

# Disease Resistance in *Crassostrea virginica*



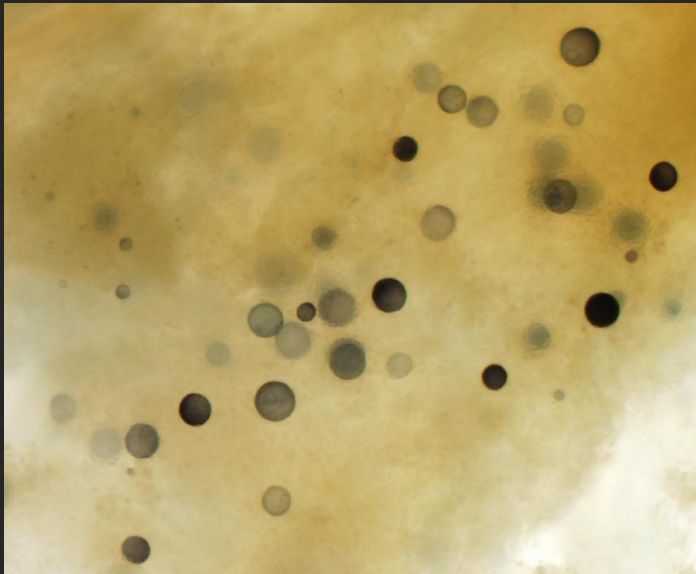
Ryan B. Carnegie

*Virginia Institute of Marine Science*



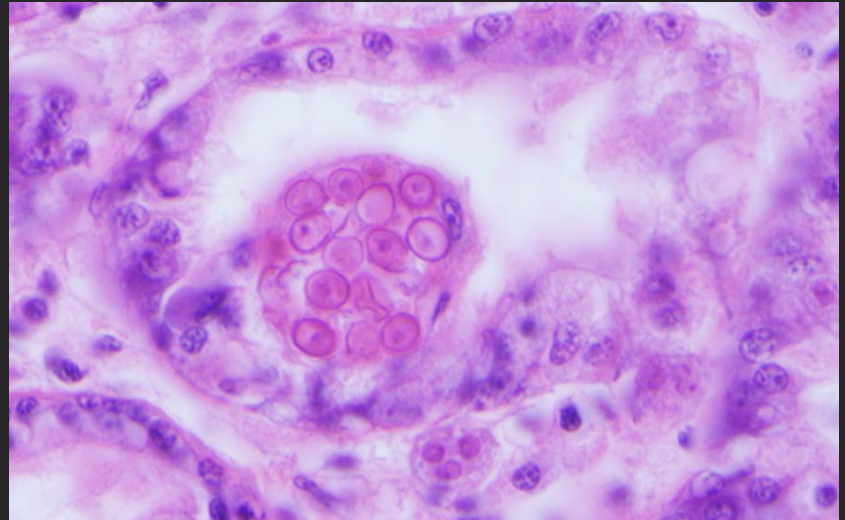
## *Perkinsus marinus*

- ❖ 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) (not known) for transmission



# What We Talk About When We Talk About Resistance

## ❖ Resistance versus Tolerance

- Resistance: oysters do not become infected at all?
- Tolerance: oysters display a lower degree of infection?

BY THE DELAWARE BAY ECOLOGY OF INFECTIOUS DISEASES GROUP

UNDERSTANDING HOW DISEASE  
AND ENVIRONMENT COMBINE  
TO STRUCTURE RESISTANCE IN  
ESTUARINE BIVALVE POPULATIONS

*Journal of Shellfish Research*, Vol. 29, No. 1, 161–175, 2010.

PERFORMANCE OF DISEASE-TOLERANT STRAINS OF EASTERN OYSTER  
(*CRASSOSTREA VIRGINICA*) IN THE PATUXENT RIVER, MARYLAND, 2003 TO 2007

Polymorphism in a serine protease inhibitor gene and its association with  
disease resistance in the eastern oyster (*Crassostrea virginica* Gmelin)

Resistance strategies limit infection (colonization, proliferation)

Tolerance strategies limit not infection, but the “fitness” consequences thereof



❖ Cobb 1894 (Aus. Gaz. N.S.W. 5:239-250)

- "It will be remembered that we proposed the terms rust-proof, rust-resistant, and rust-escaping, as descriptive of different kinds of wheat. To these three might be added a fourth, *rust-enduring*. A rust-enduring wheat is one which, though liable to rust, is able, notwithstanding the attack of the rust, to mature a fair crop of grain under ordinary circumstance. . ."

❖ Caldwell 1958 (Science 128:714-715)

- "Tolerance, enabling a susceptible plant to endure severe attack without sustaining severe losses in yield or quality. . ."

❖ Schafer 1971 (Annu. Rev. Phytopathol. 9:235-252)

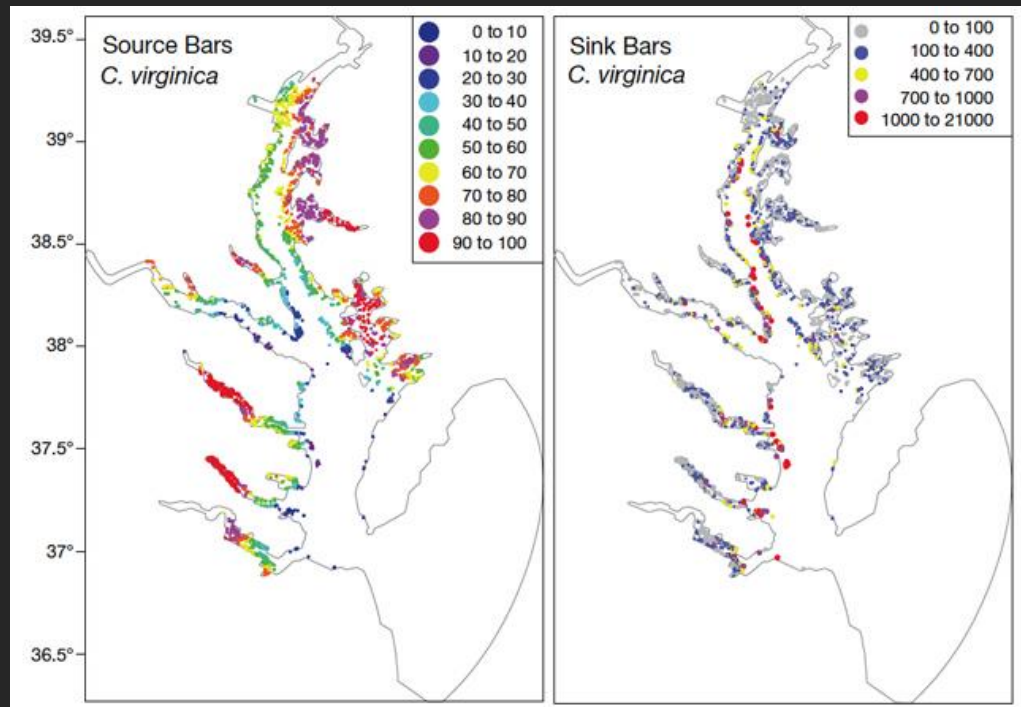
- "Tolerance may be defined as that capacity of a cultivar resulting in less yield or quality loss relative to disease severity or pathogen development when compared with other cultivars or crops."

❖ Roy and Kirchner 2000 (Evolution 54:51-63)

- "Resistance and tolerance can both improve host fitness; resistance does so by reducing infection, whereas tolerance does so by reducing the fitness loss under infection."

