

Hematodinium and the Collaboration with the National Park Service at Assateague Island Seashore



Hematodinium sp.

- Infects numerous crustaceans in a worldwide distribution
- Invades hemolymph of the host and consumes host metabolites
- Concurrent tissue damage
- Heavy infections lead to mortality

Vol. 84: 79–87, 2009 doi: 10.3354/dao02027	DISEASES OF AQUATIC ORGANISMS Dis Aquat Org	Published March 9
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Real-time PCR-based assay for quantitative detection of *Hematodinium* sp. in the blue crab *Callinectes sapidus*

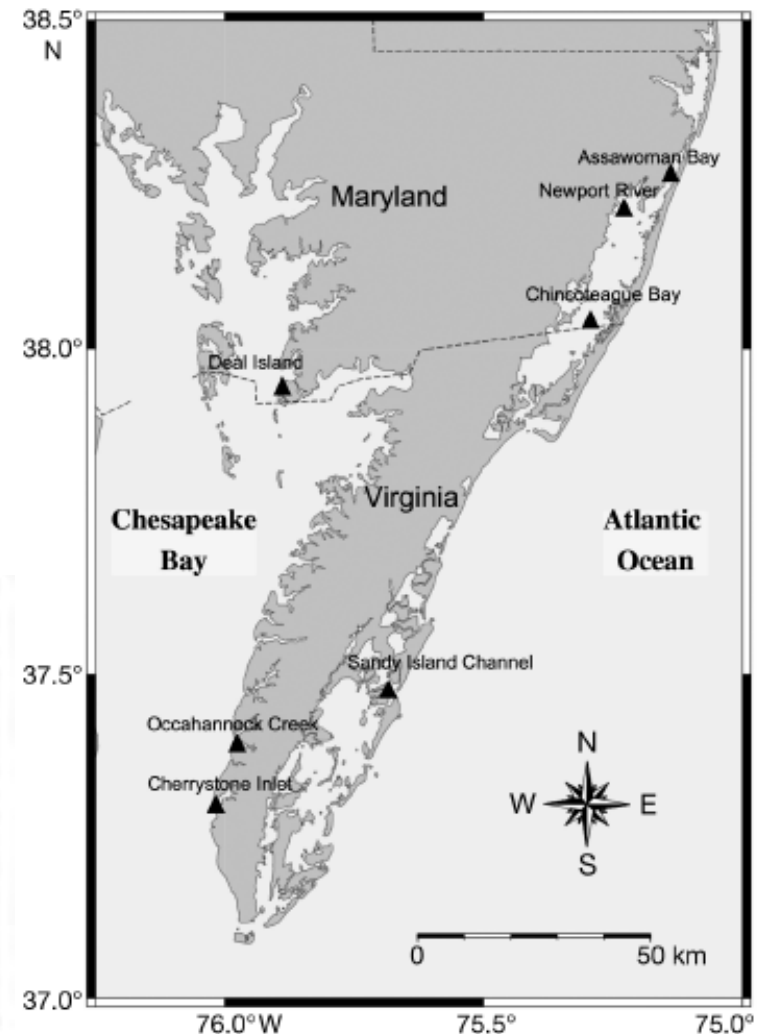
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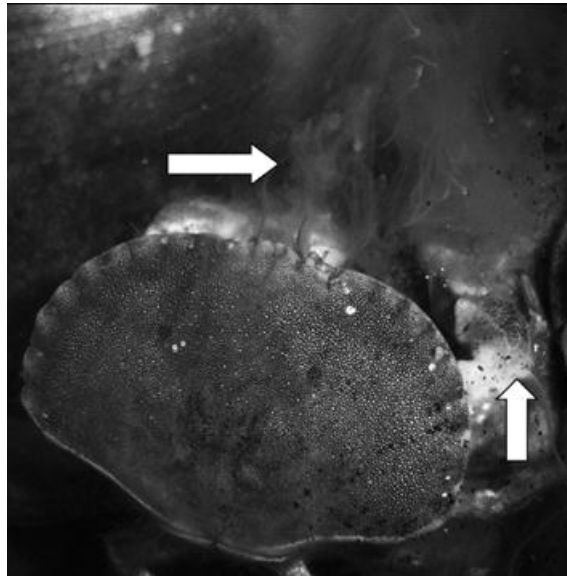
Sample site_date (mo/d)	Crab sex	Crab size (cm)	Slide parasite/hemocyt	qPCR, as cell no. equiv. per 4 ng DNA				
				Hemolymph	Muscle	Heart	Hepatopancreas	Gill
AB1_7/15	F	8.9	–	0.066	–	0.013	–	0.088
AB3_7/15	F	8.9	–	1.205	–	0.713	0.002	0.342
AB8_7/15	F	13.4	–	0.002	–	–	–	–
AB10_7/15	M	13.7	–	2.570	–	0.383	0.008	0.798
SIC2_10/4	F	11.6	–	0.030	0.007	0.003	0.001	0.063
SIC4_10/4	F	10.8	–	–	–	–	–	0.002
SIC6_10/4	F	14.6	1.13	0.001	0.383	0.045	0.018	0.040
SIC7_10/4	M	10.8	0.06	–	31.395	0.038	0.088	0.025
SIC9_10/4	M	14.6	2.03	0.026	0.273	–	0.138	0.602
AB6_10/19	F	13.3	–	3.131	0.218	0.453	0.014	4.598
AB7_10/19	M	14.0	–	0.132	0.305	0.053	0.003	0.003
AB8_10/19	F	9.5	1.68	–	0.164	–	0.013	0.508
CB4_10/27	M	11.4	–	0.135	–	0.004	0.010	–
CB7_10/27	M	9.9	0.02	–	0.040	–	0.009	–
CI4_11/1	M	9.5	–	–	–	0.007	–	0.093
CI5_11/1	F	14	–	0.206	0.010	0.059	0.002	0.088
CI8_11/1	F	12.7	0.01	0.157	0.005	–	0.002	–
CI9_11/1	F	13.3	–	0.001	–	0.013	–	–



