Histological Observation of

*Perkinsus marinus*

Ryan B. Carnegie

*Virginia Institute of Marine Science*

*Virginia, USA*
Perkinsus marinus

- Identified in the 1940s in the Gulf of Mexico

- Found in Chesapeake Bay when first sought, late 1940s
  → Perhaps a local enzootic species...

- Primary cause of oyster disease and mortality from southern New England to the Gulf of Mexico today
Dermo Disease: Basics

- A warm-season disease of *C. virginica* (and now *Crassostrea corteziensis*) where salinity exceeds 12 ppt

- *P. marinus* cells overwintering in oysters at low levels begin to proliferate in oyster tissues when temperature is > 20° C
  → Fastest at temperatures > 25° C

- Parasite levels (& disease) rise through summer and peak by early fall

- Deaths peak September-October, with peak parasite transmission coinciding with deaths of heavily infected oysters
Dermo Disease: Gross Pathology

- A wasting disease
- With infections reaching moderate or greater intensity, oysters become emaciated
Life Cycle in a Host
Histopathology

Initial infections usually observed at gut epithelium

Normal gut epithelium
Histopathology

Initial infections usually observed at gut epithelium

Light Infection
Histopathology

- As infection increases from *light* to *moderate* intensity:
  - Parasites proliferate locally and hemocytes infiltrate epithelium
  - Damage worsens from lytic secretions of *P. marinus*
Histopathology

Moderate Infection
Histopathology

- As infection becomes *heavy*.

- Parasites very abundant in epithelium

- Invasion of connective tissues, other organs

- Epithelium may slough off into gut lumen, along with abundant parasites
Histopathology

Heavy Infection
Histopathology

Heavy Infection
Histopathology

Heavy Infection
Histopathology

Heavy Infection
Histopathology

Heavy Infection
Dermo Disease: Histopathology

- With invasion of connective tissues and intensification of infection, a fibrosis of connective tissues
- Increase in brown cells and seroid
- Liquefaction of host tissues
- Death of host
Evolutionary Ecology of Oyster Disease in Chesapeake Bay

Perkinsus marinus

Crassostrea virginica

Haplosporidium nelsoni
Important Assumption

- The oyster-parasite-parasite system is static, with oysters chronically susceptible to disease
  - No evolutionary response by oysters to parasitism
  - Evolutionary changes in the parasites not considered

North et al. 2008, MEPS 359: 99-115
Is This Assumption Valid?

Are the susceptibility of the oysters and the virulence of the parasites both fixed/static?

Or is the system dynamic, with selection driving evolutionary changes among the players?
The Beginning:
Perturbation of a Stable Host-Parasite Relationship
Crassostrea virginia, the Eastern Oyster

- Ecologically and economically critical species along the North America Atlantic and Gulf coasts
**Perkinsus marinus**

- Endemic species, agent of “dermo disease” in oysters
- Directly transmissible among *Crassostrea virginica*

**Haplosporidium nelsoni**

- Introduced pathogen (Burreson et al. 2000), agent of “MSX disease”
- Complex life cycle likely requiring intermediate host(s) for transmission
Oyster and Parasite Distribution, Present

- **C. virginica**
- **P. marinus**
- **H. nelsoni**
Oyster and Parasite Distribution, 1959

- C. virginica
- P. marinus
- H. nelsoni

- MSX introduction brought sharp changes to the pre-1959 system
- Very high host mortality
- Sudden competition between two parasites, one (MSX) introduced and very virulent
Oyster Population, Industry in Virginia, Pre-1959

- Robust harvests from natural reefs
  - 1959-60: 700,000 bushels (Haven et al. 1978)
  - Recent annual: < 100,000 bushels (VMRC data)

- Large numbers of oysters transplanted from James River, planted over vast areas of lower Bay bottom
  - 1959-60: 2,533,275 bushels harvested from private leases (Haven et al. 1978)

- Oyster abundance in upper mesohaline to polyhaline waters much higher than today (Haven et al. 1978)
Perkinsus marinus in Pre-1959 Oyster Populations

What Killed Your Oysters?

By Jay D. Andrews, Ph. D.
Virginia Fisheries Laboratory
Gloucester Point, Virginia

In the past five years a new word has crept into the vocabulary of oystermen in lower Chesapeake Bay. It is the virtually unpronunciable name of the fungus which was discovered in the Gulf of Mexico and described in 1939 by Mackin, Cown, and Collin—Perkinsus marinus. Now known to be a major cause of death of oysters in salt water from Chesapeake Bay to Louisiana.

