bags) (2)
- ▢ Suspended long lines (e.g. SEAPA purses) (3)
- ▢ Racks (4)
- ▢ Rafts (5)
- ▢ Lantern nets/Pearl nets (6)
- ▢ FLUPSY/nursery system (7)
- ▢ Directly on bottom (8)
- ▢ Other: (9) ____

How much does each species grown contribute to (%) total production?

How much does each species grown contribute to (%) total production?

Q8a What kind(s) of gear do you use for Species 2? Check all that apply.

- ▢ Floating cages/bags (e.g. OysterGro, ADPI bags, Flow N Gro) (1)
- ▢ Bottom cages/bags (e.g. stacked cages, shelf cages, ADPI bags) (2)
- ▢ Suspended long lines (e.g. SEAPA purses) (3)
- ▢ Racks (4)
- ▢ Rafts (5)
- ▢ Lantern nets/Pearl nets (6)
- ▢ FLUPSY/nursery system (7)
- ▢ Directly on bottom (8)
- ▢ Other: (9)

Q8b What kind(s) of gear do you use for Species 3? Check all that apply.

- ▢ Floating cages/bags (e.g. OysterGro, ADPI bags, Flow N Gro) (1)

▢ Bottom cages/bags (e.g. stacked cages, shelf cages, ADPI bags) (2)
▢ Suspended long lines (e.g. SEAPA purses) (3)
▢ Racks (4)
▢ Rafts (5)
▢ Lantern nets/Pearl nets (6)
▢ FLUPSY/nursery system (7)
▢ Directly on bottom (8)
▢ Other: (9)

II *Now, we'd like to ask you about biofouling for the next 15 questions...*

Q9 What kind of biofouling do you encounter? Check all that apply.

▢ Acorn barnacle (1)
▢ Boring sponge (2)
▢ Slipper snails (3)
▢ Blue mussels (4)
▢ Mud/Blister worm (5)
▢ Tunicate - Didemnum (6)
▢ Club tunicate (7)
▢ Tunicate - Botryllus schlosseri (8)
▢ Tunicate - Botrylloides violaceus (9)
▢ Tunicate - sea squirts (10)
▢ ⊗Red sea weed (11)
▢ Seaweeds in general (12)
▢ Predators: clams, crabs, conchs, oyster drills (13)
▢ Other (14) ____

Q10 Can you differentiate NATIVE fouling from NON-NATIVE fouling species?

o Yes (1)
o No (2)
o Maybe/Sometimes (3)

Q11 Have you noticed more invasive species vs non-invasive biofouling species since you started your business?

o Yes (1)

o No (2)

o Maybe (3)

Q12 Can you identify which biofouling species are the most troubling to you?

____

Q13 How important is the issue of biofouling to your operation?

	Not at all important	Slightly important	Average/Not sure	Somewhat important	Very important	Not Applicable

Q14 What are the impacts of biofouling on your operation? Check all that apply.

▢ Reduced water flow through (1)

▢ Reduced growth (2)

▢ Increased size distribution and crowding (3)

▢ Increased mortality (4)

▢ Increased predation (5)

▢ Increased disease (6)

▢ Increased time (labor) (7)

▢ Increased cost of operation (8)

▢ None of the above (9)

▢ Other: (10) ____

Q15 Which species that you farm is/are most impacted by biofouling?

____

Q16 Based on your observations, has the amount encountered and timing of biofouling changed over the last 5 to 10 years without mitigation?

o Decreased a lot (1)

o Decreased a little (2)

o About the same (3)

o Increased a little (4)

o Increased a lot (5)

Q17 How has YOUR effort mitigating/cleaning biofouling over the last 5 to 10 years changed, and why?

____

Q18 How would you describe the level of biofouling (you encounter) each month of the year? Check one option for EACH month.

	Least biofouling (1)	Slightly below average (2)	Average biofouling (3)	Slightly above average (4)	Most biofouling (5)
January (1)	o	o	o	o	o
February (2)	o	o	o	o	o
March (3)	o	o	o	o	o
April (4)	o	o	o	o	o
May (5)	o	o	o	o	o
June (6)	o	o	o	o	o
July (7)	o	o	o	o	o
August (8)	o	o	o	o	o
September (9)	o	o	o	o	o

October (10)	o	o	o	o	o
November (11)	o	o	o	o	o
December (12)	o	o	o	o	o

Q19 What months of the year do you spend the most time cleaning biofouling? Check one option for each month.

