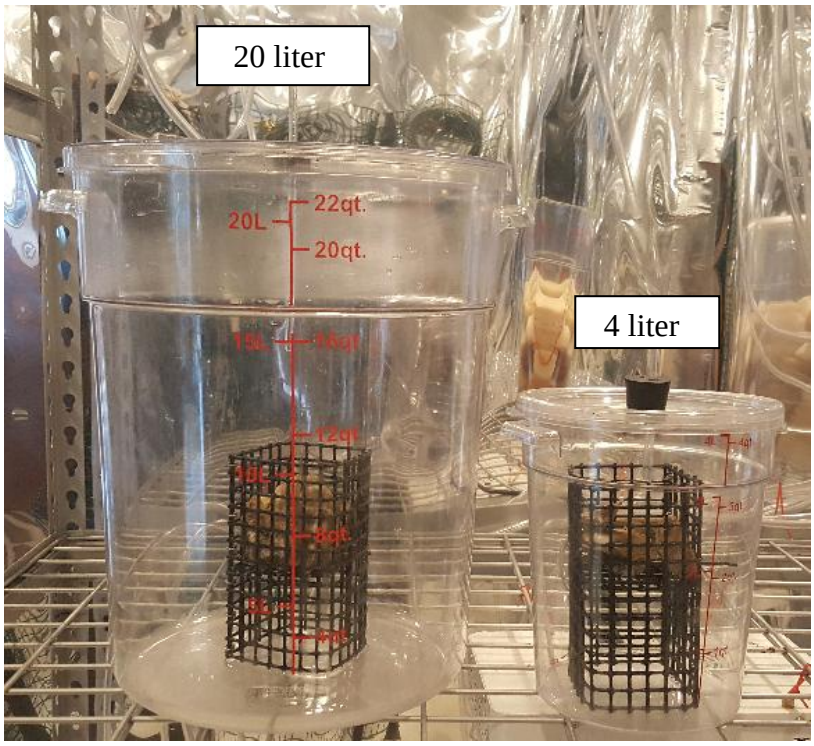
FINAL PROGRESS REPORT

Project Title	Potential gear coatings as a mitigation technique for invertebrate predators of aquacultured eastern oysters (*Crassostrea virginica*)
Reporting Period	*7/30/2019 – 6/1/2020*
Author (Project Coordinator)	Name of person submitting this report.
Key Word	Gear coating, anti-predator, aquaculture
Funding Level	Total funds allocated for this project to date.
Participants	Ward Aquafarms, LLC and ePaint ®
Project Objectives	Identify a gear coating that deter and mitigate predation by oyster drills (*Urosalpinx cinerea*) and starfish (*Asterias rubens*) on aquacultured eastern oysters via: **1) Identify if different experimental gear coatings produced by ePaint deter or attract oyster drill snails from attacking eastern oysters.** **2) Identify if different experimental gear coatings produced by ePaint deter or attract starfish from attacking eastern oysters.** **3) Identify if different experimental coatings produced by ePaint on cages housing oysters deter or attract oyster drills when the oyster drills were exposed to multiple cages with different coating simultaneously.** **4) Identify if different experimental coatings produced by ePaint on cages housing oysters deter or attract starfish when the starfish were exposed to multiple cages with different coating simultaneously.**
Anticipated Benefits	The benefits from this project are intended to identify an ecologically friendly gear coating that can be used to reduce mortality in eastern oyster aquaculture caused by invertebrate predation. Reductions in mortality caused by invertebrate predation will promote an increase in yield of eastern oysters and the potential economic income.

Project Progress	***Methods*** **1) Identify if different experimental gear coatings produced by ePaint deter or attract oyster drill snails from attacking eastern oysters.** This objective was investigated using two different experimental phases. Phase I used replicate 4 liter mesocosms filled with seawater which were equipped with air stones to provide oxygenation for the duration of each 24 hour trial. All trials were maintained at water temperature of approximately 20^{o}C. Once the 4 liter buckets were filled, a cage with an ID number identifying the coating applied was added to each mesocosm (Figure 1).  Figure 1. 20 liter and 4 liter mesocosms used during oyster drill snail and starfish predation trials in objectives 1 and 2. Note; all gear coatings reciprocating to coating ID numbers were known only by the individual(s) who applied the coating which allowed the individual(s) conducting the mesocosm manipulation to not have bias towards a result for a specific coating. The cage was then filled with approximately 200 ml of year two oysters ranging from 1-2” in shell height and 10 oyster drill snails. After 24 hours the number of snails on each cage and the oysters held within the cage were counted for each mesocosm. Once snail counts were performed, new snails and oysters