Most oystermen say that in lower Chesapeake Bay a yield of one bushel of market oysters for each bushel of seed planted is about the best that can be expected. A conservative estimate of the number of usable oysters in a bushel of James River seed is 946. There are probably an equal number more summer seasonings, many of which will be lost by predation or by cultivation by various predators. In Virginia, market oysters are marketed when the count is about 390 per bushel. The decrease from 900 to 390 signifies a loss in two-thirds of the original seed oysters.

Death Rate Is High

We have held some 38 different groups of oysters in trays, each containing of 100 to 500 oysters, and accurate counts of dead and survivors have been made frequently. Under two years of age mortality was low, but in older oysters about one-third died each year. In the trays 85 to 90 per cent of those deaths were caused by the fungus. On natural grounds the percentage of deaths from the fungus was not as high, for predators, cannibals, and other factors were killing some before the fungus could act, but even half the dying oysters examined were heavily infected.

Perkinsus marinus is a major cause of death in the saltier waters of Chesapeake Bay. The infective stage extends up the Bay in the Patuxent River, in the north of the Patuxent River, in Tangier Sound, and halfway up the Pampanosaw River. The fungus is absent from the James River area and the sea side of the Eastern Shore of Virginia and Maryland.

Activity Is Seasonal

Oysters may die from the fungus within a month after first infection, though the process usually takes longer in natural waters. The organs of the oyster are gradually replaced by the spores of the fungus until death occurs. Then the spores released by the degrading oyster are presumably carried by the waters to infect other oysters. In our laboratory experiments all oysters, regardless of size or age, were killed if sufficiently large doses of the fungus spores were fed or injected.

In nature the fungus begins to multiply in oysters in June, reaches its peak in August and September, and continues until cold weather intervenes in November. At the end of the warm season 70 to 90 per cent of the live oysters in trays and on natural grounds are infected. Many of these are light infections from which the oyster recovers but in most years about 10 per cent of the oysters in trays die from the disease, and in 1954 over half did. The spores disappear rapidly from oysters in December, January, and February, but an overwintering stage persists in a few oysters.

Care Probably Not Feasible

Treating oysters for disease is not an impossible operation, but because Perkinsus marinus attacks the living flesh, and since the fungus spores are found in nearly all seawater, any treating solution would have to penetrate throughout the body of the oyster to be effective. Furthermore, Perkinsus marinus may strike several times during a single season.

Fungus-Resistant Varieties

If care is not feasible, can the disease be prevented by breeding or selecting resistant oysters? Unfortunately, the techniques for breeding oysters artificially are not completely known and experimental crosses and segregates “farms” for testing the product do not exist. Furthermore, most adult oysters are gathered from wild estuaries with little or no control of quality by man. The mass-rearing of a large number of adult oysters for 15 to 20 years probably will prevent the segregation of varieties from one oyster ground to another.

The selection of a resistant strain of oyster is perhaps the most promising approach, but the problem of crossing the wild stock remains. There is evidence that oyster stocks will develop resistance to the fungus, for South Carolina seed grown in trays at Gloucester Point is far more resistant than native oysters, and oysters from the Pampano Saw River of Virginia and Maryland, where the fungus is absent, are more susceptible than natives. We suspect that the difference is as a result of the period of time that the fungus has been present in these waters.

Present in 1949 when oysters were first evaluated (Mackin 1951)

Perhaps long-established

An agent of chronic disease

Parasitism peaking oysters age 3 and older

Intensities generally low

Causing ≤ 30% mortality in most years

Generally manageable by industry (Hewatt & Andrews 1954; Andrews 1956; Andrews & Hewatt 1957; Andrews 1965)
Arrival of *Haplosporidium nelsoni* (MSX)

- Emerged in Chesapeake Bay in 1959 (Andrews 1962)

- Caused > 90% mortality in lower Bay reefs/grounds (Haskin & Andrews 1988)

- Planting industry abandoned in these waters (Andrews & Frierman 1974)

Post-MSX, oyster abundance far lower in (*Perkinsus*-enzootic) upper mesohaline-polyhaline waters

*Perkinsus* retreated to upriver bands of salinity ~ 10 psu

*H. nelsoni* dominated until 1986, when activity of *P. marinus*, and to a lesser extent *H. nelsoni*, intensified dramatically (Burreson & Andrews 1988)
Did the oysters respond to the increase in disease pressure?
Evolution of the Parasites
Intensification of Dermo Disease, 1980s-Present