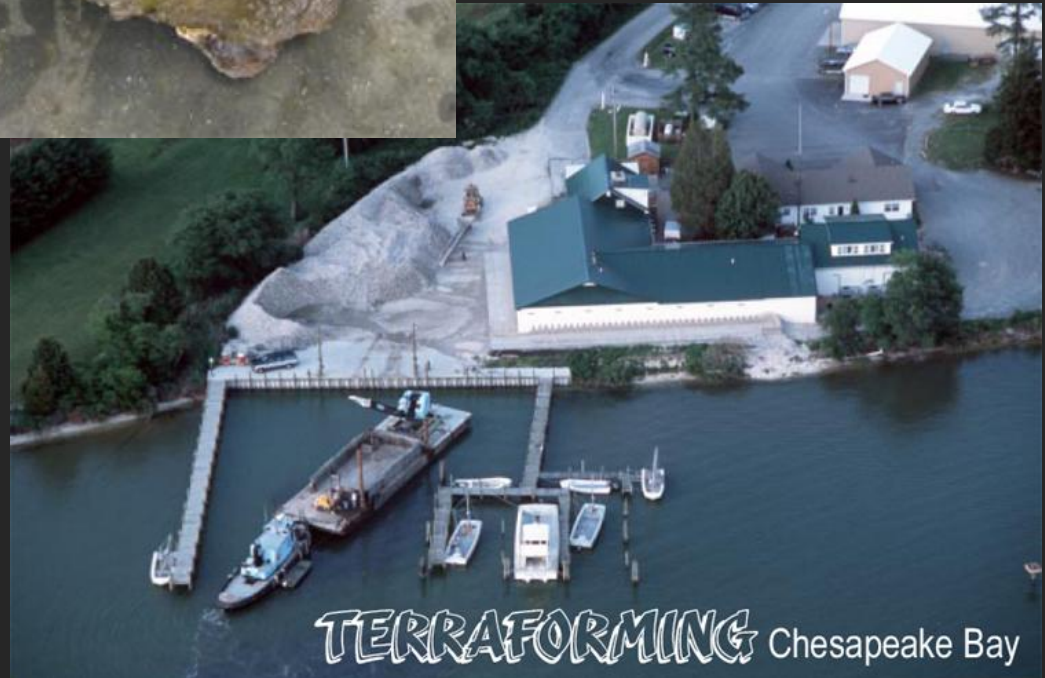
# Why Would Resistance Not Develop in Chesapeake Bay?

- ❖ Weak or inconsistent disease (selective) pressure
  - May be the case in parts of Maryland, upper parts of Virginia rivers
- ❖ Significant reproductive contributions by susceptible individuals in refugia from parasitism
  - Long assumed to be the case, and supported by physical modeling





# Is It Advantageous to Discount/Deny Resistance?



# *Haplosporidium nelsoni*

A light micrograph showing numerous oocysts of Haplosporidium nelsoni. The oocysts are spherical to oval, with a thick, pinkish-purple wall. Inside, there are several dark purple, rounded structures, likely sporozoites. The background is a light, pinkish-purple color, suggesting a tissue section or a culture medium. The overall appearance is that of a parasitic infection in a host tissue.

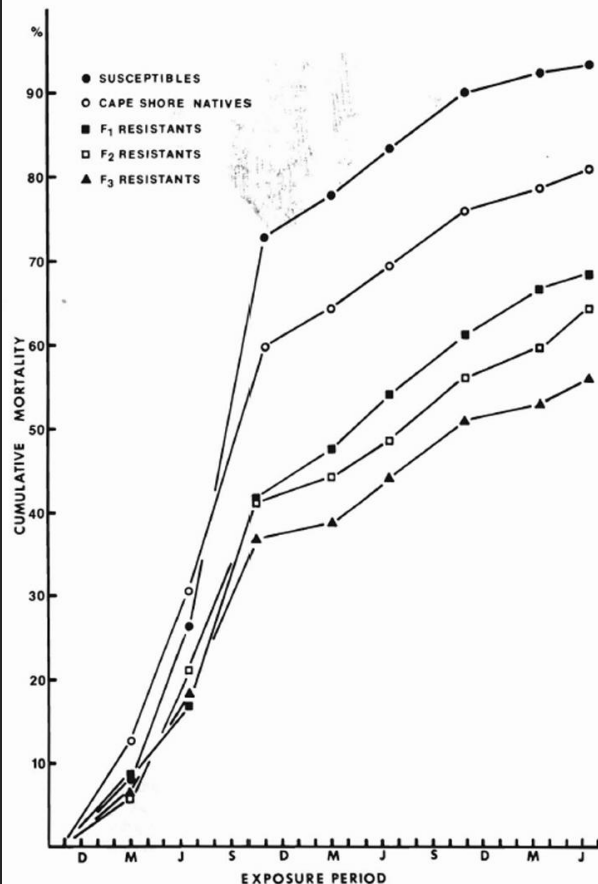
“Weak or inconsistent disease (selective) pressure”?

# Early Evidence of Resistance

## Development of Resistance to *Minchinia nelsoni* (MSX) Mortality in Laboratory-Reared and Native Oyster Stocks in Delaware Bay

HAROLD H. HASKIN and SUSAN E. FORD

Figure 2.—Cumulative mortality means for oyster stocks exposed to MSX in experimental trays on the Cape Shore tidal flats. The 33-month exposure period is shown on the abscissa.



### ❖ What about Chesapeake Bay?

- JD Andrews, memo dated 18 August 1987: Mobjack Bay "the only area in Chesapeake Bay where natural resistance to MSX has been found to be effective in most years"
- So MSX resistance developing (perhaps) but limited a quarter century after the parasite's emergence

Marine Fisheries Review, 1979



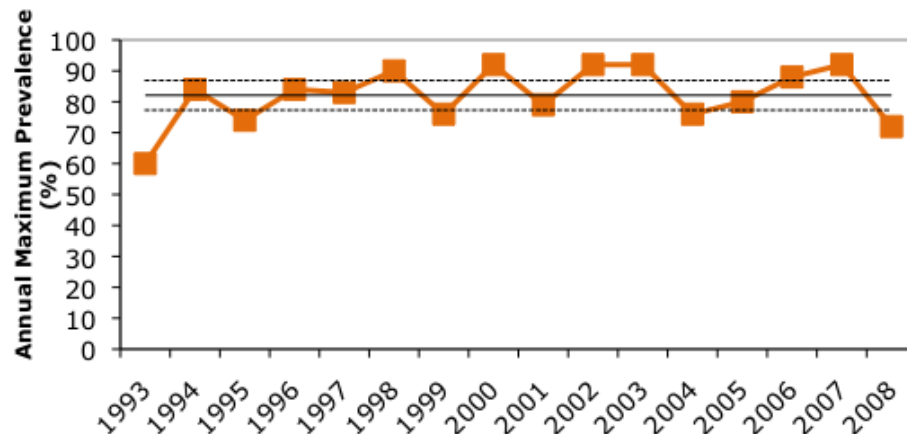
# Contemporary Resistance: What is Truly “Susceptible”?