were placed in each mesocosm. During the experimental trials using the 4 liter mesocosms, six different experimental coatings in were tested in conjunction with a "blind" control coating. Triplicates of each coating and control were tested using three experimental 24 hour trials. A second round of mesocsosm manipulations using 20 liter mesocosms (Figure 1) was then conducted to test nine new gear coatings developed by ePaint. 10 oyster drill snails and 200 ml of oysters from the same population used in 4 liter mesocosms experiment were introduced to each mesocosm. Counts of snails on each cage and the oysters held within the cage were counted for each mesocosm after 24 hours. Once snail counts were performed, new snails and oysters were placed in each mesocosm. Nine different gear coatings were tested in triplicate during the 20 liter mesocosm experiment.

2) **Identify if different experimental gear coatings produced by ePaint deter or attract starfish from attacking eastern oysters.**

20 liter mesocosms were used to test ten different experimental gear coatings developed by ePaint for their ability to deter or attract starfish predation on eastern oysters. The mesocosms and cages held within were of the same design as those used in objective one. Additionally, all ten gear coatings were those which were tested in objective one in 20 liter trials. Each cage that was place in the 20 liter mesocosms was filled 200 ml of 1-2" oysters from the same population used in the 20 liter trials in objective one. One starfish was then placed in each mesocosm. After 24 hours, whether the starfish was on or touching the cage housing the oysters was documented. After each 24 hour trial, new oysters were placed in the cages and the starfish were place back at the bottom of the mesocosm.

3) **Identify if different experimental coatings produced by ePaint on cages housing oysters deter or attract oyster drills when the oyster drills were exposed to multiple cages with different coating simultaneously.**

A 200 liter raceway tank was used to test if oyster drill snails were more attracted or deterred by different gear coatings when presented with cages with different coatings housing oysters. The 200 liter raceway tank was provided with two water pumps to provide flow and two separate air stones to provide aeration at either end of the tank. The tank was held at 20^{o}C for the duration of the experiment. Prior to placement of cages, oysters, and oyster drills, the raceway tank was separated into three sections (Figure 2).

Figure 2. 200 liter raceway tank separated into three oyster drill snail choice trial replicates.

In each section 10 randomly selected cages with different gear coatings were placed equidistant from each other in a circular pattern (Figure 2). Each cage was then filled with 200 ml of 1-2" year two oysters. Within the center of the circle of cages, 50 oyster drill snails were placed. After 24 hours of exposure to the oyster drills, the number of drills on the cages and the oysters within the cages were tallied. All snails were removed from the cages and oysters and placed back in the center of the cage circle. Measurements were taken every 24 hours for four treatments.

4) **Identify if different experimental coatings produced by ePaint on cages housing oysters deter or attract starfish when the starfish were exposed to multiple cages with different coating simultaneously.**

The same 200 liter raceway tank divided into three sections as used in objective three was used in objective four to test the if starfish were more attracted or deterred by different gear coatings when presented with cages housing oysters. The 200 liter raceway tank was again provided with two water pumps to provide flow and two separate air stones to provide aeration at either end of the tank and held at 20^{o}C for the duration of the experiment. Prior to placement of cages, oysters, and oyster drills, the raceway tank was separated into three sections. In each section, 10 randomly selected cages with different gear coatings were placed equidistant from each other in a circular pattern. Each cage was then filled with 200 ml of 1-2" year two oysters. Within the center of the circle of cages, four 6" starfish were placed (Figure 3).

Figure 3. One of three partitions in the 200 liter raceway tank for the starfish choice experiments.

After 24 hours of exposure of cages housing oysters to the starfish, the number of starfish touching the cage, on the cage and on the oysters within the cage were tallied. All starfish were the removed from the cages and oysters and placed back in the center of the cage circle. Measurements were taken every 24 hours for four treatments.