- *Perkinsus marinus* largely disappeared with the arrival of *H. nelsoni* (Andrews 1966)

- Reemerged in 1986 because of drought (Burreson & Andrews 1988)

- Very high parasite and disease levels today thought to be a function primarily of abundance, which increased greatly with 1980s droughts (Burreson and Ragone Calvo 1996)
  → More *P. marinus* ➔ higher rates of transmission

- Loss of deep winter cold temperatures contributes to this by allowing *P. marinus* to overwinter at relatively high levels (Burreson and Ragone Calvo 1996)

- *Is the story this simple?*
Temporal Trend in *Perkinsus marinus* Levels

- *P. marinus* levels in wild oysters are much higher now than in early years
  - Particularly body burdens
  - Prevalence then and now approached 100%

- Intense disease and mortality develop much more rapidly than before the arrival of MSX

A Question:
Is This Not Adaptive?

- *Perkinsus marinus* is transmitted primarily through the deaths of infected oysters (Ragone Calvo et al. 2003)—before MSX this began 2-3 years after initial exposure (Andrews 1956).

- Parasite body burdens were relatively low in the 1950s (Andrews 1980), but still ensured effective transmission among oysters at a time when oysters were particularly abundant.
With the arrival of *Haplosporidium nelsoni*:

- Oyster population density decreased dramatically
  → Decreased the transmission efficiency of *P. marinus*

- Oysters faced death within weeks-months of exposure to *H. nelsoni*
  → Theoretically putting a less virulent, slower-developing parasite (i.e., *P. marinus*) at a disadvantage

- So: do the very high contemporary levels of *P. marinus* simply reflect increased parasite abundance and warmer winters?

- Or did the introduction of *H. nelsoni* select for a *P. marinus* that generated very high body burdens very quickly, that would thrive in a more sparse MSX-enzootic oyster population?

- *That is, did the introduction of a non-native pathogen select for a more virulent native parasite?*
Is an increase in body burdens (higher weighted prevalences since the 1980s) and generally more rapid rate of infection development convincing evidence of a virulence increase?

Or can this still be explained by simple numerical abundance?

_Not a definitive sign that _P. marinus_ has fundamentally changed or evolved_
Perkinsus marinus Life Cycle

Histopathology of Infection of Crassostrea virginica (Gmelin) by Dermocystidium marinum
Mackin, Owen, and Collier
J. G. Mackin
Department of Oceanography and Texas A&M. Research Foundation,
Agricultural and Mechanical College of Texas

Fig. 1. Diagrammatic representation of the life cycle of Dermocystidium marinum. Explanation is in the text.

1951
Where are the Schizonts?

- Contemporary histology suggests far more binary division than schizogony

- Is binary division by cells that never grow to large size adaptive?

- Is *P. marinus* population growth rate increased by having a high proportion of cells going through binary division, rather than a small subset going through schizogony?
“Two types of division of cultured cells have been observed: division by schizogony and division by binary fission or budding.”

“*P. marinus* at various stages of schizogony have been observed *in vivo*, and this process is considered as the method of division *in vivo.*”

“The importance of binary fission *in vivo* is not clear. **Division by budding or fission is prominent *in vitro* in cell populations that exhibit a high growth rate (La Peyre, personal observation).**”

*Can schizogony vs. fission by manipulated experimentally, and does it relate more to genetics or to environmental influences?*
So thus far:

- Contemporary histology indicates an abandonment of schizogony in favor of binary fission, which (unpublished, anecdotal) *in vitro* evidence suggests may be correlated with high growth rates.

- Very high body burdens, developing soon after initial exposure, do characterize contemporary *P. marinus* infections.

- Suggests a transformation in *P. marinus* . . . but when did it occur?
Thomas Rock, James River, August 1986

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Remarks:
- 1984: 10.5 µm
- 1986: 20.5 µm

- No. E. indicates no evidence of disease.
- No. H. indicates high prevalence of disease.
- No. L. indicates low prevalence of disease.
- No. P. indicates possible disease.
- No. A. indicates absence of disease.

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Perkinsus marinus Conclusions

- Fundamental *phenotypic* change in *P. marinus* between 1985 and 1986
  - General abandonment of schizogony in favor of binary division by small cells

- Associated with very high body burdens, intense disease & mortality

- May hypothesize that this reflects selection for increased “virulence” induced by *H. nelsoni*, at least indirectly

- Can a genetic change by demonstrated?
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