5.2.13 Juvenile Oyster Disease of Eastern Oysters

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A. Name of Disease and Etiological Agent

Juvenile oyster disease (JOD) is a syndrome defined by macroscopic and microscopic signs, and high mortality, in cultured juvenile oysters. The etiology has not been confirmed, but bacterial involvement is highly likely. A newly described species of the α-proteobacteria Roseobacter group, *Roseovarius crassostreae*, has been found repeatedly in oysters with JOD symptoms at a variety of locations (Boettcher et al. 2000, 2005). Challenge experiments have so far failed to consistently induce characteristic symptoms.

B. Known Geographical Range and Host Species of the Disease

Geographical Range

JOD has been reported in aquaculture sites from New York to Maine, USA.

Host Species

JOD affects juvenile eastern oysters, *Crassostrea virginica*.

C. Epizootiology

JOD outbreaks occur at temperatures exceeding 20^{o} C and in relatively high salinity (≥25 ppt) sites (Bricelj et al., 1992; Ford and Borrero, 2001). It is a problem in nursery systems (upwellers, floats, lantern nets, etc.) and occurs during a period of rapid growth in midsummer, after the oysters have been deployed from the hatchery. There is no evidence of an infectious agent originating in the hatchery. At least some outbreaks have followed plankton blooms suggesting the possibility that plankton may provide surfaces and nutrients for subsequent bacterial proliferation (Lee et al., 1996). Mortality can reach 60 to 90% over a 4-6 week period in oysters 10 to 25 mm in shell height. Larger juveniles often show characteristic shell lesions, but experience much lower mortality. Decreased density and increased water flow through container systems can decrease mortality.

D. Disease Signs

Behavioral Signs

Not applicable

Gross Signs

JOD initially inhibits growth. Within a week or two, characteristic shell anomalies appear (Ford and Borrero, 2001). The soft, uncalcified, growing edge of the upper, flat valve often becomes eroded, resulting in the appearance of "lower valve overgrowth" or "uneven shell margins." The lower valve may become excessively cupped (Fig. 1) . The oyster's soft tissue retracts within the shell cavity and the mantle produces an anomalous deposit of light to dark brown organic material (conchiolin), which covers the retracted soft tissue and is affixed to the inner side of one or both valves (Fig. 2). The edge of the conchiolin deposit may be raised in a ring-like structure surrounding the soft tissue. Oysters that have recovered from JOD and resumed growth may show pronounced ridges (growth checks) on the external shell Lewis et al., 1996).

Microscopic Signs

Tissue sections show lesions of the mantle epithelium, including hemocyte infiltration, one to two weeks before shell deposits are observable. The lesions are highly correlated with subsequent conchiolin deposits and mortality. Histologically apparent mantle lesions become more pronounced as the shell deposits appear, and include exudates of hemocytes, debris, secondary bacteria and cilia, along with the anomalous conchiolin layers (Figs. 3, 4 and 5).

E. Disease Diagnostic Procedures

The fact that the etiological agent of JOD has not been confirmed causes some problems of diagnosis because different investigators have used one or more of the shell anomalies described above, and not always the same ones. This becomes a particular problem because all the signs are non-specific characteristics that can be caused by other factors. Growth inhibition can result from any adverse condition affecting oysters, and external growth checks are often present when growth resumes. "Lower shell overgrowth" can be seen in oysters affected by other diseases or adverse condition. Even the most distinctive symptom, the conchiolin deposit, can be occasionally induced by conditions other than JOD. Deposition of a conchiolin "barrier" on the inner surface of the shell is a protective "walling-off" reaction by the mantle epithelium to an irritant and can be observed from time to time in individual oysters, both adults and juveniles, from wild populations. Nevertheless, descriptions of the JOD syndrome indicate that the most consistent correlate with the disease is the conchiolin deposit and it is recommended that this be designated as the key diagnostic feature until an etiological agent is confirmed (Lewis, 2001).

Sampling Criteria

Cohorts suspected of suffering JOD should be sampled by collecting both live and dead (empty shells) oysters. The presence and extent of shell anomalies, specifically the conchiolin deposit on the inner shell, should be determined from a sample of 100 live and, if available, shells from 100dead oysters with valves still articulated

Presumptive Diagnosis

Rapid and high mortality of juvenile oysters during mid summer, associated with externally visible shell growth anomalies.

