A fatal reovirus is highly prevalent in captive blue crab, *Callinectes sapidus*

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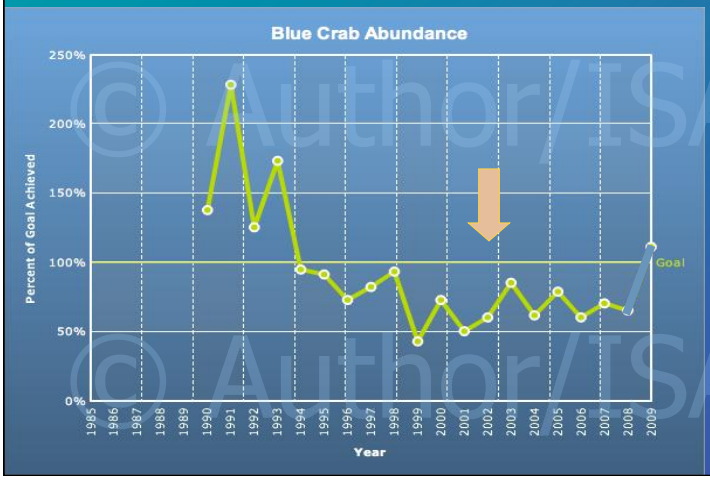
University of Maryland
CENTER FOR ENVIRONMENTAL SCIENCE
INSTITUTE OF MARINE & ENVIRONMENTAL TECHNOLOGY

Formerly known as COMB

Chesapeake Bay blue crab harvests

2002: Blue Crab Advanced Research Consortium established
- Hatchery technology
- Broodstock enhancement feasibility



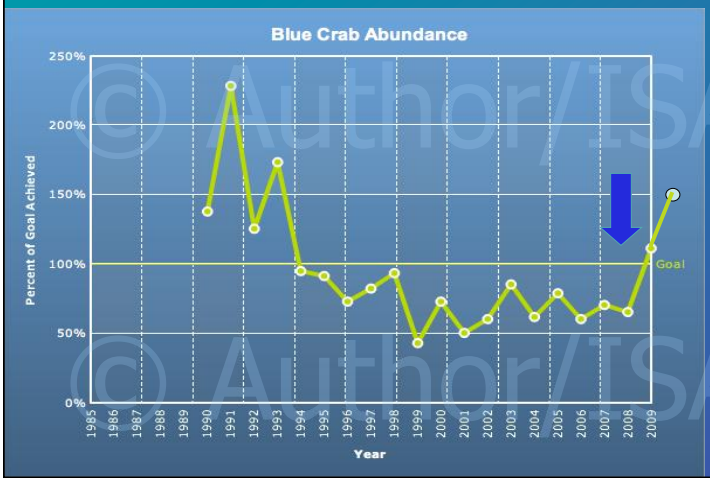
BCARC:

Funded by NOAA
- COMB (now IMET)
- SERC
- VIMS
- GCRL
- NC State
- NMFS, NCBO
- MD Watermen
- MD DNR
- Phillips Seafood

Chesapeake Bay blue crab harvests

2002: Blue Crab Advanced Research Consortium established

2008: Effective management
- MD, VA reduce harvest of females by 34%



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A blue crab hatchery for ecological and experimental studies

- **Wild broodstock**
- **Hatchery production used for:**
  - tag-release studies
  - pond aquaculture trials
  - physiology and genetics

- Disease management is crucial in aquaculture production. Viruses of particular concern.
- How can one anticipate potential viral pathogens?

Look for viruses in disease-prone aquaculture

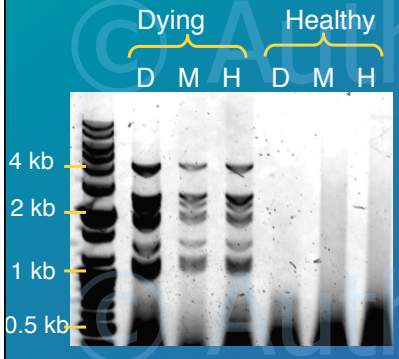
Soft shell crab production

- High mortality
- Wild harvest
- High density
- Molting stress
- Diverse settings and systems

Look for viral nucleic acid in soft shell mortalities

Detection of a blue crab reo-like virus by visualization of its dsRNA genome

double stranded RNA



reovirus schematic

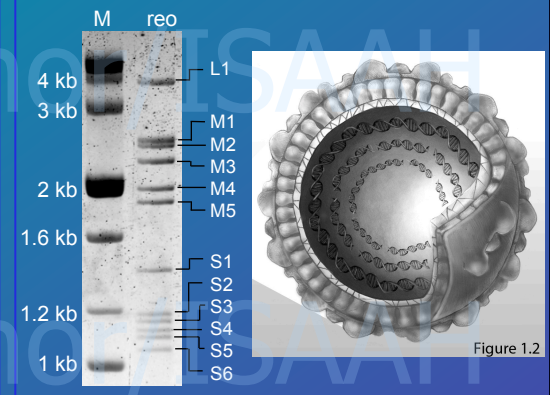
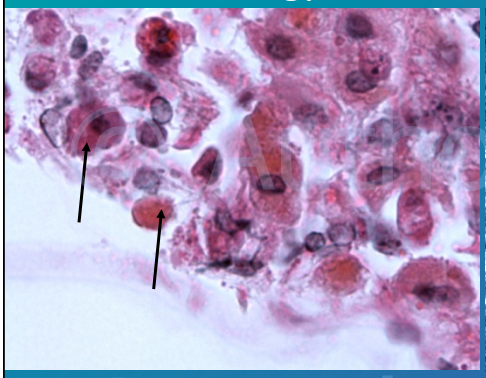


