Long-Term Evolution of Virulence in Marine Diseases

Ryan B. Carnegie, PhD
Department of Aquatic Health Sciences
Virginia Institute of Marine Science

Useful References

- EMID 2011 Workshop Report (pdf)
- Abalone biology and disease
- White spot disease in shrimp
- Climate and marine disease
- Disease virulence
- Disease transmission models
- Economics/Management of marine disease


Center for Coastal Physical Oceanography, Old Dominion University

Journal of Aquatic Animal Health 12:1–8, 2000

FEATURE

Increased Virulence in an Introduced Pathogen: *Haplosporidium nelsoni* (MSX) in the Eastern Oyster *Crassostrea virginica*

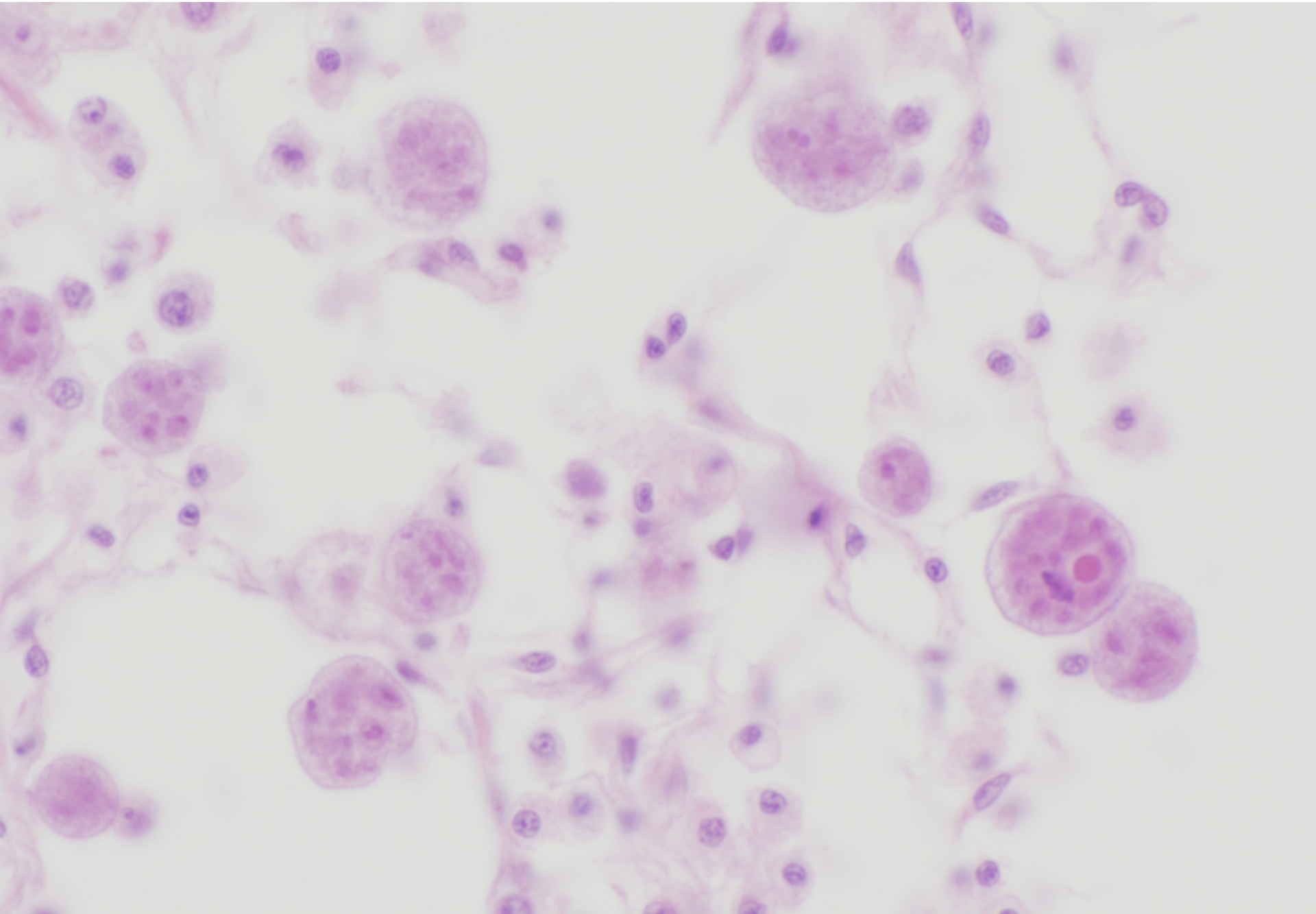
E. M. Burreson* and N. A. Stokes

Virginia Institute of Marine Science, School of Marine Science, College of William and Mary, Gloucester Point, Virginia 23062, USA

C. S. Friedman

California Department of Fish and Game, Bodega Marine Laboratory, Bodega Bay, California 94923, USA

Haplosporidium nelsoni (“MSX”)



Once-Plentiful Eastern Oyster Has Become Victim of Drought and Disease

By BEN A. FRANKLIN
Special to The New York Times

BIVALVE, Md., Oct. 30—Chincoteagues, lynnhavens, tangiers and blue points—names that once stimulated the digestive juices of fanciers of [illegible] the Democrats of fraud 20 years.

In recent years, the mollusk has been "nearly finished" in Middle Atlantic waters by two colossal blows of nature—the drought in New York and Pennsylvania and the spreading epidemic of a mysterious disease

Elizabeth Wallace, director of the Oyster Institute of North America, reported that M[illegible] had been identified also in de[illegible] oysters in Great South Ba[illegible] L. I., an area celebrated for th[illegible] famous blue points. She [illegible]

nia and New York, the "isoha-line"—the imaginary [illegible]

6 per cent of the normal har-

vention of a mechanical harvester in 1958, but oystermen heve a traditional hostility for clammers.

Prices Are Cited

Dr. Jay Andrews, a Virginia oyster biologist, and other in-

attribute a consump-prices"—$7 shell, and en on the

Chesapeake Oysters Infected As Unconquered MSX Spread

By HERB THOMPSON

BALTIMORE (AP)—Scientists offered little hope to oyster growers today that there will be any early conquering of "MSX," the destructive oyster parasite which caused heavy losses in Delaware Bay and is now attacking the lower Chesapeake.

"If the Chesapeake follows the Delaware pattern, the mortality will continue to spread to new areas," Dr. L. Eugene Cronin of Maryland's Department of Research and Education said. "The rate of spread might be rapid."

* * *

Dr. Cronin reported to growers and packers attending the annual convention of the Oyster Institute of North America. He is president of the National Shellfisheries Assn., an organization of scientists and technicians associated with the seafood industry.

Dr. Cronin said the "largest coordinated research in the history of American shellfisheries" is now going on in laboratories throughout the Middle Atlantic area to learn more about the micro-organism. His report to the convention's closing session was based on conferences with these scientists.

* * *

The organism began its destructive sweep of the Delaware Bay in 1957, appeared with less severity in Maryland coastal waters in the fall of 1958, and was found

research. Meanwhile, he added, the industry must take every step to try to keep down the spread of the micro-organism.

Working together on the research program are scientists from the New Jersey Oyster Research Laboratory, the University of Delaware Marine Laboratories, the Chesapeake Biological Laboratory, the Virginia Fisheries Laboratory and the Annapolis Laboratory of the U.S. Bureau of Commercial Fisheries.

e- nucleate sp[illegible] d by Reds
in "unknown"), has spread beyond [illegible]
Delaware Bay, virtually [illegible]

VENING SUN

ains; Science Offers Little Hope On Oyster MSX

be

xpected to
today for

Scientists offered little hope to oyster growers today that there

SUN, Wed., Aug. 3, 1960

ng Oyster MSX Unchecked

[Continued From Page 64]

uling only by careful laboratory meth-

Monday's paper says no trouble found in James River but they think there's some in York R. samples to be taken today.

Oysters Dying in Rappahannock; State Biologists Seeking Cause

By Lynn Stewart
Special Correspondent

GLOUCESTER, Aug. 27—Something deadly to oysters has invaded the waters of the Rappahannock River and marine biologists at the Virginia Fisheries Laboratory at Gloucester Point Saturday were seeking its origin.

Virtual destruction of oyster beds was reported by some Rappahannock producers this week.

Other estimates of oyster ground deaths ranged from 68 per cent to 80 per cent.

One major producer said, "We have never had anything like this in my lifetime."

Oyster growers of the middle-river area scheduled a meeting at Tappahannock Saturday night to map plans to check the unknown enemy.

Meanwhile, growers in all Virginia's tidal rivers kept an anxious eye on the James River. Its seed beds are vital to the industry.

Some growers attributed the loss to low salinity in the river after the great rains of hurricanes Connie and Diane. Others believed the oyster deaths were caused by river pollution.

Fisheries laboratory biologists studied the afflicted beds during the past two days and also have investigated the James River seed oyster beds.

This is what they found:

In the Rappahannock, Dr. W. G. Hewatt sampled a ground, belonging to John Garland Pollard, of Somers, and estimated the mortality there at approximately 80 per cent.

Dr. J. D. Andrews, surveying the James River beds, said Saturday that conditions there were "not so bad."

Dr. John L. McHugh, director of the laboratory, said that analyses of the various findings had not yet been completed but

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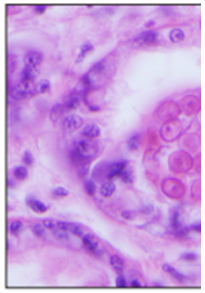
FEATURE ARTICLE:

Declining impact of an introduced *Haplosporidium nelsoni* in the *Crassostrea virginica* in Chesap

Ryan B. Carnegie*, Eugene M. Burreso

Virginia Institute of Marine Science, College of William & Mary, Gloucester Poi

ABSTRACT: Disease caused by the parasite *Haplosporidium nelsoni* has devastated *Crassostrea virginica* in Chesapeake Bay, exacerbating effects of overharvesting and adversely impacting the ecology of the bay. *H. nelsoni* is thought to persist as an impediment to oyster restoration because strong reproductive contributions from oysters in low-salinity refugia from parasitism have prevented development of disease resistance. On the contrary, long-term data indicate that while infection pressure on naïve sentinels has grown, *H. nelsoni* levels in wild oysters have fallen, with prevalence typically below 20% and advanced infections uncommon. A transplant experiment comparing naïve sentinels with oysters from disease-enzootic populations indicated that these observations represent true disease resistance, and its geographical distribution was revealed by annual fall surveys, and by intensive sampling in 2007 and 2008. Resistance is best de-