Results

1) **Identify if different experimental gear coatings produced by ePaint deter or attract oyster drill snails from attacking eastern oysters.**

For the oyster drill snail trials conducted using 4 liter mesocosms, oyster drill prevalence (% of cages for each gear coating with at least one snail present on cage or oysters within cage) ranged from 33% for gear coated in Netminder to 92% for treatment bio-based resin (Figure 4). No gear coating treatment had an oyster drill prevalence significantly different from the prevalence of 75% observed in the control treatment (GLM-TukeyHSD, Chi-square, α=0.05, two-tailed, df

= 6, n =76, p > 0.05, Figure 4). However, the prevalence of 33% observed in gear coated with Netminder was significantly lower than the 92% prevalence observed in gear coated with bio-based resin (GLM Chi-square, α=0.05, two-tailed, df = 6, n =76, p = 0.0105, Figure 4) and 83% prevalence observed in gear coated with eucalyptus oil (GLM Chi-square, α=0.05, two-tailed, df = 6, n =76, p = 0.0454, Figure 4). All snails and oysters died in trials with gear coated in pyrithione zinc.

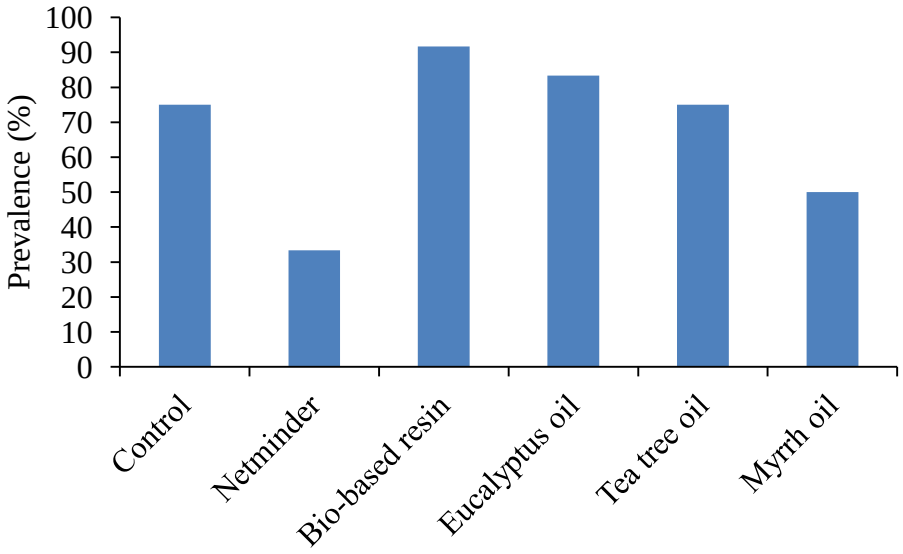


Figure 4. Oyster drill snail prevalence (% of cages for each gear coating with at least one snail present on cage or oysters within cage) for each treatment in the 4 liter mesocosms used in objective 1.

Oyster drill snail abundance (average number of snails observed on a single cage) across all trials using 4 liter mesocosms ranged from 0.58 snails/cage (0 – 3 snails per cage) for cages coated in Netminder to 2.67 snails/cage (0 – 7 snails per cage) for cages coated in bio-based resin (Figure 5).

