JOURNAL OF SHELLFISH RESEARCH

VOLUME 25, NUMBER 2 AUGUST 2006

Island Sound, NY. This site (depth = 2–3 m at low water) is significantly influenced by Block Island Sound. Maximum surface water temperature is 22°C. The adult growout system consisted of an array of longlines used to suspend lantern nets. Nets were stocked with one year old oyster seed (size range approx. 30–50 mm) obtained from Ocean Pond, Corp., a producer of seed oysters utilizing a salt pond (salinity = 12–20 ppt) on Fishers Island, NY. Growout of this large seed to market size occurs in 6–18 months. In an effort to increase the quantity of large seed oysters available to stock this system, 1mm juveniles were cultured in a land based upwelling system, grown to a size of 4 mm, and stocked in pearl nets (34 × 34 cm) at densities ranging from 200–500 oysters per net. The pearl nets, in vertical arrays of 4 nets, were deployed at the adult growout site during July for 3 consecutive years. Each year, an initial period of satisfactory growth was followed by an episode of high mortality (50–95%) during August. Conchiolin deposition was noted in live and dead oysters all three years. After three years, culture of first year oysters was abandoned at this site. Since then, seed production at the salt pond, where JOD has never been observed, has been increased and provided 100% of the 30–50 mm seed required to stock a significantly expanded adult growout system in West Harbor.

JOD AT OYSTER BAY, NEW YORK. David Relyea, Frank M. Flower & Sons, Inc., P.O. Box 88, Oyster Bay, NY 11771

In July of 1990, Frank M. Flower and Sons (FMF) experienced it's first hatchery oyster seed mortalities from Juvenile Oyster Disease (JOD). At the time it was called "Unexplained Mortality". Samples were sent to NMFS, Rutgers and VIMS. No pathogen was identified. Heavy mortalities continued in '91, '92, and '93. During that time Frank M. Flower was learning how to cope with the problem as research progressed. By 1994, oyster seed production was back to normal levels due to strategies developed by FMF in conjunction with researchers. To date it is still questionable as to what actually causes JOD.

FIELD TRANSMISSION OF JUVENILE OYSTER DISEASE ON LONG ISLAND AND FLOW/DENSITY/MORTALITY STUDIES. Gregg Rivara, Cornell University Cooperative Extension of Suffolk County, Southold, NY 11971; **Stan Czyzyk**, Long Island Mariculture Technologies, Holbrook, NY 11741

Field transmission of JOD to the Suffolk County Marine Environmental Learning Center (SCMELC) occurred with transfer of broodstock and seed oysters in 1992 and 1993 from Long Island hatcheries. In 1994 a flow/density study was undertaken at the SCMELC with support from NOAA's Oyster Disease Research Program.

A three tank, 27 silo upflow nursery system was constructed. Each tank served as a flow treatment; low (4 L/min per silo), medium (20 L/min) and high (40 L/min). Within each tank three target stocking densities were triplicated: low (one liter per silo), medium (six liters) and high (12 liters). Tanks were fed unfiltered creek water from a 3.4 m^3 head tank filled by two 2-horsepower centrifugal pumps.

Silo units were initially stocked on 11 July 1994. Shell height at this time averaged 8 mm and stocking densities were well below targets. Each week, for a period of nine weeks, the volume and mortality (number dead per 100 randomly-selected oysters) of each silo, along with shell height measurements (live and dead) were recorded. Physical parameters measured weekly included temperature, salinity, Secchi depth and a check of flow rates. As the actual volume exceeded the target volume for each silo, oysters were randomly removed to bring the volume back down to the treatment level.

Substantial mortalities (>20%) were seen by week three in low flow silos; by week four mortalities in these silos averaged 60%. At this time mortalities in high flow silos were at or under 33%, and remained at this level until the end of the experiment. Weekly volumetric increases were higher in medium and high flow silos as compared to low flow silos. Flow affected growth and survival more than stocking density.

It is not know to what degree nutritional stress caused by low flows and high densities affects the manifestation of JOD. It is that suggested that silos can be highly stocked without excessive mortalities as long as relatively high flow rates are maintained; at the SCMELC this equates to 0.03 liters of flow per square centimeter of silo screen. These results prompted the development of the axial flow floating upweller system at Cornell in 1995.