Haplosporidium nelsoni sp
heavy infection of an oyste
Chesapeake Bay

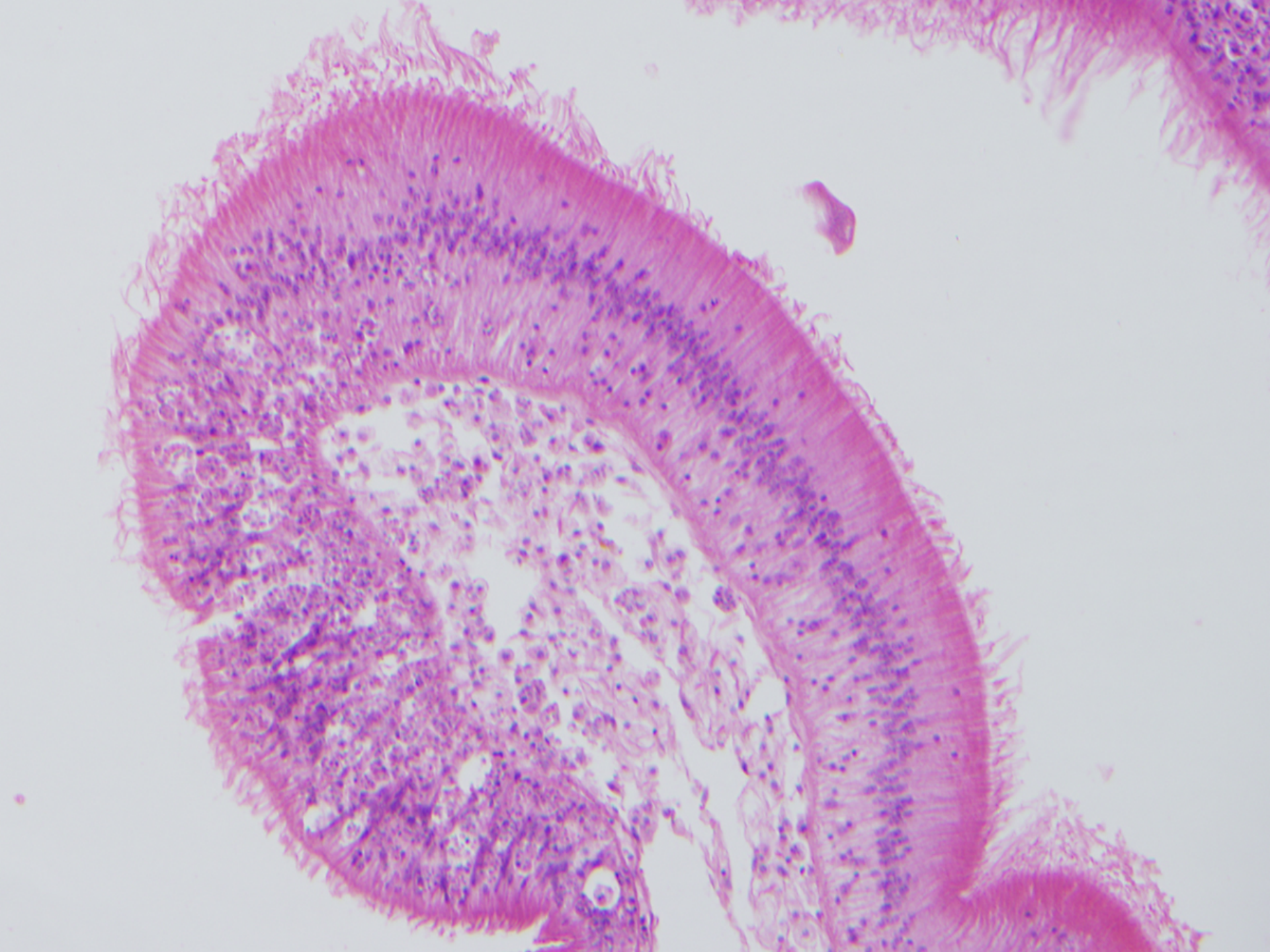
Journal of Marine Research, 70, 205–223, 2012

Development of resistance to an introduced marine pathogen by a native host

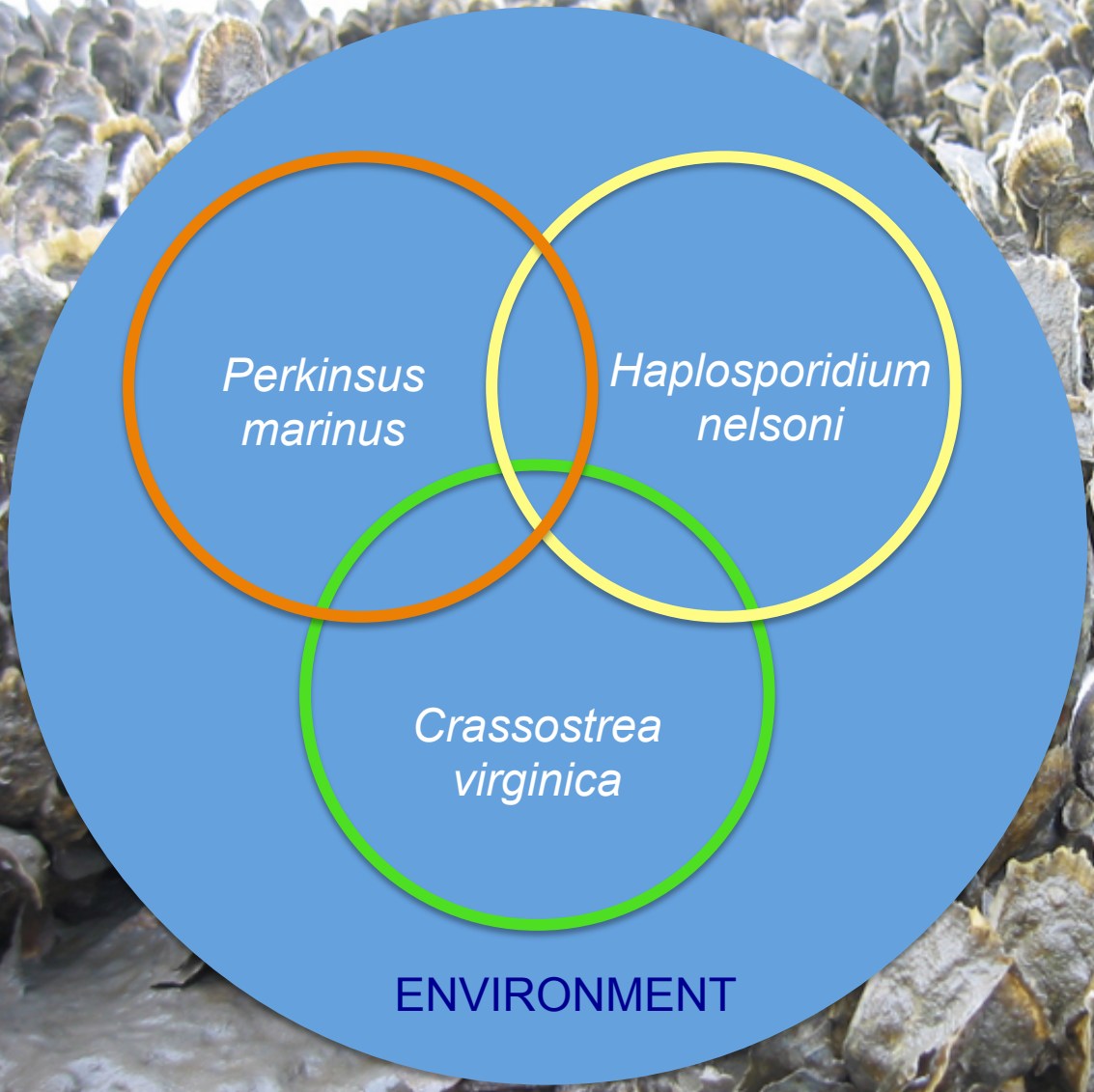
by Susan E. Ford[1,2] and David Bushek[1]

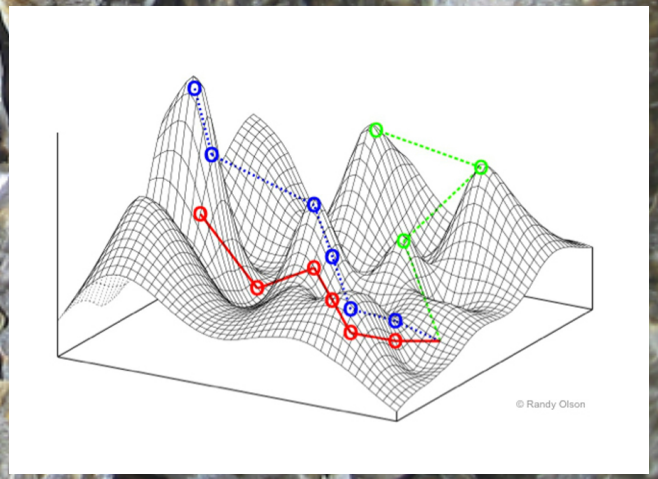
ABSTRACT

In 1957–1959, the introduced protistan parasite, *Haplosporidium nelsoni*, killed 90–95% of the oysters (*Crassostrea virginica*) in lower Delaware Bay and about half of those in the upper bay. Shortly thereafter, *H. nelsoni*-caused mortality in the wild population of the lower bay declined, approximating that of first-generation selectively bred oysters. For nearly three decades thereafter no further change in survival of the wild population was evident, although steady improvement was achieved by continued selective breeding. Survival of the wild population is thought to have plateaued because the great majority of oysters inhabited the upper bay where they were protected from *H. nelsoni* infection and selective mortality by low salinity. Consequently, they contributed most of the offspring to the bay population. From 1957 through 1987, *H. nelsoni* prevalence was cyclic, but overall high (annual maxima of 60 to 85%) in the lower bay. Since 1988, however, prevalence in wild oysters has rarely exceeded 30% anywhere in the bay, even though unselected oysters continue to become heavily infected when exposed, and molecular evidence indicates that the parasite remains present throughout the bay. This apparent "second step" in the development of resistance in the wild oysters occurred after a drought-associated incursion of *H. nelsoni* into the upper bay in the mid-1980s. Mortalities were widespread, heavy and more extreme than during the 1957–59 epizootic. Resistant survivors of the second epizootic have apparently repopulated the bay. When compared to unselected stocks, common-garden exposure to *H. nelsoni* of oysters from both upbay and downbay sites indicates that a high degree of resistance to the development of MSX disease has become widespread in the wild oyster population of Delaware Bay after two major selection events separated by nearly 30 years.