Confirmatory Diagnosis

Presence of conchiolin deposit on inner surface of one or both valves. Additional clinical signs include excessive shell cupping and uneven shell margins such that a several millimeter edge of the lower, cupped, valve is exposed and often fouled.

Procedures for Transportation and Storage of Samples

Samples collected from apparently normal, moribund, or dead oysters should be packed on ice for shipment and subsequent bacterial, histological, or macroscopic analysis. Also, live whole oysters can be fixed in Davidson's after severing the hinge ligament and propping open the valves so that the fixative enters the shell cavity. Maintaining the shell with the soft tissues is critical since the diagnosis is based on shell anomaly symptoms.

Table 1. The methods currently available for surveillance, detection, and diagnosis of JOD are listed in below. The designations used in the Table indicate: – = the method is presently unavailable or unsuitable; ? = the method is available but untested; + = the method has application in some situations, but cost, accuracy, or other factors severely limit its application; ++ = the method is a standard method with good diagnostic sensitivity and specificity; and +++ = the method is the recommended method for reasons of availability, utility, and diagnostic specificity and sensitivity.

Method	**Screening**		**Presumptive**	**Confirmatory**
	Juveniles	Adults		
Gross signs	+++	+	+++	+++
Histopathology	+	+	+	-
Bacteriology *	+	-	+	-
Transmission EM	-	-	-	-
Bioassay	-	-	-	-
Antibody-based methods	-	-	-	-
DNA probes – in situ	-	-	-	-
PCR**	?	?	-	-

**Roseovarius crassostreae* has been found in all outbreaks identified as JOD, although its etiological role has not been confirmed. It is a gram negative, motile rod (approximately 0.25 x 1.0 µm) that is oxidase positive and grows poorly in anaerobic media. See Boettcher et al. (2000) for details.