	Least time (1)	Slightly below average (2)	Average time (3)	Slightly above average (4)	Most time (5)
January (1)	o	o	o	o	o
February (2)	o	o	o	o	o
March (3)	o	o	o	o	o
April (4)	o	o	o	o	o
May (5)	o	o	o	o	o
June (6)	o	o	o	o	o

July (7)	o	o	o	o	o
August (8)	o	o	o	o	o
September (9)	o	o	o	o	o
October (10)	o	o	o	o	o
November (11)	o	o	o	o	o
December (12)	o	o	o	o	o

Q20 Which method(s) do you currently use or have previously used for cleaning biofouling from gear? Check all that apply.

- ▢ Gear type (bag, cage, rigging) (1)
- ▢ Method of grow-out (floating vs bottom) (2)
- ▢ Air drying/flipping (3)
- ▢ Brine/hot water dips (4)
- ▢ Power/pressure washing (5)
- ▢ Other: (6) ____

Q21 Of the methods you currently use, which are the most effective for mitigating biofouling?

____

Q22 How long have you been using this/these most effective method(s)?

____

Q23 What percentage of your overall time in a typical year do you ESTIMATE you and your staff spend cleaning biofouling from gear? Fill in the blank.

o % time (1) ____

III *We are interested in understanding the financial impact and costs associated with biofouling mitigation efforts. The next seven questions will ask about these aspects of your operation. Please remember that all information shared through this survey will remain confidential.*

Q24 Does biofouling increase the cost of operation?

o Yes (1)

o No (2)

Q24a What costs are affected? Check all that apply.

▢ Repair and maintenance (1)

▢ Labor (2)

▢ New gear (3)

▢ Anti-fouling equipment (4)

▢ Marketing/packaging (5)

▢ Other: (6) ____

Q25 Can you provide a ($) dollar estimate of the total cost spent on biofouling control annually? Fill in the blank.

o $ Total cost (1) ____

Q26 ESTIMATE the percent (%) of total operating costs spent on biofouling control in a typical year? Fill in the blank.

o % Total operating costs (1) ____

Q27 Is the % of total operating costs (from Q26) spent on biofouling control, more than, less than, or the same as what you spent 5 to 10 years ago?

o Less than before (1)

o Same as before (2)

o More than before (3)

Q28 Do you CURRENTLY pay more for gear that is more resistant to biofouling?

o Yes, I do (1)

o No, I don't (2)

Q29 WOULD you pay more for gear that is more resistant to biofouling?

o Yes, absolutely (1)

o Yes, but it depends on the price (2)

o No, never (3)

o Maybe, I would need to think about it more (4)

IV *Thank you for your help. We have an additional section of six questions about your opinions and ideas on biofouling mitigation...*

Q30 What do YOU think causes the majority of biofouling in your area?

▢ Equipment (e.g. cages, bags, rigging) (1)

▢ Location (e.g. water depth, flow, sediment, environmental factors) (2)

▢ Grow-out method (e.g. floating, bottom, intertidal, etc) (3)

▢ Other: (4) ____

Q31 Do you wish there was more information available on biofouling?

o Yes (1)

o No (2)

o I don't know (3)

Q32 Do you wish there was more equipment available to resist biofouling?

o Yes (1)

o No (2)

o I don't know (3)

Q33 Do you have any specific fouling problem that you would like to see addressed with better research and tools for control?

____

Q34 Anything else to consider regarding biofouling you would like to share?

____

Q35 Can we follow up with an additional phone survey or email? If so, please provide your contact information where we can reach you. (email and/or phone number)

____

Appendix C: SOP for Estimating Percent Cover in ImageJ

CRMC Biofouling Project 2022 - Finding Area Using ImageJ Software

POINT OF CONTACT:
Susanna Osinski
Shellfish Field Technician
sosinski@rwu.edu
(917) 834 1615
Center for Economic and Environmental Development
Roger Williams University
Bristol, RI 02809

OBJECTIVE
This procedure describes how to find the area of an object from a photograph. This was used with the CRMC Biofouling Research Project spanning from April 2022 - November 2022. ImageJ is used in this project to find '% Cover' of invasive species to overall fouling.