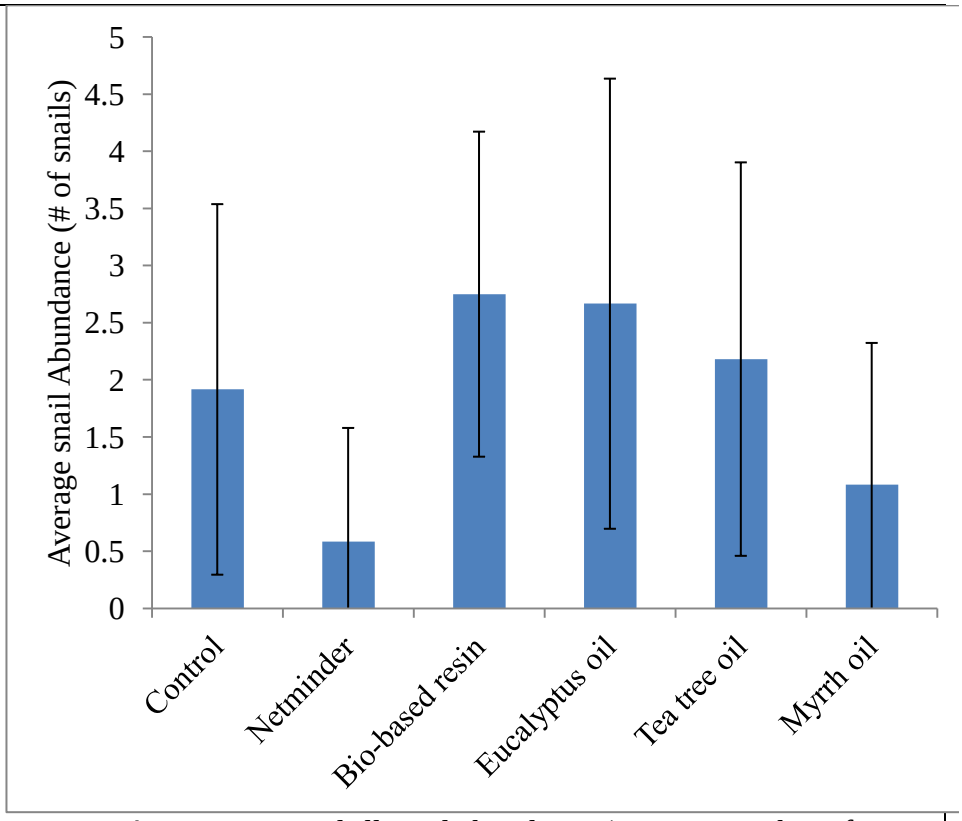


Figure 5. Average oyster drill snail abundance (average number of snails per cage for each treatment group) ± SD during the 4 liter mesocosm trials.

Average oyster drill prevalence on cages with different gear coatings used in the 20 liter mesocosm trials did not significantly vary (GLM-ANOVA, Chi-square, α=0.05, two-tailed, df = 10, n =88, p = 0.423, Figure 6), ranging from 22% prevalence on gear coated with Netminder to 78% prevalence on gear coated with capsaicum extract.

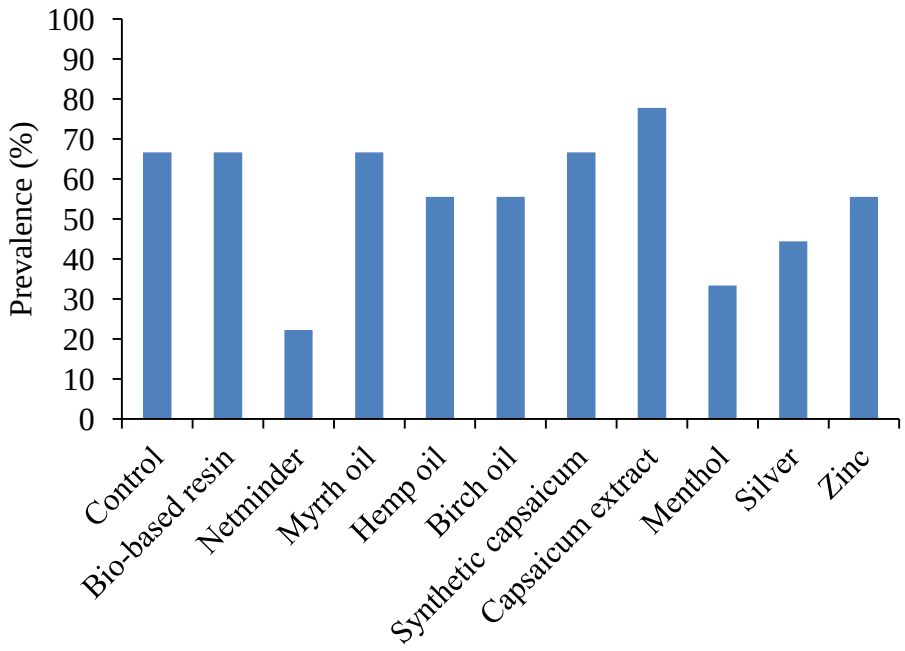


Figure 6. Oyster drill snail prevalence (% of cages for each gear coating with at least one snail present on cage or oysters within cage) for each treatment in the 4 liter mesocosms used in objective 1.

Average oyster drill snail abundance (average number of snails observed on a single cage) across all trials using 20 liter mesocosms ranged from 0.22 snails/cage (0 – 1 snails per cage) for cages coated in treatment Netminder to 2.0 snails/cage (0 – 5 snails per cage) for cages coated in treatment Capsaicum extract (Figure 7).

