The impact of aquacultural farm equipment and parasitism on *C. virginica* growth

Elsa Couvillon, Charmaine Gahan

Abstract

As the world deals with the negative consequences of overpopulation, pollution, climate change, and habitat loss, oyster farms take center stage as sustainable alternatives to current agricultural practices. In an experiment performed by Cuttyhunk Shellfish Farms, oyster farming techniques were investigated with two specific aims in mind: elucidating the types of marine farm equipment that maximize oyster growth/health, and identifying a treatment to reduce parasitic infestation of American oysters, *Crassostrea virginica*, by the mudblister worm *Polydora websteri*, which can damage oyster crops. Results from the resulting three-month experiment indicate that matrices encourage the largest change in *C. virginica* growth (in terms of length), while cages allow for the least amount of growth. Additionally, short drying intervals do not prevent *P. websteri*, however, longer and more intense drying cycles are expected to minimize infestation. A serendipitous observation from this experiment reveals that while cages may not support maximal oyster growth, they may prevent incidences of *P. websteri*. Following suit of this experiment, more research must be done on modern oyster farming techniques to support aquaculture efforts and boost the shellfishing industry.

Introduction

By the year 2050, the United Nations predicts that the worldwide human population will reach 9.7 billion [1]. As this benchmark creeps closer and closer every year, one cannot help but wonder how humanity will provide the necessary food to sustain such a large population. Big-name, industrial agricultural practices would call for the deforestation, burning, flooding, and/or leveling of more land in order to create pastures and fields for raising livestock and growing crops; however, such invasive, destructive, “deliberate maintenance” of the natural landscape would annihilate biodiversity [2][3]. Already, 38% of the Earth’s land is taken up by the agricultural industry [4], which is also responsible for 21-37% of the world’s harmful anthropogenic emissions (e.g. carbon dioxide, methane, and nitrous oxide) [5].

Because some agricultural practices lack the ecologically-friendly refinement that a climate-conscious society should endorse, certain aquaculture practices provide an interesting and more sustainable alternative. Specifically, oyster farming stands out because of its nutritional and ecological appeal. Oysters are high in protein and contain lots of important dietary minerals, namely zinc, vitamins B12 and D, selenium, and iron; additionally, diets involving oysters have been shown to benefit heart, immune, and skin health [6]. Not only are oysters a valuable delicacy, but they also act as ecosystem engineers and help minimize pollution. As filter feeders, they remove particles of dirt, nitrogen, and phosphorus from the water [7-9]. In fact, a single oyster can filter up to 50 gallons of water per day, a shocking statistic that has encouraged scientists to plant shellfish in over-polluted areas (e.g. the Chesapeake Bay) in hopes of restoring the water quality and biodiversity [10][11]. When oyster farming practices are implemented correctly and conscientiously, they have the potential to inflict minimal harm to the surrounding environment while maximizing financial, environmental, and nutritional benefits for all.

The focus of this study was to help elucidate the best oyster growing techniques as to improve and promote oyster farms, which carry great untapped potential for aiding humanity's impending food consumption crisis. The entirety of the study was conducted with the species *Crassostrea virginica* (American oysters) during the summer growing season at Cuttyhunk Shellfish Farms, located on Cuttyhunk Island, Massachusetts, USA. In this study, two experiments were conducted in parallel with one another, both of which centered on promoting healthy oyster growth and crop quality. The first experiment was designed to test how different types of oyster farming equipment impacted oyster growth, a parameter which was measured based on top shell length.

The second experiment tested the impact of drying (i.e. leaving the oysters in the intertidal for various amounts of time) on the presence of the mudblister worm *Polydora websteri*, an invasive species that burrows into oysters and creates a small, dark blister on the inside of the shell [12]. Oysters infested with *P. websteri* must divert precious energy away from physical growth and into shell repair, lest they become easy targets for predators [13]. Thus, infestations can increase the risk of oyster mortality and lower the commercial value of the crop. Not only are these blisters detrimental to the health of the oyster, but they create unsightly markings on the shell that can pop during the shucking process and ruin the meat. While little is known about *P. websteri*'s tolerances, and there is much speculation about what conditions are most conducive for infestations, one report has indicated that drying out the oyster –exposing the crop to intertidal conditions, including heat and air– can hinder *P. websteri* development [14]. Thus, drying was chosen as a potential route of experimentation.

The objectives of this dual study were to determine how to maximize oyster growth and minimize parasitic infestation by *P. websteri*, objectives which supplement the overarching goal of preserving and enhancing the oyster farming industry.

