

# Diseases Affecting Commercial Crustacean Resources

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# Disease Agents of Blue Crabs and White Shrimp

## VIRUSES

Crabs – Rheovirus and Rhabdovirus

Shrimp – WSSV virus

## BACTERIA

Crabs and Shrimp – Chitonoclastic bacteria (“black spot disease”)

*Vibrio* infections

## FUNGI

Crabs – *Lagenidium callinectes*

Shrimp – Microsporidion, *Thelubaria penaei* (“cotton disease”)

## PROTISTS

Crabs – *Paramoeba pernicioso*, *Hematodinium perezii*

Shrimp - Apostome ciliates, Phyllopharyngean ciliates ? (“Black Gill”)

## HAPLOSPORIDIA

Crabs – *Urosporidium crescens* (“pepper-spot”)

# Epizootics Caused by Transport of Crustaceans to New Sites

- Epizootic of IHHN virus that resulted in the collapse of the shrimp fishery in 1990 in Gulf of California, presumably introduced from nearby coastal shrimp farms in Mexico.
- In Europe the introduction of the American crayfish and its associated fungi, *Asphanomyces astaci*, in 1880s caused most of the stocks of the European crayfish to be wiped out.

# Crustacean Epizootics in SE US

1919 – Large die off of white shrimp off Louisiana coast due to infection by a Microspordian

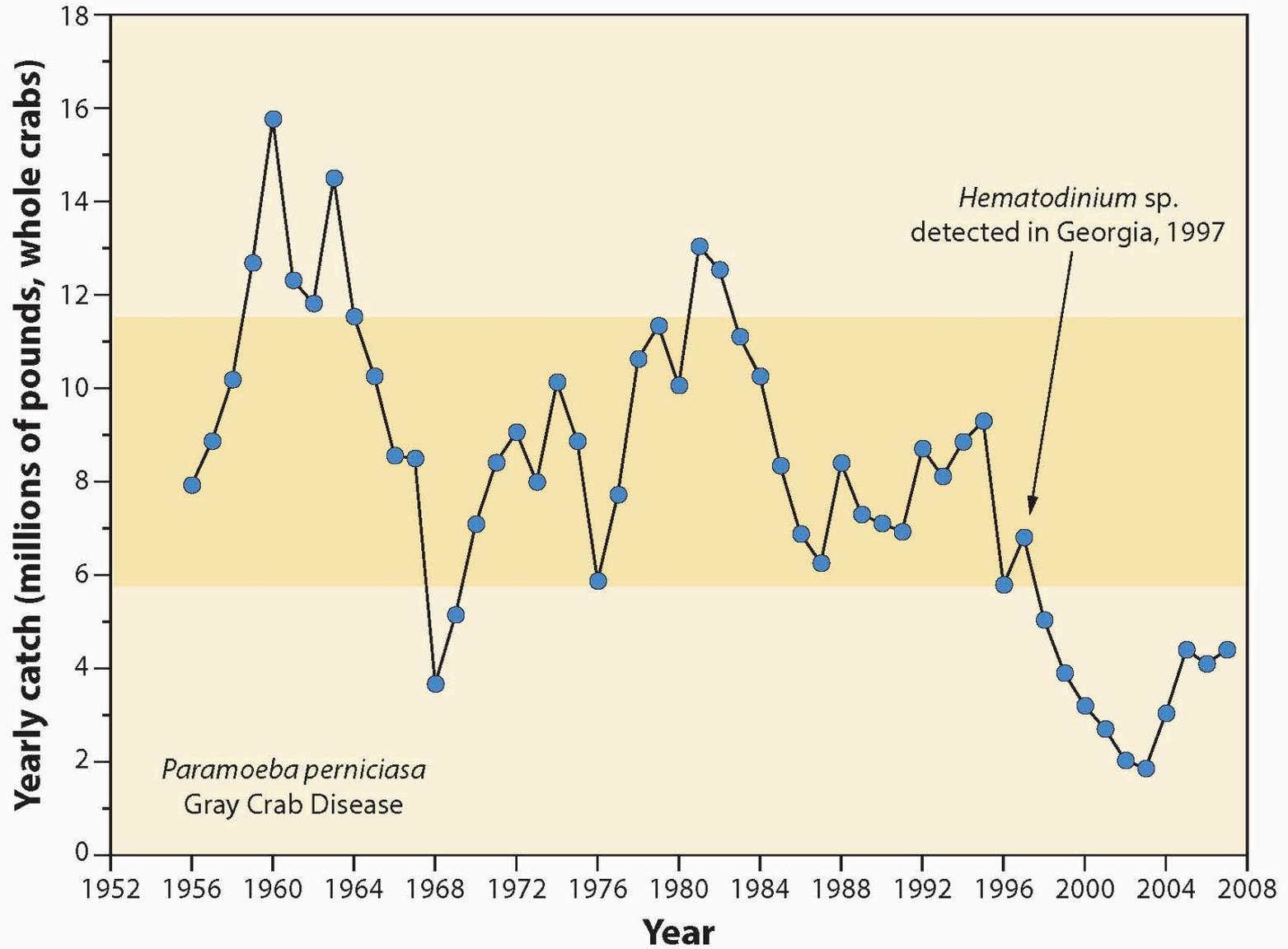
1971 - Epizootic of pathogenic amoeba, *Paramoeba pernicioso* (Gray Crab disease) in Chicoteague Bay. Decrease in crab catches in 1968 in Georgia thought to be associated with this disease.

1991 and 1992 – Epizootic of Hematodinium disease in crabs in seaside bays of MD and VA.

1998 to 2003 – Dramatic decrease in blue crab catch in GA associated with increased prevalence of *Hematodinium* and prolonged drought.

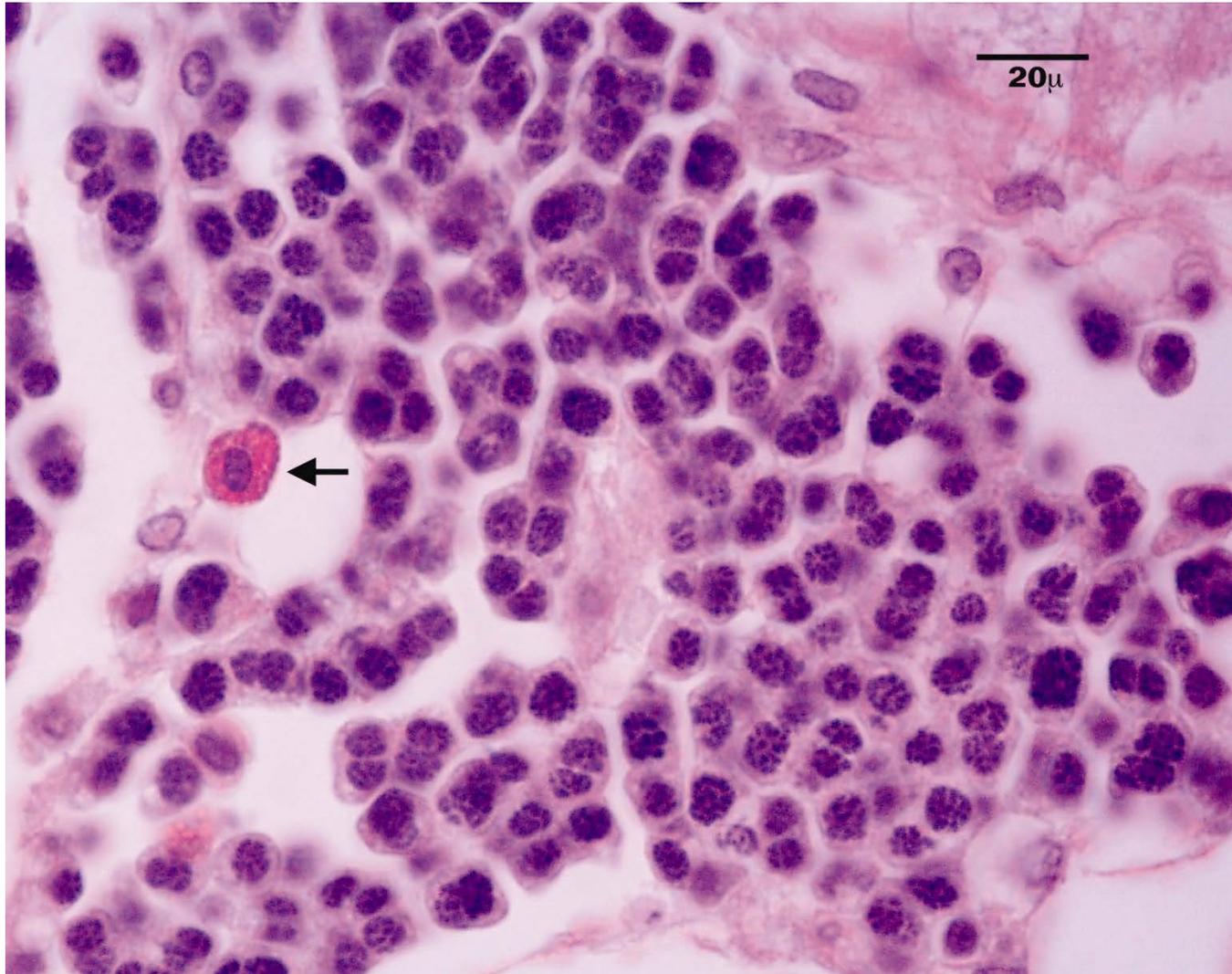
1999 to present – Major increases during the past few years in Black Gill in white and brown shrimp in SC and GA

