



The Effects of Reef Structure on Gamete Dispersal and Fertilization of the Eastern Oyster, *Crassostrea virginica*

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Abstract

Over the past several decades there has been a decline in the oyster population in Delaware Bay. Recently there has been at least four consecutive years of recruitment failure on the intensively managed seed beds. Current harvesting practices break up naturally occurring clusters of oysters and disperse them resulting in less structurally complex oyster beds. Using rotating annular flumes, we examined the effects of reef structure on gamete distribution and fertilization in *Crassostrea virginica*. By constructing oyster bed mimics in two different configurations (flat, reef) in the flumes and then injecting gametes we were able to examine and compare fertilization rates, as well as water flow patterns over two and three dimensional structure. The trials showed that there was no difference in fertilization rates between the two treatments. However, the difference in flow patterns between the treatments suggests that bed structure plays a role in egg retention and presents an opportunity for further study.

Introduction

Commercial harvesting of the Eastern Oyster, *Crassostrea virginica*, in the Delaware Bay (Fig. 1) dates back to as early as the 17th century. However, based on stock assessments of the last fifteen years, there has been a drastic decline in recruitment (Krauter, Powell, and Ford 2000). This decline is attributed to variety of factors including predation, disease, and excessive harvesting.



Fig. 1 Oyster boat and dredge in Delaware Bay

The commercial oyster industry has changed the way in which oysters grow. Oysters, which naturally grow in vertical reefs made up of long, slender "raccoon" oysters, are broken apart and spread out by dredging in order to produce oysters of a marketable size and shape (Fig. 2) (Kennedy 1995; Gosling 2003). The change in oyster beds not only affects how close oysters are to each other but also the flow of water over the bed. This research focused on the impact that the change in the structure of the oyster reef has on the reproductive success of *C. virginica*. Using annular flumes (Fig. 3) we looked at water flow, gamete distribution, and fertilization rates around two and three dimensional structures of oyster shell. The goal was to determine if gamete dispersal and fertilization changed as a function of bed complexity, and to better understand the impact dredging has on recruitment in Delaware Bay.



Fig. 3 experiments were conducted in an annular flume



Fig. 2 Natural oyster reefs made up of "raccoon" oysters

Methods



This experiment used two treatments. The first treatment was a bed of oyster shell spread out to cover the bottom of the flume to form a flat, two-dimensional structure (Fig 4, top). The second treatment was a three-dimensional oyster reef (Fig 4, bottom). Two replicates were run for each treatment. Oysters were secured from Pemaquid Aquaculture, ME.

Fig 4. Oyster bed mimics. Top panel is a plan view of the flat bed treatment (height ~5 cm). Bottom is a side view of the end of the reef treatment (height ~15 cm and ~300 cm long). There was 300 cm of flat shell between reefs



Fig. 5 Injecting colored dyes into the flume to look at flow patterns



Fig. 6 Sampling port, in flume with reef, designed to collect 10 l samples at three levels in the water column

A large part of this study was devoted to developing sampling methods capable of quantitatively collecting oyster eggs from the flume. A custom engineered sampling port was constructed from a solid pvc cylinder (19 cm x 6 cm dia.). The cylinder port sampled at three depths 8cm, 13cm, and 18 cm from the bottom of the flume. Plastic tubing carried samples away from the sampling ports to three 20 l buckets (Fig. 6). Sampling 30 l (10 l/port) took ~5 minutes. Water was replaced in the flume during sampling.

Prior to the fertilization trials, flow patterns were observed by using colored dye. The dye was injected through the oyster shell using a u-shaped pipette to simulate natural spawning event (Fig 5).

The eggs for all of the studies were obtained using strip spawning Fig. 7. The eggs were collected on a 25µm mesh screen and washed with sea water. The sperm were collected on a 25µm mesh screen and collected in sea water. Eggs were counted using a Sedgewick Counting Chamber. Sperm were counted using a hemacytometer. To determine the best ratio of sperm to eggs, a test tube study was conducted in which sperm and eggs were mixed with ratios of 1:1, 10:1, 100:1, and 1000:1. After two hours, the test tubes were preserved in formalin and the number of eggs that had reached fist cleavage or beyond was estimated.



Fig. 7 Gametes are looked at under a microscope to determine the sex. The gametes are stripped by slicing the gonad with a scalpel and gently washing the gametes away

To examine fertilization success and gamete distribution in the flume, eggs and sperm were injected simultaneously into the flumes with 10ft between the two injection points. At the same time the gametes were mixed in a beaker to serve as a base line fertilization index. The eggs in the beaker were watched under a microscope and when they started to undergo first cleavage three 10 l samples were collected from the flume at the top, middle, and bottom of the water column. The samples were rinsed into a 25µm screen and preserved in formalin. Following the first collection, samples were taken every 20 minutes for a total of three hours.