## ❖ Annual “Spring Imports”:

- Naïve sentinels collected from Ross Rock (upper Rappahannock, disease-free) around May 1<sup>st</sup> each year, evaluated monthly into fall
- Conducted annually since 1960
- Intensely affected by both parasites, with near-total mortality by fall in most years

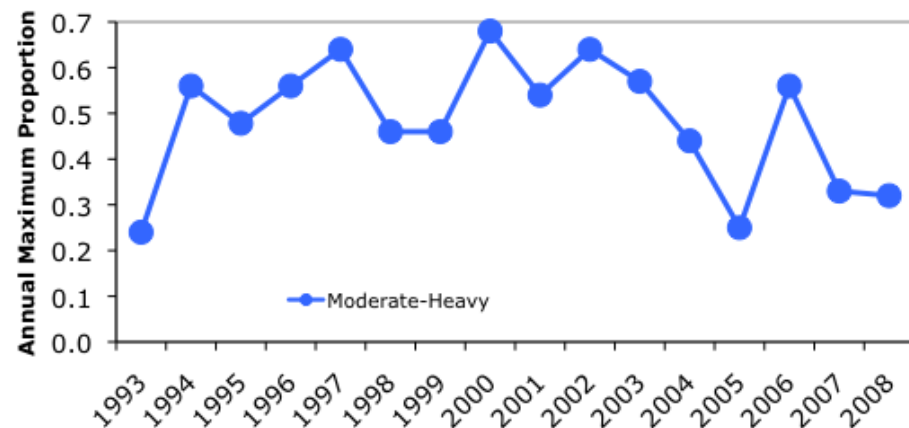


# Typical MSX Levels in Spring Imports

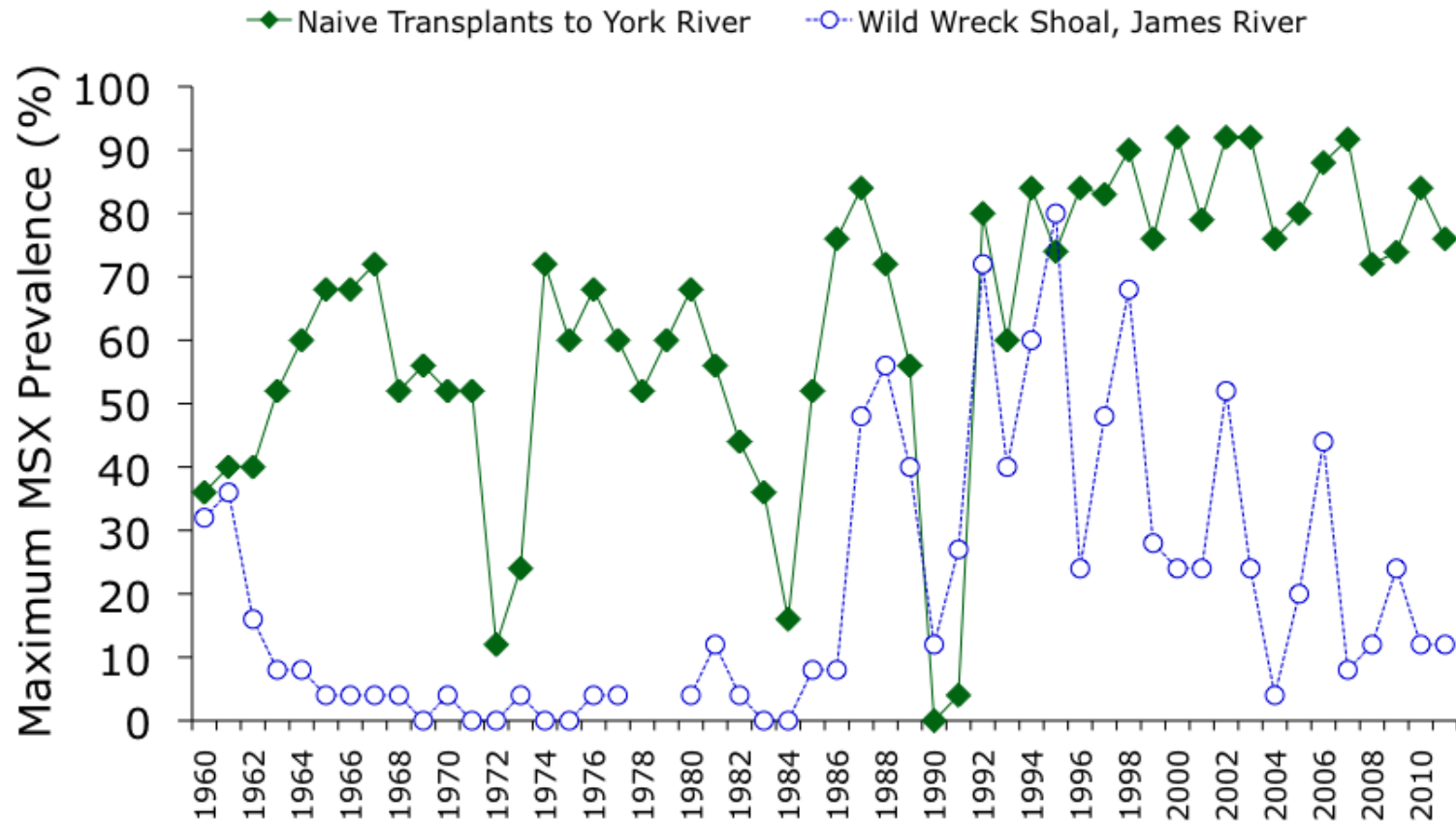


Maximum annual prevalence in monthly samples