Materials and Methods

Both experiments were conducted simultaneously over the course of a summer, from June through August, on Cuttyhunk Island, Massachusetts. The oysters used for the experiment were genetic fast-growers, around 1-2 inches from hinge to tip of the topshell after 1.25 years since the larval stage, belonging to the species *Crassostrea virginica*. More specifically, these oysters, trademarked as Cuttyhunk® Oysters and seeded from Fishers Island, New York, belonged to Cuttyhunk Shellfish Farms, which leases a 50 acre great saltwater pond on the island and operates year-round. To prepare for both experiments, genetic fast-growing oysters were separated from the rest of the crop via a sorting machine.

For the first experiment (i.e. the growth and equipment analysis), several different types of oyster growth apparatuses were compared. The following devices were utilized: bottom cages; floating matrices; bottom matrices, and lantern nets (Fig. 1A-1C).

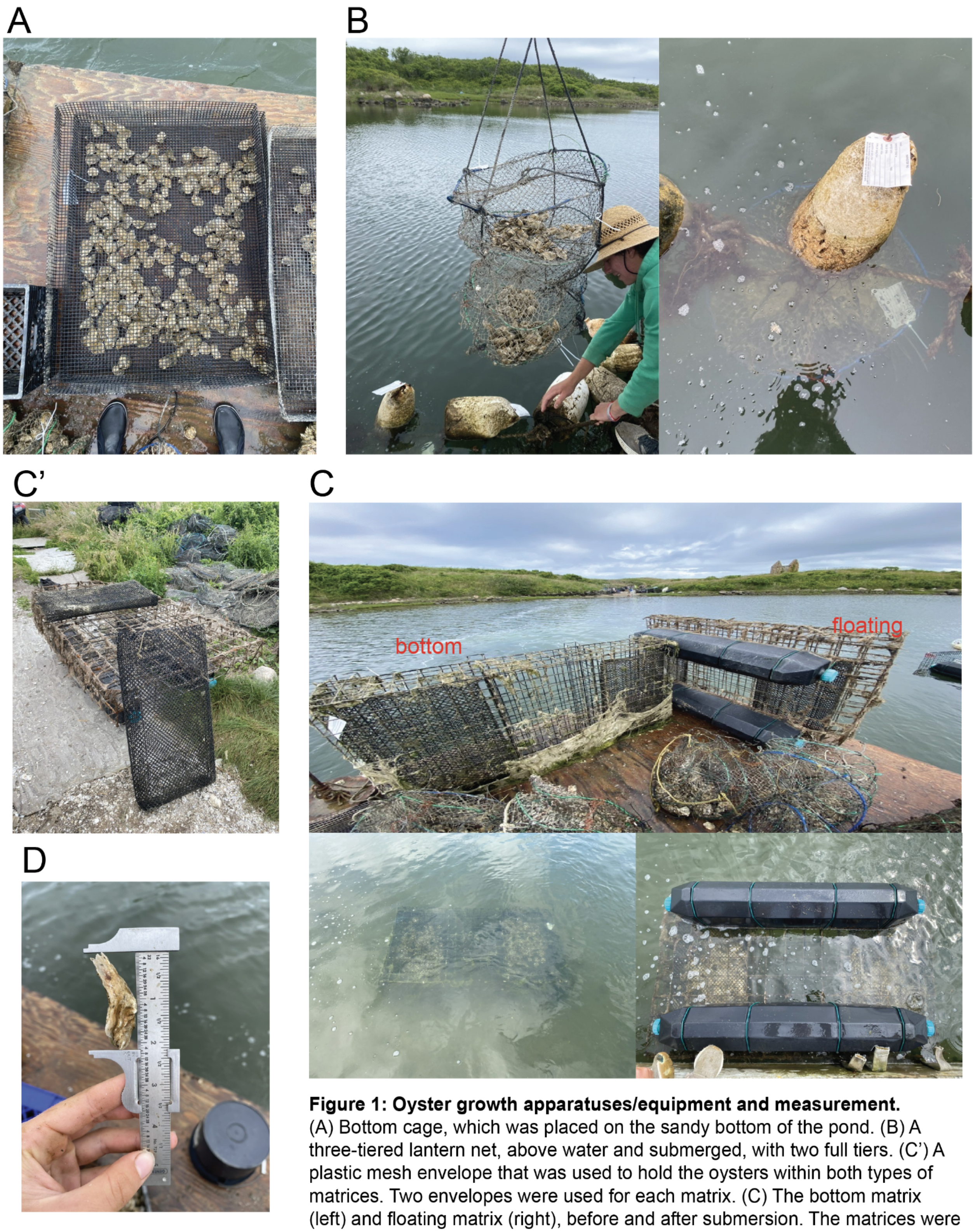


Figure 1: Oyster growth apparatuses/equipment and measurement. (A) Bottom cage, which was placed on the sandy bottom of the pond. (B) A three-tiered lantern net, above water and submerged, with two full tiers. (C’) A plastic mesh envelope that was used to hold the oysters within both types of matrices. Two envelopes were used for each matrix. (C) The bottom matrix (left) and floating matrix (right), before and after submersion. The matrices were leveled once set in the water to ensure the correct oyster density. (D) Example of oyster measurement system, from hinge to the tip of the solid top shell.