** Gene sequences are available for *R. crassostreae.*

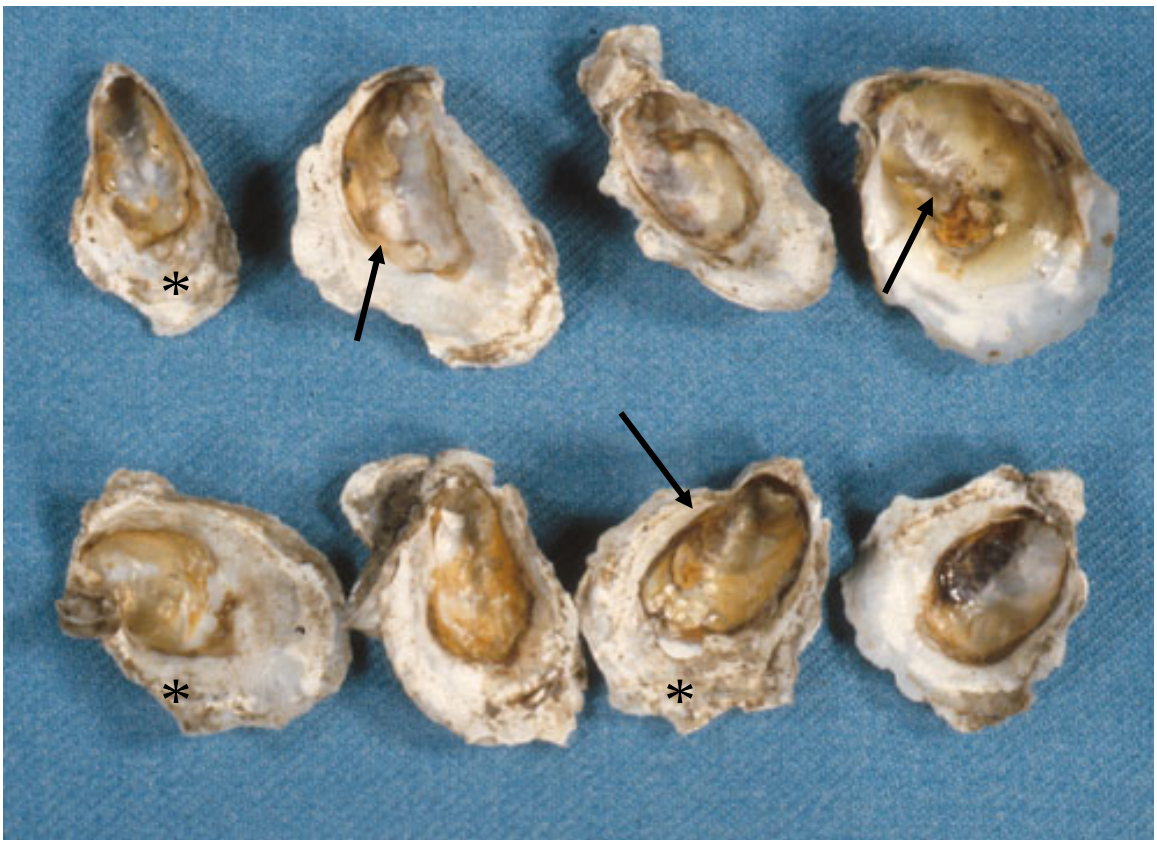

Figure 1. Inner valves of juvenile Eastern oysters affected by Juvenile Oyster Disease showing conchiolin deposits (arrows) and fouling (asterisks) on shell surface outside of deposit.

Figure 2. Top and side view of juvenile Eastern oysters affected by Juvenile Oyster Disease showing extreme cupping of lower valves and exposed inner valve edges resulting from erosion of edges of upper valves.