Maximum annual proportion of population with advanced infections

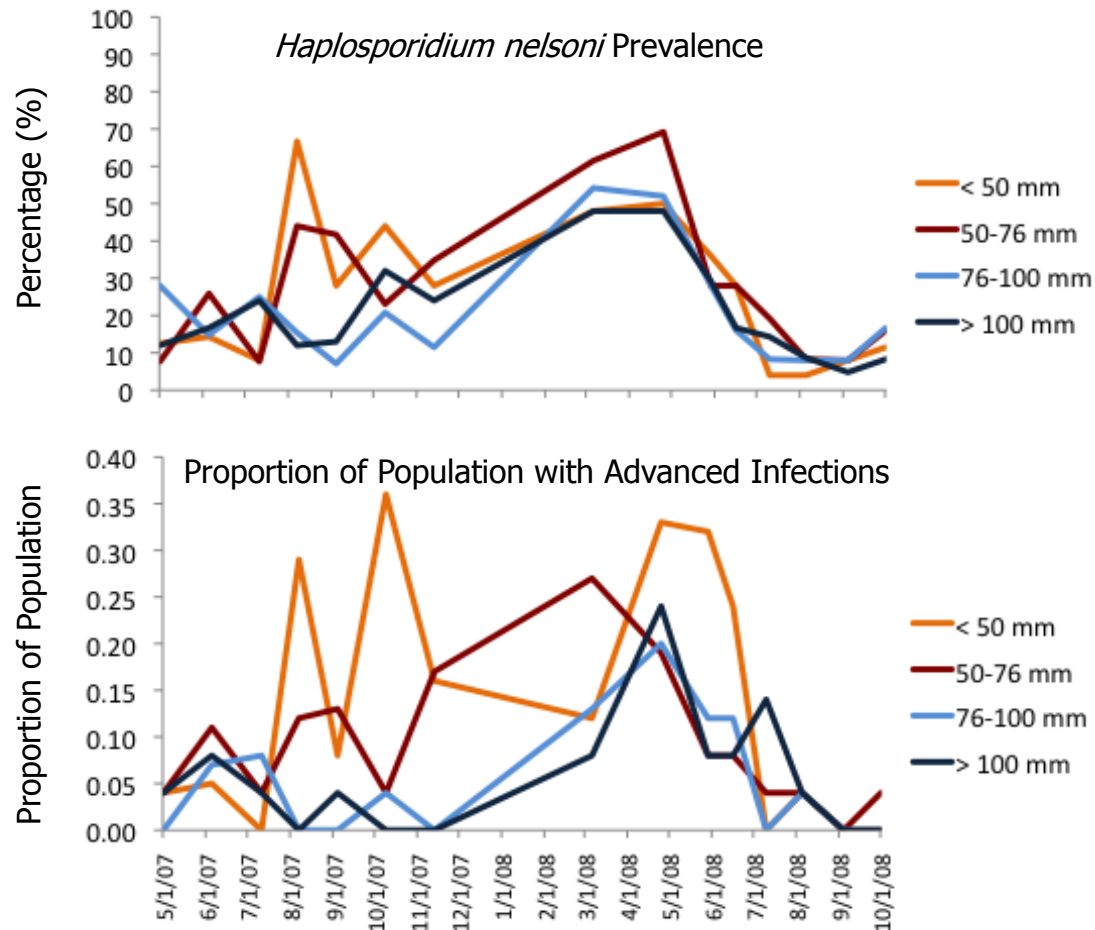


# Spring Imports Compared With A Wild Population





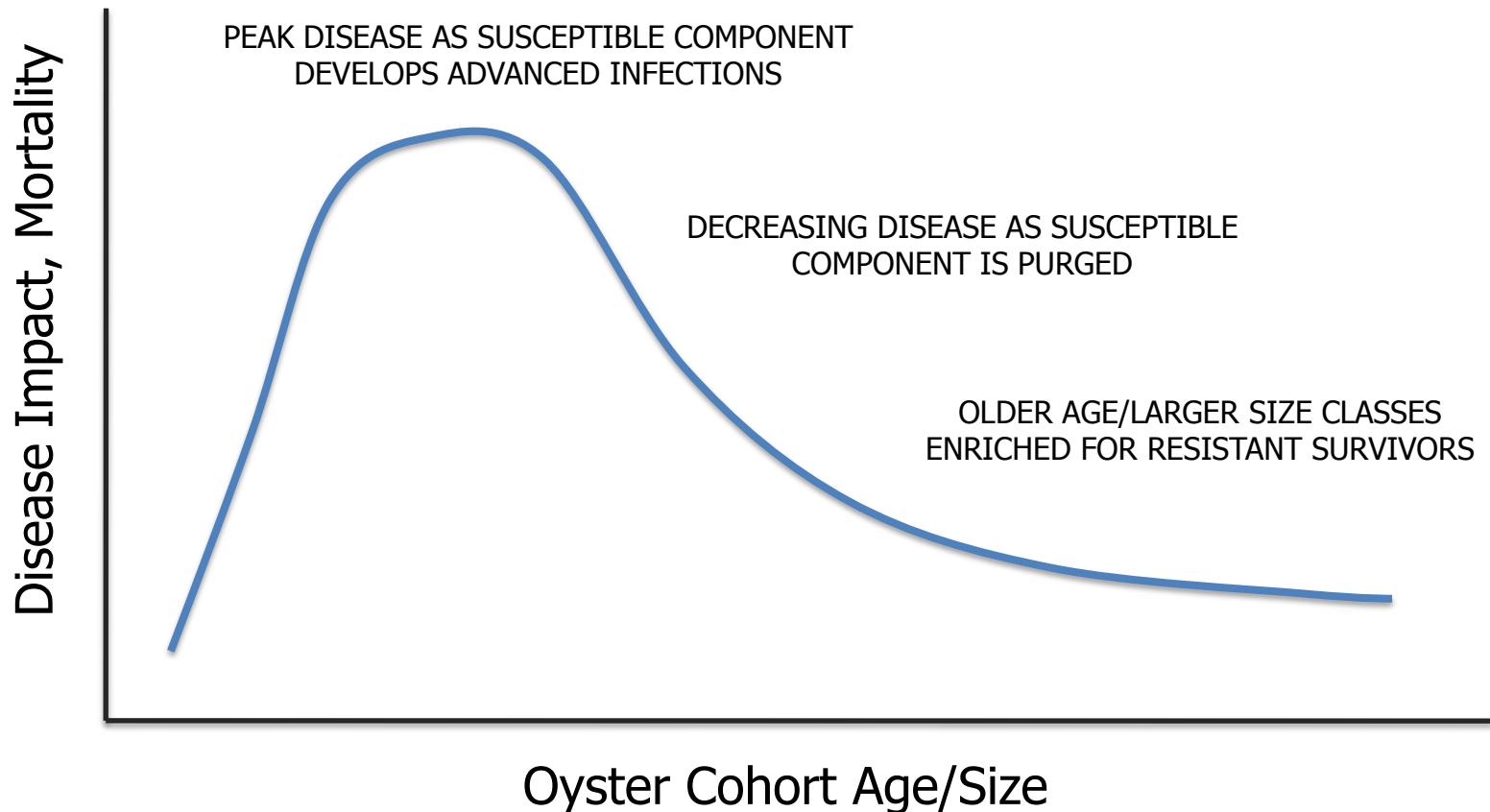
# Size-Specific MSX Impacts: Broad Creek, Rappahannock



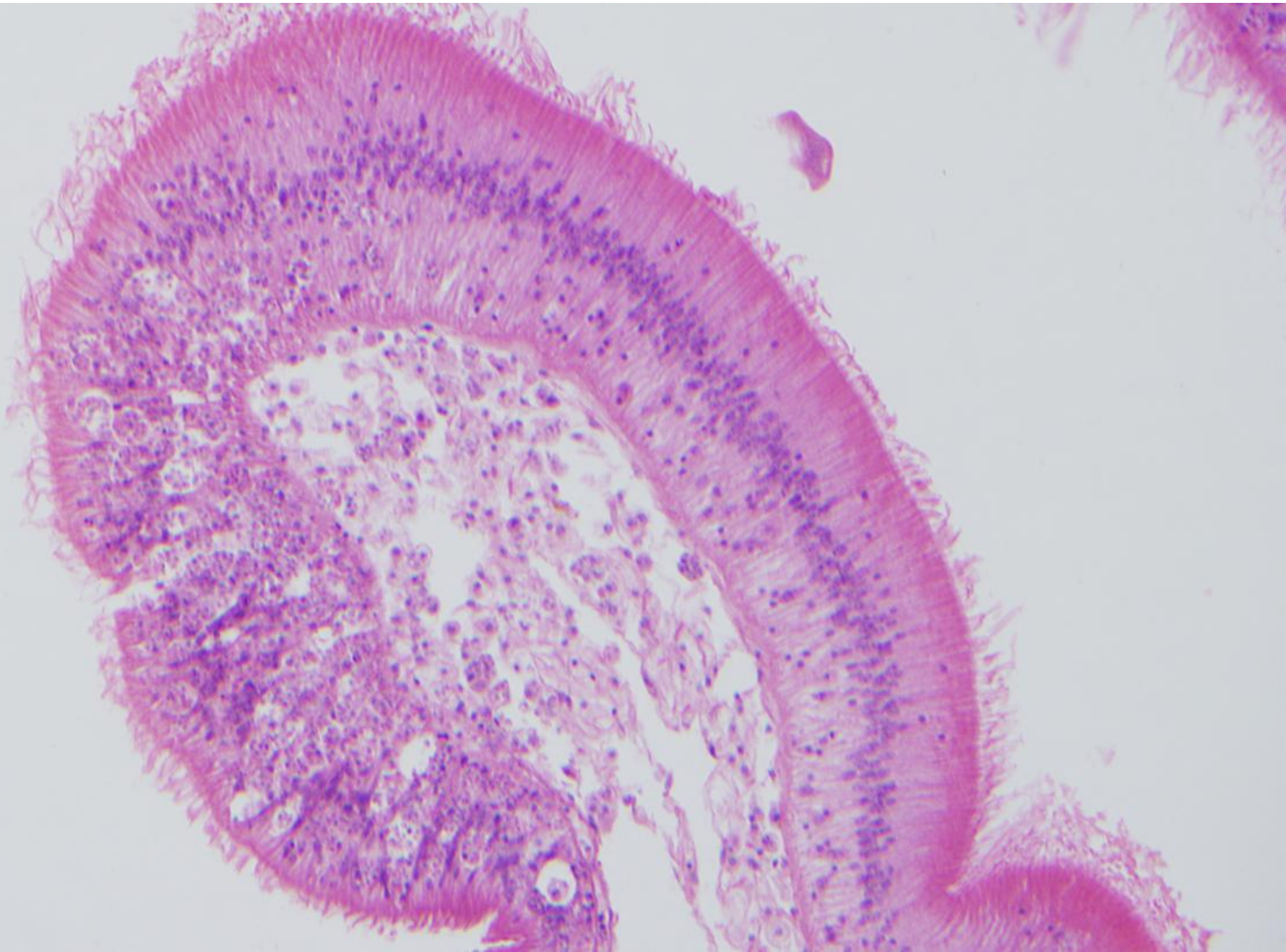
Suggests a population with resistant and susceptible components. . .  
. . . And that MSX may increasingly be a juvenile oyster disease.