# Blue crab landings from 1952 - 2007



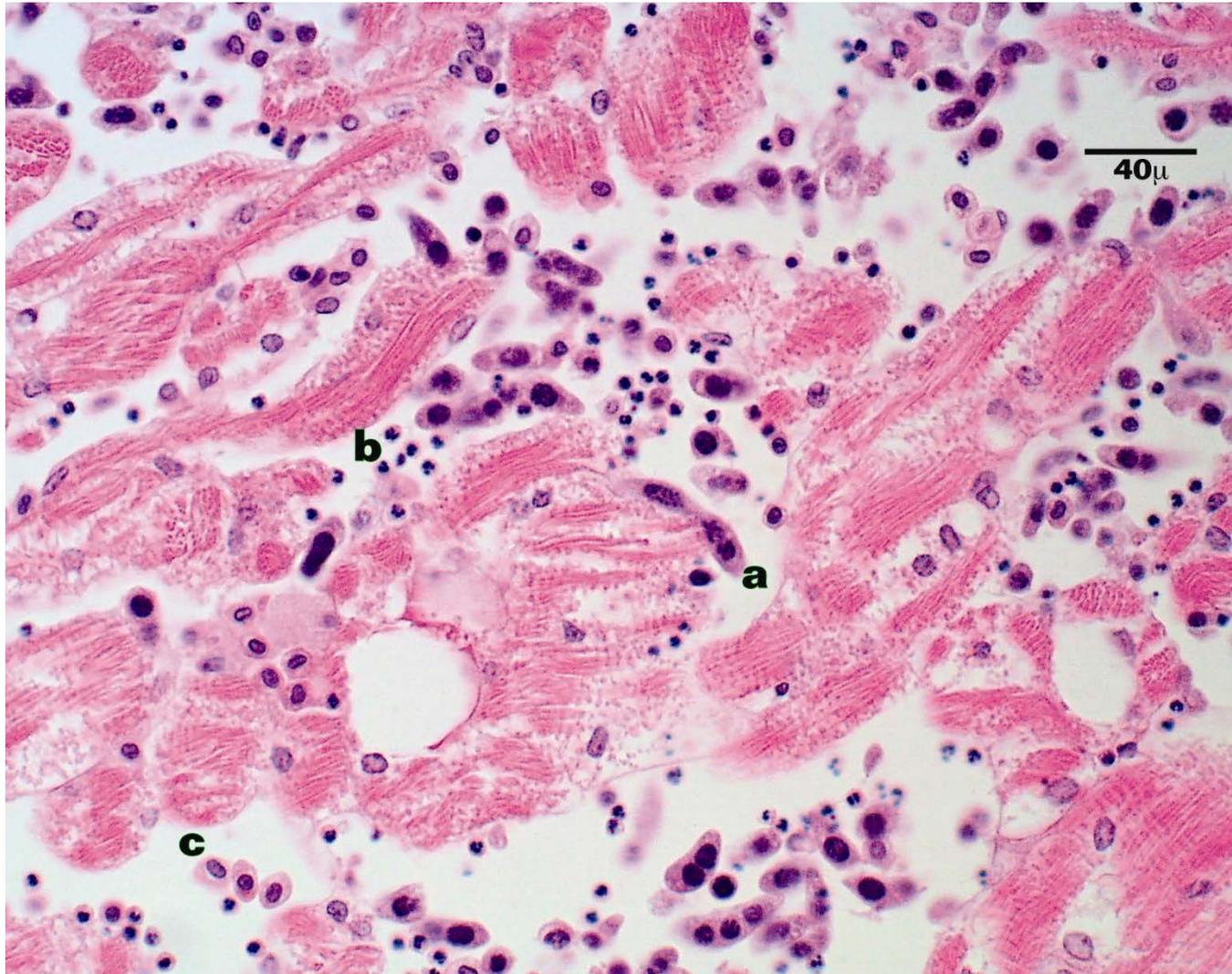
## *Hematodinium* sp. Factoids

- Parasitic dinoflagellate
- First Described in France in 1930's
- Infects Some Crustaceans But Not Others  
(Blue crabs, Stone crabs, Spider crabs and others)
- Infects Hemolymph & Other Tissues  
(cardiac & gill)

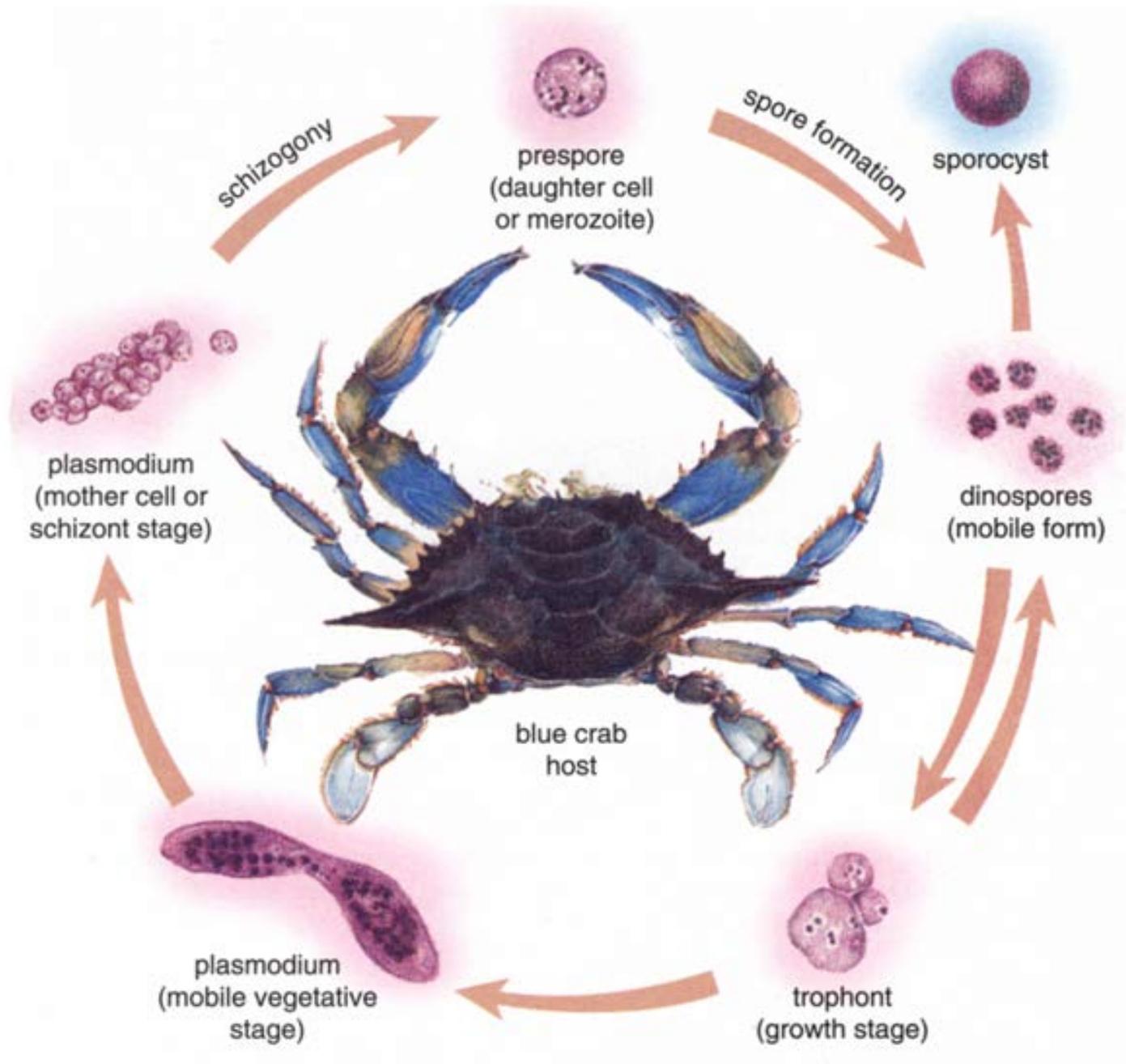


Heavy *Hematodinium* infection in vascular space.  
Arrow points to single hemocyte