To better understand the flow patterns, a laser doppler velocimeter was used to measure velocity profiles over both treatments. In the flat treatment, one profile was done. Three profiles were taken over the reef: on the upstream end, over the center, and on the downstream end (Fig. 8).



Fig. 8 Laser Doppler Velicometer takes velocity profiles at different points of reef

Results

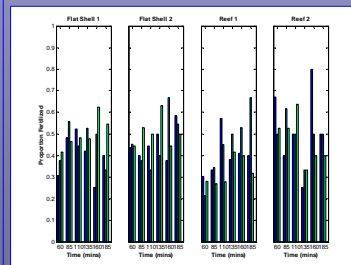


Fig. 9 Proportion of eggs fertilized vs. collection time

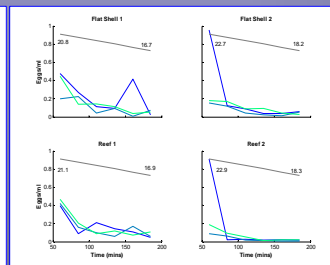


Fig. 10 Ideal egg 'catch curve' vs. observed

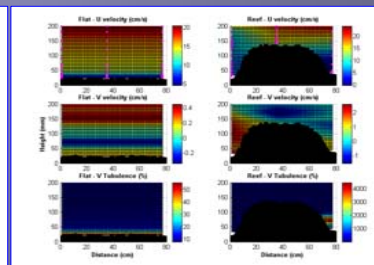


Fig. 11 Velocity and Turbulence profiles of two and three dimensional structure

- Dye injection suggested that flow in the flat treatment was less turbulent and "faster" than in the reef treatment. The time it took for a dye cloud to travel completely around the flume in the reef treatment was ~50% longer than in the flat treatment (2 versus 3 minutes)
- The sperm:egg ratios suggested that we would get ~60% fertilization at an optimal level of 100 sperm per egg. These results were entirely consistent with previous studies.
- The proportion of eggs fertilized (fertilization success) was variable across treatments and across depths (Fig. 9). Fertilization ranged from ~20 to 80% and in was typically higher in the beaker controls than in the flumes. No clear difference between treatments were drawn from this part of the experiment.
- One of the more surprising results was the rather consistent drop-off in eggs/ml as the experiment progressed. Although sample estimated egg concentrations were much less than what would be expected if eggs were evenly distributed, the drop off did not follow the curve expected due to pure dilution (dark line in Fig. 10). In all trials, there was a high initial recovery rate which immediately dropped off to low values in the second sample.
- The velocity profiles in the treatments were strikingly different (Fig. 11). In the flat shell (left, top) the U-velocity is highest near the top plate of the flume and is low near the bottom, the V-velocity (left, middle) is small. The flat structure had turbulence directly above the shell (left, bottom). In the treatment with structure, the data is more interesting. Prior to the reef there is an extremely low U-velocity (right, top). In the middle of the reef and after it the U-velocity increases to around 20cm/s. The V-velocity is positive before the reef, but becomes negative over and after the reef (right, middle). Both U- and V-turbulence values jump from about low values in front of the reef to more than 4000 behind the reef – significantly higher than in the flat treatment (V-turb shown in bottom panels).

Discussion

There appears to be no difference in fertilization success between the two treatments. This suggests that there is enough turbulence, or enough sperm, in the flume to fertilize eggs at a rate that somewhat less than in ideal conditions. This observation was surprising given the turbulence data, which indicates highly turbulent areas downstream of the reef, which we thought would increase fertilization.

Prior to running the trials, it was assumed that t eggs would be evenly distributed in the water column after 60 min, the time when the first samples were taken. However, the sharp decrease in the amount of eggs collected after the first samples suggests that something is happening to the eggs between 60 and 85 minutes. Flow around the reef, which does have small but significant negative vertical velocities, could result in 'capture' of eggs by the structure. But, this does not explain why egg numbers dropped in the two-dimensional structure as well. It has been suggested that, once fertilized, the eggs drop out of the water column in order to increase their survival and dispersion rate until they develop enough that they are competent to swim and settle. However, this is only a hypothesis and further studies need to be conducted.

While the experiments we conducted do not answer the question declining recruitment in Delaware Bay they offer new insight into the behavior of eggs after fertilization.

References:

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