# A Paradigm for MSX Disease in a Large Sub-Estuary\*

- ❖ Population a mixture of individuals varying in resistance
- ❖ Susceptible component maintained by:
  - Immigration from refugia
  - Reproduction of susceptible but pre-diseased individuals in higher salinities



# *Perkinsus marinus*





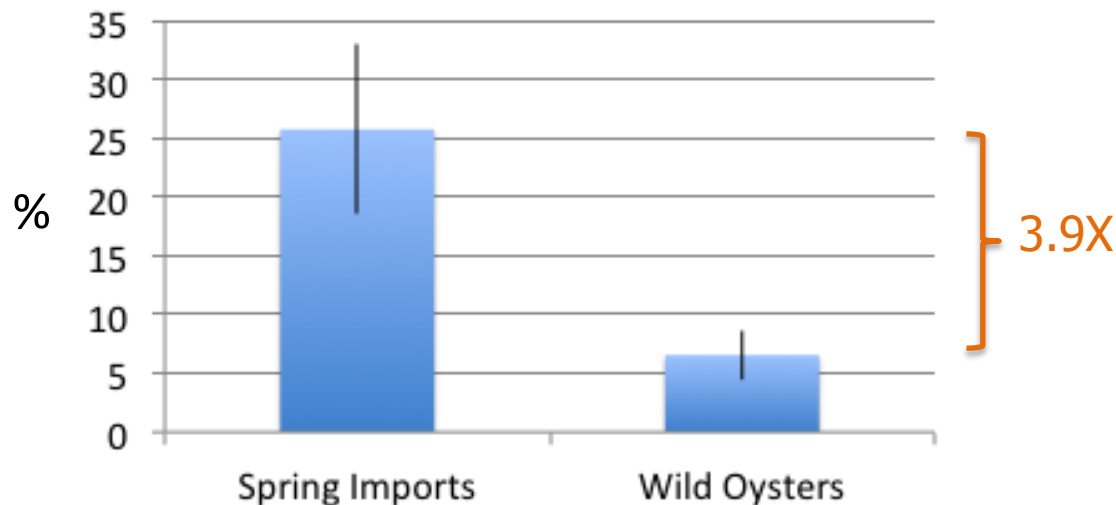
# *Perkinsus marinus*: Key Contrasts with MSX

- ❖ Causes a chronic disease, with maximum impacts in older oysters after 2-3 years of exposure (Andrews 1988)
- ❖ “Rate of death permits both resistant and susceptible oysters to breed successfully.” (Andrews 1956)
- ❖ True in the 1950s, but not today
- ❖ Great intensification of *P. marinus* activity since 1986 (Burreson and Andrews 1988) has decreased the exposure time required before serious disease develops (Burreson and Ragone Calvo 1996) in susceptible stocks
  - Spring Imports now an MSX and dermo study
- ❖ Susceptible oysters have reduced capacity for reproduction (fewer opportunities) in dermo-intense areas

# How Do Wild Oysters Compare With Naïve Sentinels?

- ❖ Prevalence is similar
- ❖ RFTM diagnoses: August-November 2006-2011
  - 1281 wild oysters from York & Pianktank Rivers and Mobjack Bay
  - 472 Spring Imports oysters evaluated

Mean Annual Percentage, Infections of MH-VH Intensity

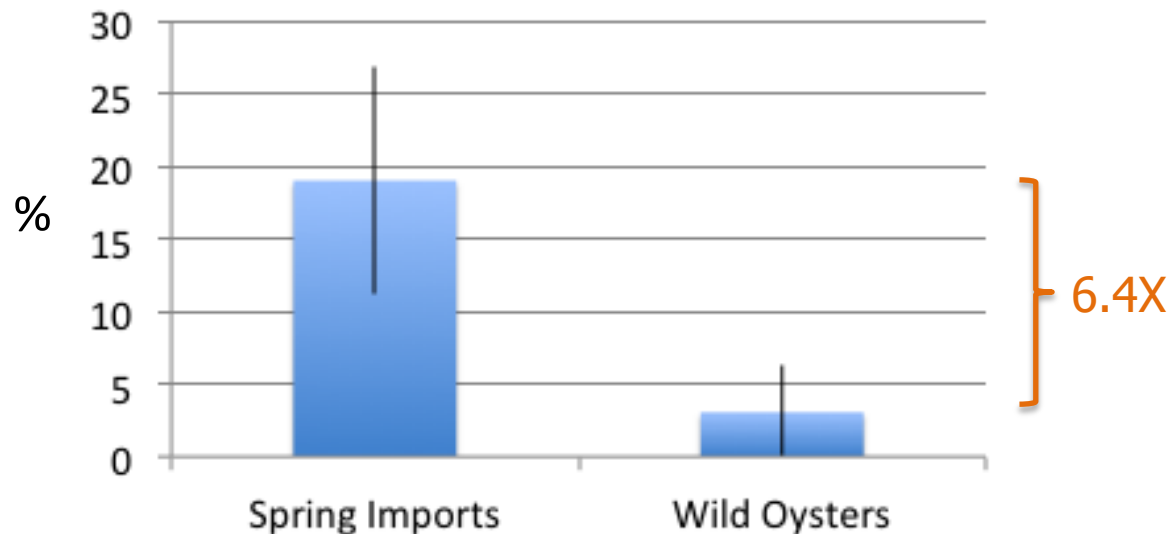


Bars = 95% C.I.s

# How Do Wild Oysters Compare With Naïve Sentinels?

- ❖ Histological diagnoses: August-November 2008-2011
  - 725 wild oysters from York & Piankatank Rivers and Mobjack Bay
  - 319 Spring Imports oysters evaluated

Mean Annual Percentage, Infections of MH-VH Intensity



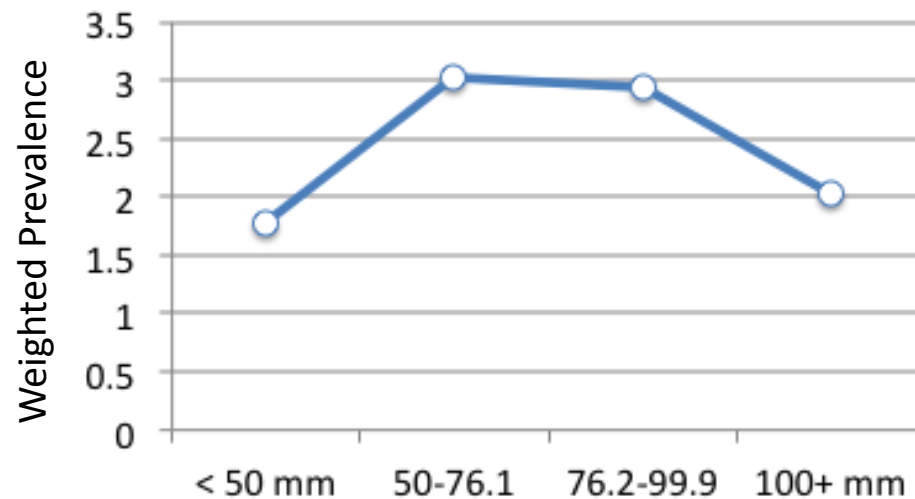
Bars = 95% C.I.s



# Size-Specific *Perkinsus marinus* Parasitism

- ❖ Does dermo disease increase with oyster size?
- ❖ Initial investigation at Pleasure House Creek, Lynnhaven River, 2006

