**The effect of the common periwinkle, *Littorina littorea*, on the biofouling and growth rate of the Eastern oyster, *Crassostrea virginica*, in aquaculture.**

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## Statement of the Problem

In this study I will measure the effect of common periwinkle snails on the biofouling and growth rates of oysters cultivated in flipped-bag surface aquaculture. Oysters will be placed in treatments of different snail densities to measure these effects. Biofouling on oysters will be measured using a Braun-Blanquet coverage scale and will be compared across the treatments. Oyster weight, volume and length will be measured over the course of a four-month study period to assess the oysters' growth rate in each treatment. I hypothesize snails will reduce biofouling growth on oysters and result in an increased growth rate of oysters.

**Rationale**

Bivalve aquaculture includes the cultivation of clams, mussels, scallops and oysters and contributes approximately 24% of total aquaculture sales in the United States, increasing total sales by 45% from 2005 to 2013 (Adams et al. 2011; USDA 2013). Bivalve aquaculture saw gross sales increase from approximately $205 million in 2005 to approximately $328 million in 2013 (USDA 2013). Oysters accounted for approximately 55% of the total bivalve gross sales in 2013 (USDA 2013). Because of steady growth in recent years, the bivalve aquaculture industry is able to provide new jobs and meet the demand for bivalve products both domestically and internationally (USDA 2016). However, bivalve aquaculture has yet to be optimized in terms of the overall profitability. Biofouling is one substantial threat to the profitability of bivalve aquaculture and its products (Adams et al. 2011; Lacoste and Gaertner-Mazouni 2015).

Biofouling in bivalve aquaculture concerns the growth of epibionts on the gear and on the cultivated bivalves themselves. Fouling organisms include sponges, barnacles, polychaetes, ascidians, bryozoans, other bivalves and many algal species (Adams et al. 2011; Carraro et al. 2012; Lacoste and Gaertner-Mazouni 2015). Biofouling can consequently pose many problems for bivalve aquaculture production (Adams et al. 2011). Fouling organisms clog gear, obstruct water flow, compete with the cultivated species for both food and nutrients, and lower the growth rate and product quality of the cultivated bivalves (Adams et al. 2011). A lower growth rate in bivalves can result in taking more time to grow to marketable size or cause them to be smaller when they are collected, consequently reducing market profitability. Biofouling has also been found to decrease the marketability of the final product due to consumer reluctance to buy products that are covered in biofouling organisms (Adams et al. 2011). Biofouling has been

shown to increase total production costs by 20-30% (Claereboudt et al. 1994; Adams et al. 2011). Because of its significant impact on aquaculture productivity, growers actively remove biofouling from the gear and cultured species (Adams et al. 2011; Lacoste and Gaertner-Mazouni 2015).

Current biofouling management procedures, such as cleaning, scraping and chemical treatment are labor-intensive and require a lot of time (Adams et al. 2011). Biofouling cleaning and removal methods can also be stressful and damaging to the bivalves (Lacoste and Gaertner-Mazouni 2015). Biofouling organisms become more abundant due to the increased substrate availability provided by the aquaculture system, and cleaning methods can release these organisms into the environment which could potentially disrupt the local ecosystem (Lacoste and Gaertner-Mazouni 2015). Overall, biofouling management is costly and must be refined to avoid high production costs (Adams et al. 2011; Lacoste and Gaertner-Mazouni 2015). One promising method for controlling biofouling is the use of biological controls. This method utilizes ecological relationships between organisms to increase production (Enright et al. 1983; Cigarría et al. 1998; Ross et al. 2004; Sterling et al. 2016). Although limited, studies involving the use of biological controls in aquaculture have shown positive effects on production rates of cultivated bivalves (Enright et al. 1983; Cigarría et al. 1998; Sterling et al. 2016).

Biological control systems incorporate a number of marine species, including sea urchins, hermit crabs and sea snails into bivalve aquaculture (Enright et al. 1983; Cigarría et al. 1998; Ross et al. 2004; Carman et al. 2009; Sterling et al. 2016). These species have been shown to decrease the amount of biofouling on aquaculture gear and species (Enright et al. 1983; Cigarría et al. 1998;

Ross et al. 2004; McKenna 2016; Sterling et al. 2016). In particular, the common periwinkle, *Littorina littorea*, has been found to reduce the amount of biofouling algal species as well as increase the growth rate of oysters in aquaculture (Enright et al. 1983; Cigarría et al. 1998; McKenna 2016). The common periwinkle is an abundant, marine gastropod that typically grazes on marine macroalgae including *Ulva* sp., *Chaetomorpha* sp., *Fucus* sp. and *Ascophyllum* sp. (Enright et al. 1983; Parr and Sachs 2011). Because of its grazing ability, positive impact on oyster growth and relative availability, the common periwinkle is potentially a cost-effective, biological control that can be utilized in oyster aquaculture (Enright et al. 1983; Cigarría et al. 1998; Parr and Sachs 2011; McKenna 2016).