MATERIALS AND EQUIPMENT
- Computer
- Photograph (.jpg file preferable)
- ImageJ software (free access)

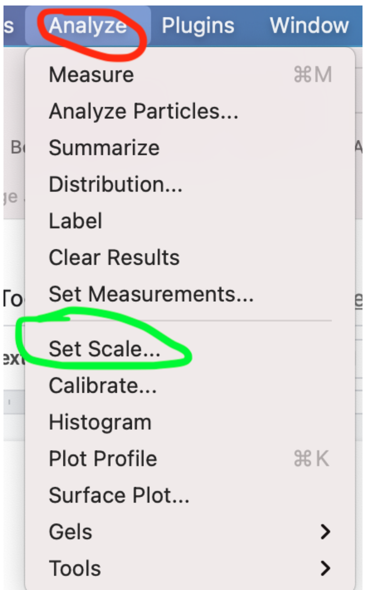
PROCEDURE
1.0 Download software and troubleshooting
- Download and install the free ImageJ software for your computer type from this website:
  - https://imagej.nih.gov/ij/download.html
- If you are having trouble with running the program or installing the software (such as your computer can't open as it is from an unidentified/unverified developer) refer to this website:
  - ImageJ user guide
  - Instructions for Mac OS X
  - Instructions for Windows
  - Instructions for Linux

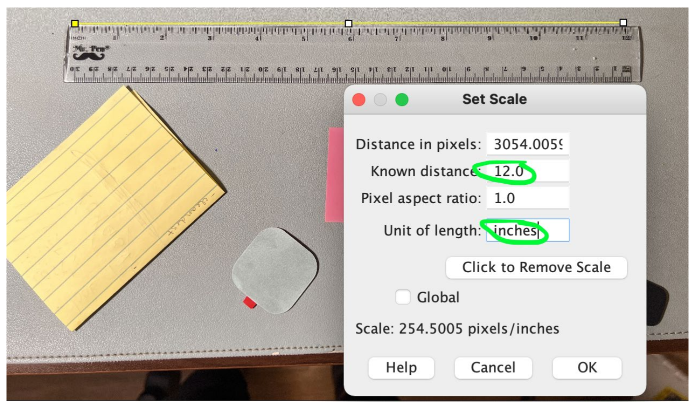
2.0 Opening your picture
- Select the image saved to your computer. It is helpful to save it as a '.jpg' file. Refer to user guide if issues arise. Images for this project were saved as Month#-Color-Bag-BagSide (ex: M3-Pu-2-FRONT) and separated via Farm folders.

- You want the image you are using to have a ruler of some sort, or item of known length in it. This length will be used to set the scale in the following steps.

3.0 Set Scale

- Click on the line icon . Create a line along the ruler in the photo.
- The line should start at the first measurement mark on the ruler and may end at any mark after. Usually we end the line at the 12 inch mark.
- Click on **Analyze** and then select **Set Scale.**

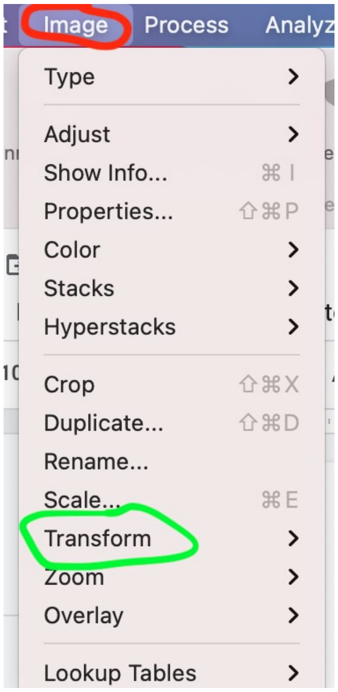




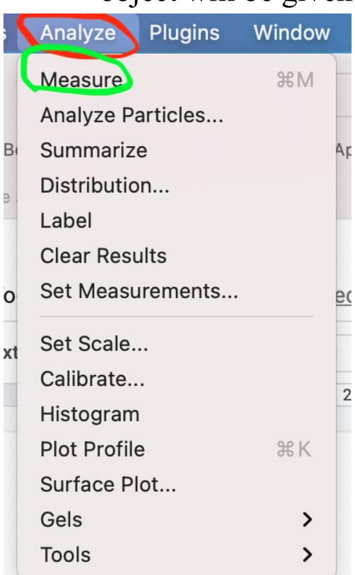
- A popup window will open for you to set the scale. FIll in **"Known distance"** with '12' and your **"Unit of length"** with 'inches' (or whatever units you are using). Refer to photo example above of what it looks like. Click "**OK**" and exit out the window. Your scale is now set.

4.0 Finding the area of an object

- If the object you are measuring is a small part of the image, you can zoom in and select the magnifying glass  to enlarge the portion of the image you are working on. You will need to zoom in to carefully select some of the smaller organisms.
- Pick the outlining icon that best fits the shape you are measuring. In most cases, the freehand selection  will be the most accurate.
- The other icons can be used if your shape is a perfect oval, square or rectangle. 
- The polygon shape can be used if the object is a perfect shape with more than two lines. 
- If the object is at an angle and is a perfect shape. Click **Image** and then select **Transform**. Then select the option that corrects the angle of your object.