Anecdotally, the authors saw free-floating oysters in silos during these experiments and in years prior. Upon observation with a dissecting scope, these oysters were found to be alive. It is hypothesized that the case of death from JOD is the self "shucking" of the oyster when at least one adductor muscle attachment site is severed by conchiolin deposition, causing the oyster to lose control of its gape and its ability to keep potential pathogens and predators out of the shell cavity.

This project was supported by NOAA award NA47FL0174.

JUVENILE OYSTER DISEASE (JOD) IN *CRASSOSTREA VIRGINICA*: SYNTHESIS OF KNOWLEDGE AND RECOMMENDATIONS. Katherine Boettcher, 11208 Beechwood Pointe, Smithfield, VA 23430; **Roxanna Smolowitz**, Marine Biological Laboratory, 7 MBL St Woods Hole, MA 02536; **Earl J. Lewis**, NOAA NOS, 904 S. Morris St., Oxford, MD 21654; **Bassem Allam,** MSRC, Stony Brook University, Stony Brook, NY 11794; **Harry Dickerson**, College of Veterinary Medicine, University of Georgia, Athens, GA 30602; **Susan Ford,** Haskin Shellfish Laboratory, Rutgers University, P.O. Box 687, Port Norris, NJ 08349; **Anwar Huq,** Center of Marine Biotechnology, University of Maryland, Columbus Center, 701 E. Pratt St., Baltimore, MD 21202; **Kimberly Reece,** Virginia Institute of Marine Science,

P.O. Box 1346 Gloucester Point, VA 23062; **Gregg Rivara,** Cornell Cooperative Extension, 3690 Cedar Beach Rd., Southold, NY 11971 and **Cheryl M. Woodley,** NOAA NOS, 331 Ft. Johnson Rd, Charleston, SC 29412

The Juvenile Oyster Disease Workshop held in conjunction with the 26th Milford Aquaculture Seminar chronicled two decades of an industry's fight against juvenile oyster disease to save their businesses; their partnership with the research community to find the causative agent(s) for this devastating disease of cultured young oysters; and their experiences in learning how to manage this disease to keep an industry alive.

THE ISSUE

Juvenile oyster disease (JOD) is a significant disease of juvenile cultured oysters in the Northeastern United States. The earliest report dates back to the mid-1980's, however the peak of the devastation experienced by most nursery operations occurred in the 1990's. Those able to withstand the economic losses wrought by this disease did so by devising effective management methods and the disease no longer affects the viability of those commercial operations. Nevertheless, as new oyster aquaculture ventures emerge in the Northeast (driven in part by the need of fishing communities to retool after the devastating collapse in numbers of feral finfish and shellfish in the area), JOD outbreaks continue to appear sporadically in other culture sites previously thought to be unaffected or those only rarely affected. The annual variability in occurrence and intensity often results in unexpected and marked economic loss to individual producers. Basic and applied research conducted over the last 20 years has resulted in significant advances in our knowledge of the disease, its etiology, and methods for its mitigation; however, questions remain. A more thorough understanding of the disease pathogenesis, including host/pathogen interactions and other factors (i.e., anthropogenic or physical and/or biological agents in the environment) that contribute to JOD-epizootics, will facilitate the development of reliable surveillance measures for early warnings, help identify threat levels for a given location, and provide a basis for growers and regulators to make informed decisions.

THE DISEASE

JOD is generally observed after water temperatures have reached 20°C and at sites where salinities are above 18 ppt and primarily affects animals between 15–25 mm in shell height. The first clinical sign of the disease syndrome is decreased growth followed by the appearance of gross signs that include extreme cupping of the left valve, loss of the growing edge of the right valve resulting in uneven valve margins with retraction of the mantle edges within the shell (mantle recession) and deposition of abnormal brownish layers of conchiolin (organic shell matrix) on the inner surface of the affected shells. Mortality, which may reach 90%, begins within a week or two of the onset of slowed growth. Conchiolin deposition (a non-specific host response by oysters) is often observed in the form of a raised ridge around the retracted mantle edge. The conchiolin layer may also interfere with attachment of the adductor muscle. Histological lesions in the mantle occur before abnormal conchiolin is deposited. Depending on the size of the animals, not all of the particular clinical signs may be present in an individual oyster (however they can be observed within a random sampling of a production lot). For example, oysters less than 15 mm shell height often die before they are able to mount the characteristic conchiolin response. Animals between 15 and 25 mm shell height typically show all gross signs of disease (including abnormal conchiolin deposition and uneven valve margins). In addition, the highest mortality rates occur among oysters that are less than 25 mm. Juveniles over 25 mm shell height often show abnormal conchiolin deposition, but growth effects (including uneven valve margins) and accompanying mortality are less common. Animals surviving JOD may develop external shell checks that result when normal growth is resumed.