McKenna (2016) found that adding periwinkles to aquaculture oyster bags decreased biofouling coverage on oysters as well as increased daily growth rate of oysters. The results of this study also suggested that flipping oyster bags may result in higher oyster growth rates. McKenna (2016) observed a positive correlation between snail density and oyster growth in the flipped bags, though only two bags of each snail density treatment were flipped in her study. In this proposed study, I plan to use thirty flipped bags (ten replicates of three snail density treatments) to have sufficient replication in my measurements of oyster growth and biofouling abundance. The goals of this study are to determine whether periwinkle snails are able to effectively reduce the biofouling coverage on oysters, and to determine the degree to which periwinkle snails are able to increase the growth rate of oysters grown in flipped-bag surface aquaculture.

**Methods**

In this study, I will measure the change in oyster volume, length, weight and biofouling abundance on oysters placed in snail density treatments of zero snails, one hundred snails and two hundred snails in flipped oyster bags. The oyster volume and weight will be calculated as averages of all oysters from each bag. The oyster length will be calculated as an average length of one hundred oysters from each bag. The relative biofouling abundance on oysters will be measured by using a Braun-Blanquet coverage scale. I will collect data over a four-month study period to measure and compare changes in oyster size across the three snail density treatments.

This study will be conducted from June 2017 to September 2017 at the Bagaduce Oyster Company aquaculture site with assistance from the growers. The oysters will be cultured in thirty off-bottom floating bags that will be flipped by the growers weekly. Flipping oyster bags is a procedure that allows the drying out of biofouling organisms on the surface of the bags exposed to the air, which effectively reduces biofouling on the exterior of the bags where the periwinkle snails cannot reach (Pangea Shellfish & Seafood Company, Inc. 2015; Moran 2017). Periwinkle snails large enough to be retained within the bags (greater than 12 mm length) will be collected at the site in the rocky intertidal zone and distributed among the oyster bags. The bags will be stocked with snail densities of zero snails, one hundred snails and two hundred snails (ten replicates of each density treatment) per bag. There will be 250 oysters of uniform size (approximately 5 cm length) placed in each of the thirty bags at the beginning of the study.

Growth and biofouling abundance measurements will be taken at four-week intervals. On each sampling date, I will collect all thirty oyster bags and individually submerge them in a hundred-

gallon tank equipped with a meter stick to measure the total volume of each bag. The displacement of water measured with the meter stick will be multiplied by the surface area of the tank to calculate the total displacement volume (L) of each bag. The volume of the empty bag and calculated volume of snails will be subtracted from this total volume, and the data will be standardized as the mean oyster volume (mL) for each bag. On the first and last sampling dates, I will haphazardly select one hundred oysters from each bag and measure the total length (mm) across the longest distance of their shell. This will be done to compare the change in oyster length to the change in oyster volume. Oyster growth is not uniform (some grow longer, wider or taller), and comparing growth rates of individuals using only length measurements is difficult (Moran 2017). However, oysters reach marketable size when they reach a certain length (Moran 2017). I suggest comparing the two measurements because volume may more accurately describe an oyster's overall growth, whereas measuring length can show how many oysters reach marketable size by the end of the study. A Wixey WR100 digital caliper will be used to measure length. These digital calipers measure length to the nearest 0.05mm and up to 150.00 mm. I will also measure the weight of all 250 oysters in each bag using an Adam Equipment - CPWplus 35M electronic bench scale. This scale measures weight to the nearest 0.01 kg (10 g) and has a load capacity of 35 kg. I will place the oysters in a tared five-gallon bucket and weigh them on the scale. I will then calculate the average mass (g) of each oyster within each bag for later analysis. A Braun-Blanquet coverage scale will be used to measure the relative abundance of biofouling organisms on each of the hundred oysters sampled from each bag (Wikum and Shanholtzer 1978). This scale quantifies the relative coverage abundance in categories 1-6 based on the percentage of total cover (Table 1) (Wikum and Shanholtzer 1978).