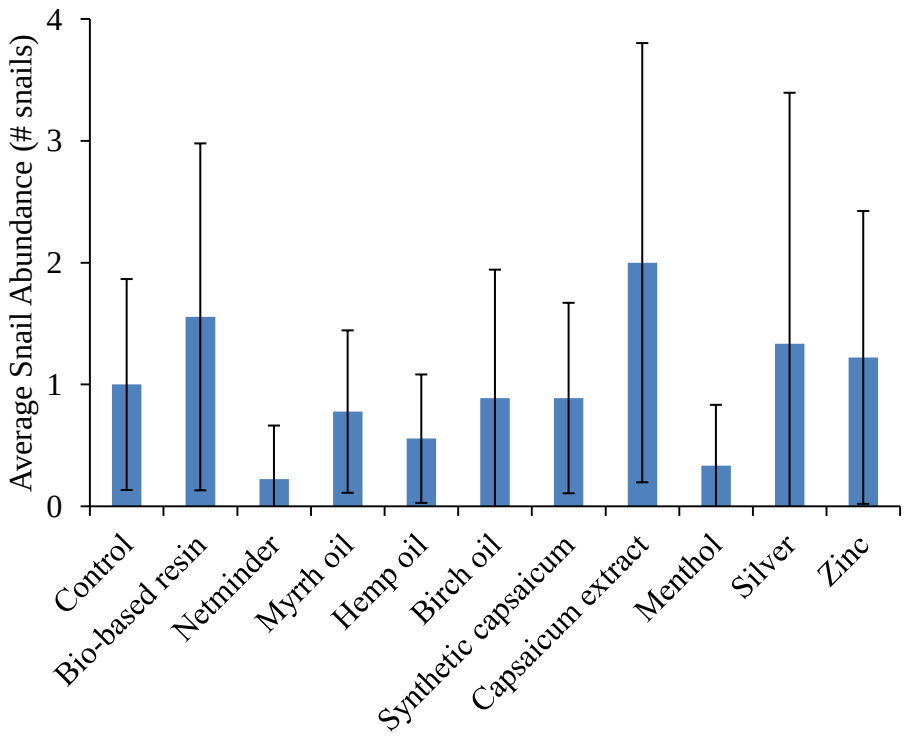


Figure 7. Average oyster drill snail abundance (average number of snails per cage for each treatment group) ± SD during the 20 liter mesocosm trials.

2) **Identify if different experimental gear coatings produced by ePaint deter or attract starfish from attacking eastern oysters.**

No starfish were observed on cages housing oysters regardless of gear coating. All starfish in the mesocosm experiments were observed near the surface of the water on mesocosm itself.

3) **Identify if different experimental coatings produced by ePaint on cages housing oysters deter or attract oyster drills when the oyster drills were exposed to multiple cages with different coating simultaneously.**

For the oyster drill snail choice trials conducted using the 200 liter raceway system, oyster drill prevalence ranged from 0% for the control to 53% for gear coated with myrrh oil (Figure 8). The only treatment which had an oyster drill snail prevalence that was significantly higher than any of the other treatments was the aforementioned 53% for gear coated with myrrh oil (GLM-TukeyHSD, Chi-square, α=0.05, two-tailed, df = 10, n =88, p = 0.0030, Figure 8).

