

The frequency and distribution of genetic strains of *Hematodinium*, a parasitic dinoflagellate of the blue crab (*Callinectes sapidus*), along the Delmarva Peninsula, VA

Katrina M Pagenkopp, Jeffrey D Shields, Jie Xiao, Terrence L Miller, Hamish J Small, Allen R Place, and Kimberly S Reece

The Host: *Callinectes sapidus*

The American blue crab: *Callinectes sapidus*

Euryhaline

Omnivorous: bivalves, crustaceans, worms, fish, snails, plants, and detritus

Range: Nova Scotia to Brazil

Major fisheries: USA eastern seaboard and the Gulf of Mexico



Photo: A. Huntley



nationalgeographic.com

The Parasite: *Hematodinium*

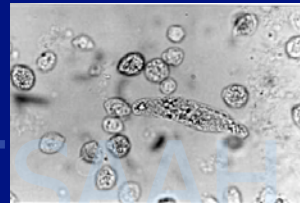
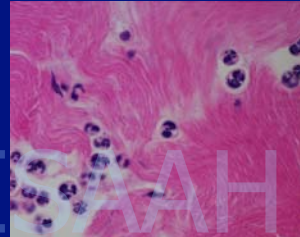
Parasitic dinoflagellate from the Family Syndinidae

Newman and Johnson (1975): first reported *Hematodinium* infections from North Carolina, Georgia, and Florida

Experimentally infected blue crabs live ~40-55 days post-inoculation

Higher prevalence at higher salinities and temperatures

Hematodinium reported from various US states with economically important fisheries from New Jersey to Texas



Both photos: J. Shields

Proposed Life Cycle of *Hematodinium*

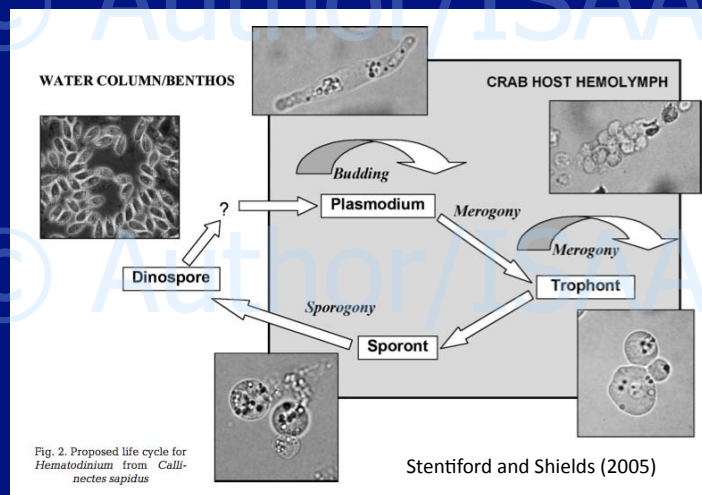
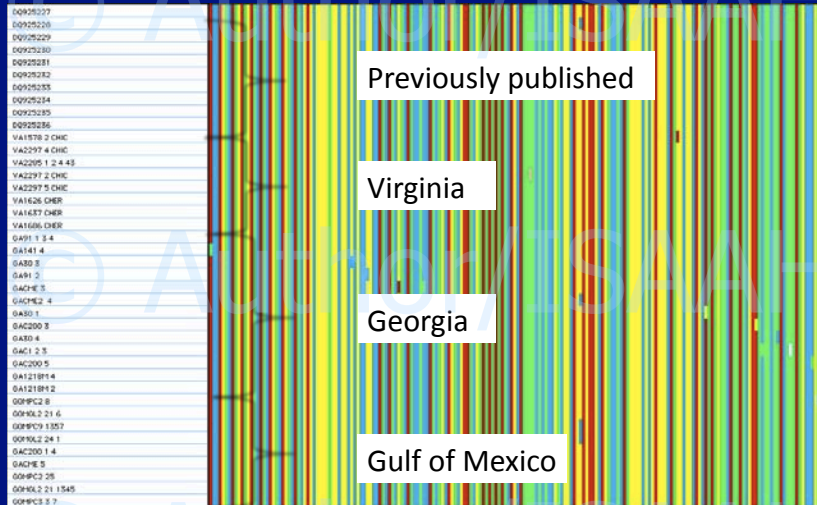


Fig. 2. Proposed life cycle for *Hematodinium* from *Callinectes sapidus*

Stentiford and Shields (2005)

Ploidy? Sexual Reproduction? Multiple Infections?

A single *Hematodinium* species

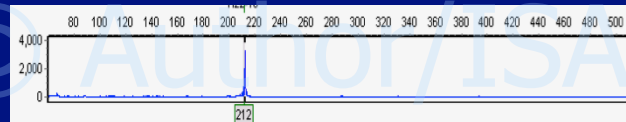


Hematodinium from infected blue crabs collected from VA and the Gulf of Mexico. Georgia blue crab samples from Marc Frischer and Richard Lee (SKIO).

Microsatellites for *Hematodinium*

Microsatellites = simple sequence repeats, usually selectively neutral, high mutation rates, and distributed throughout the nuclear genome

```
GTGAAAAGGAAGACTGGTTGATGAAAACCGAAAATGAATATAACGCACTGCTGGAAGATT
TAGCATTGACATATAATGTATAGATTTTTTGGATTGTGTGTGTGTGTGCGTCAGAAAAC
AAAAACCTATCAACTGGTAGAAAAAGCCGAAGTCACCCGTCCCGTCGCGTCAGCTCTGGT
```



Developed microsatellite library with non-clonal *Hematodinium* culture created from infected blue crab collected from Wachapreague, VA

What we expected to find...