- First take an area measurement of the whole bag/tray.
- Then pick one organism to start working on and select all visible areas. When selecting multiple objects of the same organism species that are not connected, use the shift button to pick up your cursor and move to a different part of the image and continuing to select.
- After all objects have been outlined, click **Analyze** and select **Measure**. The area of the object will be given as the output in a separate pop up window.



5.0 Saving measurements

- Using the measurement provided from the pop-out window, select the total area and copy and paste into a separate document.

- Record the photo label and the total area, then make a label for each species found and insert the recorded area found from the ImageJ analysis.
- When done, identify known species. You can refer to identification books to help. You can also reach out to Brian Wysor at RWU who specializes in macroalgae as well. Summer interns were trained by Kevin Cute from CRMC to identify invasive species.
- Once done with collecting all species, add the different species together and multiply by the total area from the bag to find percent cover. You can multiply just invasive species coverage by total area to find the invasive species percentage as well.

Additional Notes:

- The more fouling on the bags, the longer each photo identification will take.
- It is preferred to have a professional in AIS identify the different species found to save time.
- Be sure to save each area measurement with its species before analyzing the next species in order to keep results organized.

Example of photograph used in ImageJ analysis:

Example of close up photographs used for species identification:

Example of collected fouling to be dried:

Appendix D: Standard Operating Procedure (SOP) and Data Sheet for Collections

SOP for collection adapted for Chessawanock Island Oyster Company. SOPs were adapted for each particular farm and the three other SOPs can be provided upon request.

POINT OF CONTACT:
Susanna Osinski
Shellfish Field Technician
sosinski@rwu.edu
(917) 834-1615
Center for Economic and Environmental Development
Roger Williams University
Bristol, RI 02809

OBJECTIVE

This procedure describes the steps for collecting the fouling from the Flow N Gro bags at Chessawanock Island Oyster Co. for the CRMC Biofouling Research Project spanning from April 2022 - November 2022. Collection is to be done every month (~5 week span) at Hog Island, Bristol, RI.

MATERIALS AND EQUIPMENT

- RWU truck and key
- Work gear:
    - Grundens, waders, boots, gloves, hat, jacket, sweater, etc.
- Field notebook and Sharpie/pencil (Pencil preferred)
- Clipboard and data sheet to be filled out
- Field backpack (black and green - in Susanna's office)
- Field dry box (2x)
    - Small
    - Big
- Calipers, forceps, scrapers (2x each)
- Fish totes with holes (3x), without holes (2x)
- 5-Gallon buckets (4x)
- Power washers (gas)
    - Garden hose
    - Wand tips
    - Gas can
    - Brown tarp
- PVC collection frame

    - Rubber pipe connector
    - 1in clamps (10x)
    - Green spat bags (9x)
    - 6ft x 6ft plastic sheeting

- Several zip ties of 4-different colors
    - 4” zip ties
    - 8” zip ties
- Tote hook, flathead screwdriver, pliers/wrench, ruler
- YSI (DI, water temp) - borrow from Dave Taylor
- Refractometer (salinity)
- Folding table
- DI water/fresh water to rinse testing equipment
- Camera (use your phone if possible) or bring GoPro
- Walkie talkies (optional)
- Bug spray/Sunscreen/Drinking water
- Invasive species identification booklet
- Clean and dry ADPI bags (9x) (optional)

PROCEDURE

1.0 Reserve RWU pickup truck and YSI/Refractometer ahead of time

- Email Kevin Cute (from CRMC) to confirm his availability.
- Request truck if Kyle will bring us to the farm. Request boat if we will be going without Kyle.
- Check with RWU faculty and staff to reserve the truck for the day of collection (can go through Slack).You can use your own vehicle if necessary.
- Get the truck key from Susanna’s office.
- Email Dave Taylor to coordinate pickup of YSI.

2.0 Pack and load up truck/boat

- Accumulate the buckets, fish totes, power washers, extension cords/gas can, garden hose, green spat bags, tote hook, tools, and folding table from the shoreline shed.
- Accumulate PVC collection frame, rubber connector, clamps, plastic sheeting, tarp and DI water from the Tool Shed/Outside.
- Print out data sheet and put it in the clipboard with pen/pencils and field notebook. Get YSI from dry lab.
- Grab the small dry box and fill with zip ties, and refractometer from Susanna’s office. Put clipboard, small dry box, and work gloves in the field backpack.