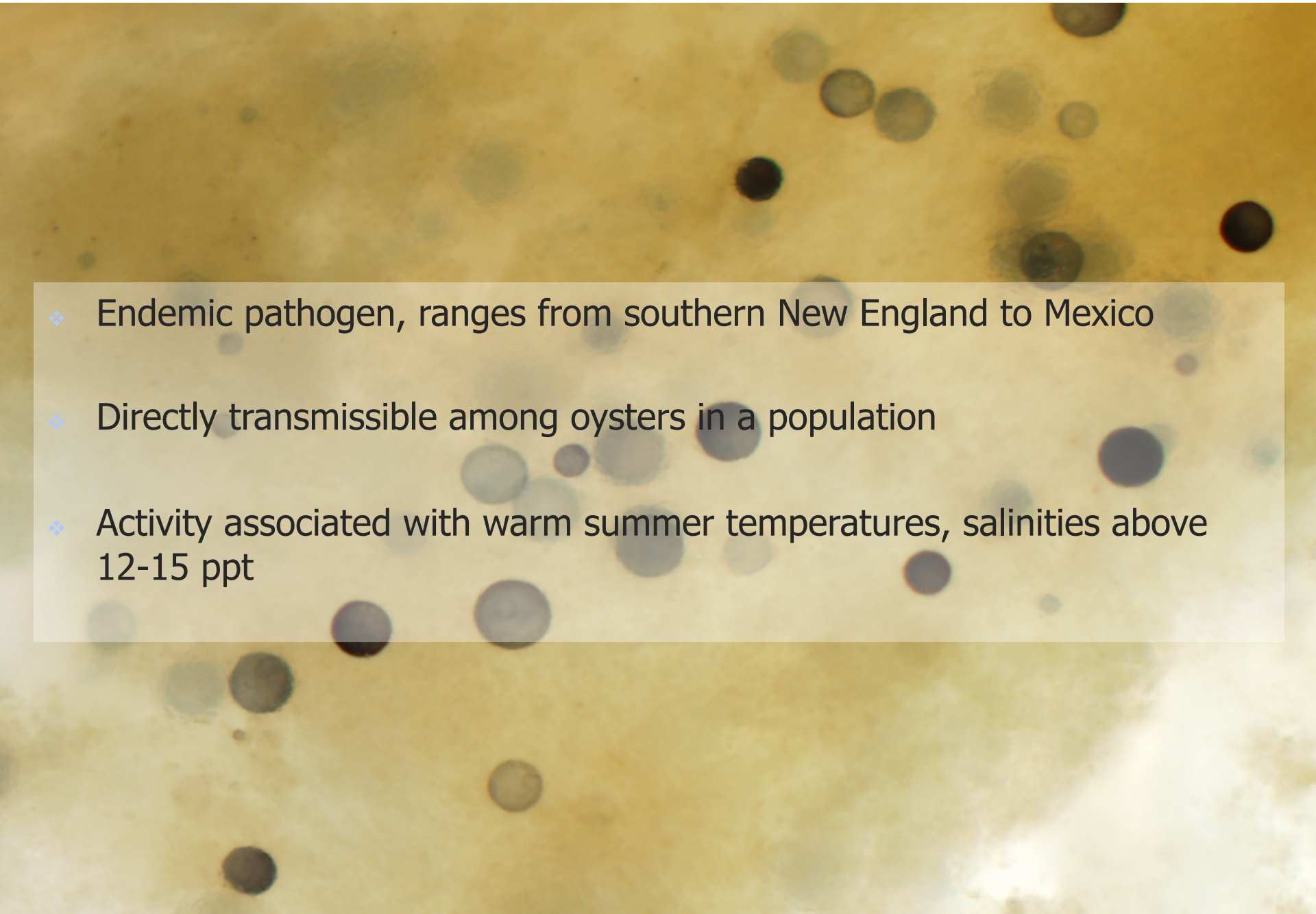


Evolutionary Ecology of Oyster Disease





Perkinsus marinus



- Endemic pathogen, ranges from southern New England to Mexico
- Directly transmissible among oysters in a population
- Activity associated with warm summer temperatures, salinities above 12-15 ppt

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 unpronounceable name of the fungus which was discovered in the Gulf of Mexico and described in 1950 by Mackin, Owen, and Collier — *Dermocystidium marinum* — now known to be a major cause of death of oysters in salty waters 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 900. There are probably as many more spat and yearlings, many of which will be lost by smothering or by drill predation. In Virginia most oysters are marketed when the count is about 300 per bushel. The decrease from 900 to 300 signifies a loss of two-thirds of the original seed oysters.

Death Rate Is High

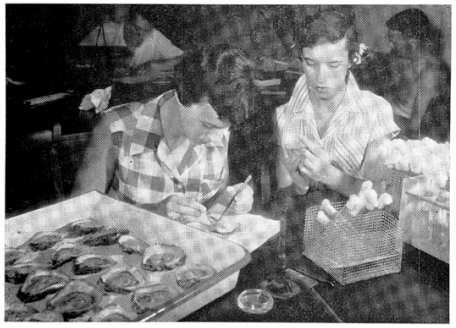
We have held some 60 different groups of oysters in trays, each consisting of 100 to 800 oysters, and accurate counts of dead and survivors have been made frequently. Under two years of age mortality was low, but in older oysters about one-third died each year. In the trays 85 to 90 per cent of these deaths were caused by the fungus. On natural grounds the percentage of deaths from the fungus was not as high, for predators, smothering, and other factors were killing some before the fungus could act; but over half the dying oysters examined were heavily infected.

Dermocystidium is a major cause of death in the saltier waters of Chesapeake Bay. The infective area extends up the Bay to the Patuxent River, to the mouth of the Potomac River, to Tangier Sound, and half-way up the Rappahannock River. The fungus is absent from the James River seed area and the sea side of the Eastern Shore of Virginia and Maryland.

Fungus 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 disintegrating 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 100 per cent of the live oysters in trays and on natural grounds are infected. Many of these are light infections from which the oysters recover; but in most years about 30 per cent of the oysters in trays die from the disease, and in 1954 over half died. The spores disappear rapidly from oysters in December, January, and February, but an overwintering stage persists in a few oysters.



Pat Burke and Sally Gantt, lab workers, remove bits of oyster meat for culture in nutrient-filled test tubes. Cultures are held warm for two days before examination.

Cure Probably Not Feasible

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

Fungus-Resistant Varieties

If cure 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 underwater "farms" for testing the product do not exist. Furthermore, most seed oysters are gathered from a wild crop with little or no control of quality by man. The free-swimming larval stages prevent the segregation of varieties from one oyster ground to another.

The selection of a resistant stock of brood oysters is perhaps the most promising approach, but the problem of replacing 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 sea side of the Eastern Shore of Virginia and Maryland, where the fungus is absent, are more susceptible than natives. We suspect that the differences in susceptibility are related to the period of time that the fungus has been present in these waters.

Contributions from the Virginia Fisheries Laboratory, No. 65.

Reprinted from PROCEEDINGS OF THE OCEANS '88 CONFERENCE
Baltimore, Maryland, October 31-November 2, 1988

UNUSUAL INTENSIFICATION OF CHESAPEAKE BAY OYSTER DISEASES DURING RECENT DROUGHT CONDITIONS

Eugene M. Burreson and Jay D. Andrews

Virginia Institute of Marine Science
School of Marine Science
College of William and Mary
Gloucester Point, Virginia 23062

ABSTRACT

Two protozoan parasites of oysters, Haplosporidium nelsoni (MSX) and Perkinsus marinus, have severely limited the harvest of oysters in lower Chesapeake Bay during the past 30 years. The distribution and abundance of both parasites appears to be regulated by salinity and the three consecutive drought years from 1985-1987 have resulted in a dramatic increase in mortality from these diseases in Chesapeake Bay. Oyster mortality from MSX in monitoring trays at VIMS was 77% and 78% for 1986 and 1987 respectively, the highest values ever recorded in 28 years of continuous monitoring. In addition, Perkinsus was at record high levels and, for the first time in history, both diseases invaded and caused serious mortality in the upper James River seed-oyster area. The increased salinity resulting from low runoff over the watershed allowed both diseases to spread deep into the Maryland portion of the Bay in 1987 and cause serious mortality. Bay-wide oyster stocks are now severely depressed and prospects for rapid recovery are poor.

INTRODUCTION

Two protozoan parasites of oysters, Haplosporidium nelsoni, popularly called MSX, and Perkinsus marinus, popularly, but improperly, called Dermo, cause serious annual oyster mortality along the Atlantic and Gulf coasts of the United States. MSX causes oyster mortality from Massachusetts to Chesapeake Bay (1), and an organism similar to MSX has been reported as far south as Florida (2). Perkinsus causes mortality throughout Chesapeake Bay, south along the Atlantic coast, and in the Gulf of Mexico (3).

MSX first appeared in Delaware Bay in 1957 and spread to the lower Chesapeake Bay in 1959. Perkinsus has probably always been endemic in high salinity portions of the Bay and has caused significant, but tolerable, mortality. However, within a few years of the appearance of MSX the annual Virginia oyster harvest decreased from an average of about 3.5 million bushels to less than 1 million bushels (Figure 1). The industry in Virginia has never recovered. During the 1960s mortality was high on public oyster grounds, but hardest hit were the private planters who had been producing about 85% of the annual harvest. MSX causes about 50% annual mortality in the endemic area (4), and this rate is intolerable for transplanted seed stocks that require two or three years to mature to marketable size. All of the traditional private growing areas in the lower James River and Mobjack Bay have been abandoned since the early 1960's because of the continued presence of MSX.

The distribution and abundance of both diseases appear to be controlled by salinity. In years with normal rainfall, MSX is restricted to the lower Bay and the lower portions of tributaries south of the Rappahannock River (4). During periods of drought, MSX may spread throughout upper Virginia and many Maryland tributaries into populations of highly susceptible oysters, but it recedes as rapidly when salinity returns to normal. MSX requires about 15 ppt salinity to infect oysters and usually does not cause serious moralities unless summer salinity reaches 18 to 20 ppt (4, 5). However, these limitations are coupled with seasonal fluctuations in salinity that allow expulsion of the parasite in spring if salinity drops below 10 ppt for ten days or more (6, 7).

Perkinsus marinus requires only about 12 to 15 ppt salinity to infect oysters and cause mortality (3, 8). Therefore, it has a far wider endemic area than MSX and persists in nearly all Virginia tributaries and also in the lower portions of Maryland. Furthermore, Perkinsus is suppressed but not easily exterminated by low salinities. It can persist several years at low seasonal salinity without causing appreciable mortality. Nearly all oyster growing areas reach 12 to 15 ppt salinity during average summers, which allows some multiplication of Perkinsus.

METHODS

The annual abundance of MSX in the endemic area is determined by placing susceptible oysters from the upper James River seed area into trays in the lower York River. Replicate trays of 500 oysters each are established on May 1st each year. Counts of live and dead oysters are made semi-monthly through December. Dead oysters are removed. Samples for histological diagnosis of MSX

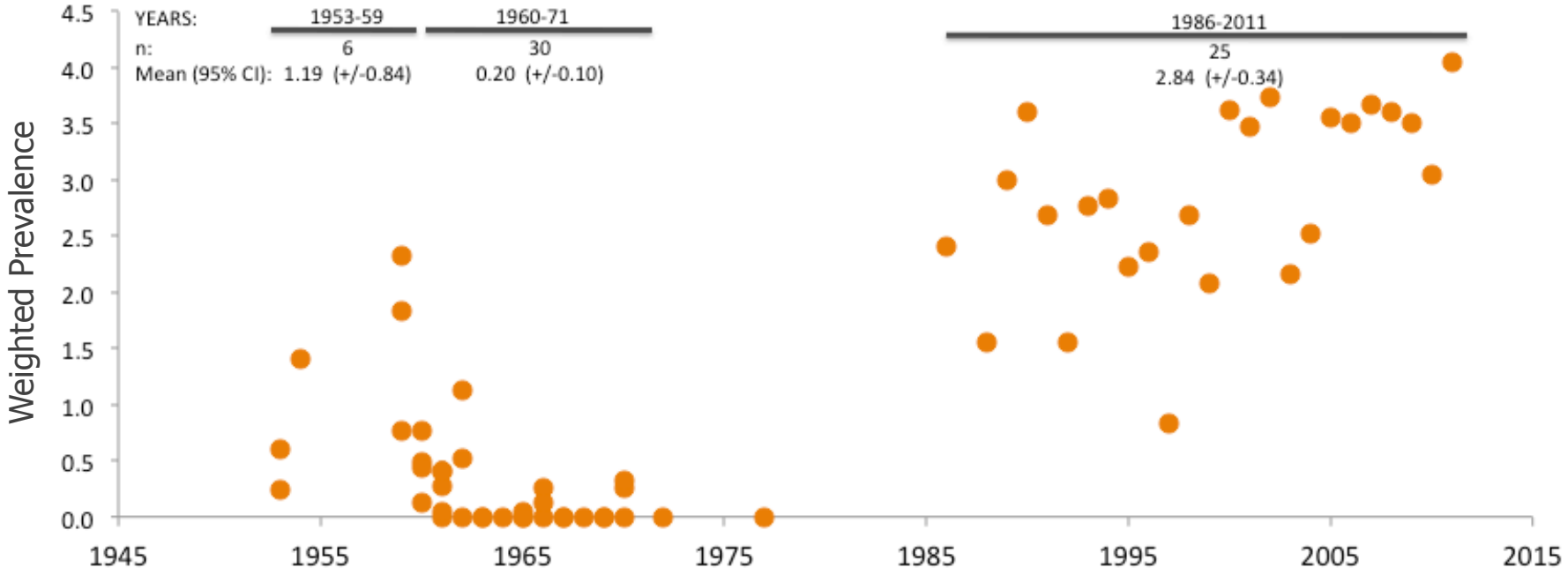
CH2585-8/88/0000-

Dermo Disease Then And Now

- Initially, 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
- Since mid-1980s, an acute pathogen
  - Acute disease and mortality within just months of exposure
  - Intensities very high
  - Mortality > 70%
  - Unmanageable by industry (until recently through selective breeding)