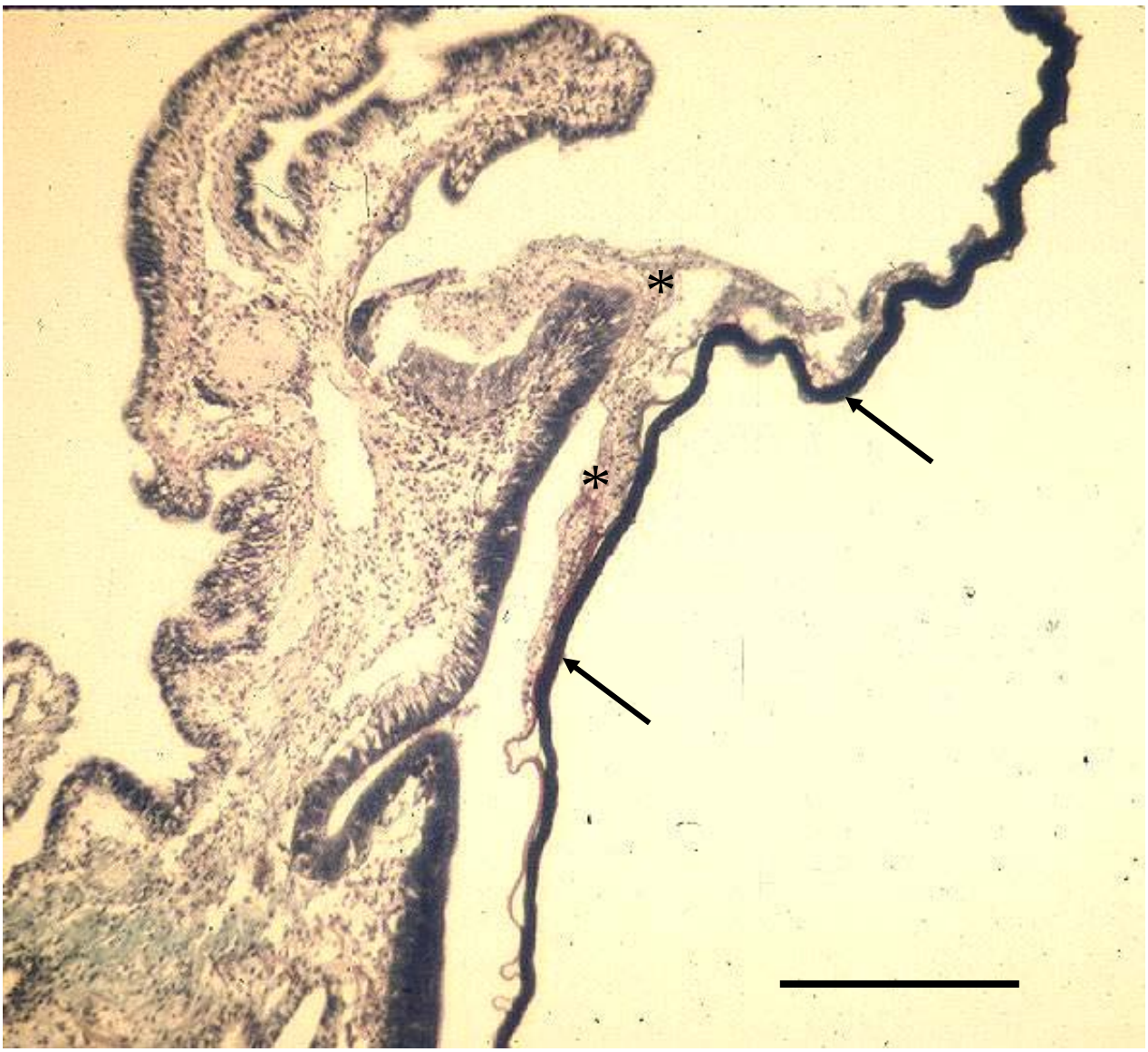

Figure 3. Histological section of an oyster affected by Juvenile Oyster Disease showing relationship of mantle edge, exudate of hemocytes (*), and anomalous conchiolin deposit (arrows) Hematoxylin, aniline blue, acid fuchsin. Scale bar = 100 µm.