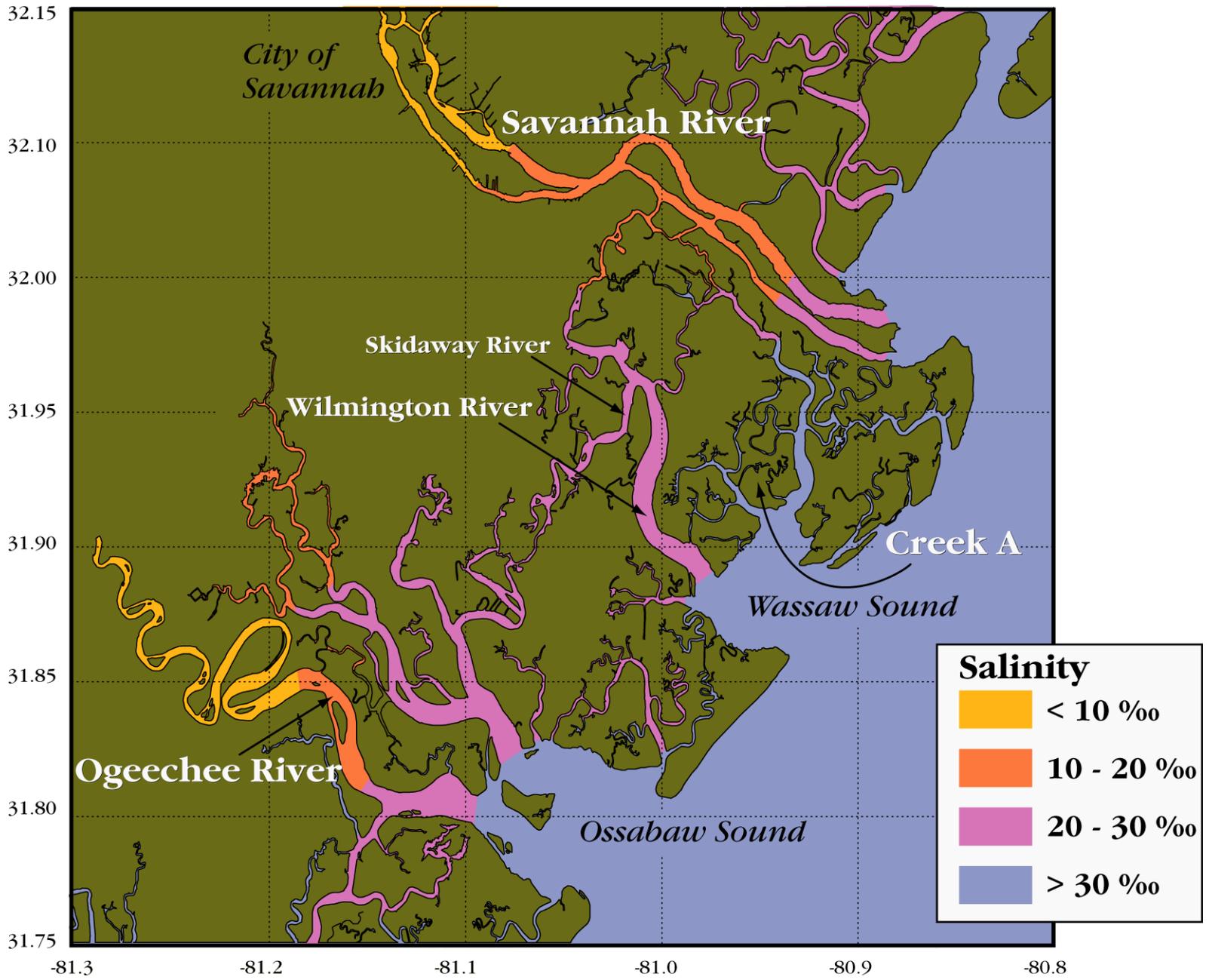
# Infected Cardiac Muscle



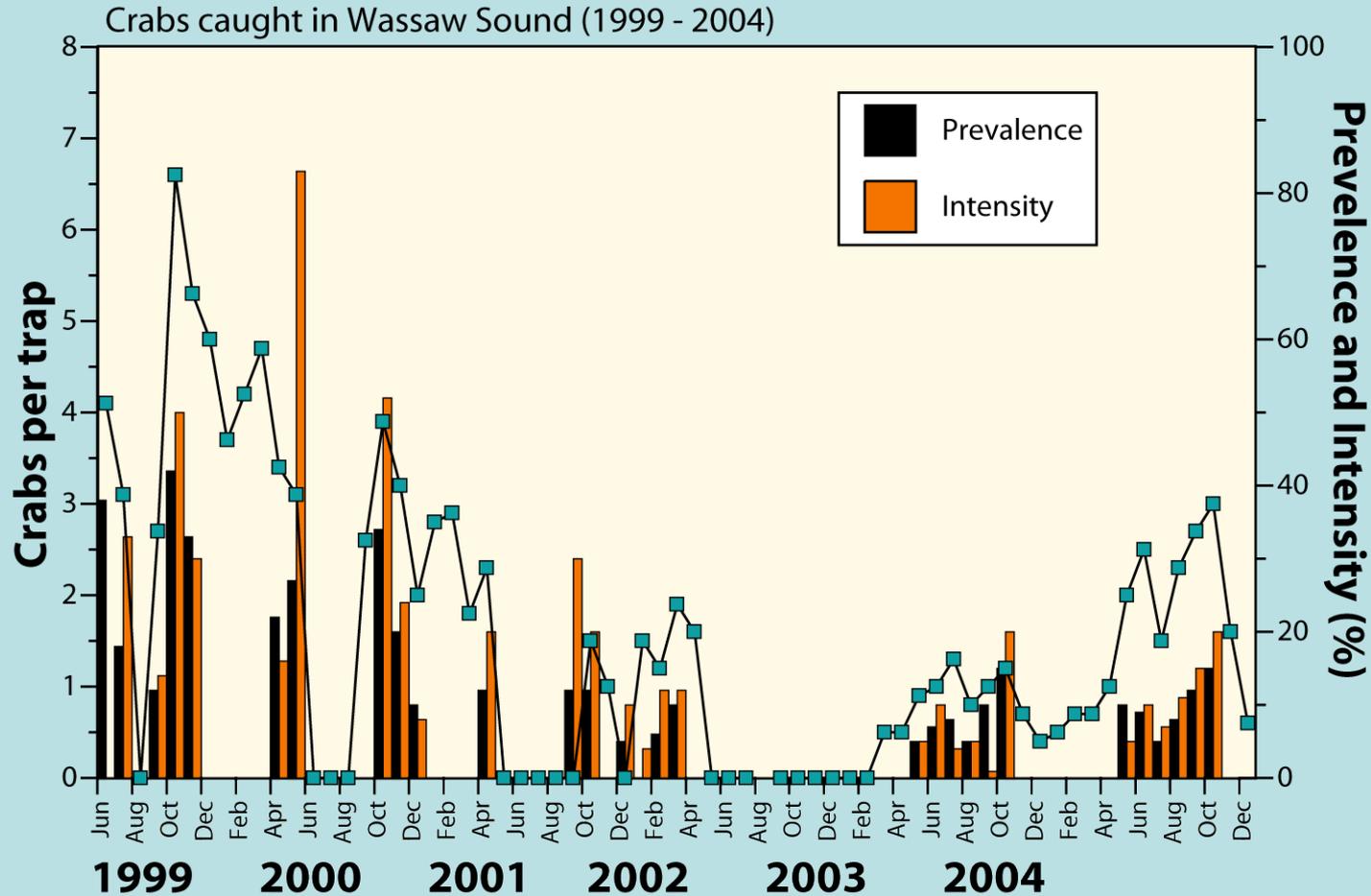
a – plasmoidal form; b- dinospores; c - hemocytes





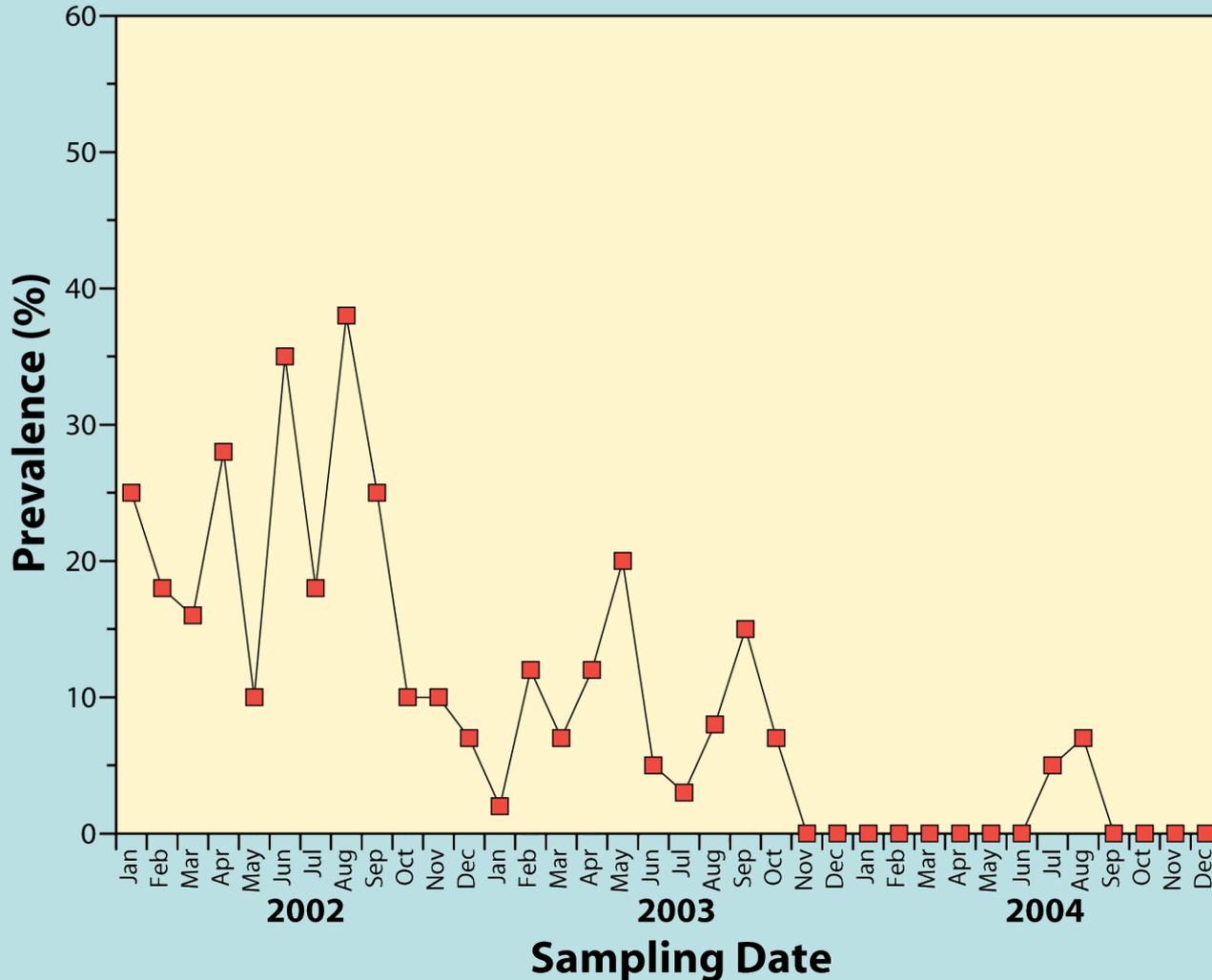


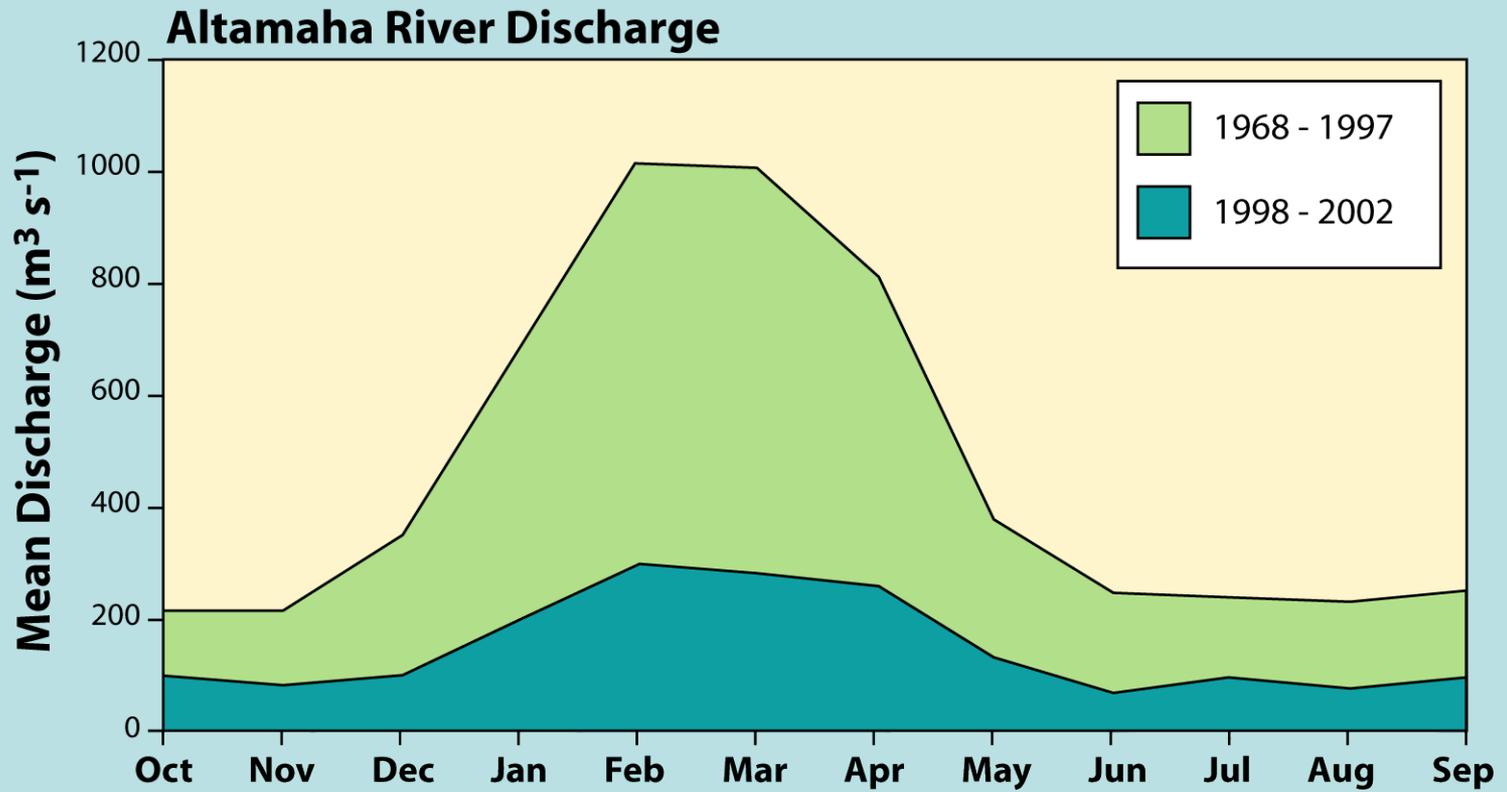
# Changes in Crab Population and Prevalence/Intensity of Hematodinium Infection in Wassaw Sound Crabs from 1999 thru 2004





# Changes in the Prevalence of Hematodinium Infection (2002-2004) in Blue Crabs from Doboy Sound, GA



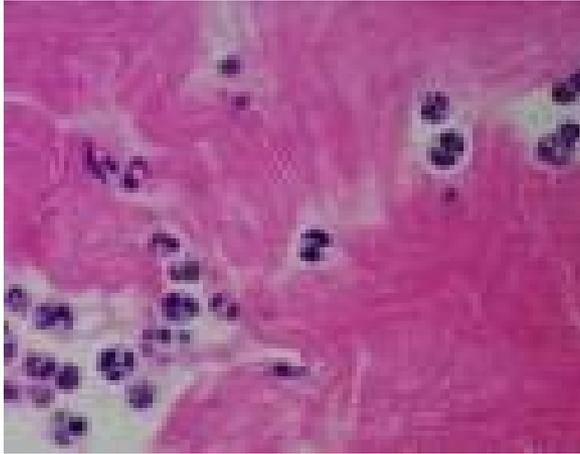


# Outbreaks of Disease in Marine Animals Linked to Meteorologic Events

- Blue crab hematodinium disease linked to drought and resulting high salinities in coastal Georgia.
- Low prevalence of the oyster diseases MSX and Dermo linked to years with cold winters and increased prevalence in dry years in eastern US (Paraso et al., 1999; Ragone and Burreson, 1993)
- Outbreaks of paramoebiasis in sea urchins linked to unusually warm water in eastern Canada (Scheibling and Hennigar 1997)