Dermo Disease in "Spring Imports" Sentinels

After 4-5 months of exposure in York River



High levels of disease developing much more rapidly than in the past

Warming Climate and Dermo Disease

Estuarine, Coastal and Shelf Science (1998) **46**, 587–597

The Relationship Between Increasing Sea-surface Temperature and the Northward Spread of *Perkinsus marinus* (Dermo) Disease Epizootics in Oysters

T. Cook[a], M. Folli[a], J. Klinck[b], S. Ford[c] and J. Miller[a]

Climate Change Influences on Marine Infectious Diseases: Implications for Management and Society

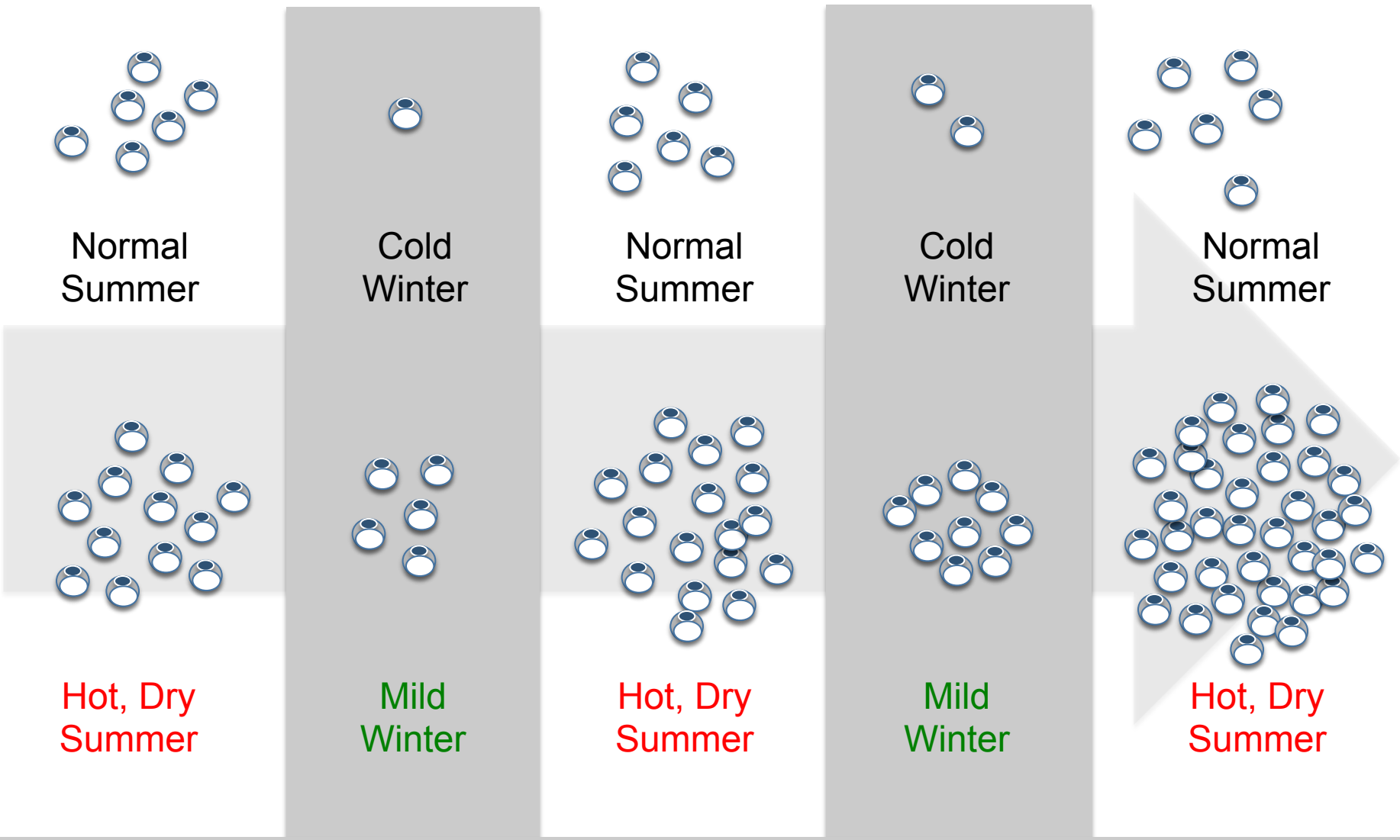
Colleen A. Burge,[1] C. Mark Eakin, Carolyn S. Friedman, Brett Froelich, Paul K. Hershberger, Eileen E. Hofmann, Laura E. Petes, Katherine C. Prager, Ernesto Weil, Bette L. Willis, Susan E. Ford, and C. Drew Harvell[1]

[1]Department of Ecology and Evolutionary Biology, Cornell University, Ithaca, New York 14853; email: cab433@cornell.edu, cdh5@cornell.edu*

Intensification of Dermo Disease, 1980s-Present

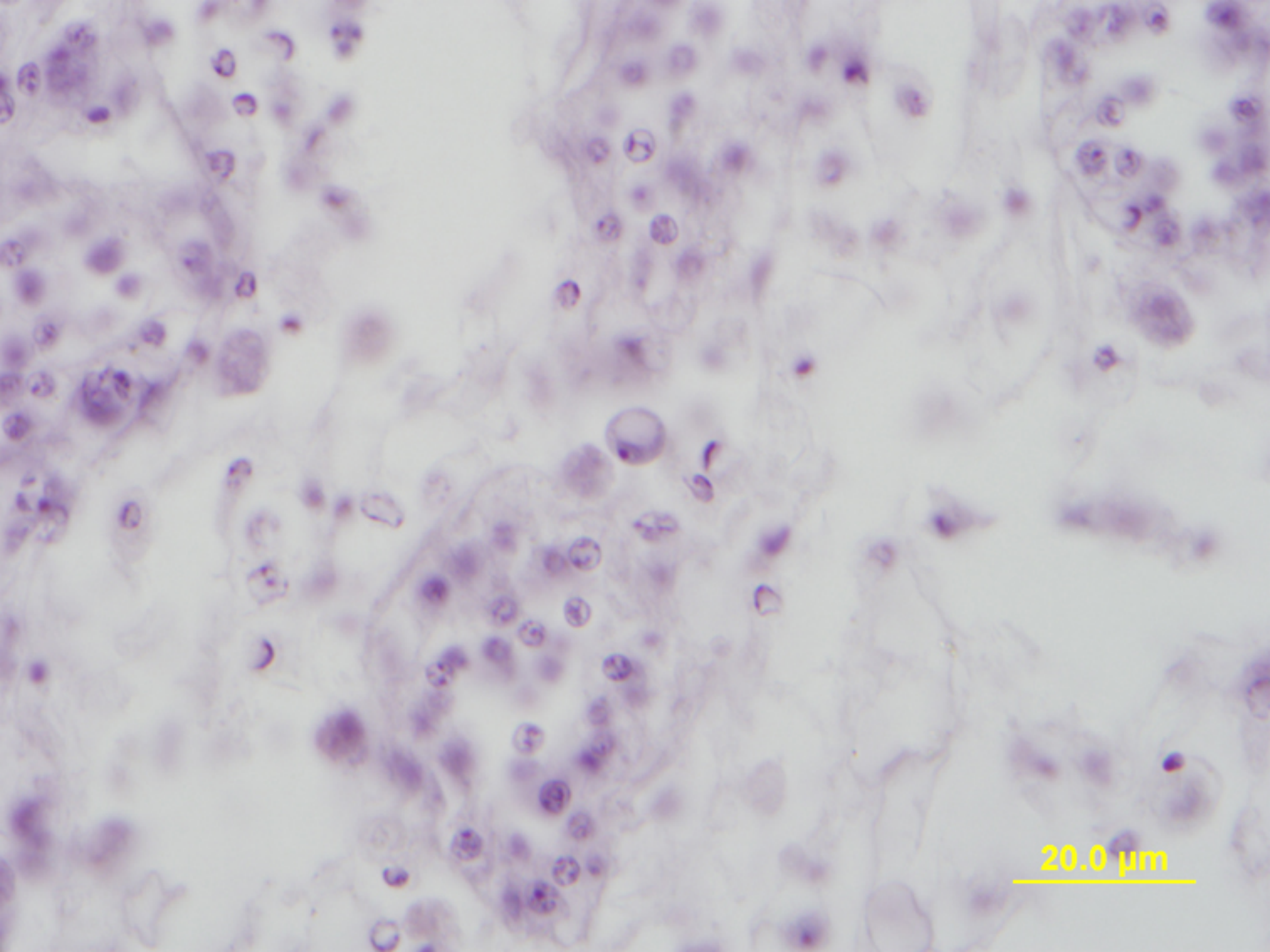
- *Perkinsus marinus* largely disappeared with the arrival of *H. nelsoni*, its range restricted to upper parts of the rivers (Andrews 1966)
- Reemerged in 1986, during, and presumably because of, a multi-year drought (Burreson & Andrews 1988)
- Very high parasite and disease levels today thought to be a function primarily of abundance, which increased greatly with 1980s droughts (Burreson and Ragone Calvo 1996)
  - More *P. marinus* ➔ higher rates of transmission ➔ more *P. marinus* . . .
- Loss of deep winter cold temperatures had contributed to this by allowing *P. marinus* to overwinter at relatively high levels (Burreson and Ragone Calvo 1996)

Environmental Drivers of Intensification



Drought conditions increased abundance, mild winters favored higher overwintering