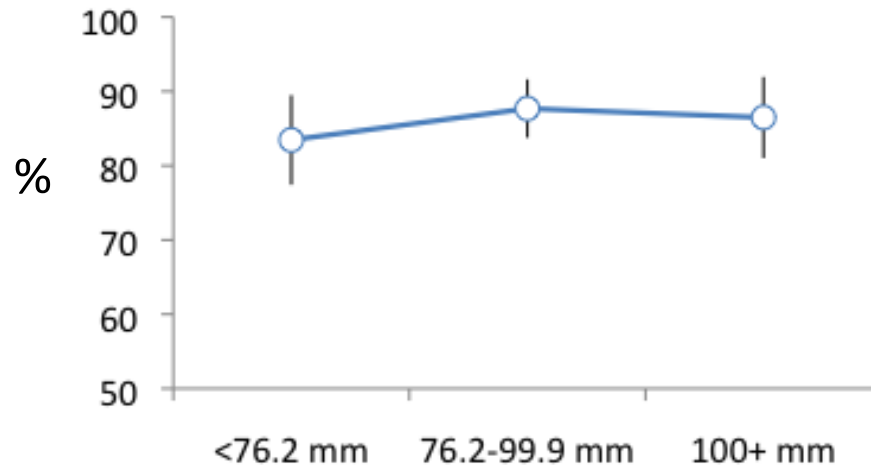
Weighted Prevalence of *Perkinsus marinus*, By Size Category



# Size-Specific *Perkinsus marinus* Parasitism

- ❖ Twenty “expanded” disease samples, 2006-2011 (2374 diagnoses)
  - Great Wicomico, Rappahannock, Piankatank, James, Lynnhaven, Seaside ES
  - Oysters < 76.2 mm, 76.2-99.9 mm, ≥ 100 mm

Mean Percent Prevalence By Size Category

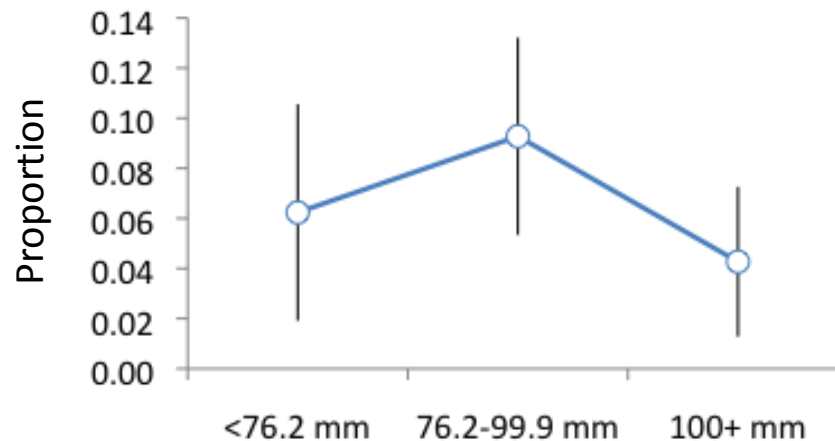


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Mean Proportion, Infections of MH or Greater Intensity



Bars = 95% C.I.s

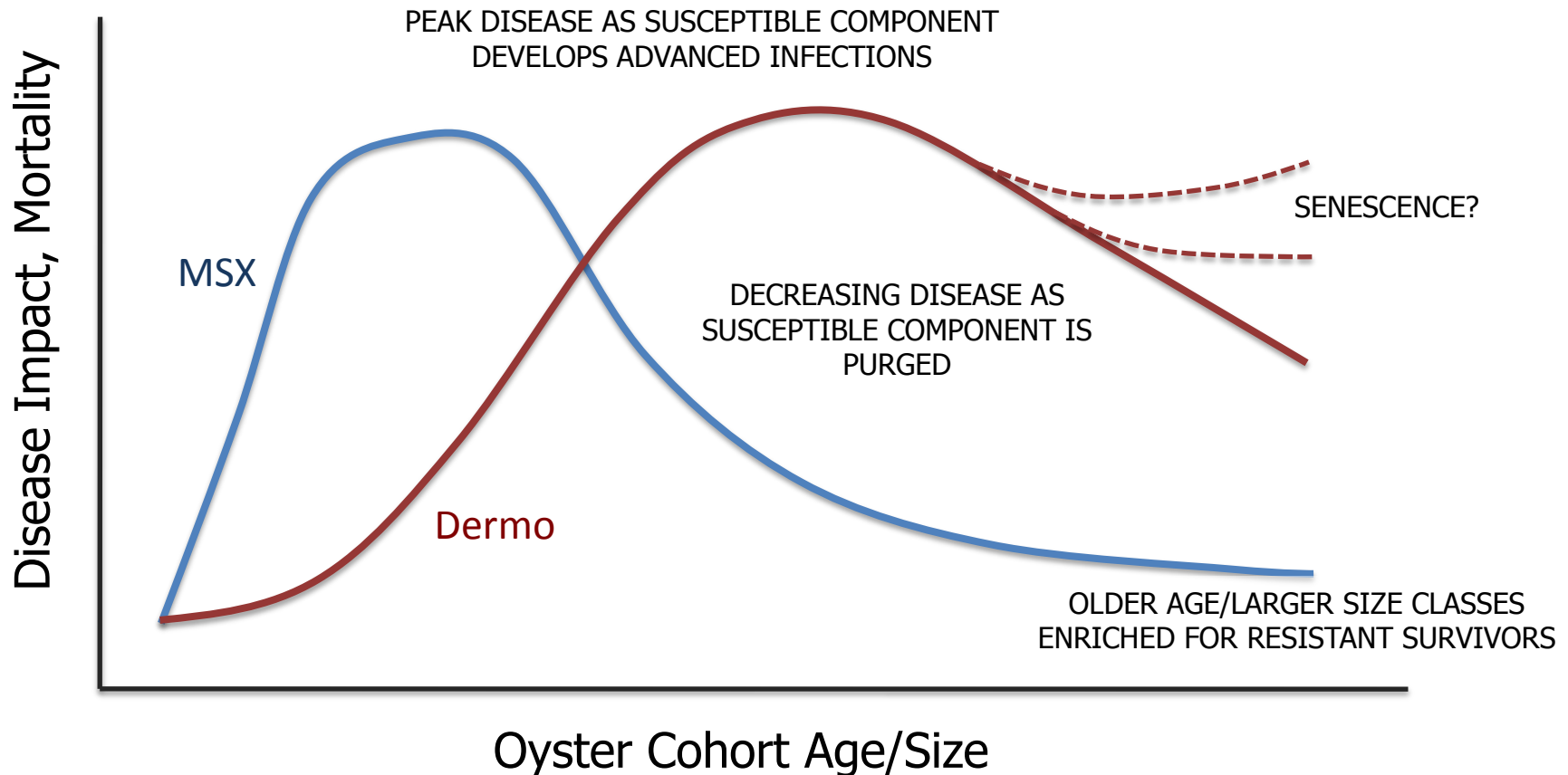
# Resistance to MSX and Dermo: Summary

- ❖ Clear resistance to both *H. nelsoni* and *P. marinus*
  - Better developed in the former than the latter
  - Need to remember it is all relative—oysters aren't MSX- and dermo-*proof*
- ❖ Resistance to *H. nelsoni* expressed as a prevention of tissue colonization, period, and then a restriction of the parasite to gill epithelia
- ❖ Resistance to *P. marinus* expressed as a restriction of infections to gut epithelia; perhaps more effective killing by hemocytes
  - Prevalence of infection is still high
- ❖ Differences between the two relate to:
  - Fundamental differences in host-parasite interactions at a molecular-cellular level
  - The slightly less acute nature of dermo disease, giving susceptible individuals more opportunities to reproduce