Major Ecological Questions in the Field

- a) How many species exist in nature, and what is their geographical range?
- b) How do crustaceans become infected?

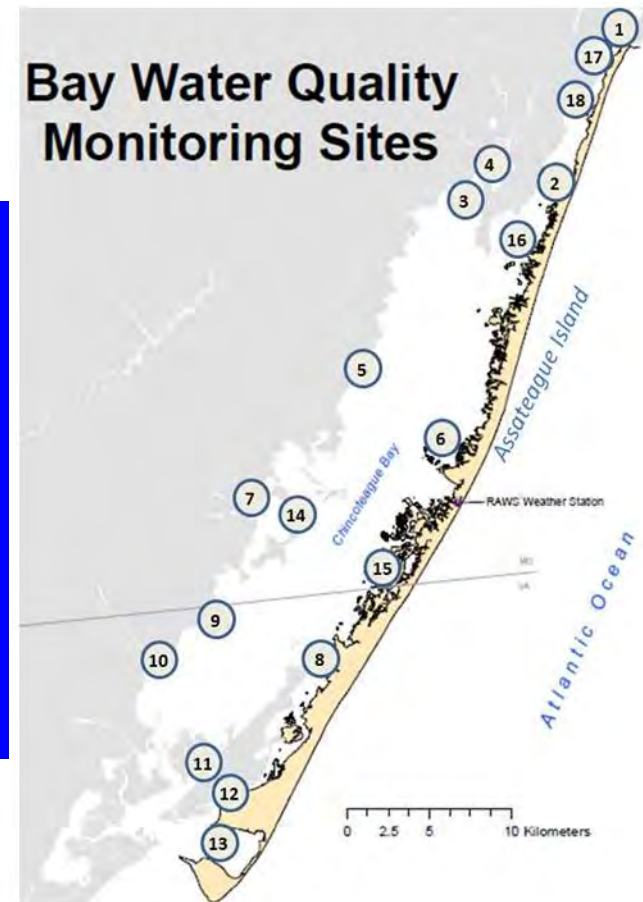
Sporulation Event in *Cancer pagurus*



Dr. Sue MARRS in Stentiford and Shields *DAO*,
66: 47-70, 2005

<http://www.vims.edu/~jeff/biology/stentiford%20and%20shields%202005.pdf>

Searching for Environmental Reservoirs



- Collaboration with National Park Service in search of *Hematodinium* sp. reservoirs in water and sediment samples.
- Historically the coastal bays have shown high infection rates.