Increased transmission efficiencies ➔more *P. marinus* ➔increased transmission. . .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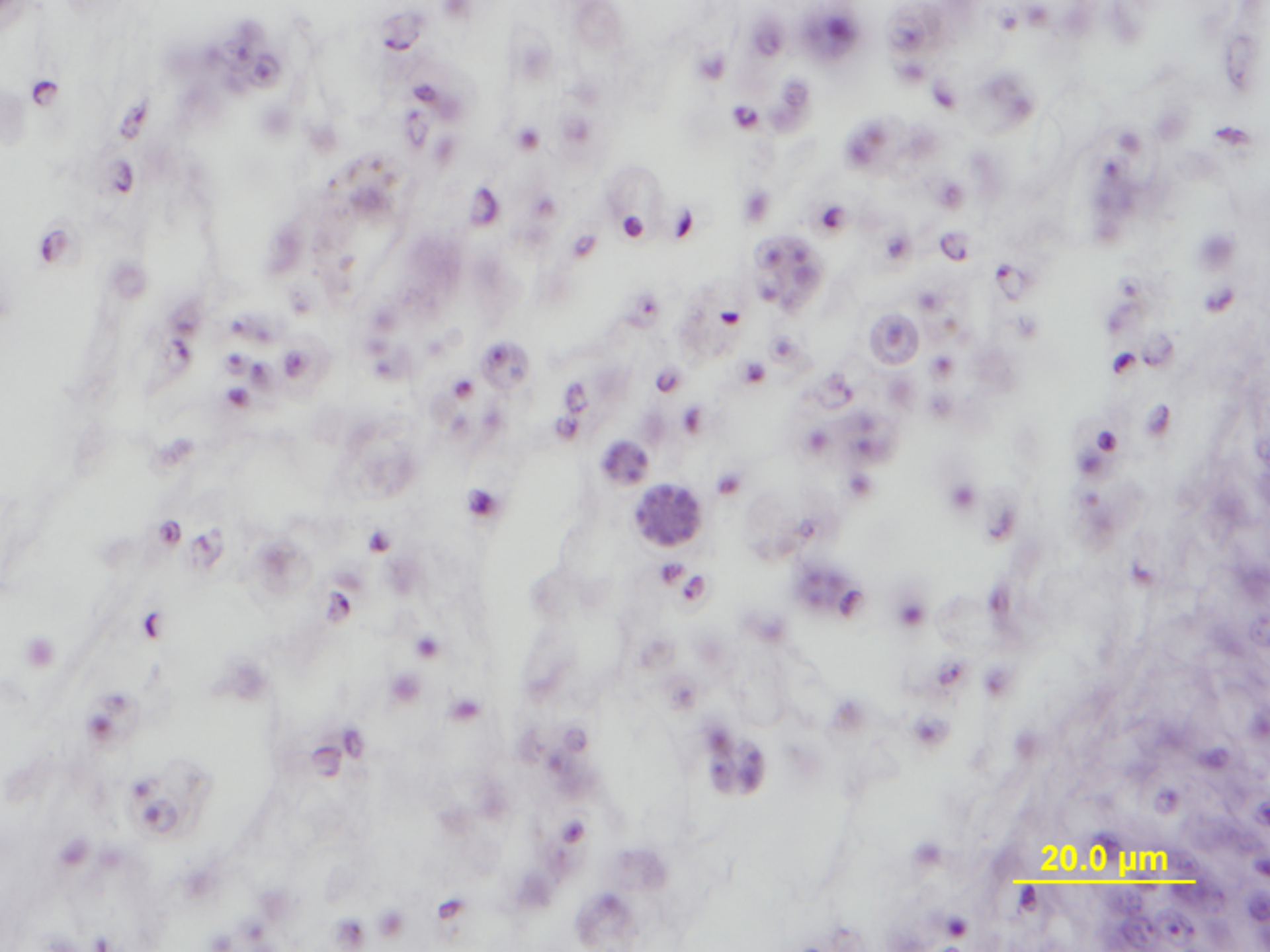
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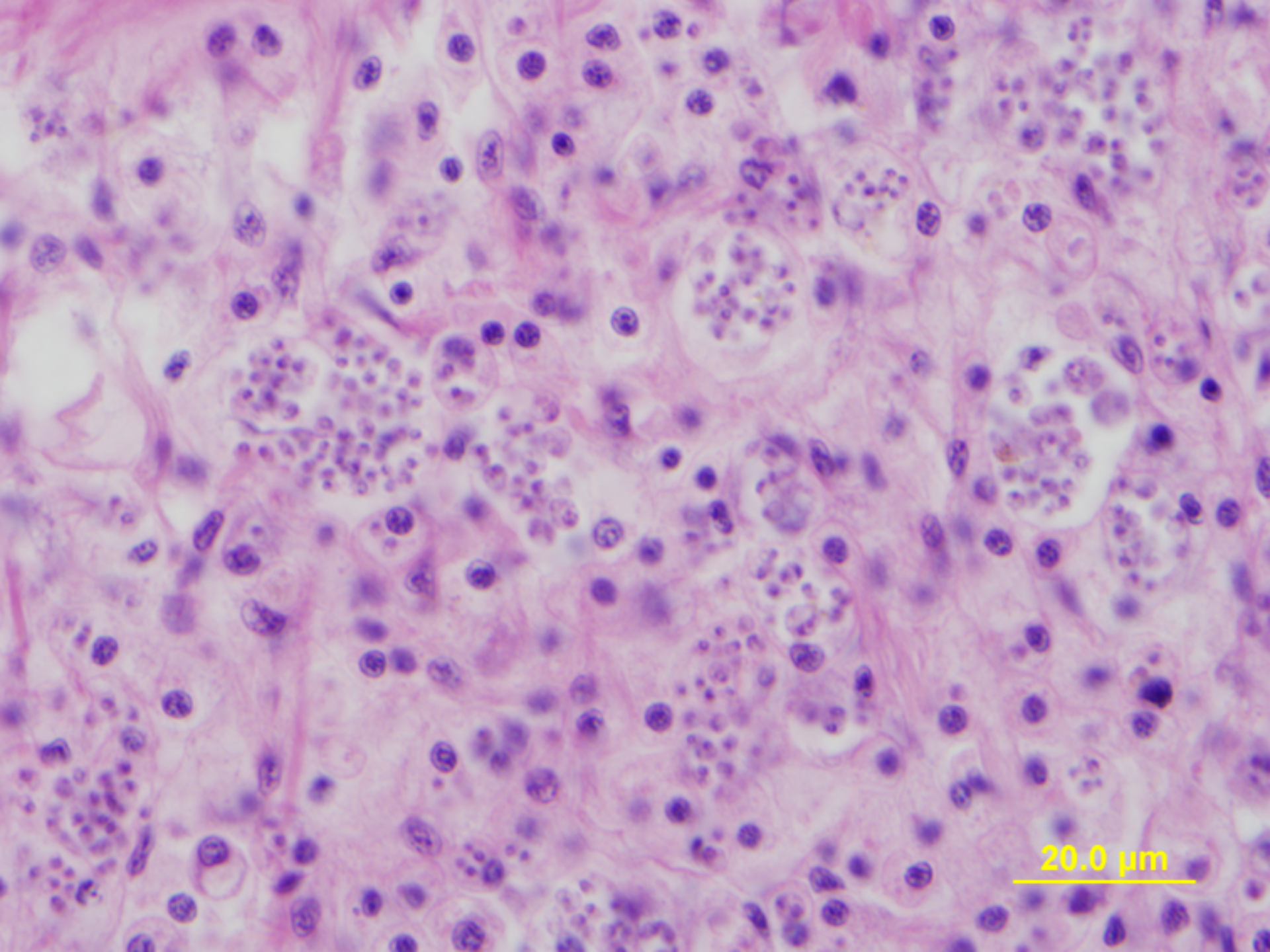
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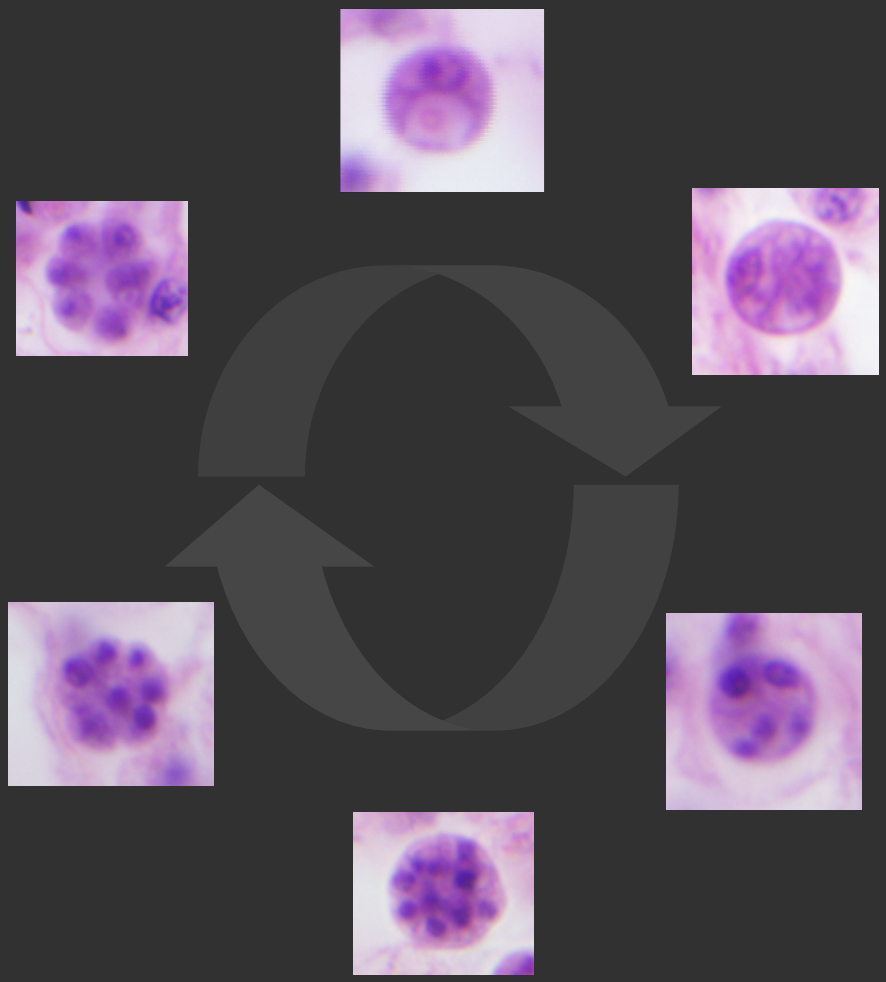
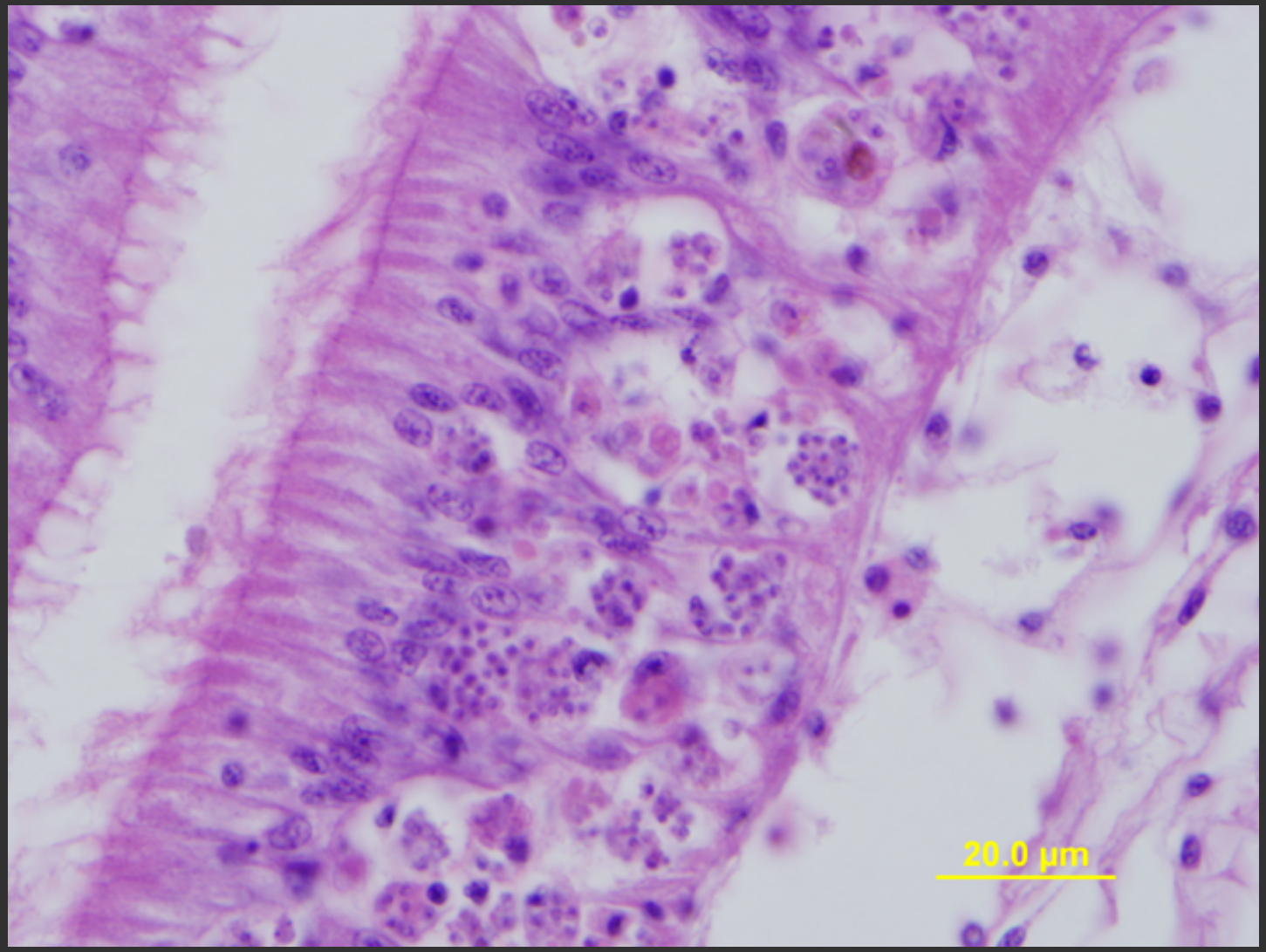
Nature of the Phenotype Change: Shortened Life Cycle