**Table 1**. Braun-Blanquet cover-abundance scale (Wikum and Shanholtzer 1978).

| *Category* | *Total coverage (%)* |
|---|---|
| 1 | <1 |
| 2 | 1-5 |
| 3 | 6-25 |
| 4 | 26-50 |
| 5 | 51-75 |
| 6 | >75 |

On the date of the last measurements, I will also count the number of living oysters in each bag as well as the number of snails in each bag to calculate overall survivorship of each species.

For the analyses of my data, I plan to use a one-way analysis of variance test in Microsoft Excel to compare the mean change in oyster volume across the three treatments. Mean change in oyster volume will be calculated for each month of the study period. I will use a linear regression test to compare the change in oyster volume to the change in oyster length for the first and last sampling dates. I will use a one-way analysis test to compare mean relative biofouling abundances among the three treatments for each month of the study period. If statistical differences are found, I will use post hoc tests to compare the results among the three treatments. Because some of the oyster weight may include biofouling organisms, I will not include a statistical analysis of the mean oyster weights. Instead, the mean oyster weight will be graphically compared to the mean relative biofouling abundance for each sampling date. I will graphically analyze the survivorship rates of both the oysters and snails for each of the three treatments.

**Budget**

| **Items to be purchased** | | | | | |
|---|---|---|---|---|---|
| *Item* | *Description* | *Quantity* | *Cost/unit* | *Vendor* | *Total cost* |
| Adam Equipment® - CPWplus 35M - Bench Scale | Electronic bench scale for measuring oyster weights | 1 | $350.00 | H & C Weighing Systems (affordablescales.com) | $350.00 |
| Tyvek® Self-Seal White Envelopes - 6 x 9" | To be cut into labels that will be used to mark oyster bags | 1 case (100 units) | $27 per case | Uline (www.uline.com) | $27 |

| **Materials** | | | |
|---|---|---|---|
| *Item* | *Description* | *Quantity* | *Source* |
| Oyster bags | Floating oyster cultivation bags | 30 | Provided by the Bagaduce Oyster Company |
| Launch boat | Boat to get back and forth between the bags and the aquaculture floats | 1 | Provided by the Bagaduce Oyster Company |
| Large tank | For measuring the displacement volume of each bag | 1 | Provided by the Bagaduce Oyster Company |
| Digital calipers | For measuring the length of oysters (to the nearest 0.05mm) | 1 | MMA Ocean Studies Department |
| 5-gallon buckets | For holding the oysters selected out of each bag | 12 | MMA Ocean Studies Department |
| 1-liter glass beaker | For measuring the displacement volume of the snails | 1 | MMA Ocean Studies Department |
| Roll of masking tape | For labeling each bucket with treatment-replicate number | 1 | MMA Ocean Studies Department |
| **Organisms** | | | |
| *Species* | *Description* | *Quantity* | *Source* |
| Oysters (*Crassostrea virginica*) | 250 oysters will be placed in each of 30 bags | 7500 | Provided by the Bagaduce Oyster Company |
| Periwinkle Snails (*Littorina littorea*) | 100 snails in each of 10 bags; 200 snails in each of 10 bags | 3000 | Collected in rocky intertidal zone by the Brooksville bridge |

**Timeline**

| Date | Event |
|---|---|
| 4/27/17 | Make purchase orders for the scale and waterproof labels for the bags |
| 6/13/17 | Assemble supplies for first sampling |
| 6/16/17 | Collect 3000 snails in the rocky intertidal zone by the Brooksville Bridge |
| 6/17/17 | Sampling Date #1 |
| 7/15/17 | Sampling Date #2 |
| 8/12/17 | Sampling Date #3 |
| 9/9/17 | Sampling Date #4 [final] |
| 9/16/17 | Write Rationale section |
| 9/30/17 | Analyses of data |
| 10/7/17 | Write Methods section |
| 10/21/17 | Write Results section |
| 11/4/17 | Write Discussion section |
| 11/5/17 | Begin creating poster |
| 11/16/17 | Final Research Paper due |
| 11/27/17 | Make poster |
| 12/5/17 | Poster presentation to instructors |
| 12/9/17 | Refine poster |
| 12/14/17 | Poster presentation to Ocean Studies department |

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