Procedures



YSI



Ponar **Grab**



20 micron plankton trawl



DNA Isolation kits

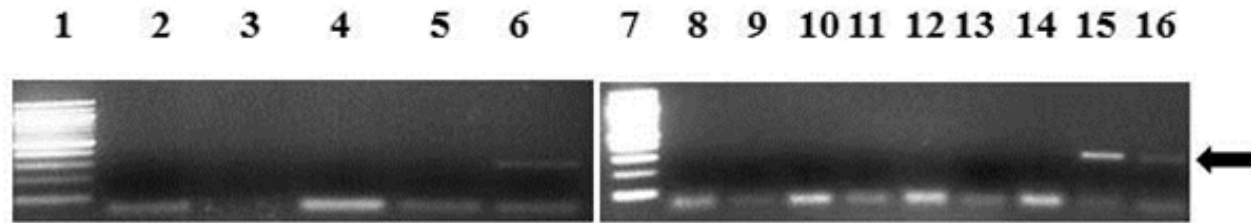


traditional PCR

QPCR



Endpoint PCR Analysis for Environmental Detection



		Water Collection Dates	Sediment Collection Dates
Site 1	Commercial Harbor	7_10	8_11
Site 2	Verrazano Bridge	9_10	4_10; 5_10; 7_10; 8_11
Site 3	Newport Bay	* 6_10; 9_10	6_10; 8_11
Site 4	Trappe Creek	4/10; 7/10	8_11
Site 5	Public Landing	6_10; 9_10	5_10; 8_11
Site 6	Whittington Point		8_10; 8_11
Site 7	Taylor's Landing	4/10; 6/10; 7/10	
Site 8	Wildcat Point		8_10; 8_11
Site 9	Greenbackville	6_10	8_11
Site 10	Sinnickson	* 4_10; 6_10; 7_10; 8_10; 10_10	5_10; 7_10; 8_10; 10_10; 11_10
Site 11	Chincoteague Channel	7_10	
Site 13	Tom's Cove	* 6_11	8_10; 10_10; 8_11
Site 14	Johnson's Bay	4_10; 6_10	
Site 15	Cedar Island	7_10	
Site 18	Snug Harbor	10_10	4_10; 6_10

Pitula, Dyson, Bakht, Njoku, and Chen. *Aquatic Biosystems* 8:16, 2012.

- 48 of 546 (8.8%) of environmental samples from the Maryland and Virginia coastal bays were positive for *Hematodinium* sp.

Sequence Analysis of *Hematodinium* sp. Clones

	Identical Sequence	Percentage
Sinnickson		
p/w April 2010	13/16	81%
Sed August 2010	3/3	100%
Sed November 2010	25/27	93%
Tom's Cove		
p/w June 2010	12/14	86%
Sed August 2010	16/20	80%
Sed November 2010	25/36	69%
Newport Bay		
p/w June 2011	5/5	100%
Sed June 2010	10/12	83%
Total	109/131	83%

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+
Caap
TC810a
TC1110a
TC1110b
TC1110c
CATCCACGTTTGCITTTCCATAAACAACAACATCTCT--AATTTAGCTATTTCATCTTGCTCTGCT
CATCCACGTTTGCITTTCCATAAACAACAACATCTCT--AATTTAGCTATTTCATCTTGCTCTGCT
CATCCACGTTTGCITTTCCATAAACAACAACATCTCT--AATTTAGCTATTTCATCTTGCTCTGCT
CATCCACGTTTGCITTTCCATAAACAACAACATCTCT--AATTTAGCTATTTCATCTTGCTCTGCT
CATCCACGTTTGCITTTCCATAAACAACAACATCTCT--AATTTAGCTATTTCATCTTGCTCTGCT
CATCCACGTTTGCITTTCCATAAACAACAACATCTCTCTAATTTAGCTATTTCATCTTGCTCTGCT