Shift Toward Epithelial Tropism



When Did The Change In *Perkinsus marinus* Occur?

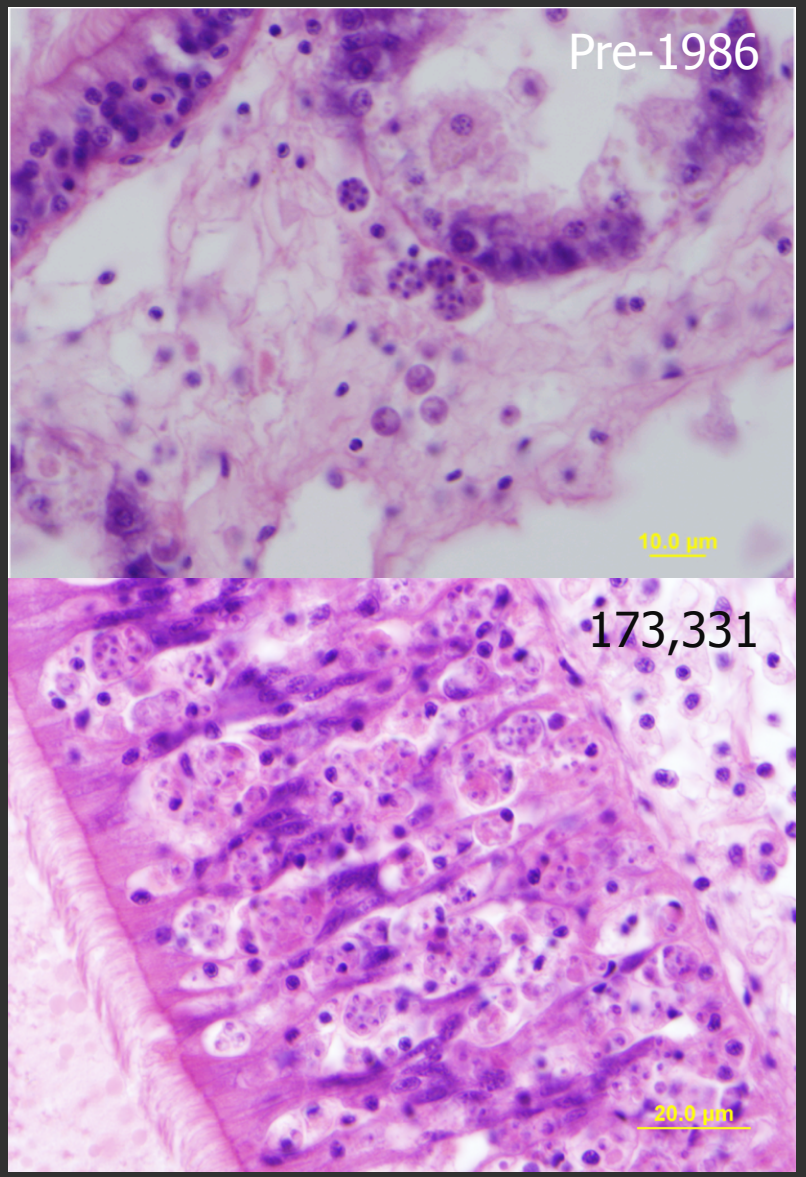
VIMS OYSTER DISEASE MONITORING

Collection Date 12 Aug 86
Location Thomas Bolt
Tray Number
Temp Salinity
Fixative

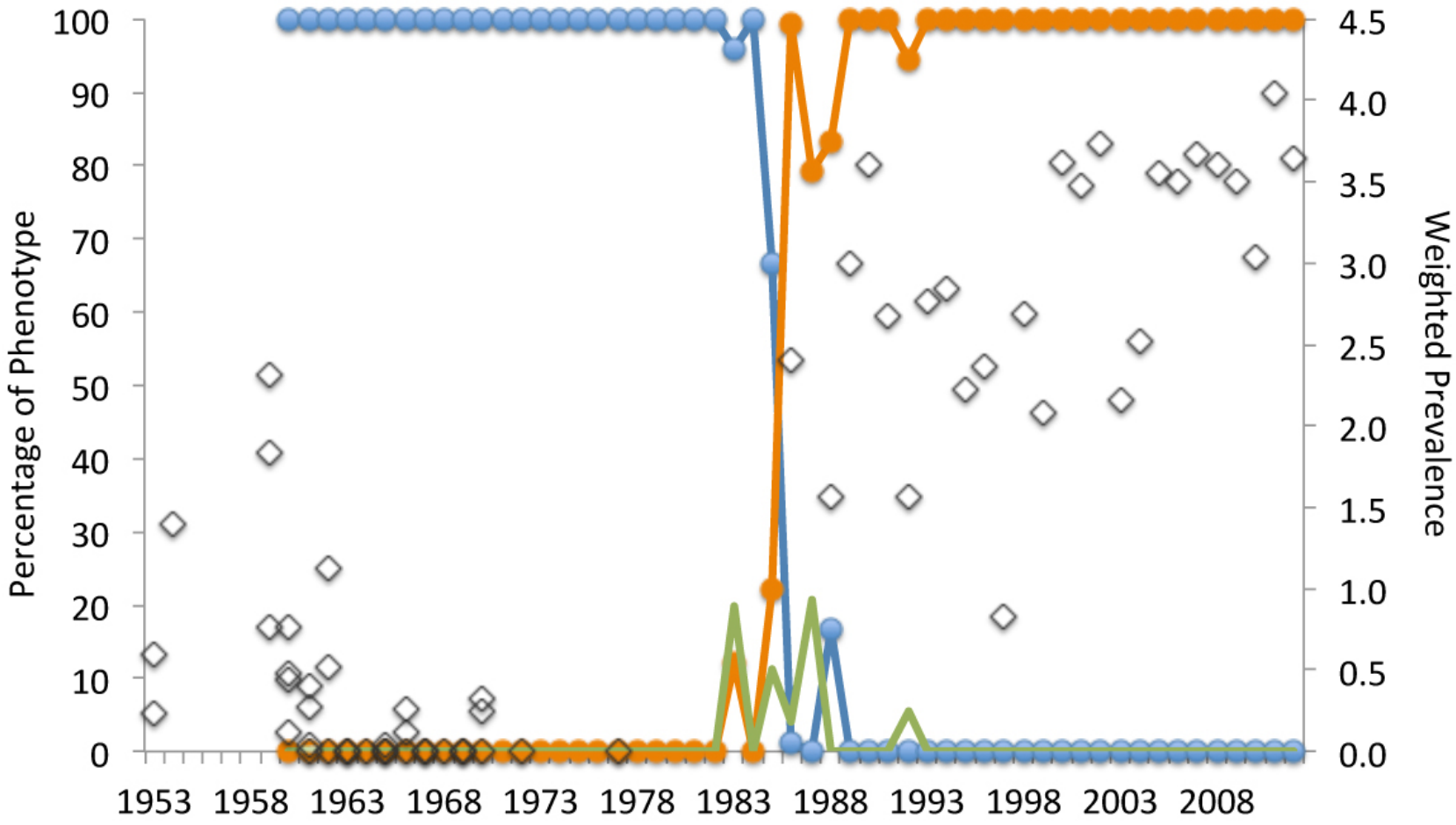
Prevalence MSX 0
Prevalence SSO 0
Prevalence Perkinsus 6 in state
not thio. test