# Detection of parasites:

## -Histological diagnostic techniques



Hematodinium

Requires highly trained parasitologist,  
Laborious, not very sensitive



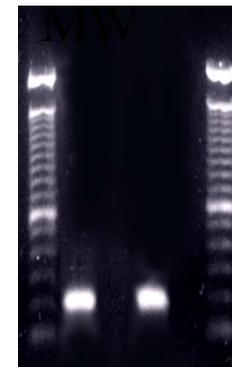
Paramoeba

Photo Credit - Jeff Shields

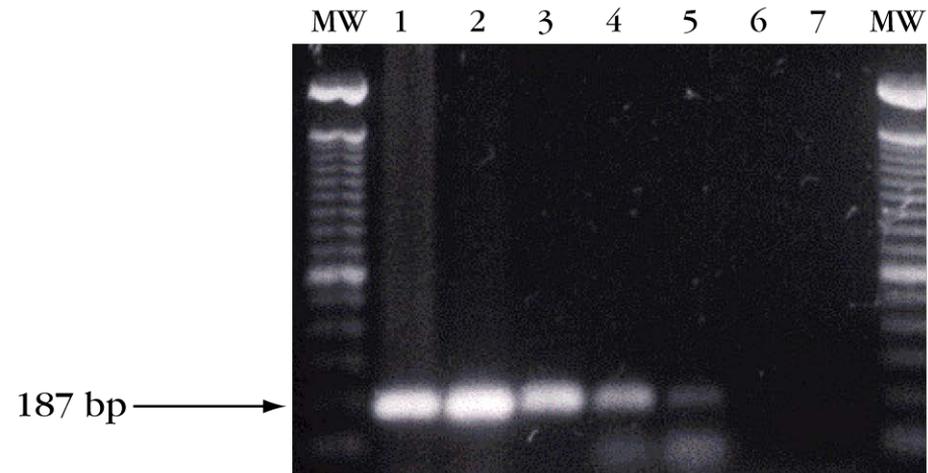
## -Molecular diagnostic techniques

Extremely sensitive and relatively  
simple, but requires *a priori* sequence  
knowledge (PCR) or specific antibodies

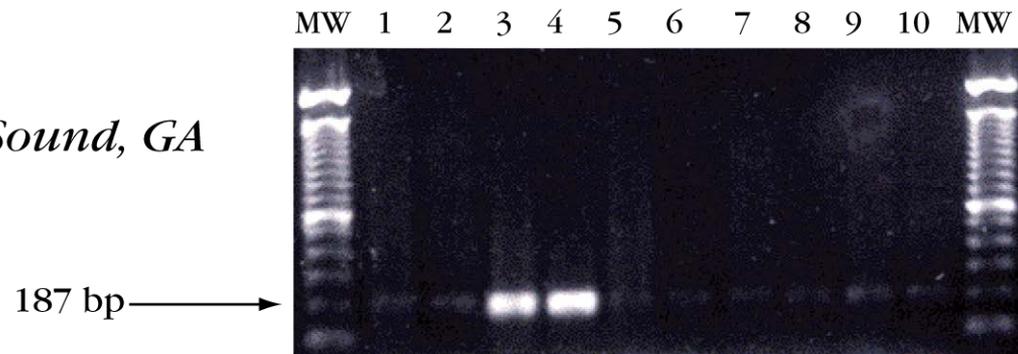
Pos Control  
Healthy Crab  
Sick Crab  
*P. marinus*



# Detection sensitivity of *Hematodinium* by PCR technique



*Wassaw Sound, GA*



# Molecular Approache for the Detection and Quantification of Protozoan Parasites in Crustaceans

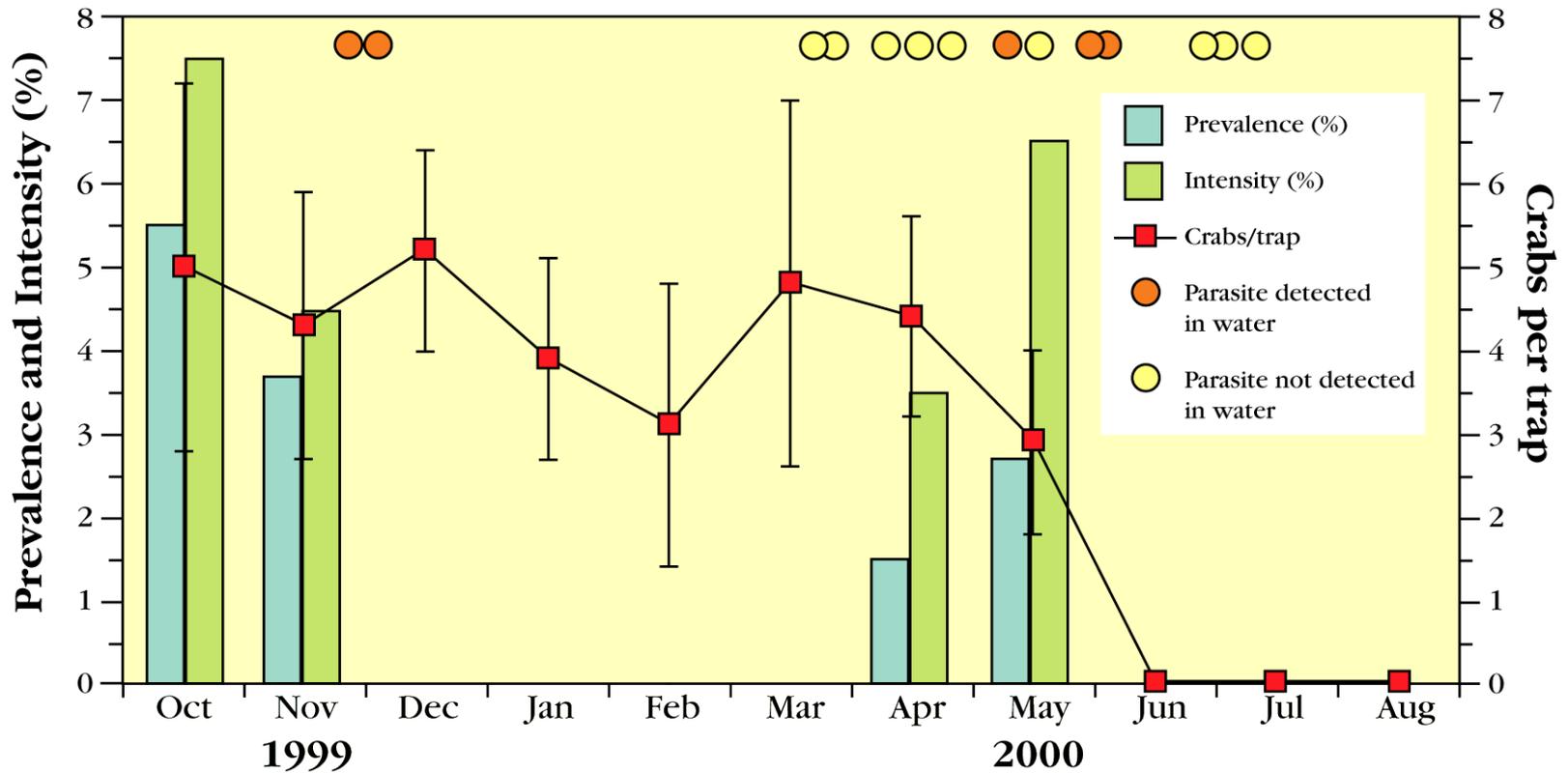
- PCR Based Diagnostic Approach – Specific Pathogen

Based on specific fragments of the 18S RNA gene

Procedure – Use PCR primers on isolated genomic DNA from host that amplifies a specific fragment. Visualized and sized by agarose gel electrophoresis. Can be used in water and different hosts.

In hemolymph assay can detect 1 parasite cell among 300,000 host cells

- Real time PCR – quantification based on gene copies of parasite



## *Hematodinium* in Water and Crabs from Wassaw

Can be transmitted via the water - Frischer et al. Harmful Algae (2006)

# Summary

- *Hematodinium* infection positively correlated with salinity and temperature, negatively correlated with rainfall and river discharge
- A molecular based diagnostic method allows detection and quantification of *Hematodinium* in various hosts, life cycle forms and water/sediment
- *Hematodinium* has caused mass mortalities of crabs at several sites worldwide
- The blue crab hematodinium disease in coastal GA appears to have been an example of a weather driven epidemic. The disease resulted in the smallest reported blue crab landings in 47 years of record keeping.

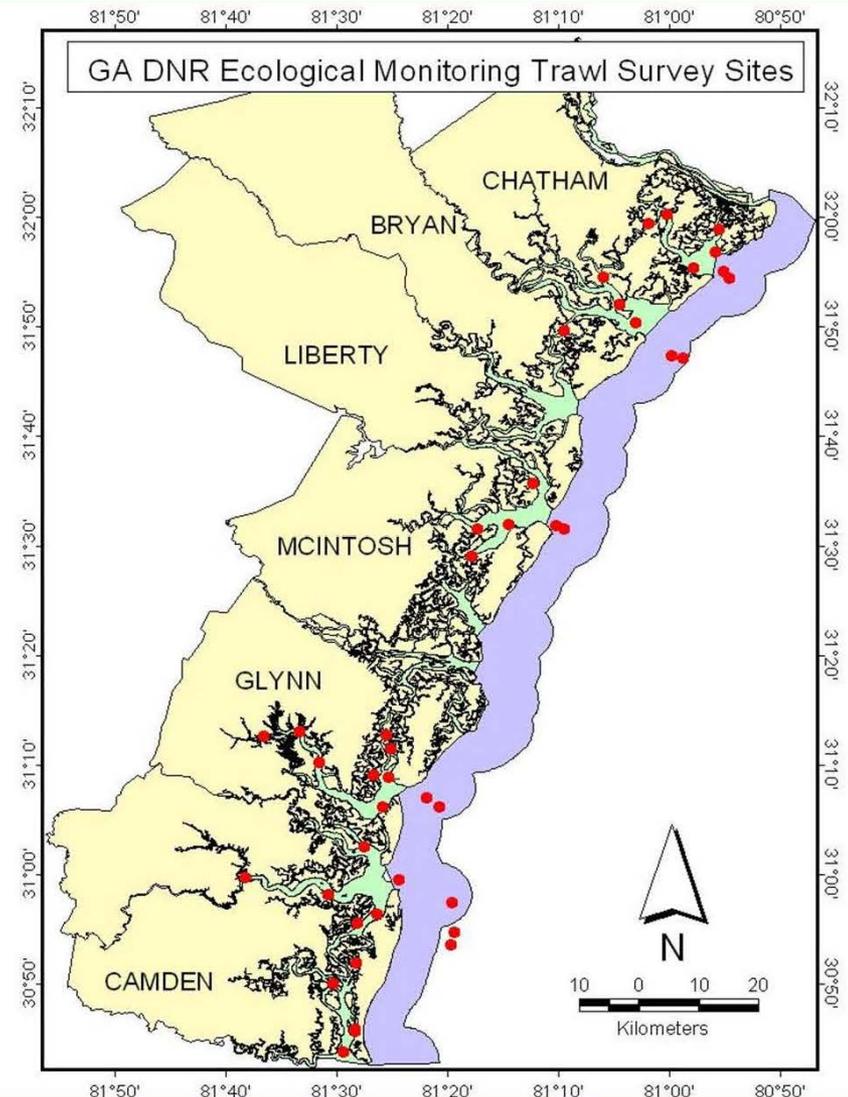
# GA DNR Ecological Monitoring Trawl Survey (EMTS)