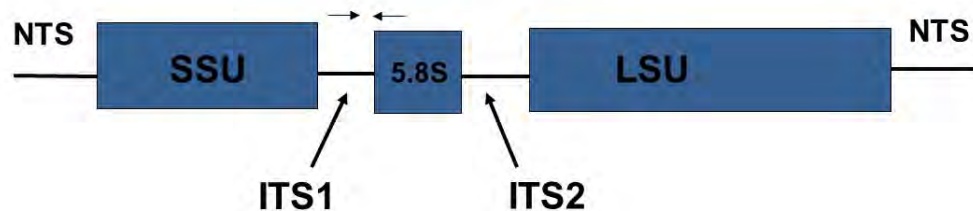
+
Caap
TC810a
TC1110a
TC1110b
TC1110c
CCCTTTTCGCGGGGATAGGGCTTTCTTCAAACGATGACTAGAAAAATTTAGCGATGAATGCCTCG
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CCCTTTTCGCGGGGATAGGGCTTTCTTCAAACGATGACTAGAAAAATTTAGCGATGAATGCCTCG

+
Caap
TC810
TC1110a
TC1110b
TC1110c
GCTCGGTTACGGTGAAGGACGCAGCGAATTCGATTAAGCAATGCGAATTGCAGAATTCGGTGAA
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GCTCGGTTACGGTGAAGGACGCAGCGAATTCGATTAAGCAATGCGAATTGCAGAATTCGGTGAA

+
Caap
TC810a
TC1110a
TC1110b
TC1110c
TCATCAGATTTTGAACGTACTCTACGCTCTCGGGTATCCCTGGGAGCATGT
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TCATCAGATTTTGAACGTACTCTACGCTCTCGGGTATCCCTGGGAGCATGT
TCATCAGATTTTGAACGTACTCTACGCTCTCGGGTATCCCTGGGAGCATGT

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Ribosomal Gene Cluster



That the population is relatively homogenous ranging from April to November, at three distinct sites, supports a hypothesis that one species of *Hematodinium* is responsible for infections within the ecosystem.

	1	10	20	30	40	50	60	70	80	90	100
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----										
consensus	TCGCCTACCACTGAACCTCCATCCCACGTTTGCTTTCCATAAACACACACATCTCTAATTTTCAGCTATTTCATCTTGCTCTGCTCCCTTTTCGCGGGGATA										
2006clone	TCGCCTACCACTGAACCTCCATCCCACGTTTGCTTTCCATAAACACACACATCTCTAATTTTCAGCTATTTCATCTTGCTCTGCTCCCTTTTCGCGGGGATA										
2006gulfofnexico	TCGCCTACCACTGAACCTCCATCCCACGTTTGCTTTCCATAAACACACACATCTCTAATTTTCAGCTATTTCATCTTGCTCTGCTCCCTTTTCGCGGGGATA										
Consensus	TCGCCTACCACTGAACCTCCATCCCACGTTTGCTTTCCATAAACACACACATCTCTAATTTTCAGCTATTTCATCTTGCTCTGCTCCCTTTTCGCGGGGATA										
	101	110	120	130	140	150	160	170	180	190	200
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----										
consensus	GGGCTTTCTTCAAACGTATGACTAGAAAATTTTAGCGATGAATGCCTCGGCTCGGGTTACGGTGAAGGACGCAGCGAATTGCGATAAGCAATGCGAATTG										
2006clone	GGGCTTTCTTCAAACGTATGACTAGAAAATTTTAGCGATGAATGCCTCGGCTCGGGTTACGGTGAAGGACGCAGCGAATTGCGATAAGCAATGCGAATTG										
2006gulfofnexico	GGGCTTTCTTCAAACGTATGACTAGAAAATTTTAGCGATGAATGCCTCGGCTCGGGTTACGGTGAAGGACGCAGCGAATTGCGATAAGCAATGCGAATTG										
Consensus	GGGCTTTCTTCAAACGTATGACTAGAAAATTTTAGCGATGAATGCCTCGGCTCGGGTTACGGTGAAGGACGCAGCGAATTGCGATAAGCAATGCGAATTG										
	201	210	220	230	240	250	260	270	280	290	294
	-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----+-----										
consensus	CAGAATTCGCGAATCATCAGATTTTGAACGACTCTACGCTCTCGGGTATCCCTGGGAGCATGTCTGGTCTCAGCGTCTGTTCAA										
2006clone	CAGAATTCGCGAATCATCAGATTTTGAACGACTCTACGCTCTCGGGTATCCCTGGGAGCATGTCTGGTCTCAGCGTCTGTTCAA										
2006gulfofnexico	CAGAATTCGCGAATCATCAGATTTTGAACGACTCTACGCTCTCGGGTATCCCTGGGAGCATGTCTGGTCTCAGCGTCTGTTCAA										
Consensus	CAGAATTCGCGAATCATCAGATTTTGAACGACTCTACGCTCTCGGGTATCCCTGGGAGCATGTCTGGTCTCAGCGTCTGTTCAA.....										