In this first experiment, two of each type of growth apparatus were filled with enough oysters to maintain a consistent density of 0.3264 oysters per square inch of equipment (e.g. per square inch of cage, per square inch of net, etc.) The apparatuses were then placed in the water. Every two weeks, 10 oysters from each apparatus (10 per apparatus, two of each "type" of growth apparatus, so n=20 per "type") were measured from hinge to tip of the topshell to determine average oyster size (Fig. 1D). When measuring, the caliper was pushed through the growth edge of the oyster; the growth edge is a flimsy, more delicate ring of shell that has not yet solidified into a protective layer. This was done to ensure that the measurement would be taken strictly from hinge to the tip of the actual top shell, and therefore played a role as a standardization technique. To prevent biofoul (like tunicates, barnacles, and seaweed) from growing in and around the apparatuses, and thus interfering with healthy oyster growth, each net, cage, and matrix was shaken and/or left above water to dry as-needed. Growth in each apparatus was compared to the growth of a control group of fast-growers, which were scattered on the bottom of the pond in a sandy, secluded spot with an approximate density of 0.3264 oysters per square inch and an average depth around four feet (image not shown).

For the second experiment, which focused on the impact of drying on *P. websteri* infestation, five different nets –each representing a different duration and frequency of drying– were selected and filled with 100 oysters per tier (100 oysters per tier, three tiers per net, one net per condition, and five conditions). The control net experienced no drying at all throughout the entirety of the experiment; it was left alone, expect for a couple times throughout the three-month period where the net was "rolled" (i.e. cleaned by thrashing it around as to fling off the biofoul and break up oysters that may have started to grow together). A summary of the different conditions is as follows:

- ➔ Net A was left above the surface to dry for 2 hours, once a day, every 2 weeks
- ➔ Net B was left to dry for 3 hours, once a day, every 2 weeks
- ➔ Net C was left to dry for 2 hours, once a day for 3 days, every 2 weeks
- ➔ Net D was left to dry for 3 hours, once a day for 3 days, every 2 weeks.

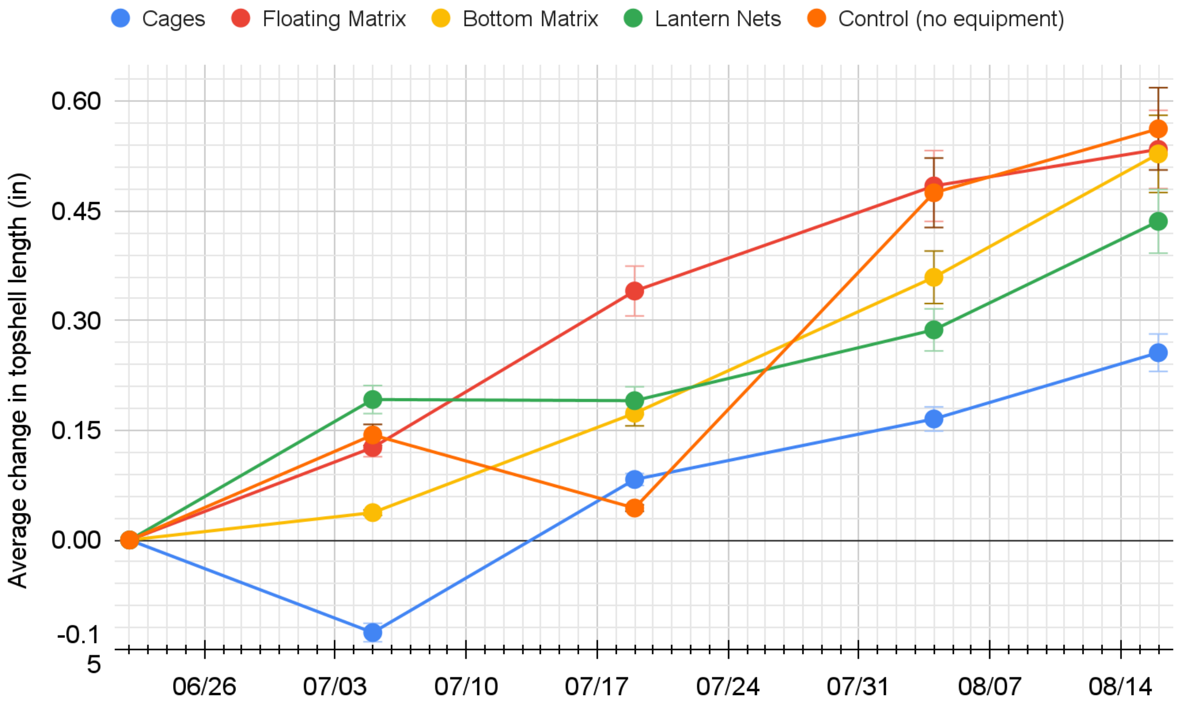
Both experiments began on June 22-23rd and concluded on August 16th, thus lasting for eight weeks. At the end of the eight week period, oyster shell-length measurements from the first experiment were compared to see which apparatus/equipment type proved most conducive for maximal positive change in oyster growth. For the second experiment, five oysters from each net were shucked and visually checked for varying signs of *P. websteri* activity at the end of the eight weeks. Unfortunately, Net D –which dried for 3 hours, 3 days in a row, every 2 weeks– was lost somewhere in the pond on August 7th and never located, meaning that no data from that condition could be collected at the conclusion of the experiment.