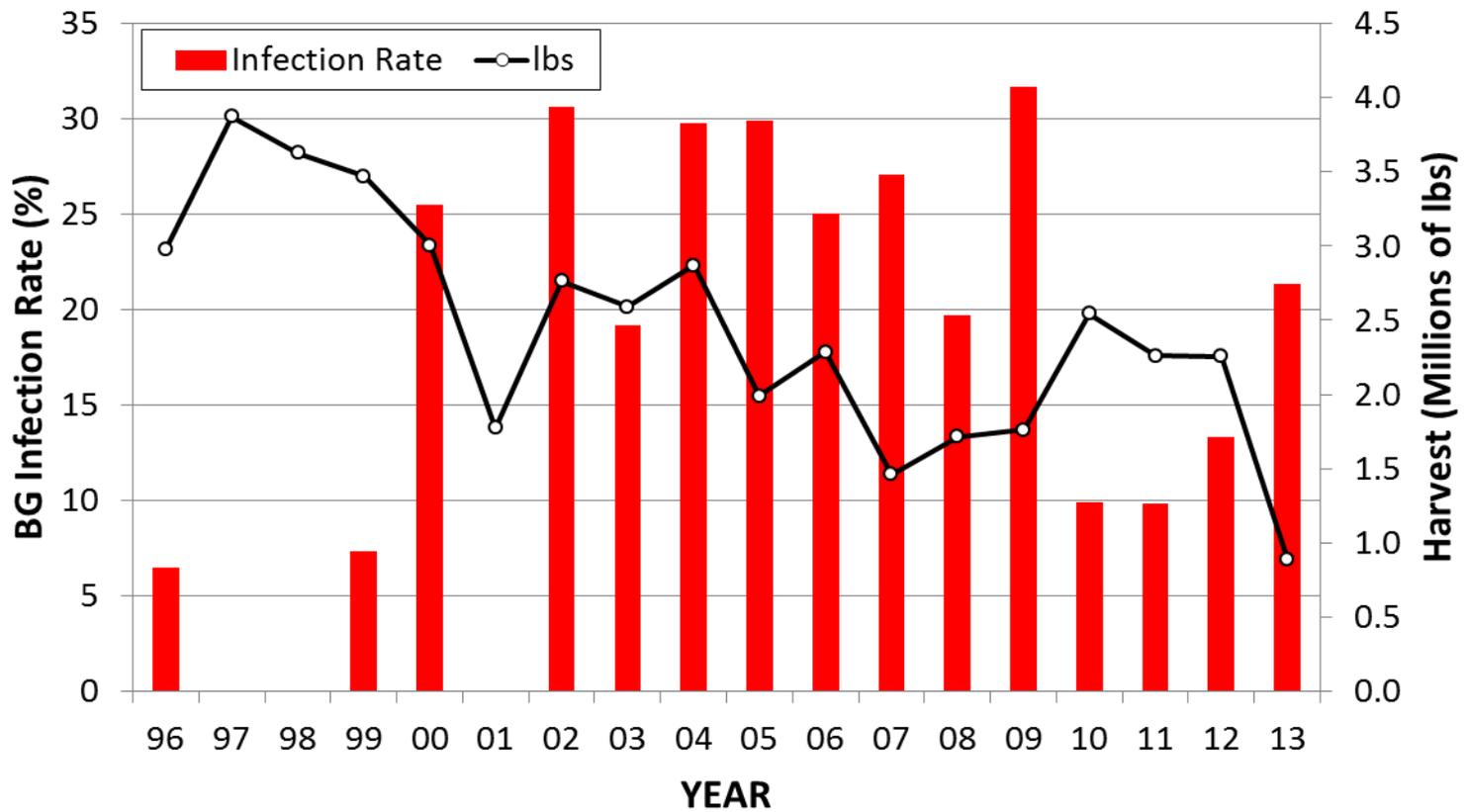
## Statewide Monitoring for Black Gill since 1996

- Over 15,000 trawls since 1976.
- 42 monthly stations across 6 sounds (WA, OS, SP, SS, SA, Cu).
- Gear: 40 ft flat trawl net towed for 15 min.
- Monitor abundance, size, and condition of all species.

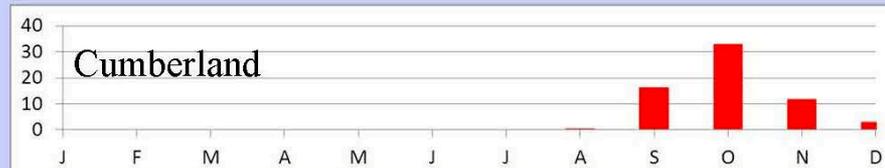
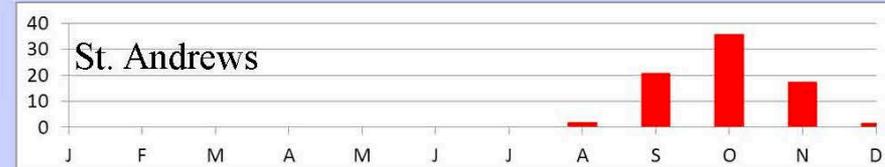
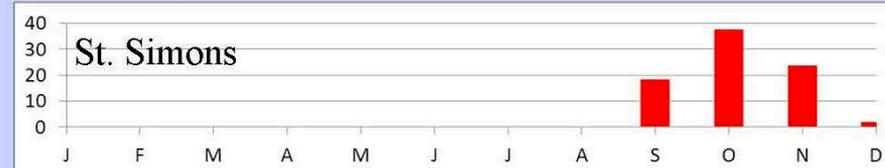
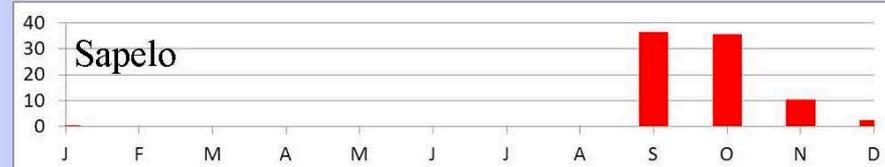
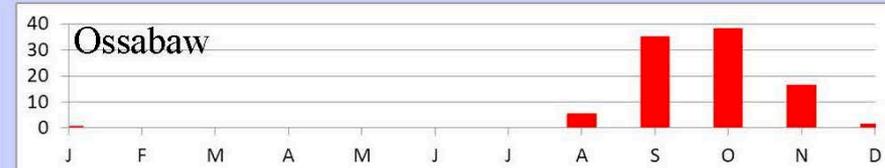
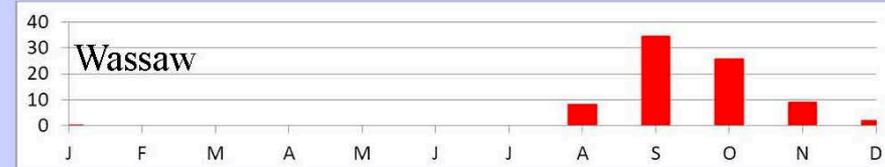
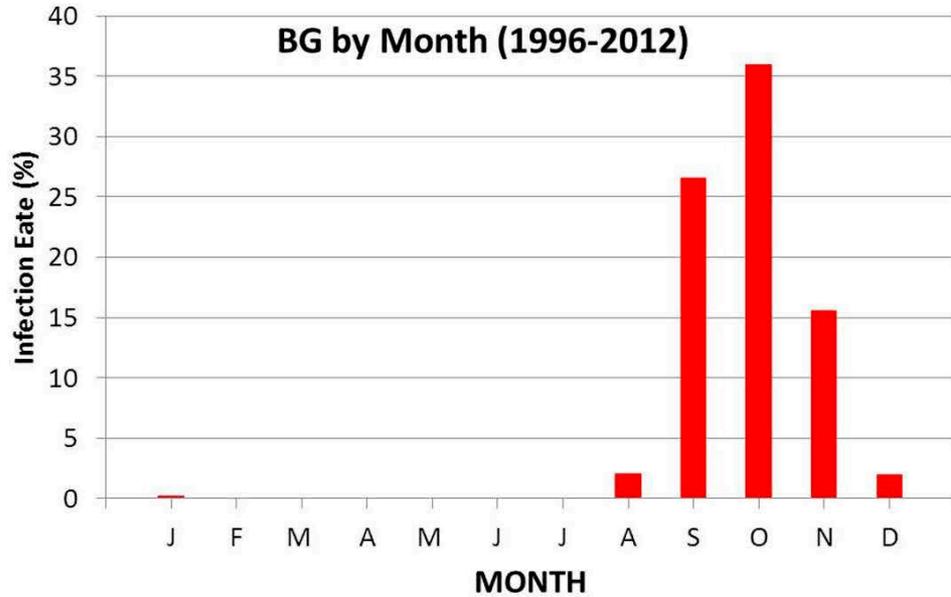
### Purpose:

- 1) Manage shrimp and crabs
  - 2) Provide estimates of finfish abundance
- Shrimp Data Collected per sample:
    - Total weight and number
    - Count size and CPUE
    - Sex and gonadal development
    - Disease condition
    - Lengths on a representative subsample