Typical histological findings with JOD begin early in the disease process. Initially, there is increased hemocytic inflammation in the sinusoidal tissues of the mantle underlying the shell epithelium that is associated with attenuation and cuboidal metaplasia and/or hyperplasia of shell epithelium. In more advanced lesions, necrosis of shell epithelium occurs, resulting in ulceration and severe hemocytic inflammation. Other histological findings include diapediasis of hemocytes across the epithelium into the extra-pallial space (located between the epithelium and shell), accumulation of necrotic cellular debris and bacteria between layers of abnormal conchiolin in that space and occurrence of small coccoid bodies in the affected epithelium. Although the precise cause of death is unknown, these lesions may result in destruction of the adhesion between the shell and adductor muscle causing a critical loss of function. Secondary infections, loss of hemolymph and generalized physiological dysfunction also may contribute to death.

Advances in management practices that are effective in helping to control JOD include:

- Increasing water flow in nurseries, including the use of floating upweller systems and mesh sizes of 6 mm or greater in grow-out containers.
- Decreasing stock density.
- Selective breeding has resulted in some stocks of oysters that demonstrate enhanced tolerance to JOD.

THE AGENT

No set of criteria can provide absolute proof of causation but that guideline can, and should, be used to weigh evidence (Sir Austin Hill, 1965)

In the late 1800's Robert Koch recognized the need for defining a causal relationship between a microbe and a specific disease. His postulates have since been used to guide the collection of evidence to determine if a given organism is the cause of a disease with the proof lying in the concordance of the evidence. Ideally, one should show 1) the parasite occurs in every case of the disease and under circumstances that can account for the pathology and clinical course of the disease; 2) the parasite occurs in no other disease as a fortuitous and nonpathogenic parasite; and 3) after being isolated from the body and repeatedly grown in pure culture, the parasite can again induce the disease and be reisolated. These postulates illustrate the underlying principles:

- Demonstration of a specific association with disease serves to rule out a commensal relationship.
- Isolation in pure culture on laboratory medium proves the existence of an independent living organism and provides a pure inoculum.
- The ability to reproduce disease by exposure to the pure culture of the microorganism demonstrates pathogenicity.
- The ability to re-isolate the microorganism in culture from an experimentally infected host strengthens the case and demonstrates replication within the animal.
- The microbiological evidence should also be in agreement with the pathological and circumstantial data.

Based on these postulates and additional supporting evidence, *Roseovarius crassostreae*, has been established as the etiological agent of JOD. This novel species is a gram-negative bacterium within the *Roseobacter* clade of the marine Alphaproteobacteria, and is the numerically dominant bacterium isolated from JOD-affected oysters. Their relative abundance was confirmed using a culture-independent approach (i.e. sequence analysis of 16S rDNA libraries from affected oysters). The association is consistent regardless of time, location or size of the animals. Thus, *R. crassostreae* is the only organism that completely satisfies Koch's first postulate. In agreement with Koch's second postulate, *R. crassostreae* is not found associated with any other oyster disease and is not found in healthy individuals. (Interestingly, a close relative has been implicated in black-band disease of corals, but the bacterium has not been isolated in culture). Both mortalities and JOD-like conchiolin have been induced in healthy oysters under laboratory conditions when challenged by injection with *R. crassostreae*, and the bacteria were re-isolated from affected individuals. *R. crassostreae* has also been detected in oysters coincident with the earliest microscopic lesions and at least one week prior to the appearance of gross JOD-signs. The bacteria appear to use polar fimbriae to preferentially colonize the inner shell surface, including the conchiolin deposits. Consistent with previous histological studies, *R. crassostreae* do not appear to invade the oyster soft tissues.