Results

After the eight-week growth period, both the floating matrix oysters and the bottom matrix oysters tied with the control oysters (grown without equipment; scattered on the sandy bottom) for the largest change in growth, which was just above 0.50 inches of topshell length increase (Fig. 2). The lantern net oysters showed the second largest increase in length, around 0.40 inches of shell growth, followed by the cage oysters, which only seemed to grow around 0.25 inches (on average) by the end of the experiment (Fig. 2).

As for the second experiment, evidence of *P. websteri* infestation was found in all the shucked oysters, regardless of drying frequency or duration (Fig. 3). On both the top shell and bottom shell, we found *P. websteri* burrows –black, thick, vein-like splotches filled with waste and dirt– just under the shell’s surface. Additionally, most of the shells showed yellow discoloration in the enamel, which indicated that the oysters were under stress. Of all the shucked oysters, those dried for 2 hours, 3 days in a row, once every 2 weeks showed the least signs of *P. websteri* in the bottom shells (Fig. 3B’, left side); however, the top shells still showed discoloration and contained burrows. No single treatment produced consistently “clean” oysters.

Figure 2: Oysters grown in matrices experience the largest increase in size.



Average change in oyster length (as measured from hinge to tip of topshell) from 07/05/2022 through 08/16/2022 as observed for four types of growth apparatuses (n=20 per apparatus).

Figure 3. Shucked oysters still exhibit convincing signs of *P. websteri* regardless of different short drying intervals.

(A-D) Five shucked oysters from each drying treatment (and the untouched control net) harvested at the end of the three-month experimental period. (A’-D’) A close-up of the boxed oyster with *P. websteri* burrows outlined in red. Note the yellow discoloration of the shells.

Discussion

Based on the results from the growth experiment, matrices seem to be the best equipment for enhancing oyster growth in terms of size (Fig. 2). Interestingly, matrices encouraged the most growth regardless of whether they were located on the surface of the pond (suspended in the water column) or placed on the sandy floor in the shallows. This observation suggests that the benefit of the matrix lies not in its placement, but in its design. Matrices are set up by pouring oysters into rigid, plastic, mesh envelopes, which are then slid into a larger, metal matrix. The entire apparatus is leveled (i.e. shaken side-to-side until the oysters are evenly spaced) and placed in the water (floating on the top or standing on the bottom) (Fig. C', C). If oyster length and growth is not due to placement, then, perhaps it has to do with the structure of the matrix.

The matrix has two layers of security and spacing that the cages and nets lacked, which could contribute to the favorable growth conditions. The cages, for example, were often found to contain lots of crabs, which preyed on the oysters. Logically, increased risk of predation could cause the oysters to divert more energy into strengthening and repairing their shells, rather than growing larger. While crabs could easily squeeze through the gaps between the cage top and bottom, they were rarely found in the oyster-filled envelopes of the matrices, which were well-secured and made of a smaller mesh. The issue with the lantern nets, on the other hand, might involve space and nutrient flow. While oysters were filled to the same density in both the nets and the cages, the nets became overgrown with biofoul more quickly, which often choked up the mesh and could prevent the flow of nutrients and oxygen to the oysters within. In fact, on measuring days, it was often noted that the net oysters looked dirtier (i.e. were covered in more settled silt and marine detritus, crawling with small shrimp and other microorganisms, etc.). The matrices, on the other hand, required very little maintenance and still produced clean-looking oysters. This might be because the two-structure system of the matrix (envelope and metal skeleton) provides the oysters another layer of separation from any biofoul.