173,325-49

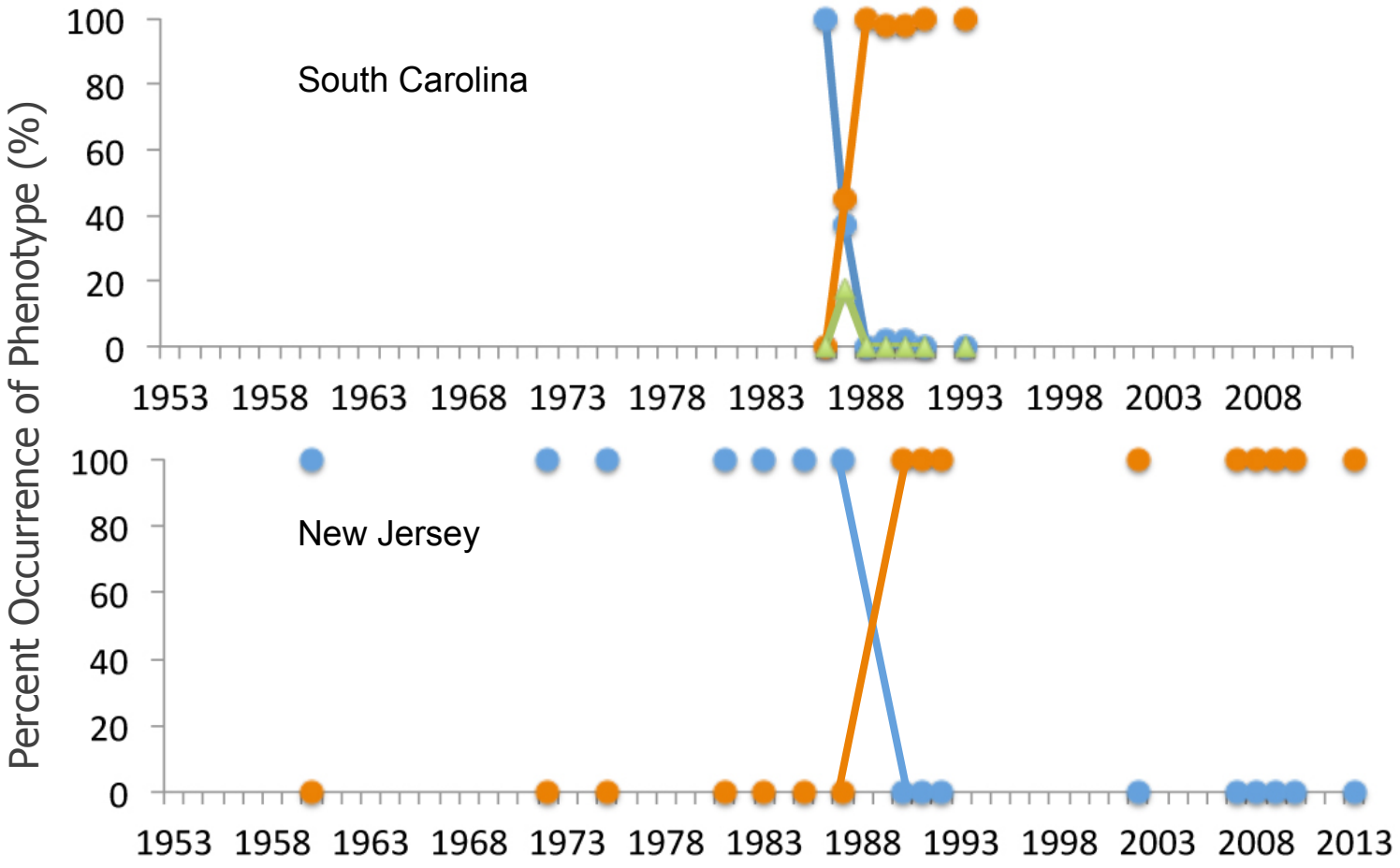
Accession #	Sex of Oyster	MSX Intensity N,R,L,M,H	Systemic/Local	SSO - plas/pre/spore	Perkinsus - slide: +,-	Perk. - Thio: R,L,M,H	Nematopsis: +,-	Bucephalus: +,-	Chlamydia: +,-	Hemocytosis: N,L,H	Ceroid: N,L,H	Blood Smear	Remarks
173,325	♂	N	–	–	–	–	+	–	–	L	L		
26	–	N	–	–	+	–	+	–	–	H	L		purple cells
27	♀	N	–	–	–	–	+	–	–	L	L		
28	♂	N	–	–	–	–	+	–	–	L	L		
173,329	♂	N	–	–	–	–	+	–	–	N	L		
30	♀	N	–	–	–	–	+	–	–	N	L		pink cells
31	–	N	–	–	+	–	+	–	–	L	L		* see L. gut epith - Dens?
32	♀	N	–	–	±	–	+	–	–	L	L		purple cells, *
173,333	♂	N	–	–	–	–	+	–	–	L	L		
334	♀	N	–	–	+	–	+	–	–	H	L		sev L gut, V sick epith
35	♀	N	–	–	–	–	+	–	–	L	L		
36	–	N	–	–	+	–	+	–	–	H	L		V. Sick epith
173,337	♀	N	–	–	–	+	+	–	–	H	H		
38	♀	N	–	–	–	–	+	–	–	L	L		
39	♀	N	–	–	+	–	+	–	–	H	L		pink cell
40	♂	N	–	–	–	–	+	–	–	L	L		
173,341	♂	N	–	–	–	–	+	–	–	L	L		
42	–	N	–	–	–	–	+	–	–	L	L		
43	♂	N	–	–	–	–	+	–	–	L	L		
44	♂	N	–	–	–	–	+	–	–	L	L		
173,345	–	N	–	–	–	–	+	+	–	N	L		
46	♀	N	–	–	–	–	+	–	–	L	L		
47	♂	N	–	–	–	–	+	–	–	L	L		abnl
48	♀	N	–	–	–	–	+	–	–	N	L		
49	–	N	–	–	+	–	+	–	–	L	L		



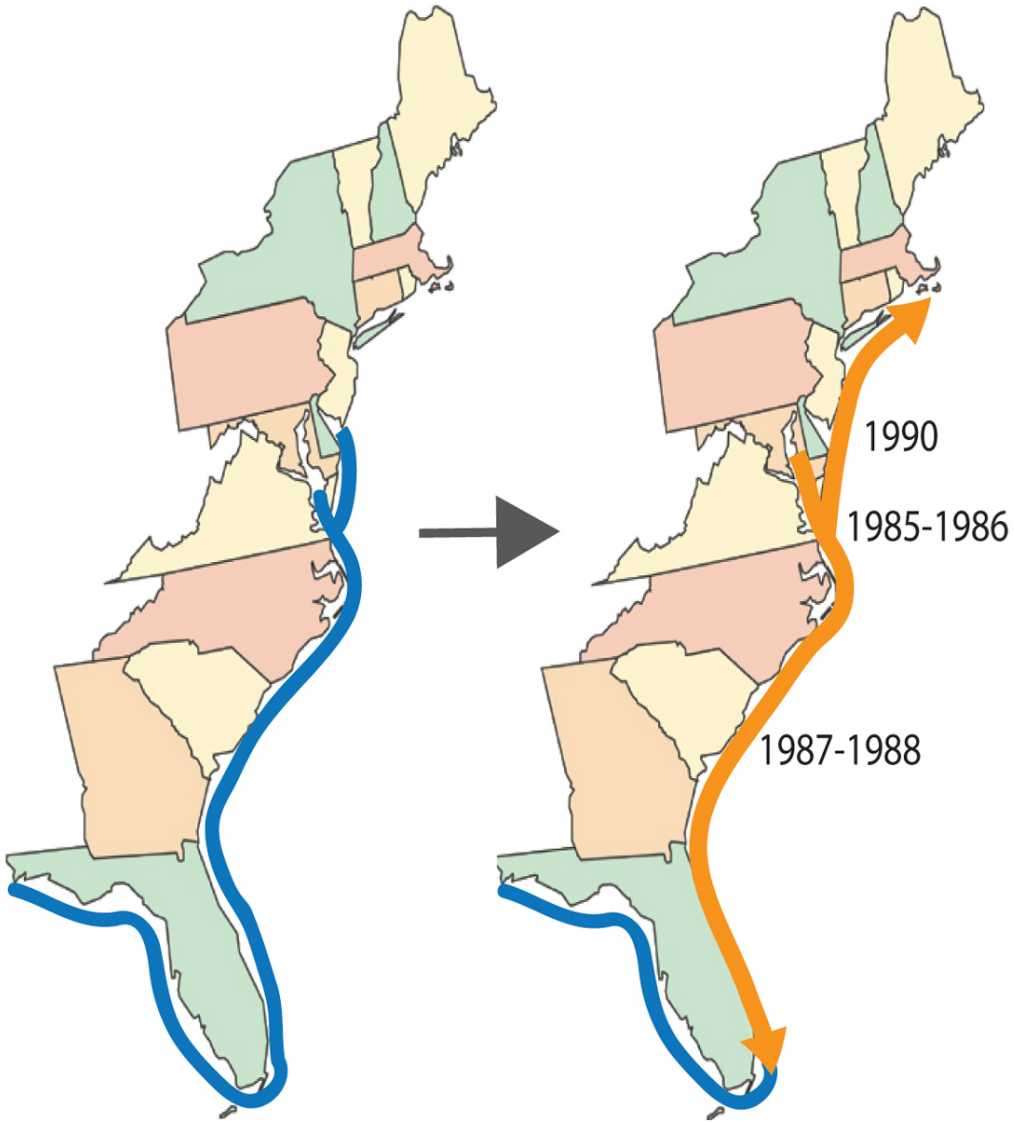
Disease Intensification and the Phenotype Shift



Did a Similar Phenotype Shift Occur in Other Locations?