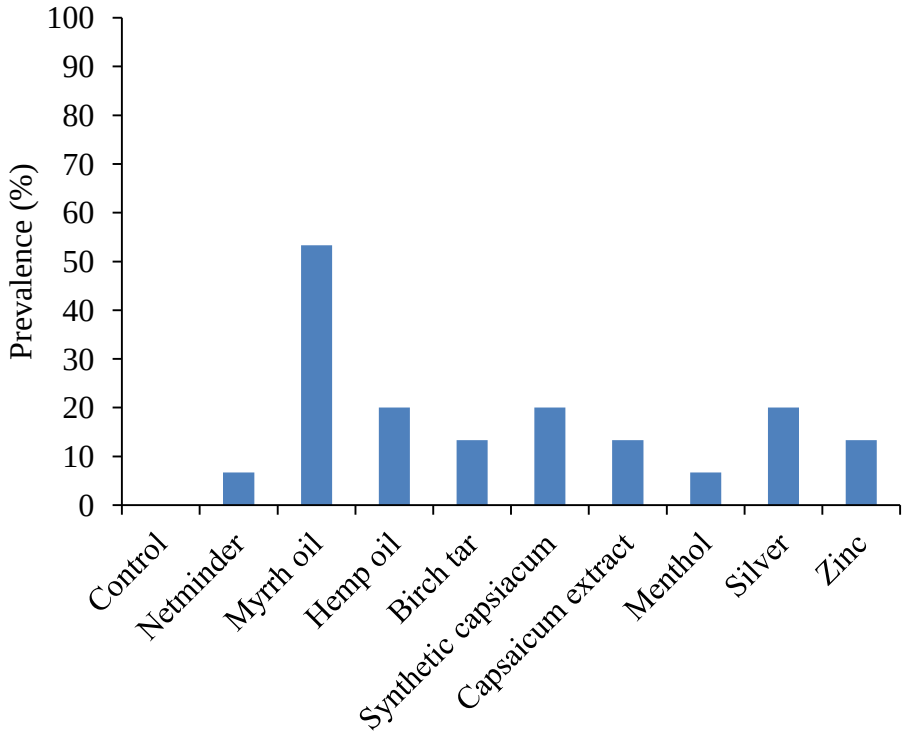


Figure 8. Oyster drill snail prevalence (% of cages for each gear coating with at least one snail present on cage or oysters within cage) for each treatment in the 200 liter raceway system used in objective 3.

Oyster drill snail abundance (average number of snails observed on a single cage) across all trials conducted in the 200 liter raceway system ranged from 0.0 snails/cage for cages coated in the control treatment group to 0.8 snails/cage (0 – 2 snails per cage) for cages coated in treatment myrrh oil (Figure 9).

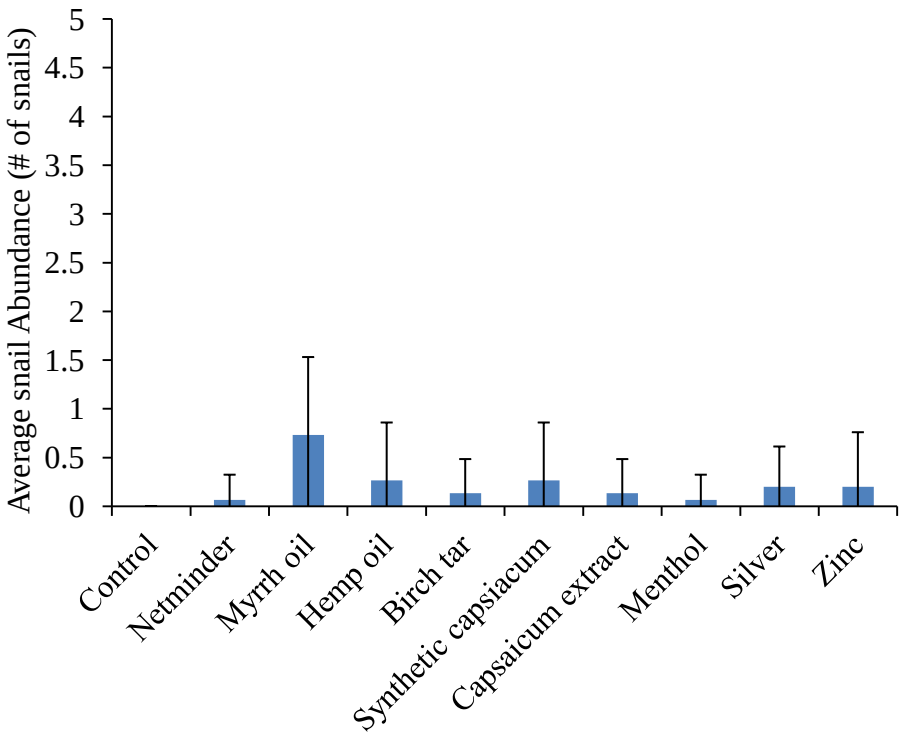


Figure 9. Average oyster drill snail abundance (average number of snails per cage for each treatment group) ± SD during the 200 liter raceway system trials in objective 3.

4) **Identify if different experimental coatings produced by ePaint on cages housing oysters deter or attract starfish when the starfish were exposed to multiple cages with different coating simultaneously.**

In the choice trials conducted with starfish in the 200 liter raceway system the prevalence of starfish on or touching a cage did not significantly vary between gear with different coatings (GLM-ANOVA, Chi-square, α=0.05, two-tailed, df = 9, n =80, p = 0.07234, Figure 10). Starfish prevalence ranged from 11% for menthol, silver, and zinc to 56% for treatments myrrh, hemp oil, synthetic capsaicum and capsaicum extract (Figure 10).

