Diseases Affecting Commercial Crustacean Resources

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Disease Agents of Blue Crabs and White Shirmp

**VIRUSES**
Crabs – Rheolike and Rhabdolike viruses
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 perniciosa, Hematodinium perezi*
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 perniciosa* (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

- Yearly catch (millions of pounds, whole crabs)

- Hematodinium sp. detected in Georgia, 1997

- Paramoeba perniciosa
  Gray Crab Disease

Year

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 – plasmodial form
- b – dinospores
- c – hemocytes
Locations of Heavy Crab Mortality Due to Hematodinium Infection

Crabs caught in Wassaw Sound (1999 - 2004)

- Prevalence
- Intensity
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

- Molecular diagnostic techniques

Extremely sensitive and relatively simple, but requires *apriori* sequence knowledge (PCR) or specific antibodies
Detection sensitivity of *Hematodinium* by PCR technique

[Image of gel electrophoresis with bands at 187 bp for two locations: Wassaw Sound, GA.]

MW 1 2 3 4 5 6 7 MW

187 bp

Wassaw Sound, GA

MW 1 2 3 4 5 6 7 8 9 10 MW

187 bp
Molecular Approach 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

BG by Month (1996-2012)

Department of Natural Resources
Coastal Resources Division
Crustacean Immune System

Cellular Components, Hemocytes

- Phagocytosis
- Encapsulation
- Formation of nodules

Humoral Components

- Anticoagulant proteins
- Agglutinins
- Phenoloxidase enzyme
- Antimicrobial peptides
- Free radical
#368 (X400) small brown shrimp with BG; lots of ciliates and the nearby gill lamellae appear to be necrotic.
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

BG White Shrimp from Pamlico Sound, NC

BG White Shrimp from Charleston Harbor, SC.
Collected by South Carolina DNR (Aug 2013)
Visual vs. Quantitative Histology

October 2013, EMTS Survey

Ciliates/10 HPF

Visual Infection Rank

\[ r^2 = 0.67, \ n = 183 \]
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?

![Graph showing infection intensity over time and methods]

- **Infection Intensity (Ciliates per 10 fields)**
- **X-axis:** 0 Days, 3 Days, 7 Days
- **Y-axis:** 0 to 50
- **Legend:**
  - Reference
  - Indirect
  - Direct

The graph indicates a significant increase in infection intensity from 0 days to 7 days, particularly for the Indirect method.
What is Causing BG in Georgia Shrimp?
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
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?

*Chlamydodón sp.* swimming form. (Phyllopheryngean 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 Phyllophyseryngean ciliate.
- Agent is infectious.
- Molecular diagnostics in development.
- Environmental triggers unclear (precipitation?).

YET ANOTHER BURDEN FOR A FISHERY IN PERIL
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