Evaluation of resistance to *Roseovarius* Oyster Disease of lines of *Crassostrea virginica* in Rhode Island coastal waters

Nevan Richard[1], Kathryn Markey[2], Marta Gomez-Chiarri[2]

[1]Coastal Fellow, University of Rhode Island, [2]Department of Fisheries and Animal Veterinary Science, University of Rhode Island

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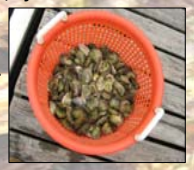
Enter the Oyster

For decades *Crassostrea virginica* (the eastern oyster) has been a staple of raw bars and seafood restaurants in New England. In recent years, however, oyster fisheries have had to deal with challenges to the industry, such as habitat loss and over-harvesting (*Schulte, 2009*). New diseases have also affected production on the local level.



The Emerging Threat

Roseovarius Oyster Disease (ROD) has become a concern for oyster farmers in the Northeast in the past couple decades. ROD was only recently renamed from Juvenile Oyster Disease after the etiological agent, *Roseovarius crassostreae*, was identified (*Maloy, 2007*). ROD primarily affects oysters <25 mm in length (Fig. 1). Following a 1-2 week lag period, mortality rates can reach as much as 90% (*Ford, 2001*). Clinical signs of ROD include:

- Shrunken mantle
- Conchiolin rings along inner shell (Fig. 1a)
- Flaking upper valve= exaggerated cup

Fig. 1- A 25mm juvenile oyster
Fig. 2- Brown rings of conchiolin form around the mantle to protect it from bacteria.

Goals of the study

R. crassostreae thrives in warm summer waters above 20 degrees Celsius, the same conditions ideal for oyster growth. The inability of farmers to raise juvenile oysters without losses due to ROD is a serious problem in the Northeast.

We sought to evaluate some of the factors that influence juvenile oyster susceptibility to ROD, namely the environmental conditions at the growing sites and the genetics of the oyster. In order to do that, we tested the performance of three lines of oysters at four different farms.

Experimental Design

Three lines/stocks of *C. virginica* were used:

- Green Hill Pond (GHP) = Local wild stock
- New England High Survival (NEH) = Hatchery reared line selectively bred for resistance to parasitic infection
- Hybrid (HYB) = A cross between NEH and GHP

- Broodstock was spawned at the Roger Williams University hatchery in March 2009 and moved to upwellers in June.

- Seed oysters over 6 mm in shell height were deployed in July 2009 to two Coastal Pond (CP) farms and two Narragansett Bay (NB) farms as shown in Fig. 2.

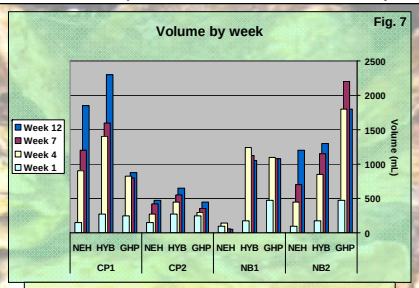


Fig. 7- Except for CP2, all farms had at least one line decrease in volume during the study. Despite being the only farm with consistent growth, CP2 had the least growth overall. The top performers were HYB at CP1, NEH at CP1, and GHP at NB2.

In the Field

Oyster performance was evaluated every three weeks from June – October 2009.

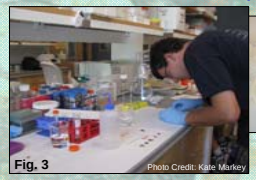
1. Shell height of 100 random oysters for each line.
2. Total volume (mL) for each line.
3. Live and dead counts for two 100 mL groups from each line.

When clinical signs of ROD were evident, ~10 juveniles were taken back to the lab to be tested for *R. crassostreae*.

In the Laboratory

The presence of *R. crassostreae* was tested using standard protocols (*Maloy, 2007*).

1. The oysters were dissected, with the bodies and mantles removed and preserved (Fig. 3).
2. Bacterial swabs were taken from the inner valve, then cultured on SWT plates (Fig. 4).



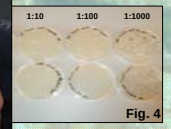
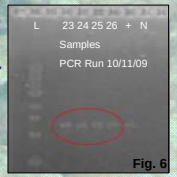


Fig. 4- Plated dilution series give individual colonies room to grow.