After screening >100 colonies, found 24 microsatellite sequences

Created primers for and optimized 12 microsatellite markers

Genotyped *Hematodinium* from infected blue crabs collected from six sites along the Delmarva Peninsula, VA

Total number of samples collected (2008-2009): 286

Total number of samples analyzed: 227

38 of the 59 (64%) samples excluded were light infections or the host was dead

Of the 12 microsatellite markers:

8 were polymorphic

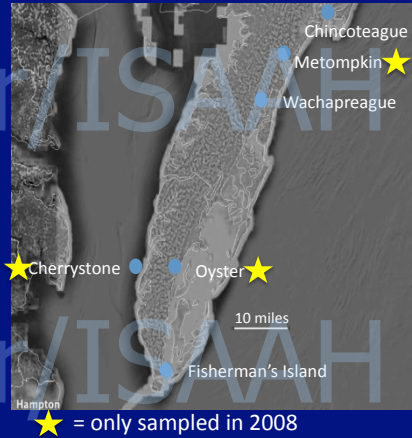
4 were monomorphic

Samples with 1 allele at every locus: 58%

Samples with 2 alleles at some loci: 20%

Samples with 3 or more alleles at some loci: 22%

****Up to 7 alleles at a single locus!!!**



Results: Allele frequencies

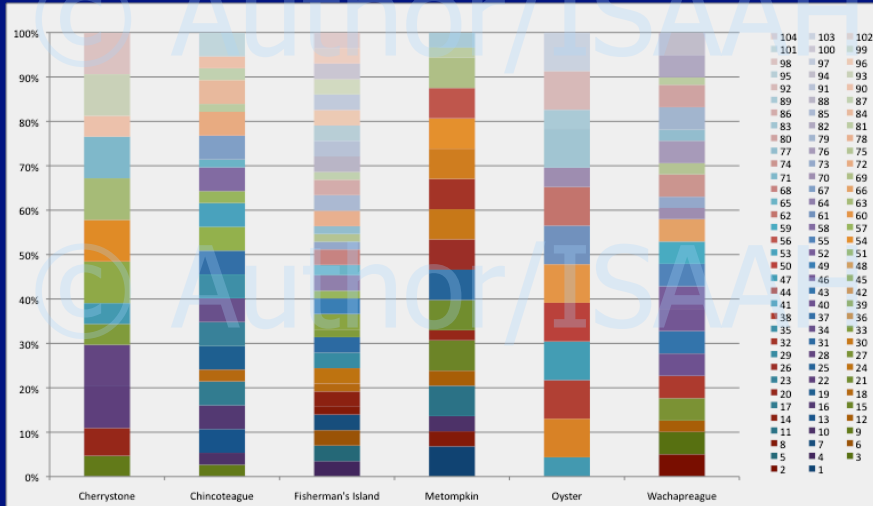
Most of the alleles appear at all sites, indicating some mixing among all sites

Private alleles

- Wachapreague = 4 (blue)
- Metompkin = 2 (red)
- Chincoteague = 2 (orange)
- Fisherman's Island = 1 (green)
- Oyster = 1 (purple)
- Cherrystone = 1 (yellow)



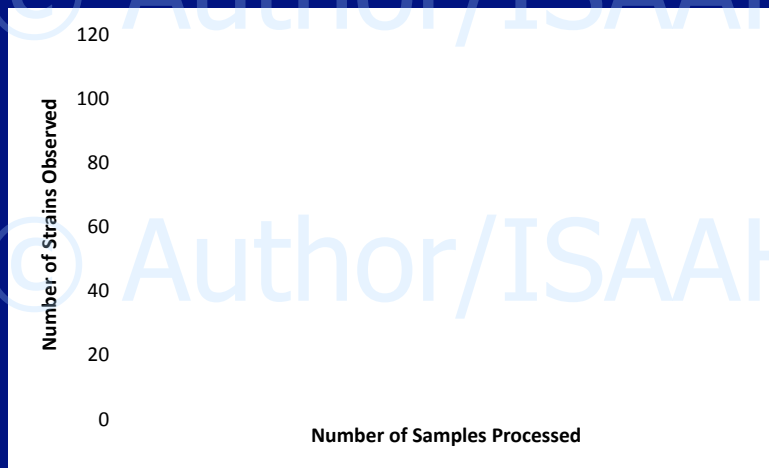
Results: Genotype frequencies



104 total multi-locus genotypes observed: 81 (78%) are unique

Fisherman's Island had the most MLGs and the most unique MLGs, but Oyster had the highest percentage of unique MLGs

Results: Saturation Curve



Apparently, we have only scratched the surface...

Conclusions

- ◆ *Hematodinium* appears to be haploid in the host.
- ◆ The frequency of multiple infections in VA waters is relatively high (42%).
- ◆ Distribution of alleles along the Delmarva Peninsula shows both mixing between sites (as same alleles are seen in all sites) and probably isolation by site (as all sites have at least one private allele).
 - ◆ Private alleles artifact of sampling?
- ◆ High level of genotypic variation is comparable to what has been reported in free-living dinoflagellate species and indicates a sexually reproducing life stage.
- ◆ Ongoing work is comparing these results to *Hematodinium* microsatellite data obtained from alternate hosts and blue crabs from other geographic areas.

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Sample collection

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Shields Lab: Kersten Wheeler, Dr. Caiwen Li, Dr. Jessica Moss-Small, Tom Dolan, Anna Huntley, Peter Coffey, Kelly Delaney

Sample processing

Reece Lab: Gail Scott, Alanna MacIntyre, Georgetta Constantin, Dr. Jan Cordes

Data Analysis

Dr. Jan McDowell