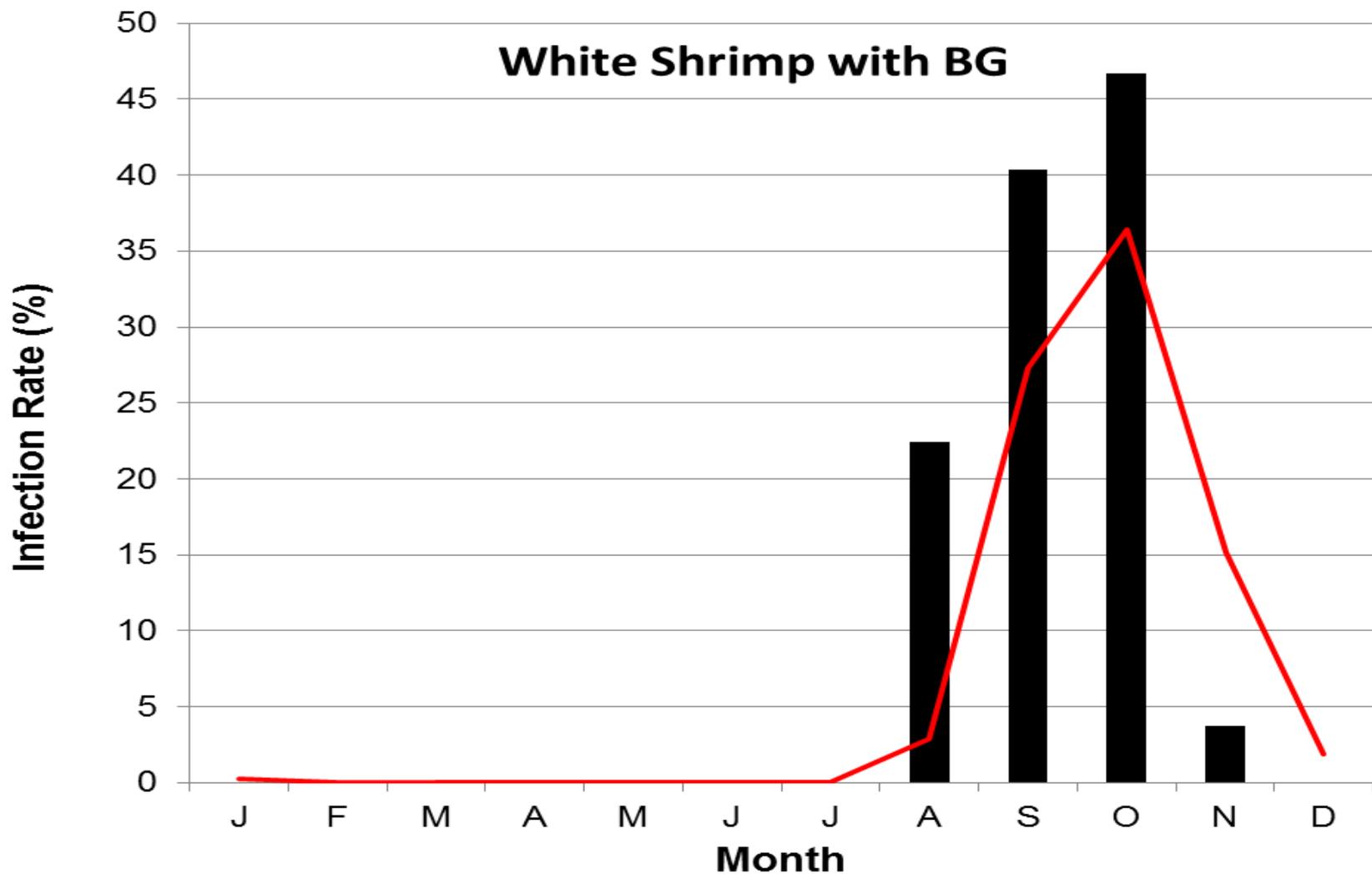




# BG Infect Rate by Sound System and Month 1996-2013



# White Shrimp with BG



■ 2013      — Long Term (96-13)

# Crustacean Immune System

## Cellular Components, Hemocytes

- Phagocytosis
- Encapsulation
- Formation of nodules

## Humoral Components

- Anticoagulant proteins
- Agglutinins
- Phenoloxidase enzyme
- Antimicrobial peptides
- Free radical

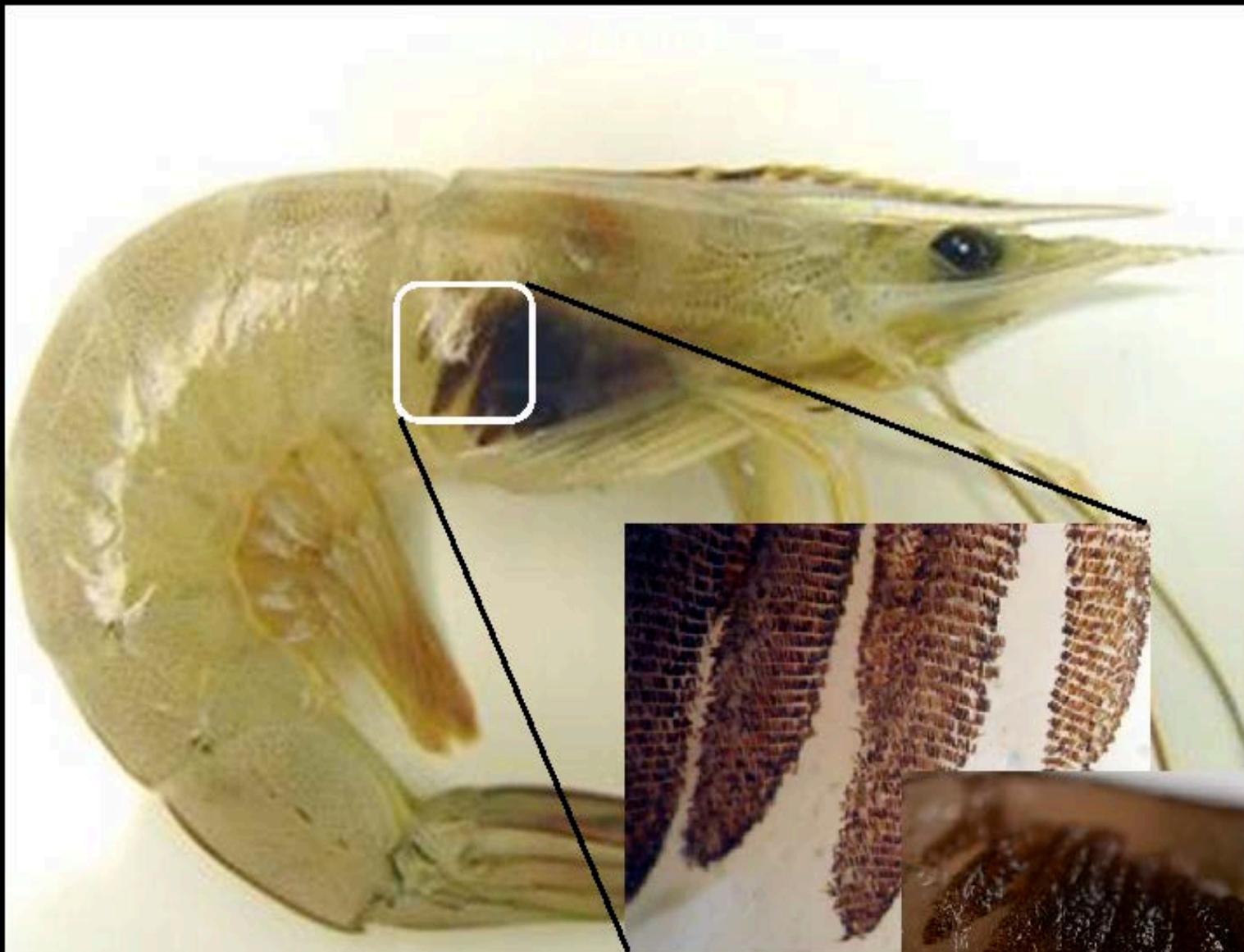
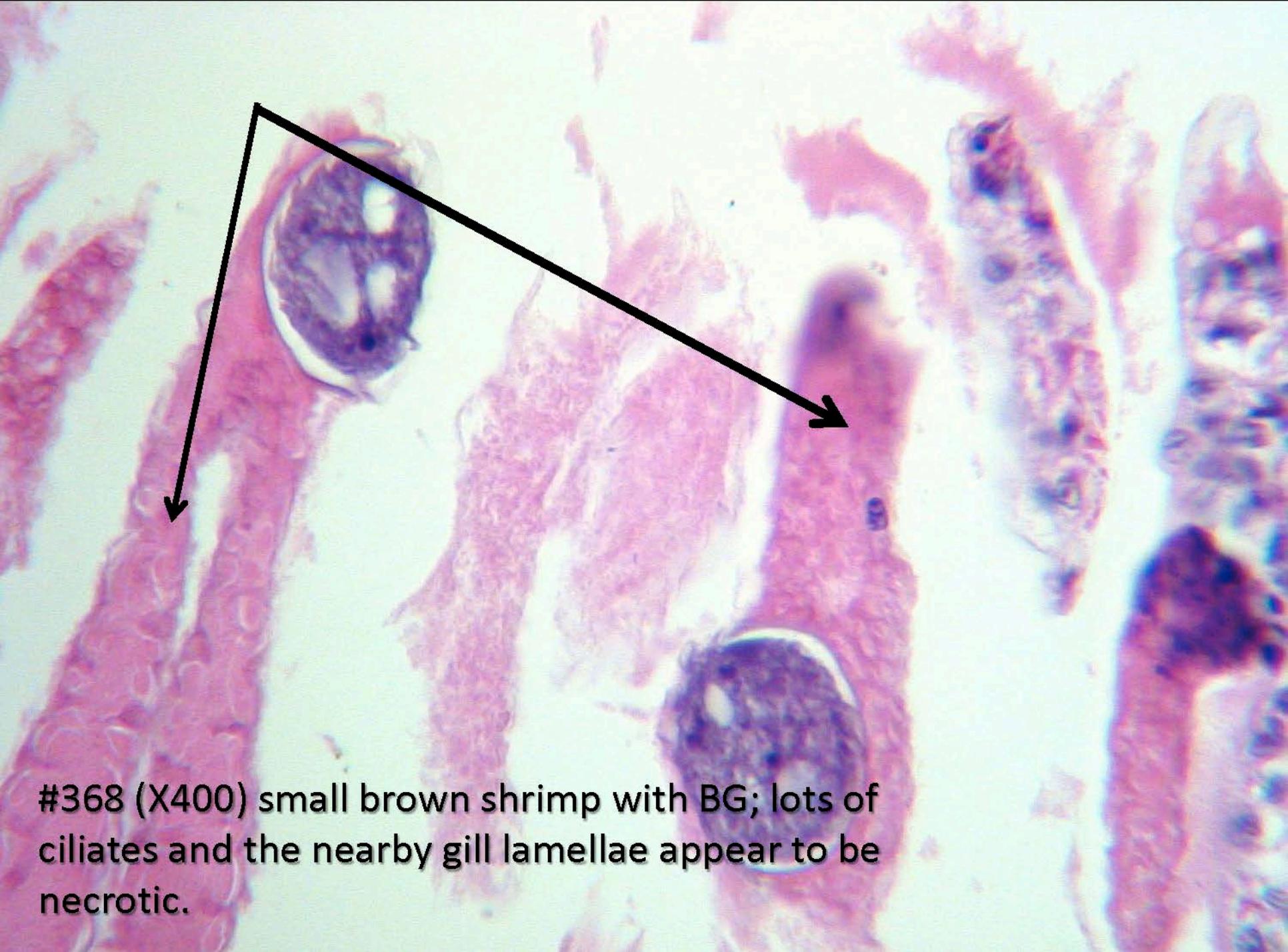