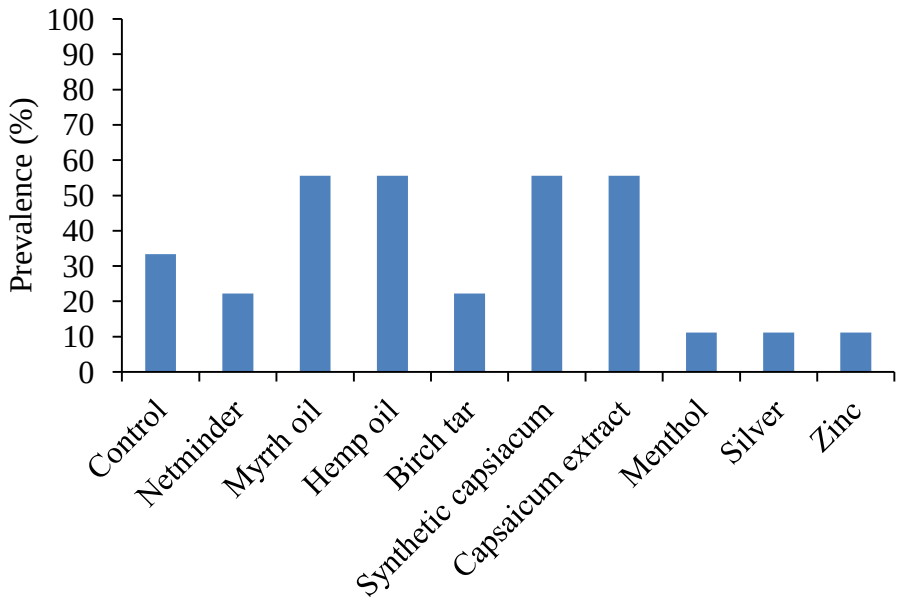


Figure 10. Starfish prevalence (% of cages for each gear coating with at least one starfish on or touching a cage containing oysters) for each treatment in the 200 liter raceway system used in objective 4.

Starfish abundance (average number of starfish observed on or touching a single cage) across all trials conducted in the 200 liter raceway system ranged from 0.1 starfish/cage (0 – 1 starfish per cage) for cages coated in menthol, silver, and zinc to 0.9 starfish/cage (0 – 3 starfish per cage) for cages coated in treatment myrrh oil (Figure 11).

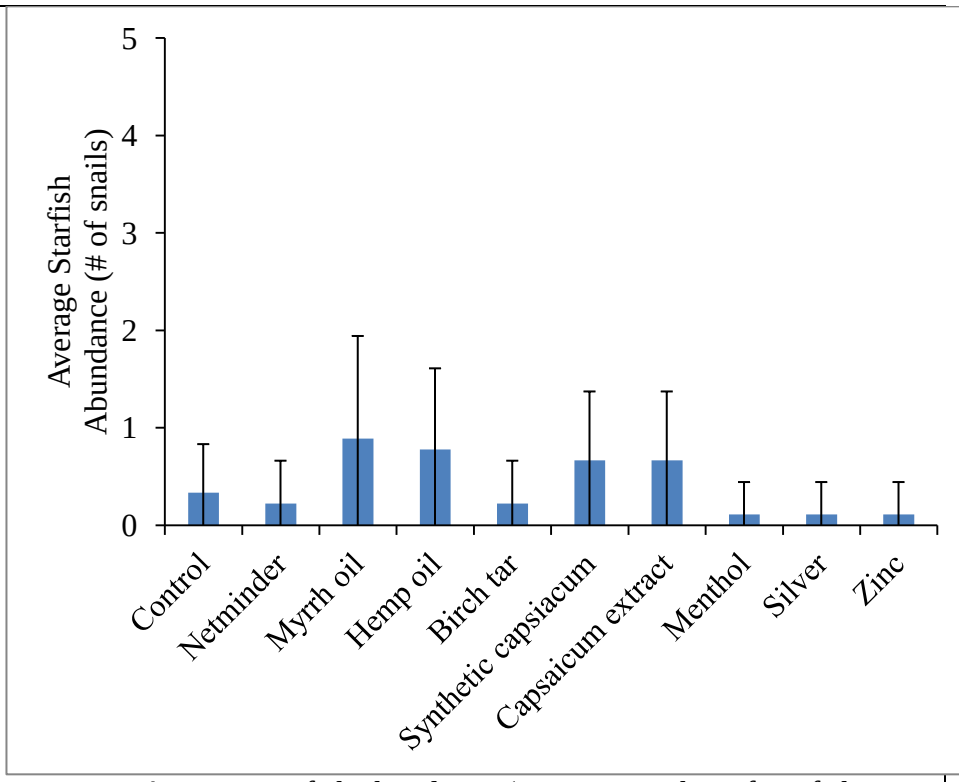


Figure 11. Average starfish abundance (average number of starfish on or touching a cage for each treatment group) ± SD during the 200 liter raceway system trials in objective 4.