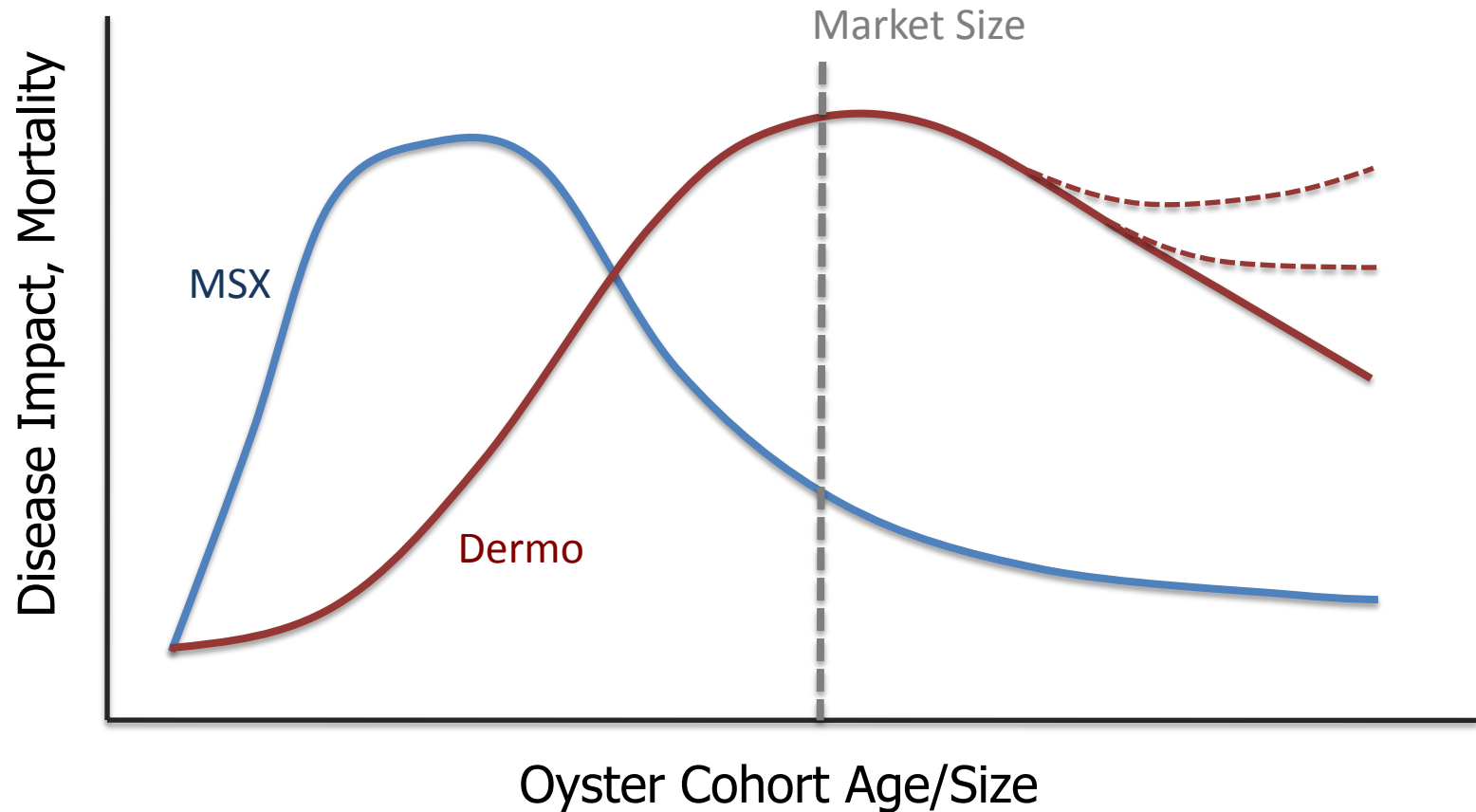


# A Dual-Disease Paradigm

- ❖ Population a mixture of individuals varying in resistance
- ❖ Susceptible component maintained by:
  - Immigration from refugia
  - Reproduction of susceptible but pre-diseased individuals in higher salinities



# If We Can Accept This Model...What Do We Do Next?



- ❖ Resistant component cannot fully exert its advantages (longevity, fecundity) if harvested, which argues for protection (sanctuaries, etc.)

# Other Considerations: Where To Site Sanctuaries?

- ❖ Everywhere (North et al. 2008)
- ❖ Certainly, higher-salinity, high-disease environments should not be ignored
- ❖ Oysters there cannot be substantially sustained by recruitment from low-salinity refugia
- ❖ Oysters in lower-salinity environments are ephemeral anyway



# Other Considerations: Role of Habitat/Elevation

- ❖ Can a reef be engineered to provide a habitat more conducive to oyster health (and advance the development of resistance)?
- ❖ *Are oysters on higher reefs healthier?*
- ❖ Collaboration with Paynter Lab (UMCES)
  - Sampling Bland Point (Piankatank), Shell Bar Reef (GWR), fall and spring
  - Base and crest transects, 4 replicate samples (each  $n = 20-25$ ) at each
- ❖ At peak dermo season, no differences between base and crest



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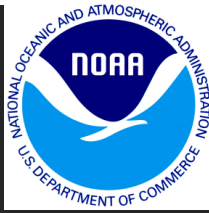


WILLIAM  
& MARY  
VIRGINIA INSTITUTE OF MARINE SCIENCE





# A Bay-Wide Integration of Oyster Disease Analyses



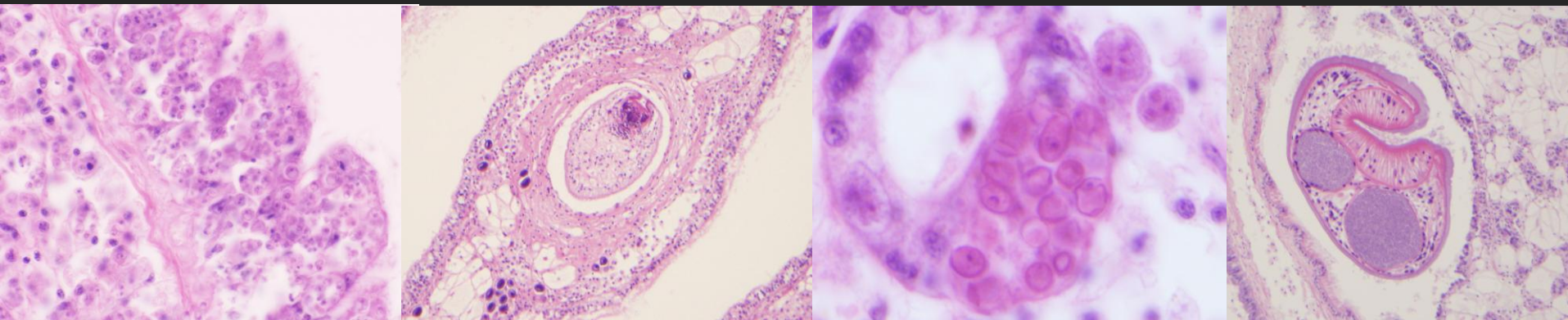
Ryan B. Carnegie<sup>1</sup> and Christopher F. Dungan<sup>2</sup>