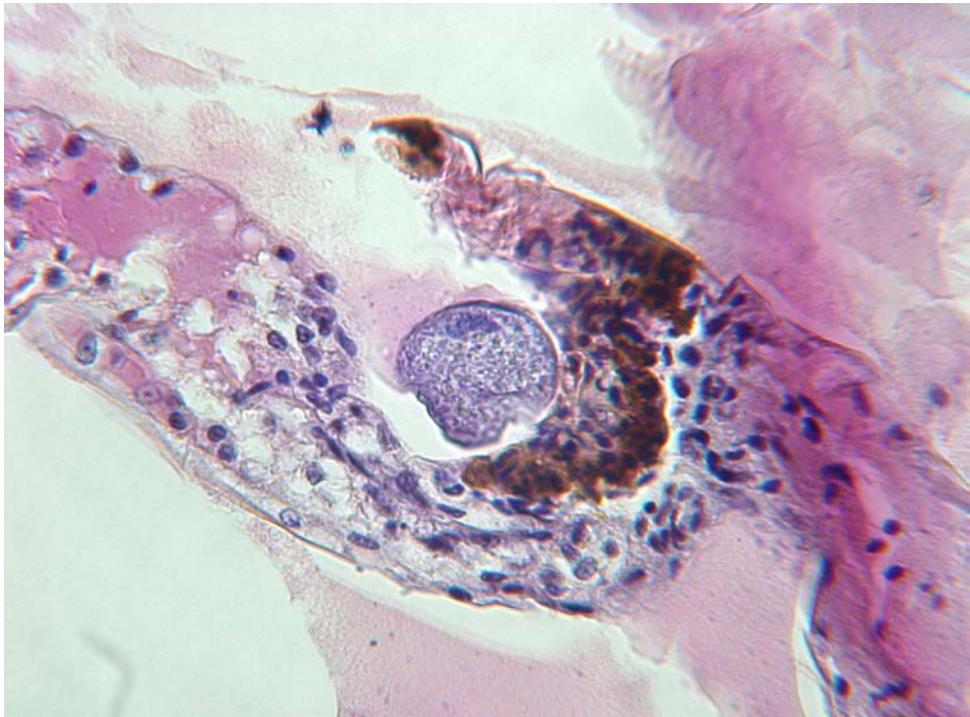
Figure courtesy of R. Overstreet (all rights reserved)

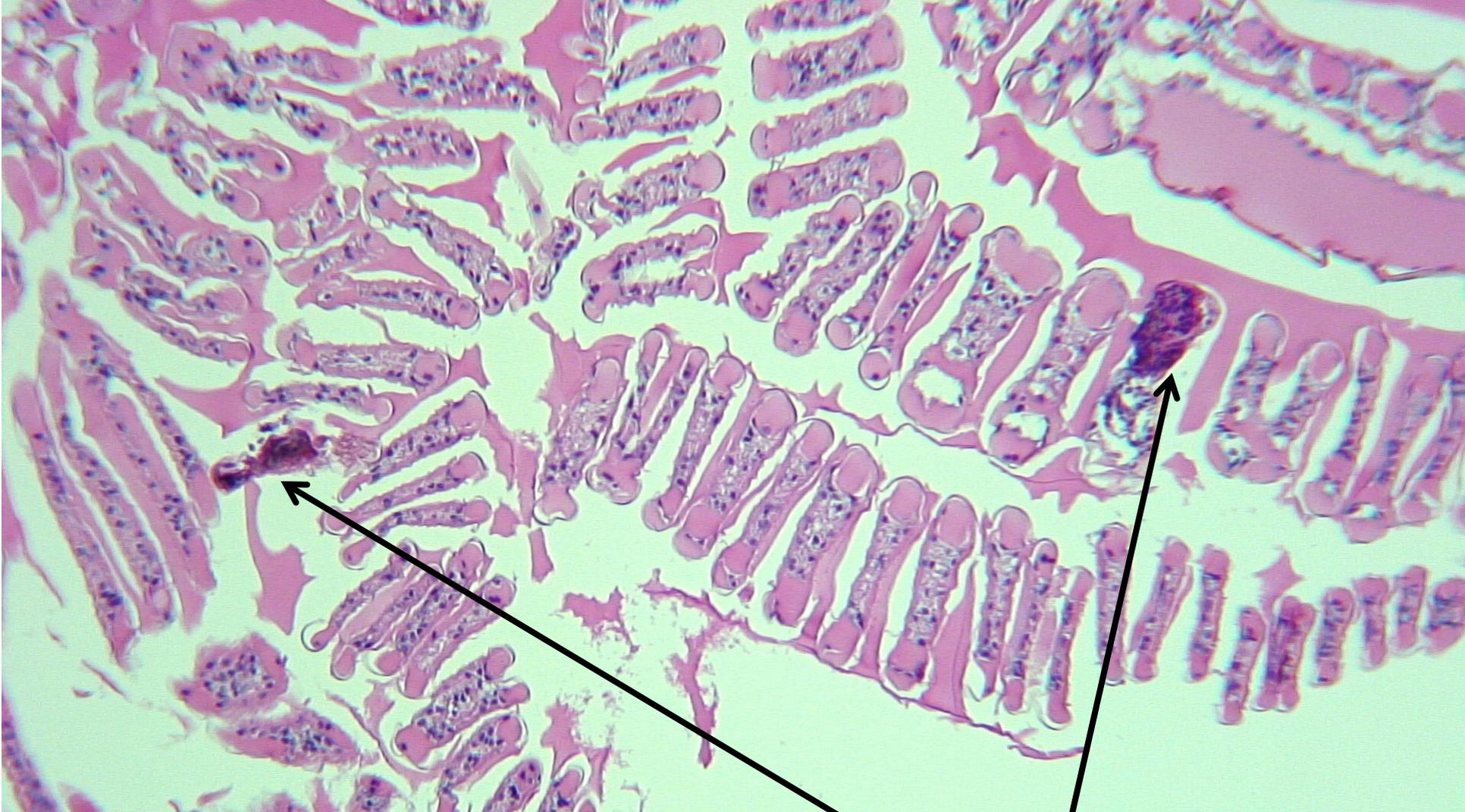


**Tissue Invasion?**



#368 (X400) small brown shrimp with BG; lots of ciliates and the nearby gill lamellae appear to be necrotic.



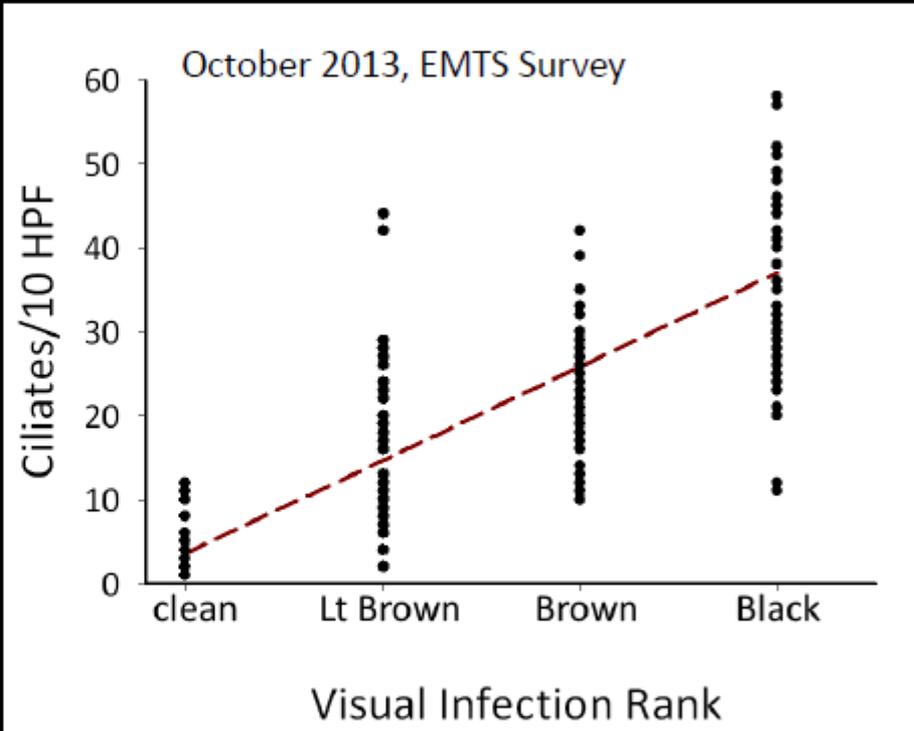


**Gill tissue from Shrimp # 370 (X100)**  
**Macroscopically the shrimp was free of disease, but there are a few nodules visible at the microscopic level. Does this mean a shrimp can harbor an asymptomatic infection that**

# Similar Appearing Organism Present in North and South Carolina

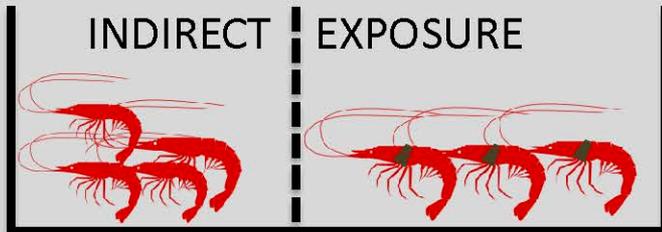


## Visual vs. Quantitative Histology



$$r^2 = 0.67, n = 183$$

# Transmission – Is BG Infectious?



## Exposure Study (Oct 2013)

Live white shrimp (*L. setiferus*) with & without BG collected from Wassaw Sound, GA 5 Oct 2012  
*Black Gill present (62%).*

Animals (ca. 15 per tank) were maintained in aquaria with running seawater & aeration

### Treatments

Control – BG-free shrimp only

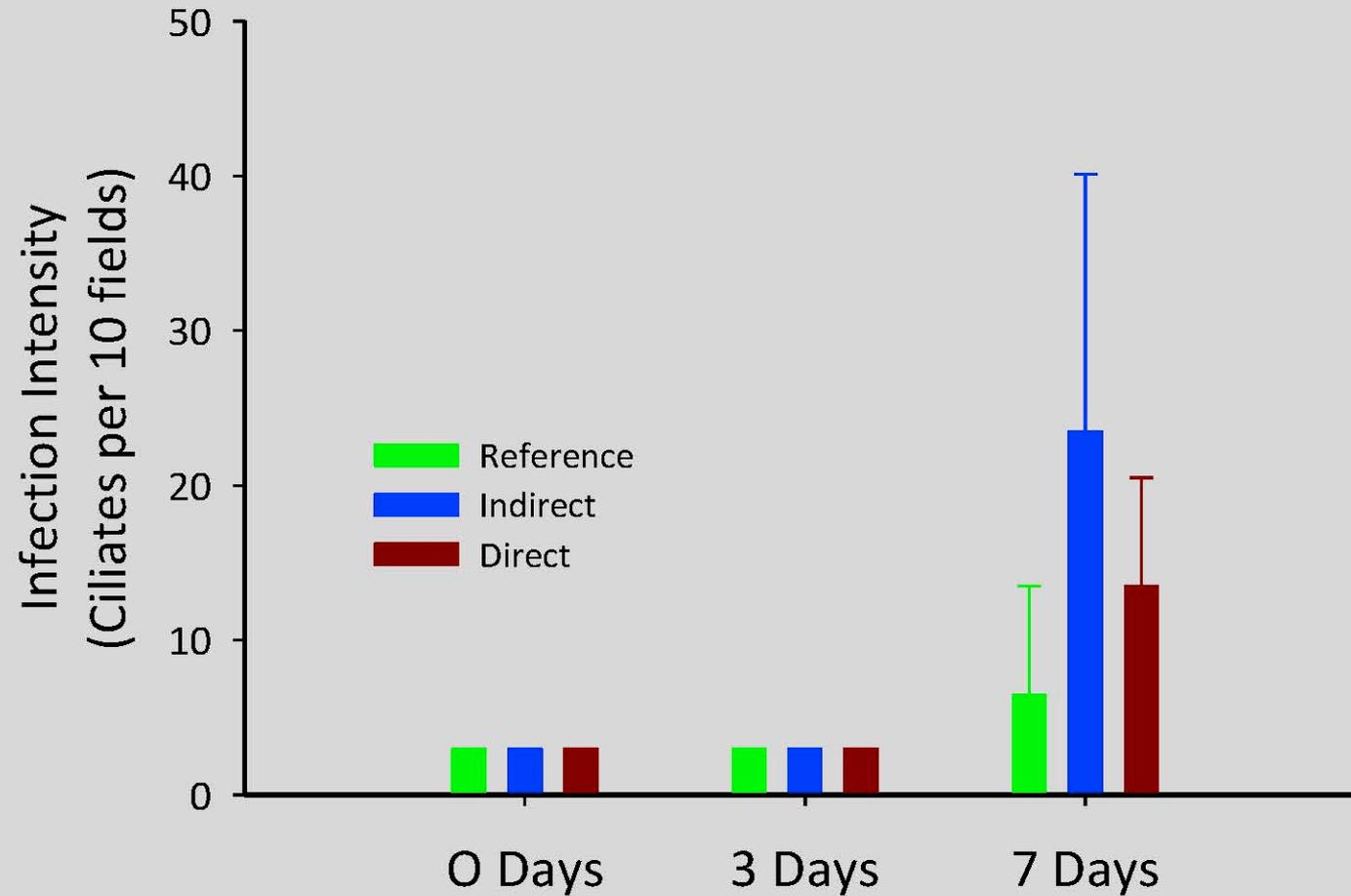
Direct – BG-free shrimp + 5 BG shrimp heads