+ : Consensus sequence of 2012

2006 Gulf of Mexico: Schott,E.J., Zimmerman,N. and Messick,G.A;

- The 2011 consensus sequence in the ITS1/5.8S region is 99.6% identical to a blue crab isolate from the coastal bays in 2006, suggesting that the same species is responsible for infection throughout this time period
- It was also identical to a 2006 isolate from the Gulf of Mexico



RESEARCH

Open Access

Temporal distribution of genetically homogenous 'free-living' *Hematodinium* sp. in a Delmarva coastal ecosystem

Joseph S Pitula^{1*}, Whitney D Dyson¹, Habibul B Bakht¹, Ihuoma Njoku² and Feng Chen²



RESEARCH

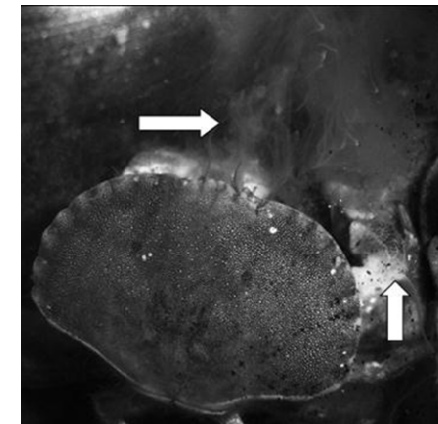
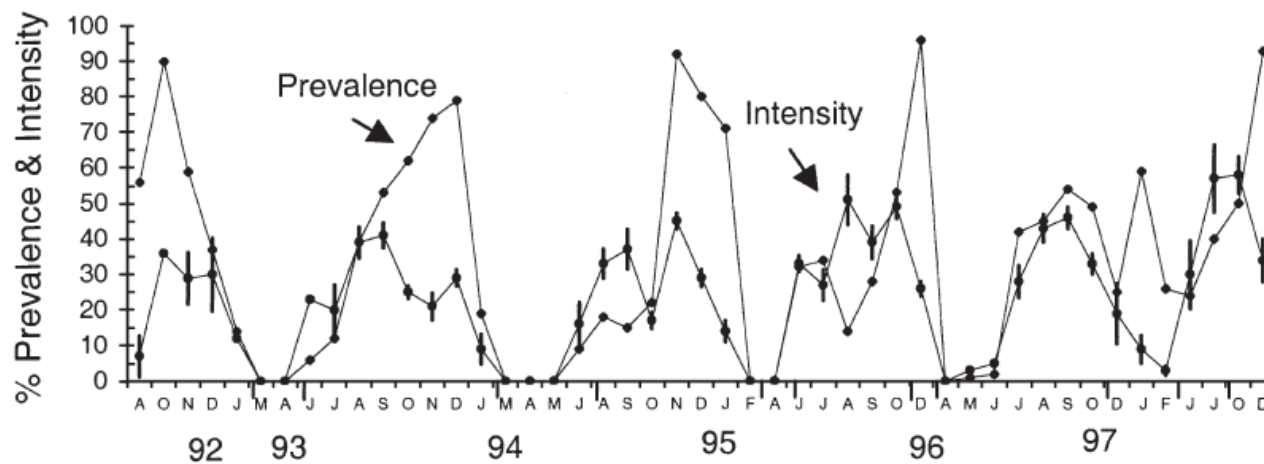
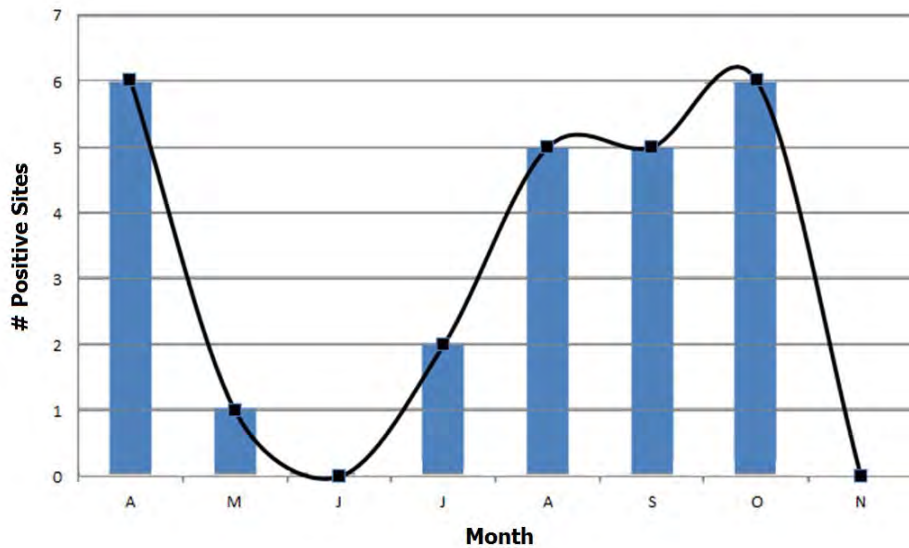
Open Access