The disease can be spread directly among animals, but factors influencing its emergence are not well understood. Minor genetic variations in the 16S–23S rDNA internal transcribed spacer (ITS) region sequences of *R. crassostreae* strains have been identified and used for epizootiological applications. The data indicate that widespread dispersal of genotypes occurs more quickly than the time required for even slight divergence in the ITS. Some data also suggests that most new cases of JOD arise from indigenous *R. crassostreae* and that prolonged use of shellfish culture sites may contribute to their enrichment in those sites. Some *R. crassostreae* introductions may have resulted from direct transfer of adults or seed oysters.

It is still possible that other bacteria and/or protists may play a role in predisposing oysters to JOD or in the disease process itself. For example, JOD frequently occurs soon after exposure of the seed to a phytoplankton or zooplankton bloom, which in turn may be followed by a bloom of *Vibrio* spp. Whereas no particular *Vibrio* species has ever been associated with JOD, increases in total *Vibrio* levels in the environment (and in the oysters themselves) have been associated with at least some JOD outbreaks. Elevated *Vibrio* levels, which appear before JOD symptoms and persist as the disease develops, may also contribute to mortality. In a recent molecular study into a possible protistan involvement in JOD (using cloned 18S ribosomal RNA sequences), a ciliate, *Mesanophrus magna,* was identified in a population of JOD-affected animals. Subsequent PCR-based screening of those same oysters with primers specific for *M. magna* and *R. crassostreae* showed highly significant correlations with the presence of these two microbes in diseased tissues.

CONCLUSIONS

After reviewing the history and findings presented during this workshop the invited external panel and members of the workshop recognize that significant advances in understanding the etiology of JOD have occurred. Nevertheless, they emphasize that many questions remain unanswered. They were asked to 1) identify salient gaps in our understanding of this disease and its process that will improve the management and eontrol of JOD and 2) make recommendations as to the strategic research needs. The recommendations they put forth are as follows:

- Characterization of the environmental, physical and biological conditions that drive the disease process (e.g., salinity, temperature, and the potential involvement of algal blooms) will provide information for early warning and devising better management strategies.
- Development of additional (e.g., quantitative) diagnostics for early detection and surveillance of potential areas which may be at risk are needed to limit the spread of the disease.
- Elucidation of the pathogenesis of JOD is needed and includes determining the conditions that facilitate colonization of hosts by *R. crassostreae*, the minimum infectious dose, the identification and regulation of virulence factors, and a more thorough examination of the host responses to the infection.
- Identification of factors associated with oyster development and their role in susceptibility to JOD may provide insight into the restricted period during which the animals are susceptible.
- Understanding defense mechanisms (host response) of infected oysters is needed for insight into the biological basis of resistance.
- The ecology of *R. crassostreae* needs to be further investigated (e.g., factors influencing its distribution and abundance, reservoirs, and possible intermediate hosts).
- Further evaluation is needed of *Stappia stellulata* as a potential bacterial probiotic to prevent *R. crassostreae* colonization of oyster seed and subsequent mortalities.
- Future efforts directed at development of additional disease-resistant strains using information from research as well as survivor breeding will provide excellent management methods for this disease.

ACKNOWLEDGEMENTS

We would like to thank all of the Juvenile Oyster Disease Special Session speakers whose input was invaluable to the synthesis process: Rocco Cipriano, USGS, Kearneysville, WV; Richard Clime, Dodge Cove Marine Farm, Newcastle, ME; Christopher Davis, Pemaquid Oyster Company, Waldoboro, ME;

Javier Gómez-León, University of Rhode Island, Kingston, RI; Richard Karney, Martha's Vineyard Shellfish Group, Oak Bluffs, MA; Richard Langan, University of New Hampshire, Durham, NH; Mats Lundqvist, NOAA NOS, Charleston, SC; Steve Malinowski, Fishers Island Oyster Company, Fishers Island, NY and David Relyea, Frank M. Flower and Sons Inc., Oyster Bay NY. Acknowledgements are also due to Aaron P. Maloy, Letterkenny Institute of Technology, Donegal, Ireland who worked with Katherine Boettcher on much of the *Roseobactor* work and Dr. Christopher Groocock, US Department of Agriculture/APHIS (retired) for his review of this manuscript.

This workshop was sponsored by the National Oceanic and Atmospheric Administration, National Ocean Service under grant number: NA86RG0052, Am. 11 and by the Cooperative Oxford Laboratory, Oxford, MD.