Accomplishments:	
Outreach Overview	Results will be relayed to appropriate audiences via regional aquaculture conferences and potential publications if applicable. Results will be extended via the aforementioned avenues once restriction and complications with the Covid-19 virus have subsided.
Targeted Audiences	Target audience will be shellfish growers, primarily operations which culture eastern oysters in waters where starfish and oyster drill snail predation are an issue or a concern.
Outputs:	Outputs are reports, presentations to shellfish growers, and future proposals to continue development work following the successful minigrant project.
Outcomes/Impa	Results from this study will aid in the development of techniques to mitigate

cts:	predation of starfish and oyster drill snails on cultured eastern oysters. Eastern oysters are a sessile invertebrate, meaning they are immobile and unable to move away from potential predators, such as starfish and oyster drill snails. Although much of the gear used in oyster aquaculture keeps oysters from being directly in the sediment, portions of the gear typically extend to the sediment, such as legs on cages or rebar racks in intertidal aquaculture. With oyster aquaculture gear extending to the sediment, organisms such as starfish and oyster drills are able to climb to the location of shellfish, such as oysters, housed in said gear. Since starfish and oyster drills typically have to come into contact with gear prior to having access to forage on aquacultured oysters, identify a gear coating that deters starfish and oyster drills will help decrease oyster mortality and increase production. Results from this study illustrate multiple coatings that could help in reducing predation by starfish and oyster drills on eastern oysters housed in aquaculture gear. **Oyster drills:** When gear was coated with the 2016 Netminder formula, oyster drill prevalence and abundance were significantly reduced compared to other treatments. Application of Netminder on aquaculture gear which houses eastern oysters in areas where oyster drill predation is common could help increase oyster survival and the revenue of the operation. **Starfish:** Results from this study suggest that menthol, zinc and silver could be potential options for reducing starfish predation on aquacultured eastern oysters. Although the observed reduction in starfish prevalence on gear coated with menthol, zinc and silver was not statistically significant, coating aquaculture gear in menthol, zinc and silver could have a significant impact if applied to gear on a commercial scale.
Impacts Summary	1. **Relevance:** Issue – what was the problem? Starfish and oyster drill snails are known predators of eastern oysters, and can have serious economic impacts for oyster aquaculture operations. Identify potential strategies to mitigate starfish and oyster drill snail predation on eastern oysters could increase oyster survival, which would increase yield and revenue.

	2. **Response:** What was done? Fifteen different experimental gear coatings on cages housing eastern oysters were tested for their ability to deter starfish and oyster drill snail predation. 3. **Results:** How did your work make a difference **(change in knowledge, actions, or conditions)** to the target audiences? Results from this study indicate that Netminder could significantly reduce oyster drill snail predation in eastern oyster aquaculture, increasing survival and revenue. Additionally, results from the study indicate that coating gear in menthol, zinc or silver could help deter starfish predation on eastern oysters housed in gear coated with the aforementioned gear coatings. 4. **Recap:** One- sentence summary If applied to aquaculture gear on a commercial scale, several of the experimental gear coatings tested in this study could significantly reduce oyster drill snail and starfish predation on eastern oysters increasing oyster survival and potential farm revenue.

Publications	Follow the format to list publications in the following categories:
	• Presentations:
	  o Oral
	  o Posters
	• Peer-reviewed:
	  o Print (journal, etc.)
	  o Digital (websites, videos, etc.)
	• Non-Peer-reviewed:
	  o Extension factsheets
	  o Popular articles

Students/Participants:	Provide the following information for **every** student that worked with you during the reporting period: • Name: • Whether Degree was completed during the reporting period (name, yes/no): • New or Continuing Student: • Capstone/Thesis Title (actual or anticipated): • Date of Graduation: • Provide link to thesis/dissertation document:

Partnerships	List any partners that you worked with on your project. Provide the following information for each Partner:

Specific Type	**Level**	**Nature of Partnership**
ePaint	Co-PI	Developed and produced novel coatings for aquaculture