Variation in spatial and temporal incidence of the crustacean pathogen *Hematodinium perezii* in environmental samples from Atlantic Coastal Bays

Ammar W Hanif¹, Whitney D Dyson², Holly A Bowers³, Joseph S Pitula², Gretchen A Messick⁴, Rosemary Jagus¹ and Eric J Schott^{1*}

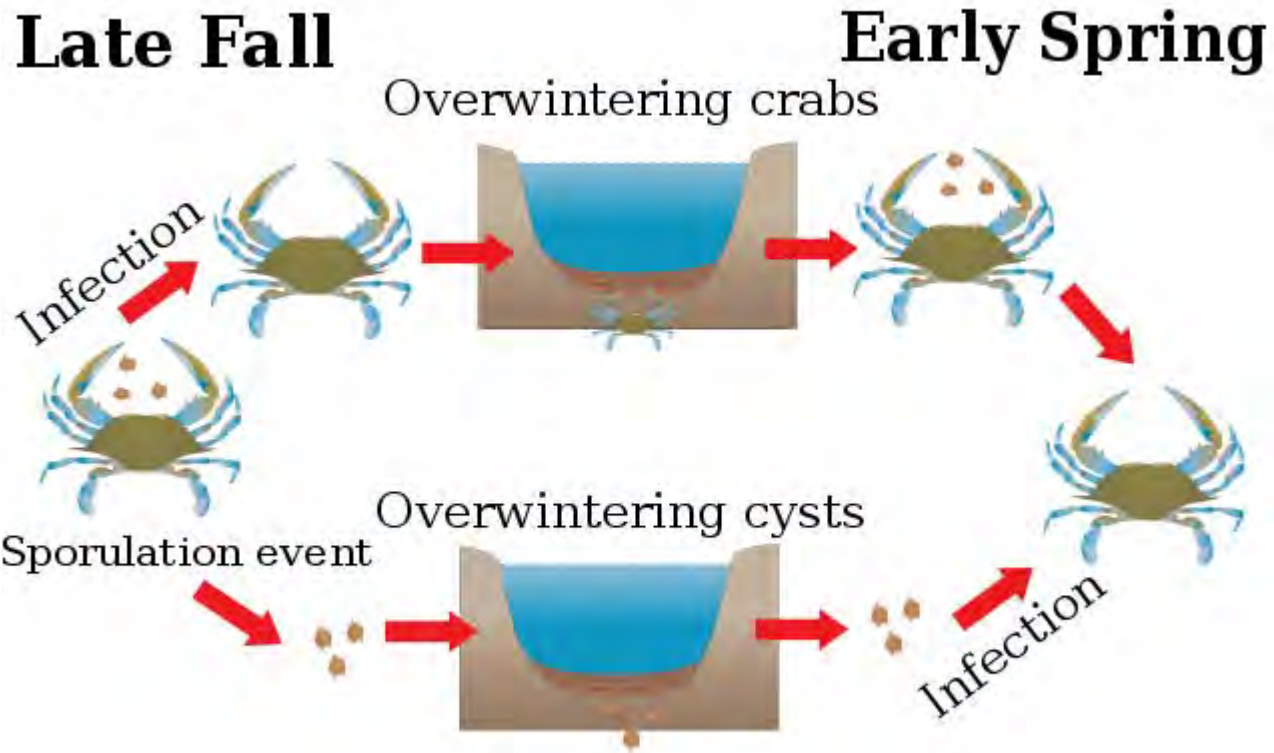
Current Observations Concerning Life Cycles and Disease