Indirect - BG-free shrimp + 5 BG shrimp

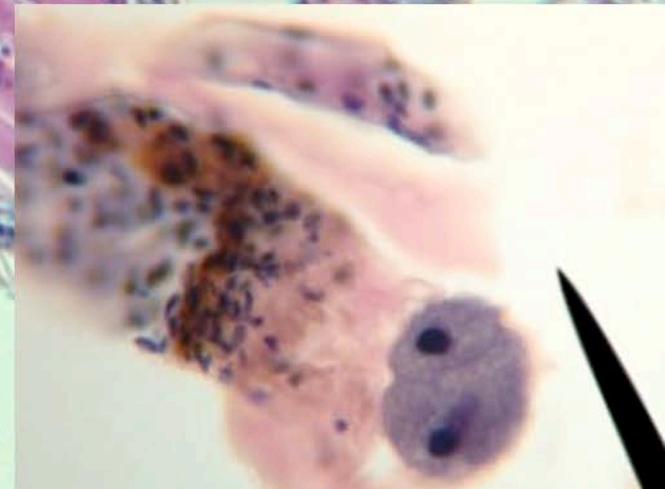
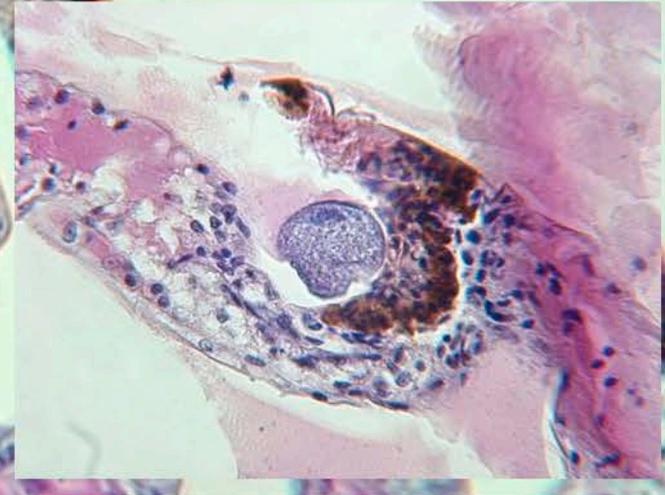
Sample (4) shrimp initially (T=0) and after 3 and 7 days

Visual and histological observation of BG

# Transmission – Is BG Infectious?



# What is Causing BG in Georgia Shrimp?



100 μm

# Apostomatia

## *Hyalophysa chattoni*



*Hyalophysa chattonii*  
in grass shrimp



# Molecular (18S rRNA)-Based Identification

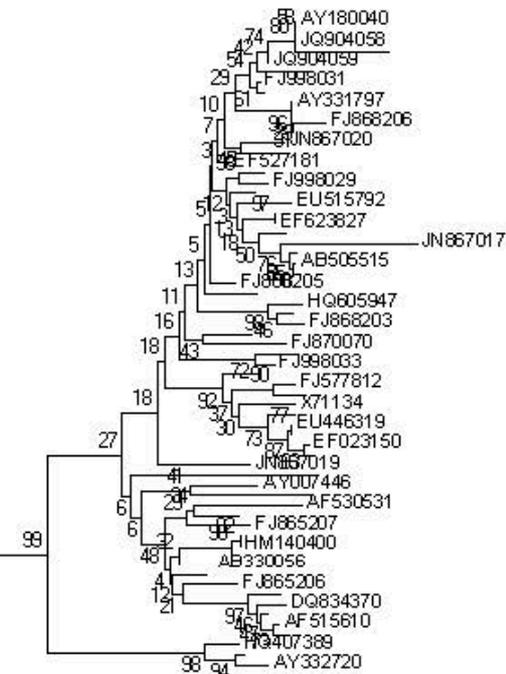
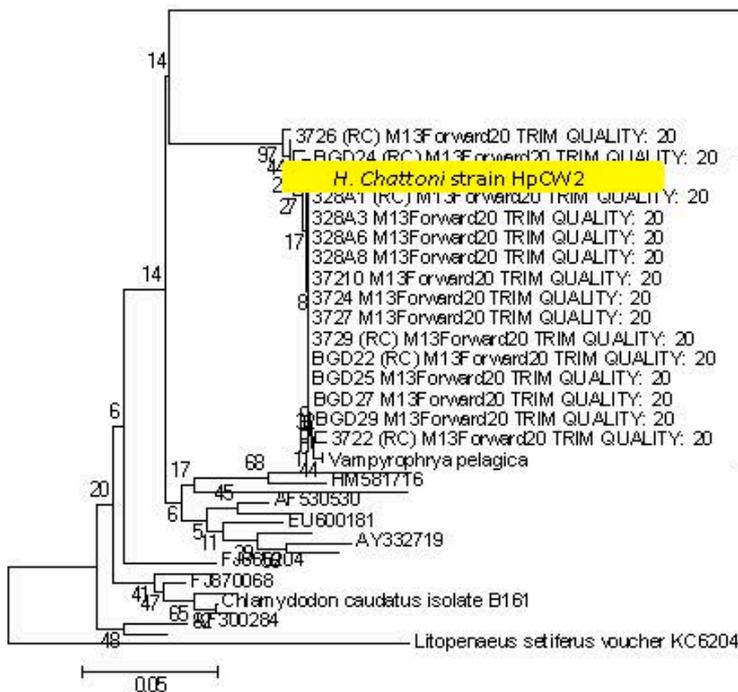
- “universal” ciliate PCR 18S rDNA targeted primers (~ 513 bp)
- 4 BG shrimp (2 severe white, 1 mild white, 1 severe brown)
- 31 ciliate sequences retrieved

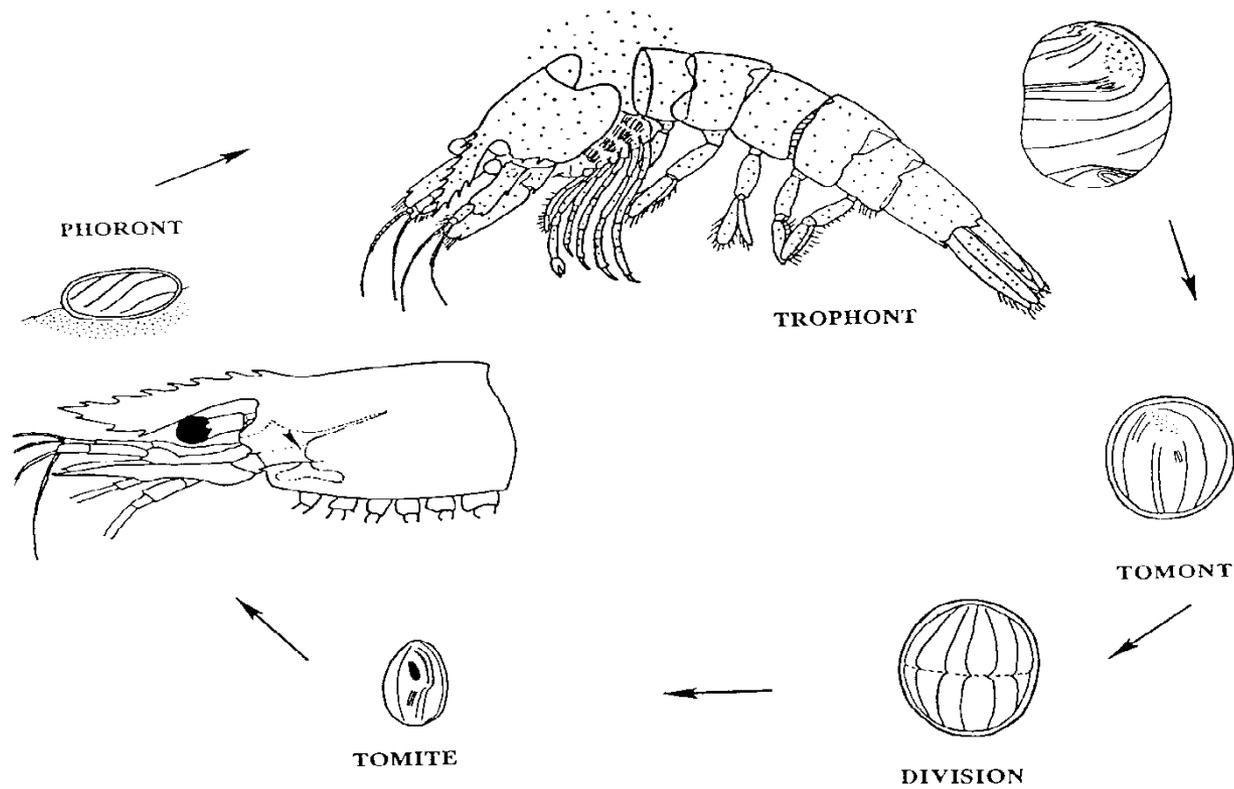
Phyllopharyngean Ciliates

GA Black Gill Shrimp

Apostomate Ciliates

31 of 31 ciliate sequences  
retrieved from 4 specimens





Apostomes have an encysted phoretic stage (the phoront) and at molting change to a structurally different feeding stage, the trophont. The trophont feeds on exuvial fluid in the molted exoskeleton and encysts into the tomont stage and then forms swimming tomite that searches out a new host.

# Causative Agent of Shrimp Black Gill?



*Chlamydodon* sp. swimming form. (Phyllopharyngean ciliate)  
Hongwei Ma, University of Akron



# SHRIMP BLACK GILL DISEASE IN GEORGIA

- Widely distributed, appears to be caused by the same disease agent.
- Causative agent still unknown, but likely the common Apostomate ciliate *Hyalophysa* sp. or an unknown Phyllopheryngean ciliate.
- Agent is infectious.
- Molecular diagnostics in development.
- Environmental triggers unclear (precipitation?).



YET ANOTHER BURDEN FOR A FISHERY IN PERIL



# Acknowledgments

Marc Frischer	SkIO -UGA
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