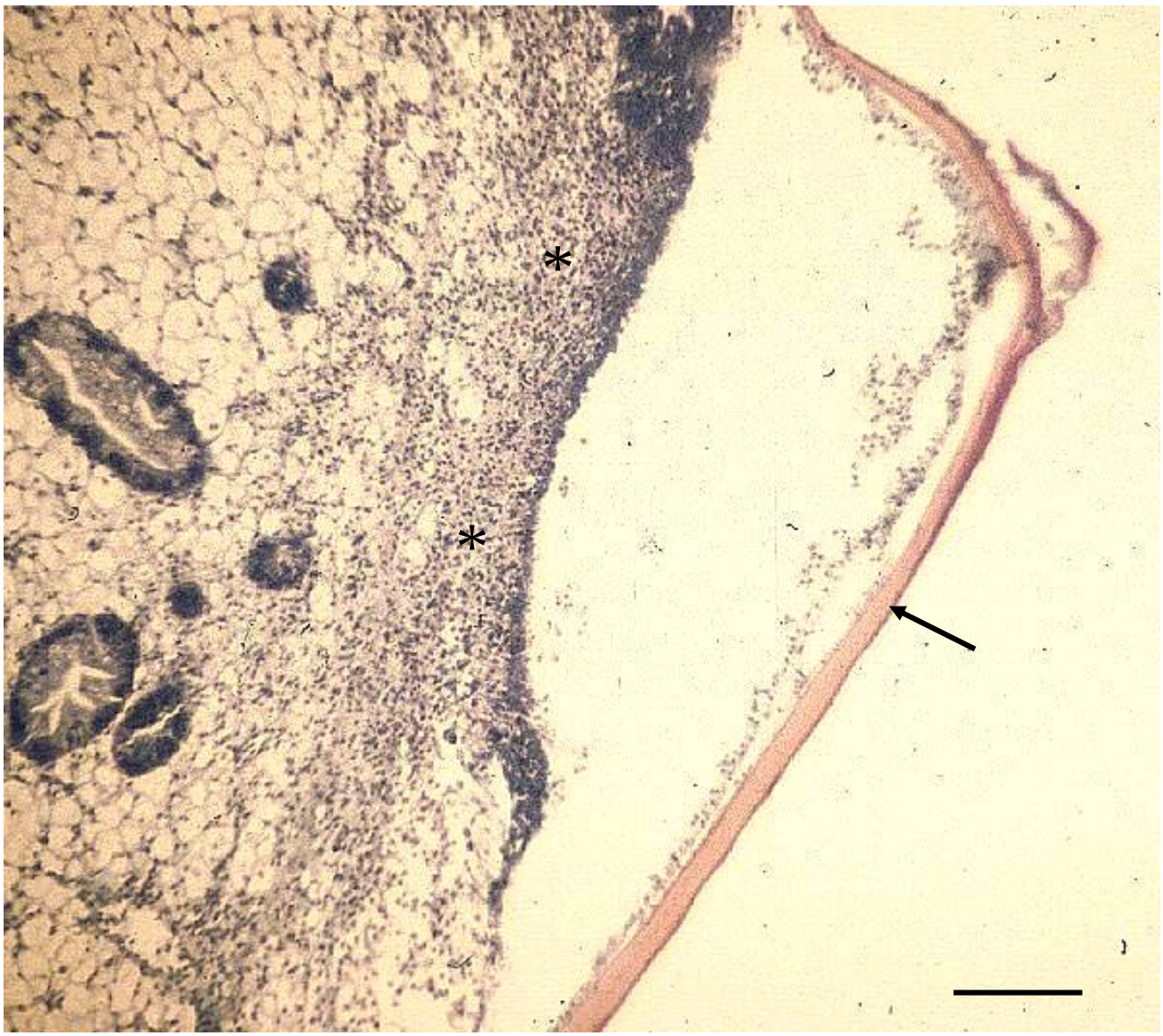

Figure 4. Histological section of oyster affected by Juvenile Oyster Disease showing lesion on mantle surface associated with hemocyte infiltration (asterisk) and anomalous conchiolin deposit (arrow). Hematoxylin, aniline blue, acid fuchsin. Scale bar = 50 µm.

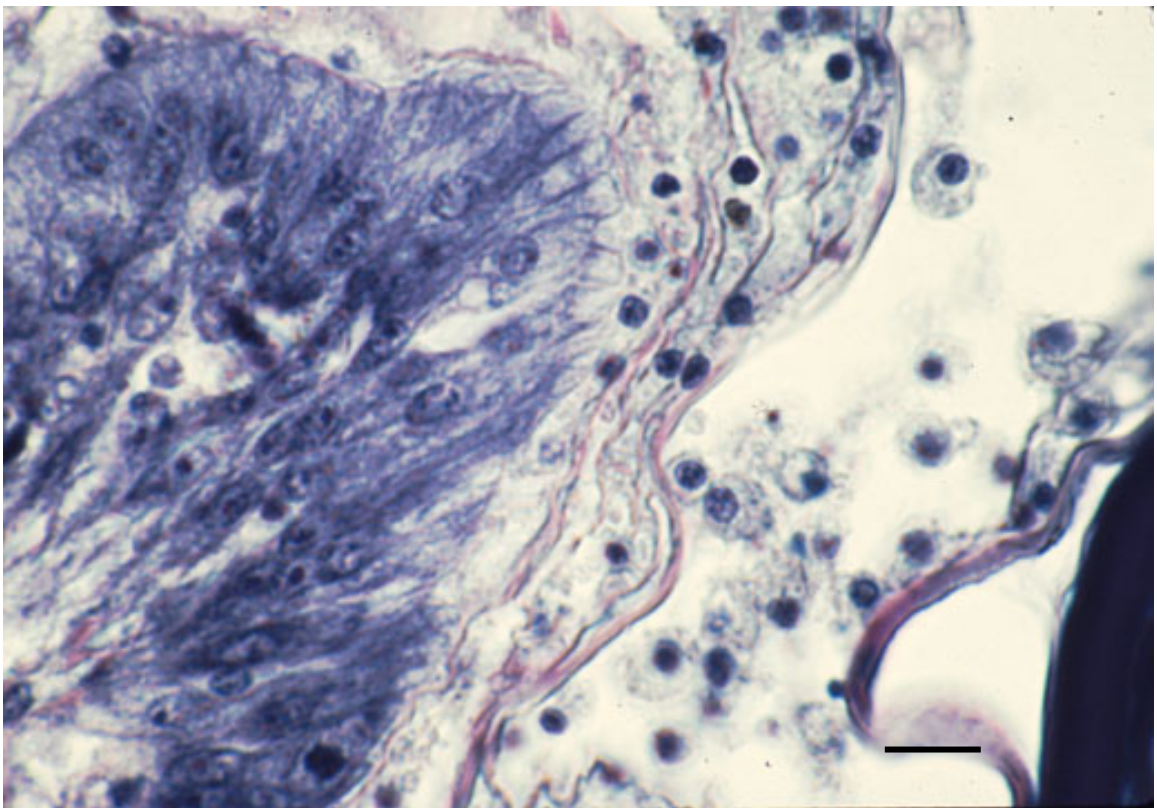

Figure 5. Higher magnification of exudate in Figure 3 showing hemocytes interspersed between layers of secreted conchiolin. Hematoxylin, aniline blue, acid fuchsin. Scale bar = 10 µm.

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