Detections in Water Column During 2012



Messick and Shields 1994, *Diseases of Aquatic Organisms*

Overwintering Conceptual Model





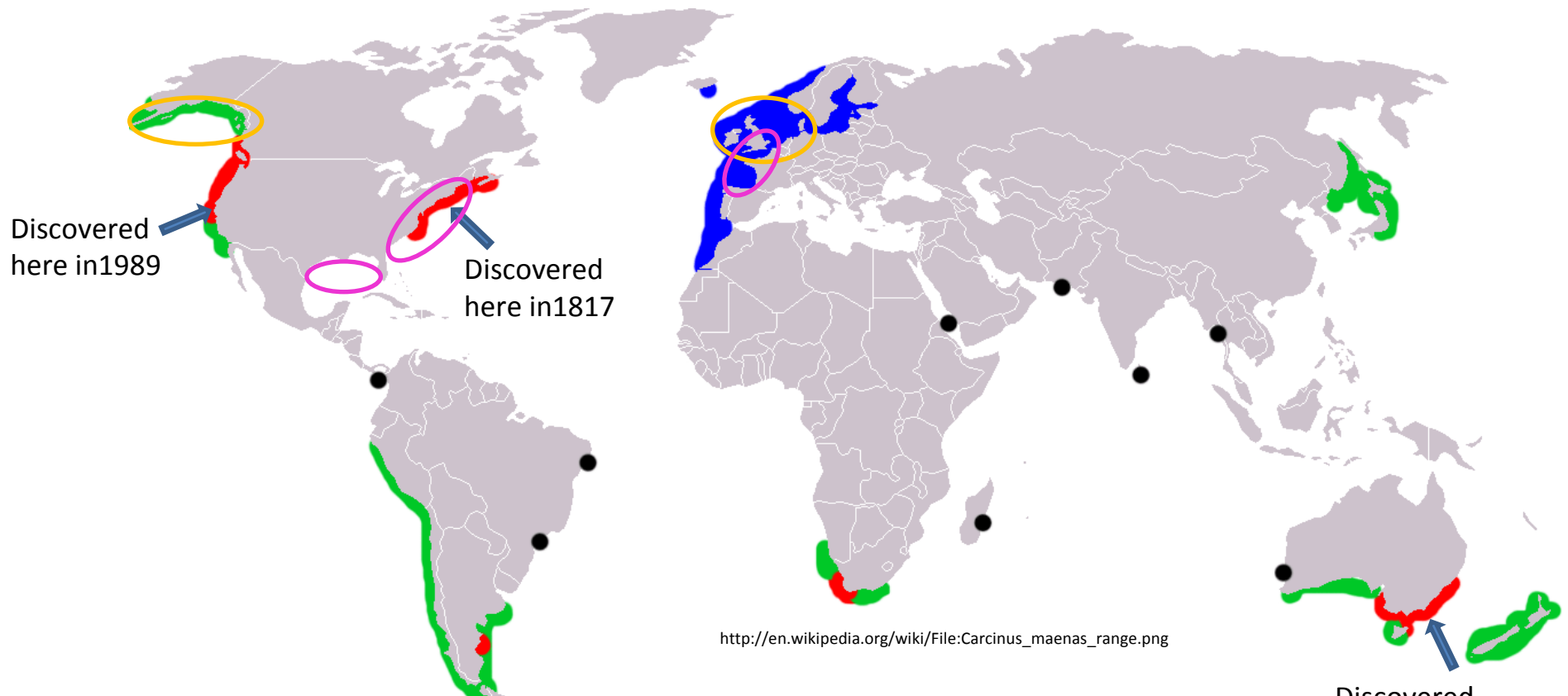
[\(ian.umces.edu/imagelibrary/\)](http://ian.umces.edu/imagelibrary/)