Another interesting result was that the control-group oysters, which were simply scattered on the bottom of the pond and grown without any equipment, enlarged just as much as the matrix oysters and also beat out the cage and net oysters. Rationale for this could be that the control oysters were rarely disturbed throughout the experiment. While oysters in different growth apparatuses/forms of equipment require handling in order to keep them clean, oysters on the sandy bottom maintain themselves, and thus can grow quietly on their own, without any potential stress that handling may cause. Additionally, because these oysters were not contained within a finite space, they could each grow independently of any diseases or predatorial massacres that might have seriously impacted equipment-grown, confined oysters.

In regards to *P. websteri*, no reliable conclusions can be made as to whether or not drying has an impact on infestation. As previously mentioned, while some of the oysters that were dried for 2 hours, 3 days in a row, once every 2 weeks appeared to have slightly decreased signs of *P. websteri* in their bottom shells (Fig. 3), this observation was necessarily true for all the shucked oysters in this group, nor can it be reliably applied to the entire crop from this condition. However, it should also be noted that the drying intervals (2 hours, 3 hours) were extremely short. In another species of oyster, *Crassostrea gigas* (Pacific oyster), it was found that long freshwater treatments of 12 hours followed by short heat-shock treatments and a

2-month saltwater recovery period helped decrease instances of a different species of mudworm in the *Polydora* genus [14]. Going forward, it would therefore be worthwhile to re-attempt a similar drying experiment with longer intervals, over a longer period of time, to see if a more intertidal-like, intense drying cycle could effectively reduce *P. websteri* impact. In this particular experiment, the timing of the drying cycle was limited by the availability of employees and length of the work day.

While the cage oysters may have shown the least positive change in growth, when shucked at the end of the eight week experiment (data not shown), they appeared to have very few signs of *P. websteri* (i.e. very few burrows, no to slight shell discoloration). This serendipitous observation, while informal, suggests that cage growth might actually discourage *P. websteri* infestation. Other reports from other oyster farmers help support these findings. For example, farmers in Maine claim that the sediment helps protect oysters from the mudworm by blocking its access to the shell [12]. New experiments, then, might look to merge the two dual studies performed at Cuttyhunk Shellfish by exploring what types of equipment, if any, prove best for protecting against *P. websteri* infestation.

Going forward, future experiments should look to measure oyster depth –not just length– to better determine the impact of equipment on oyster quality. Additionally, more thought should be given to quantifying *P. websteri's* upper and lower limits: for example, the ranges of desiccation and salinity the worm can tolerate. This way, shellfish farmers could have a more concrete idea of how to preserve their crop and their revenue.

Conclusion

Overall, very little information is available on oyster growth preferences; even farmers who have spent decades working with the bivalves, like those working at Cuttyhunk Shellfish Farms, still wonder how they can improve the quality of their crop, whether that is by changing up the equipment they use, trying new techniques for oyster handling and/or treatment, etc. The scientific community can and should contribute to this quest for aquaculture knowledge by delving more into the science of shellfish and their parasites. This experiment sought to take some of the early steps into this quest. Over the course of a summer, we found that matrices, whether floating or bottom-based, seem to encourage the most oyster growth. While we could not make any solid conclusions on how to combat *P. websteri* with short drying intervals, we have stumbled across new ideas for experiments going forward that might produce more operational results. Research will continue at Cuttyhunk Shellfish, and we implore other farms and labs to join us.

So much is known about protecting other types of crops and livestocks. For example, scientists have created various pesticides and GMO strains to enhance plant growth. They have extensively researched different diets to feed cows and chickens and have filled books with information on different diseases that might harm farms. Now, it is time to consider the oysters.

Acknowledgements

We would like to extend special thanks to the Southeastern Massachusetts Agricultural Partnership (SEMAP) in South Dartmouth, MA for funding this project via their 2022 TIE Award.

Additional thanks to Charmaine Gahan, current owner of Cuttyhunk Shellfish Farms, and Seth Garfield, founder, for their assistance in designing these experiments. We would also like to acknowledge the dedicated staff of Cuttyhunk Shellfish, including (but not limited to) Andrew Frothingham, Charlie Anderson, Sam Heintz, Sawyer Devoe, and Sam Hinson for their help maintaining the experimental oysters and the farm.

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