- Grab the big dry box and fill with extra pens/pencils, calipers, forceps, scrapers, walkie talkies, invasive species booklet, towel, extra nozzle tips, bug spray, ruler and tools from Susanna's office.
- Accumulate all necessary gear (check weather beforehand).
- Load everything into the truck the day before, if possible.(Load the boat the morning of if necessary).

3.0 Arrive at farm

- Once at the farm, unload the truck into Kyle's boat. Put on gear,
- Go to farm and take YSI and refractometer measurements as well as other data points Record on data sheet.
- Pick up all bags carefully and remove bags from each cage putting them gently into a fish tote. Use a different fish tote for each cage and bring back to shore.
- Keep marked bags of oysters separate from each other!!
- Lay out brown tarp on ground. Put single bag on tarp with ruler next to it (NOT on top of it). Take pictures of EACH side of each bag with fouling and oysters inside before cleaning. Be sure to have whole bag in each picture.
- Count live and dead oysters of each bag, record in field notebook. Discard dead oysters.
- Take 25 random oysters from each bag and measure the length and width with calipers and record in field notebook.
- Set up collection frame and power washer. Attach spat bag to frame and put a bucket underneath. Attach colored zip tie to spat bag of corresponding bag's color.
- Power wash **9 bags** until fouling is completely gone. Change spat bag for each bag. Leave control cage's bags (tagged ORANGE) alone; only measure and count oysters for the control cage's bags.
- Put oysters back in cleaned bags. Put back in cages. Put control oysters back in DIRTY bags, put back in cage. Take pictures throughout process to document.
- **IF NOT CLEANING ON SITE: bring 9 clean bags to farm and exchange dirty bags for clean bags. Do not exchange control bags. Bring dirty bags back to RWU and clean there.**

4.0 Finishing up

- Put all materials back into the truck, and make sure you clean up your work area.
- Check that the **data sheet and field notebook** have been properly filled out.
- Rinse YSI and refractometer with DI water. Rinse again thoroughly upon return to RWU.
- Unload the truck, clean, and put away all items. Return truck key to Susanna's office.

Additional notes:

- Full collection on this farm takes a few hours with only one person.
- If short on time, bring extra bags and finish clearing at RWU. You can also measure less bags if necessary.

- It is recommended you have at least one additional person to help.
- Try to split up work, so 1-2 people are measuring while 1 person powerwashers.
- Be sure to pack warm clothing as it is often windier and colder on the water.
- If we are **borrowing** equipment, be sure to properly clean and store equipment before returning it.
- If bringing RWU boat, be VERY CAREFUL of submerged rocks. Go at low tide.

Data Sheet for Collections:

Farm Name:____________________________ Farm Location:______________________ **Time Start:**________________ Date:______________________ **Time End:**_________________

Name of Researchers: ____

Air Temp(F)	Water Temp(C)	% DO	Salinity (ppt)	Wind (mph) & Direction	Cloud Cover (%)	Rain (last 24hrs)	% Cover of invasive
Other Comments:							

AT SITE CHECKLIST:

- Take pictures/videos of Cage, Bags, and or Trays throughout deployment
- Take YSI data and salinity data and rinse with fresh water
- Remove trays/bags of 4 replicates - and take pictures
- Count LIVE and DEAD oysters
- Measure 25 oysters from **each** bag/tray
    - Length and width of 25 in each **bag**/**tray**
    - If short on time, 25 from each replicate
    - If no time, 25 from whole experiment
- Set up collection unit and wash 3 of 4 replicates **leaving control alone**
- Put oysters back in bags/trays and tag properly and deploy

LOAD RWU TRUCK CHECKLIST:

- GEAR: grundens, waders, boots, gloves, hat, jacket, sweaters, etc
- Field notebook and pencil
  BRING EXTRA PENCILS
- Clipboard and data sheets
- Calipers, forceps, scrapers (2x each)
- Fish totes w/ & w/o holes (>2x each)
- Buckets (4x)
- Zip ties (several of 4 diff colors)
- YSI and Refractometer
- DI water/Fresh water
- Tote hook
- Pliers, wrench, flathead screwdriver
- Camera (phone or GoPro)
- Bug spray/Sunscreen
- Folding table
- Invasive species booklet
- Power washers (gas and electric)
  - Garden hose
  - Wand tips
  - Extension cord
  - Gas can
  - Brown tarp
- Collection frame
  - PVC pipes
  - 1in clamps (10x)
  - Spat bags for collection (9x)
  - Plastic sheeting (6'X6')
  - Black rubber connector
- Walkie talkies (optional)
- Clean and dry ADPI bags (9x)