<sup>1</sup>Virginia Institute of Marine Science, Gloucester Point, Virginia

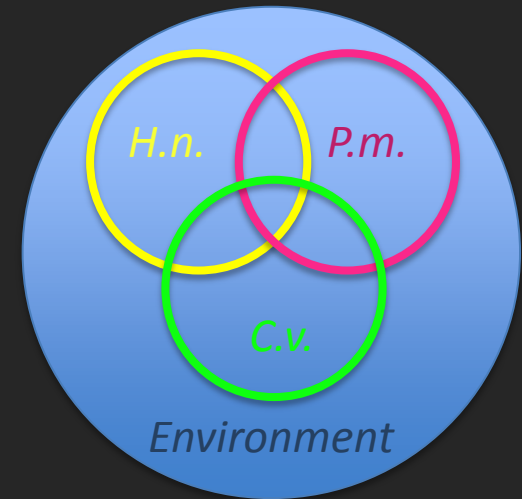
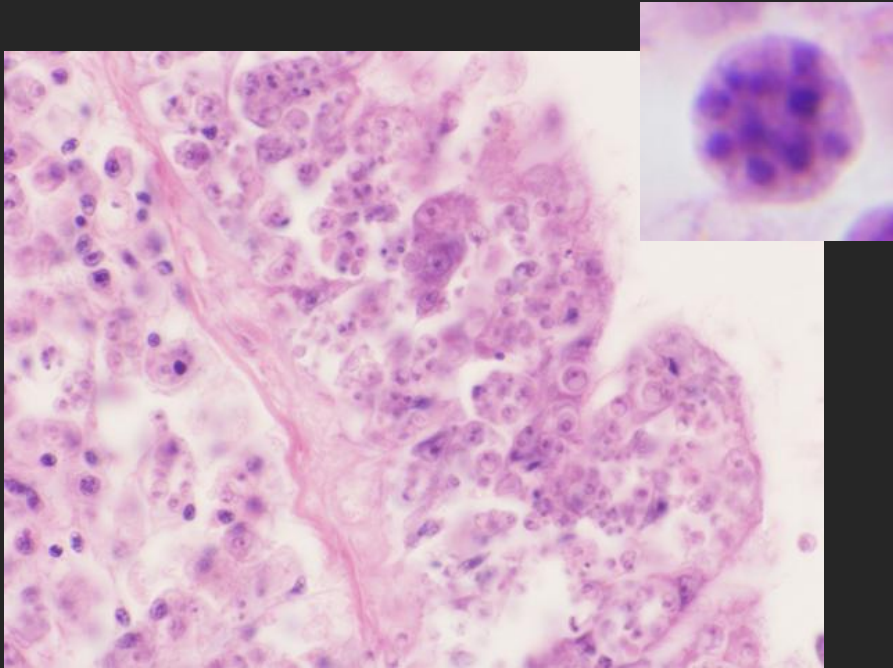
<sup>2</sup>Maryland Department of Natural Resources, Cooperative Oxford Laboratory, Oxford, Maryland

**Background and Rationale** – Virginia and Maryland have long maintained separate and incompatible datasets on oyster disease. Integrating these analyses – developing unified rating systems for the diseases, and applying them to present samples but also retrospectively – will yield a dynamic bay-wide portrait of oyster disease. It will allow incorporation not only of the oyster but of the pathogens as well into ecosystem models of Chesapeake Bay.

In addition to the core monitoring and analyses, evaluation of size-specific disease levels in oysters ranging from small sub-markets (< 50 mm) to large survivors (> 100 mm) will provide insight into the development and distribution of disease resistance among oysters in Virginia and Maryland.



# Evolutionary Ecology of Oyster Disease





# Acknowledgments

Corinne Audemard

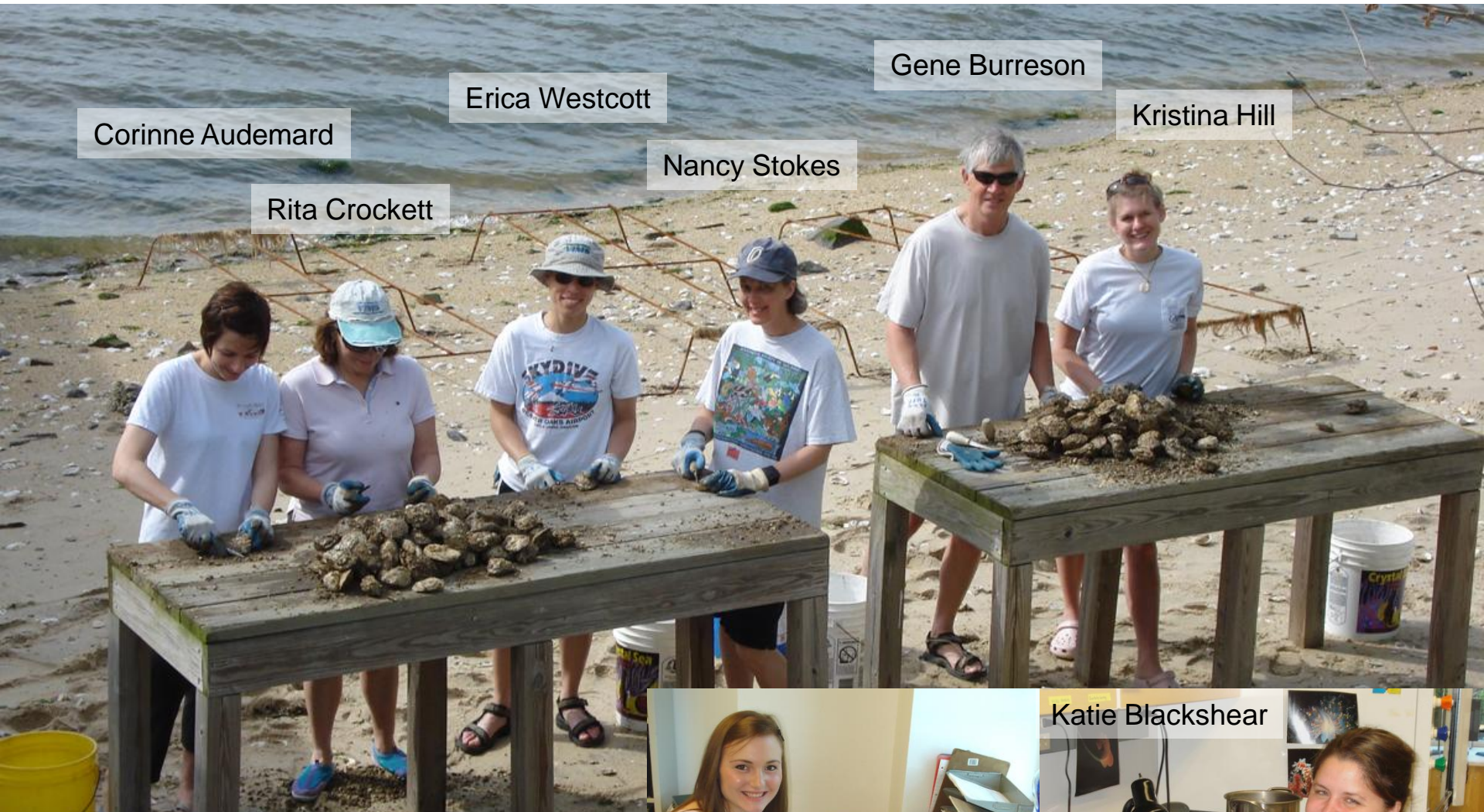
Erica Westcott

Gene Bureson

Kristina Hill

Rita Crockett

Nancy Stokes



Amy Grinnell



Katie Blackshear