Table 2 Dinoflagellate species present in water samples from Sinnickson, VA in 2010

<i>Hematodinium</i>	Other species	
April	15/16	1 unidentified nanoflagellate
June	2/13	10 <i>Heterocapsa rotundata</i> , 1 <i>Peridinium sp.</i>
July/August	13/25	3 <i>Gymnodinium sanguineum</i> , 1 <i>Gymnodinium sp.</i> , 3 <i>H. rotundata</i> and 5 unidentified eukaryotic clones.
October	10/16	2 <i>Pentaparsodinium tyrrhenicum</i> , 1 <i>G. simplex</i> , 1 <i>Gymnodinium sp.</i> , 1 <i>H. rotundata</i> and 1 <i>Dinophyceae sp.</i>

Shown are sequencing results from analysis of libraries generated using dinoflagellate-specific 18 S rRNA.

Clade A: 
Clade B: 



- Blue areas are the native range of *C. maenas*
- Red areas are the introduced or invasive range
- Black dots represent single sightings that did not lead to invasion
- Green areas are the potential range of the species

Detection Using an ITS-2 QPCR-Based Assay

Variation in Temporal and Spatial Incidence of the Crustacean Pathogen *Hematodinium perezii* in Environmental Samples from Atlantic Coastal Bays

Aquatic Biosystems; under revision

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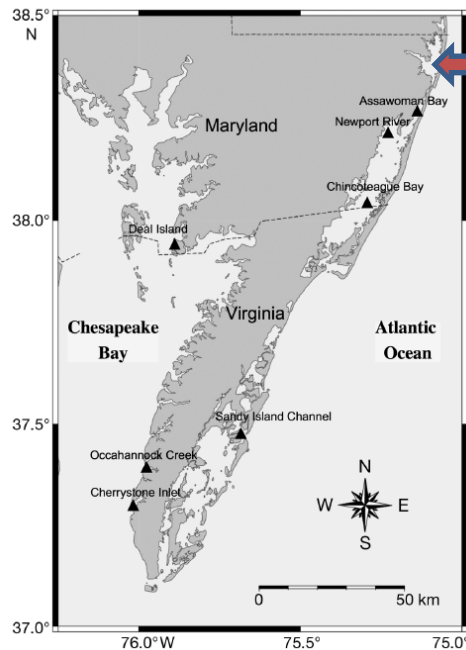
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Year	Species	N	% Prevalence
2008	<i>Callinectes sapidus</i>	19	79
	<i>Ovalipes ocellatus</i>	1	100
	<i>Carcinus maenas</i>	1	100
	<i>Palaemonetes pugio</i>	12	0
	<i>Ilyanassa obsoleta</i>	12	0
2009	<i>Orchestia grillus</i>	7	0
	<i>Palaemonetes pugio</i>	10	0



Isolated here

Ovalipes ocellatus: Lady crab; *Portunidae*
Carcinus maenas: Green crab; *Portunidae*

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UMCES-IMET

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