3. Suspect colonies (Fig. 5) were picked based on size and color, then grown in SWT media overnight at 20 degrees Celsius.

4. Bacterial DNA was extracted and a fragment of the Internal Transcriber sequence unique to *R. crassostreae* was amplified.
5. The amplicons were run in a 1.5% agarose gel. Bands confirmed the presence of the bacterial DNA (Fig. 6).



Results

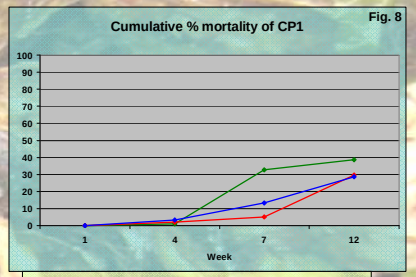


Fig. 8- CP1 had the lowest mortalities (<40%) of all four farms. GHP experienced the highest mortalities, starting between weeks 4-7 after deployment.

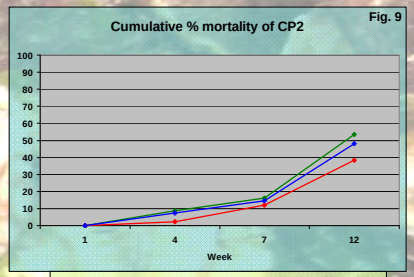


Fig. 9- All three lines saw uniform mortality over the course of the study. The greatest increase in mortalities occurred between weeks 7 and 12 after deployment (late summer).

= GHP
= NEH
= HYB

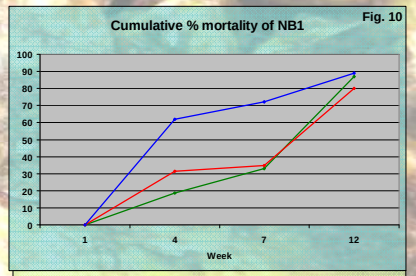


Fig. 10- NB1 exhibited the highest mortality rates of all farms, affecting all lines. NEH oysters showed high mortalities by week 4. Most of the mortalities for the GHP and HYB lines occurred between weeks 7 and 12.

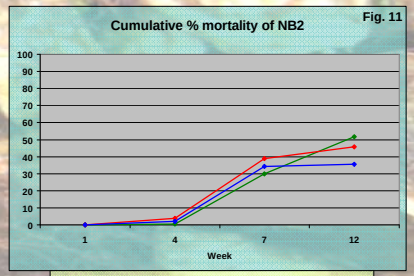


Fig. 11- Mortalities for all three lines increased the most from Weeks 4-7. NEH and HYB mortalities leveled off after Week 7, while GHP mortality rates continued increasing.

Discussion

- These results suggest the influence of both genetics and environment on performance:
  - No line was universally superior at all the farms.
  - HYB had the highest volume at both CP farms, followed by NEH.
  - GHP had the highest volume at both NB farms, followed by HYB.
- Mortality did not completely explain differences in performance. For example, GHP had the highest mortality at almost every farm, but grew faster at some sites leading to higher volumes.
- The timing of mortalities played a large role in performance. For example, NB1 NEH suffered higher mortalities than the other lines early in the season, and, although it experienced faster growth afterwards, it was not enough to outperform the other lines.
- Despite clinical signs including conchiolin deposits and stunted growth in all lines at each farm, *R. crassostreae* was only isolated and cultured from five bacterial isolates, all from NB2. There are several potential explanations:
  1. That another pathogen with symptoms similar to ROD but not *R. crassostreae* is killing juvenile oysters.
  2. *R. crassostreae* grows very slowly on plates, oftentimes being overrun by other bacteria. Thus, identifying colonies by eyesight alone is difficult and imprecise.

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Sources: 1. Schulte et. al. 2009 "Unprecedented Restoration of a Native Oyster Metapopulation"
2. Maloy et. al. 2007 "*Roseovarius crassostreae*, the etiological agent of Juvenile Oyster Disease...in *Crassostrea virginica*"
3. Ford et. al. 2001 "Epizootiology and Pathology of Juvenile Oyster Disease in the Eastern Oyster, *Crassostrea virginica*"