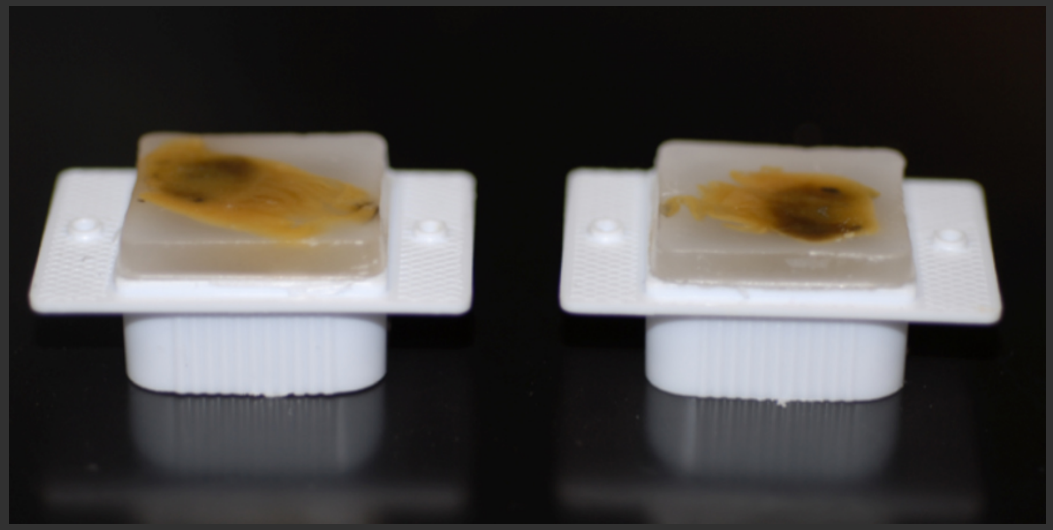


Emergence, Dispersal of Hypervirulent *Perkinsus marinus*

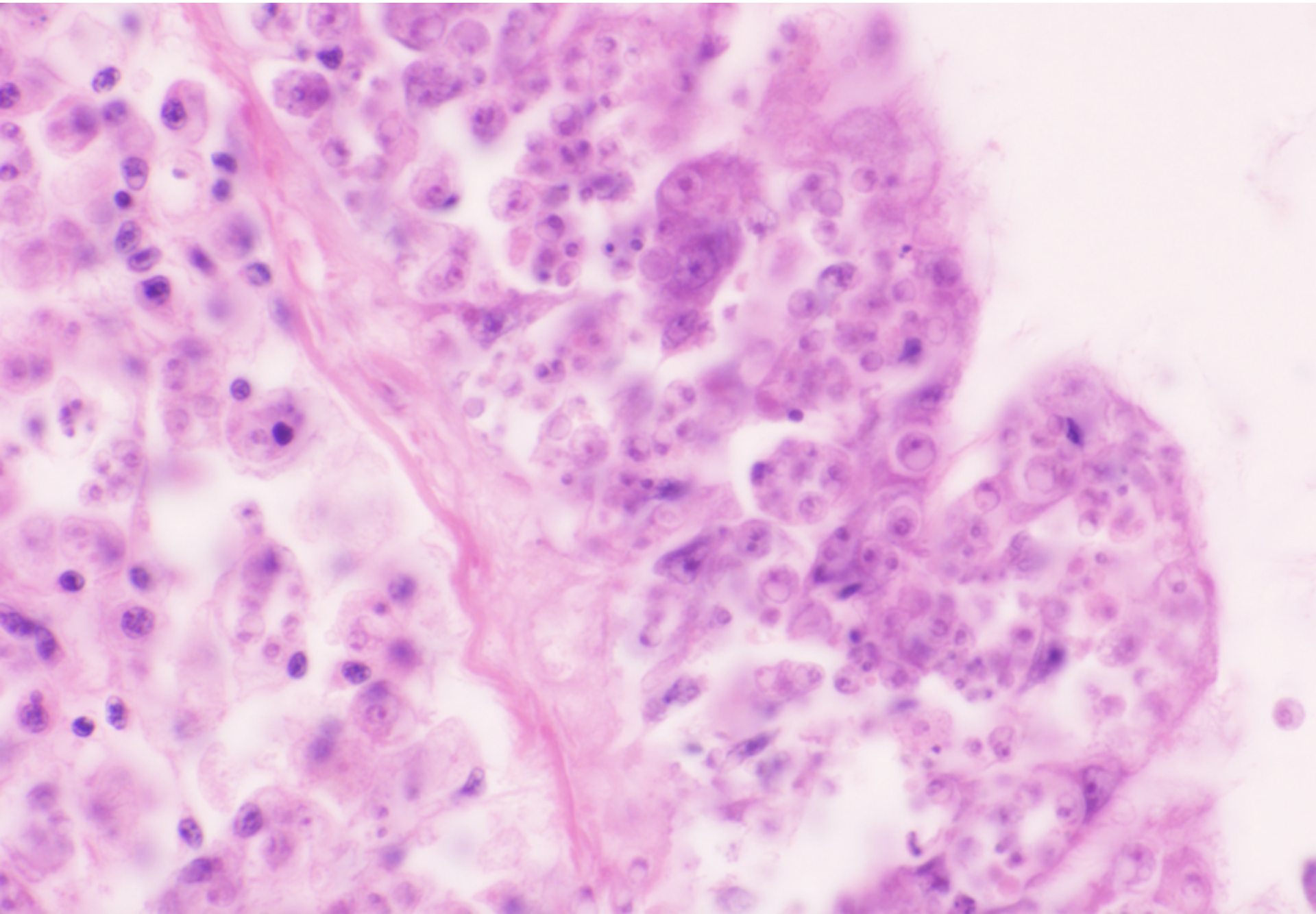



Some Questions…

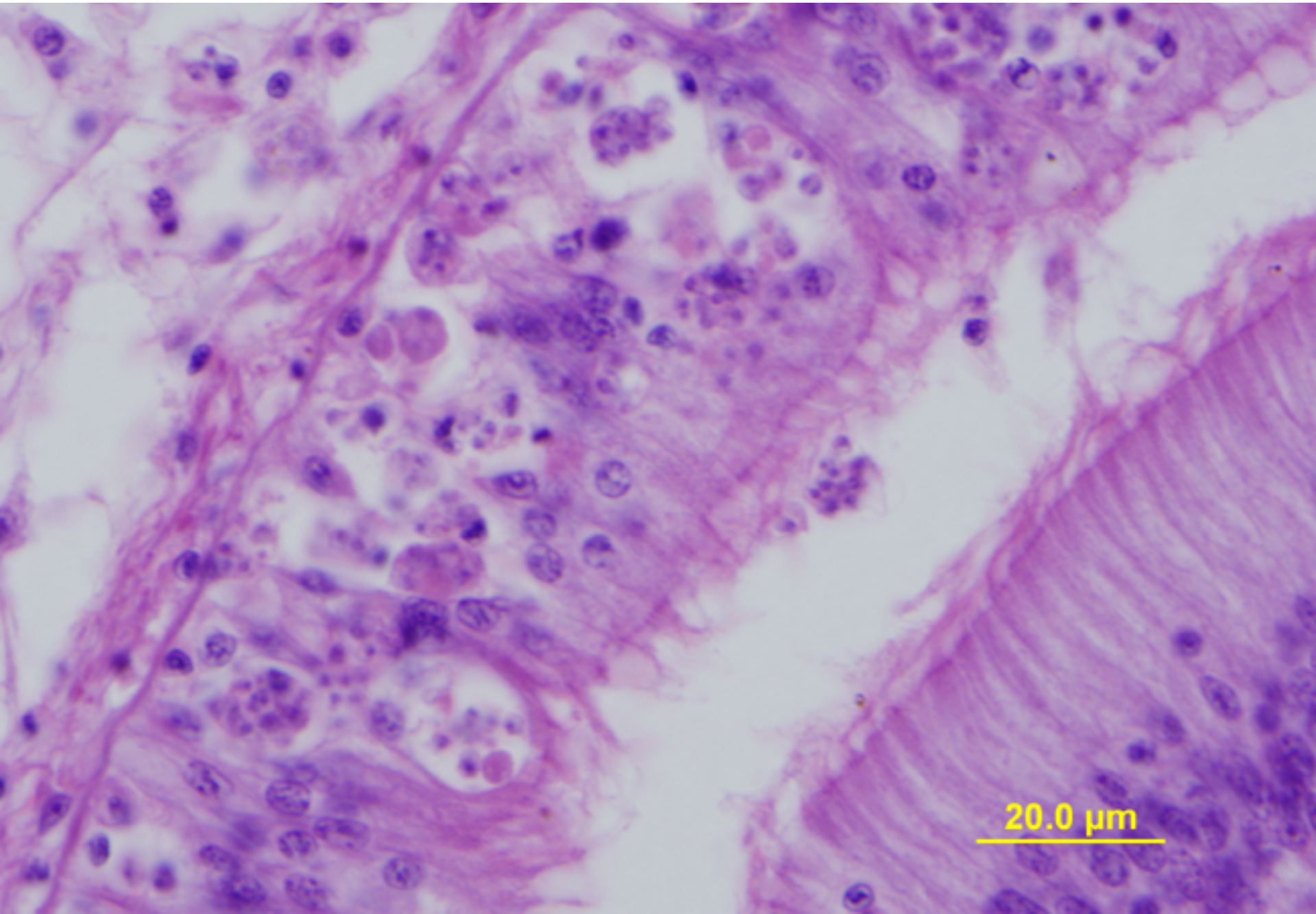
Is There a Genetic or Epigenetic Basis for the Change?



Timing of Changes in Division, Cell Size, Tropism?



Significance of Epithelial Tropism as an Adaptation?



How Did *Perkinsus marinus* Change So Rapidly?

A "winter paradox" of *P. marinus* parasitism?

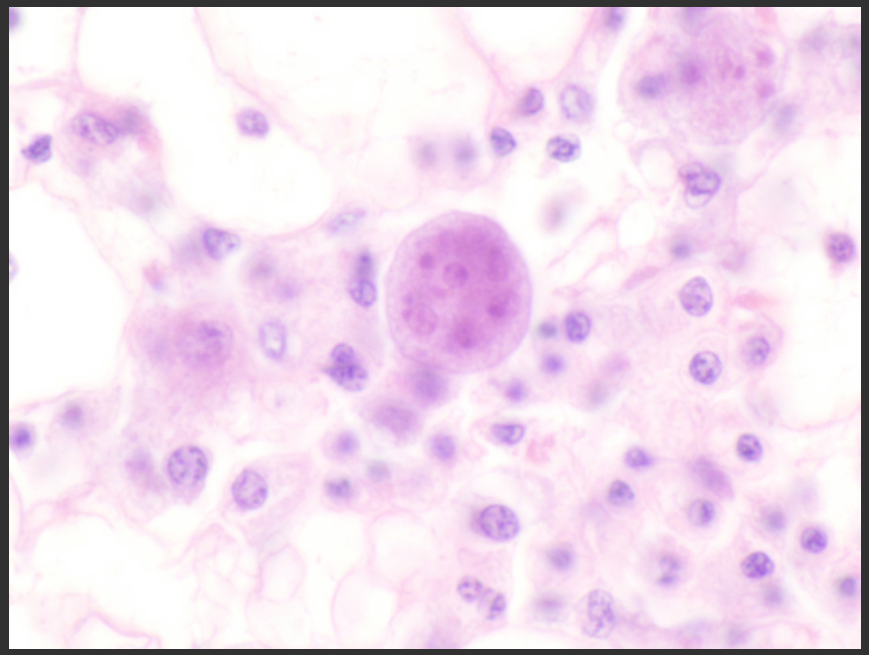
What Drove the Phenotype Change?

Oyster Population, Industry in Virginia, 1950s

- 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 waters > 12-15 ppt much higher than today (Haven et al. 1978)

With the Arrival of *Haplosporidium nelsoni*

- 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 (distribution, densities) far lower in upper mesohaline-polyhaline waters

Oyster *longevity* was also reduced as MSX killed quickly

- Both disadvantageous for a parasite that required a host that was 1) at high density for optimal transmission, and 2) long-lived

- Question: Did the *H. nelsoni* invasion select for a *P. marinus* that could generate high infection intensities very quickly so as to transmit successfully in a more host-sparse system?


TERRAFORMING Chesapeake Bay

“Unless these diseases can be substantially controlled -- and no evidence suggests they can -- the outlook for *C. virginica* is bleak. . .”

C. Ronald Franks, MD Department of Natural Resources, c. 2005

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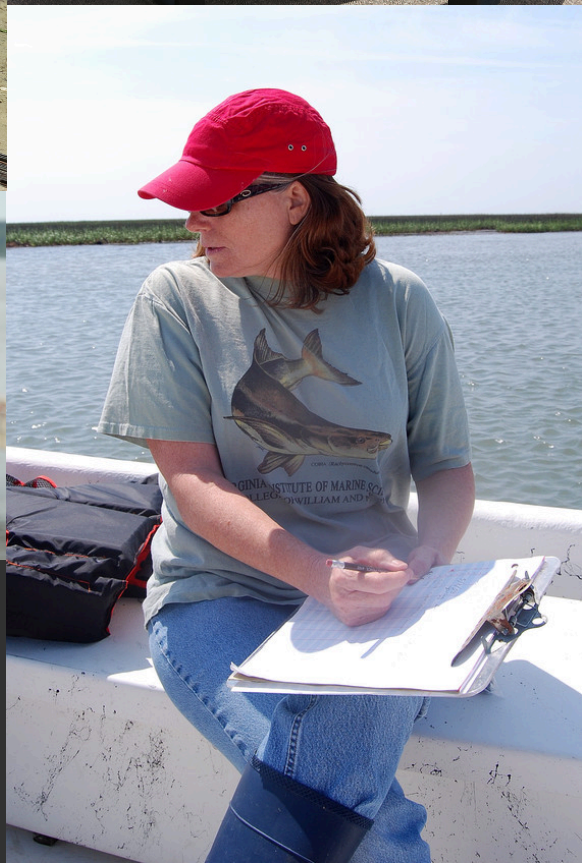
- Tidewater Oyster Gardeners Association (TOGA)



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