Illustration: Tara Rose, JHMI

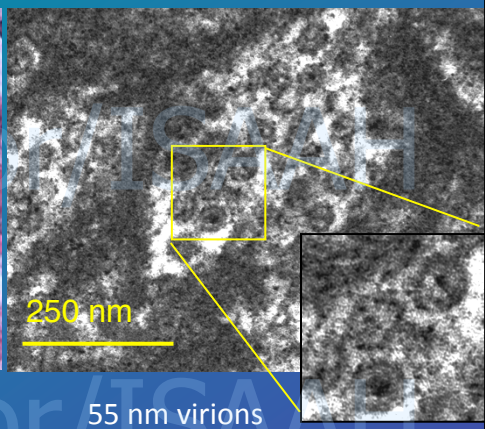
Microscopy of the putative reo-like virus

Histology



Cytoplasmic inclusions in gill

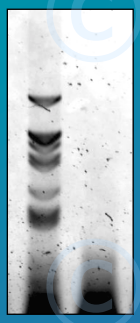
E.M.



55 nm virions in hemocytes

RLV dsRNA is associated with mortality in captive crabs

dsRNA assay



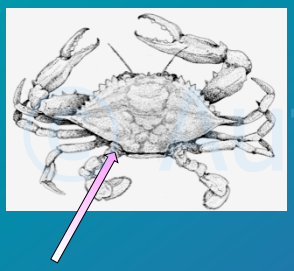
Prevalence of reovirus dsRNA in premolt and intermolt crabs

	No. tested	dsRNA+ %
soft shell systems*		
Live crabs	8	0
Dead crabs	41	73
2008 fresh harvest		
Inter-molt, live	42	0
Pre-molt, live	16	0
2008 hatchery		
COMB recirculating hatchery, dead	9	44

* Statistically significant association between dsRNA and mortality

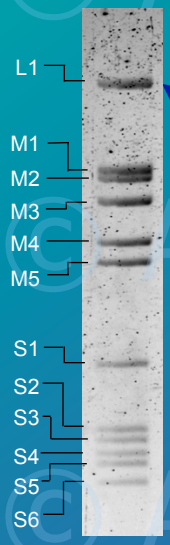
Passage of RLV by injection

- Inject 10 crabs with virus from an infected soft shell crab
- Sacrifice injected crabs and assess dsRNA at days 0-16



Crab drawing: K. Forrest.VA Inst Mar Sci

Partial sequencing of the viral genome Part I



RNA dependent RNA polymerase
-partial sequence
-useful for taxonomic placement

Predicted RdRp sequences from RLV and mud crab reovirus

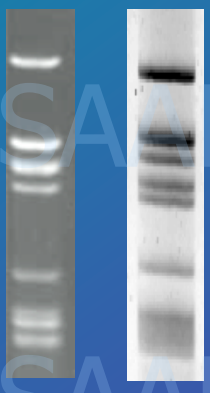
```
                 10        20        30        40        50        60
                  |         |         |         |         |         |
RLV      TTGGGTGATATCATCATTTTGCAACATGGTAATAGGTCAGGTGTACCCTGGACTGGAACT
          : :: ::::::::::::  : :: ::::: :: ::::: :: :: :: ::::: :: :::
Scylla   CTTGGAGATATCATCATACTACAGCATGGCAACAGGTCTGGGGTTCCTTGGACGGGCACT
           L  G  D  I  I  I  L  Q  H  G  N  R  S  G  V  P  W  T  G  T

                 70        80        90        100       110       120
                  |         |         |         |         |         |
RLV      CAGAATGACTTAGTCAATGTGAGTAATCACCATATGGCTTACGATGAGTACAAAAAACGT
         :: :::::: : :::::::: :::::::: :::::::: :::::::::::::::::::::
Scylla   CAAAATGACCTTGTCAATGTTAGTAATCATCATATGGCCTACGATGAGTACAAAAAACGT
           Q  N  D  L  V  N  V  S  N  H  H  M  A  Y  D  E  Y  K  K  R

                        130       140
                         |         |
RLV      GTTGCGGAATTACAGCGACAAGGAAG
         :: :: :: ::::: :: :: :: :
Scylla   GTAGCTGAGTTACAACGTCAGGGCAA
           V  A  E  L  Q  R  Q  G  R
```

DNA: 75 %
Protein: 100 %

Scylla: GenBank EF523476

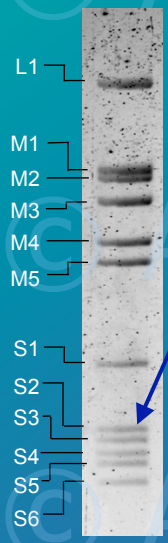
Reovirus of mud crab, *Scylla serrata*

Implicated in mass mortalities of mud crab in pond culture, Guangdong, China

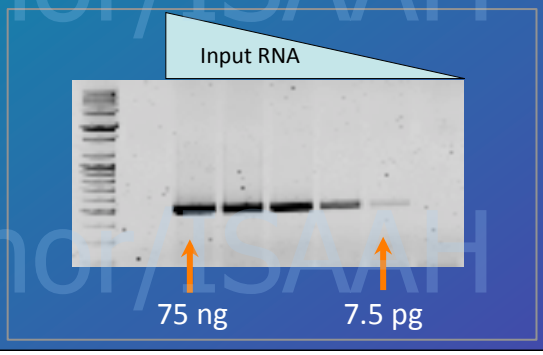


Weng et al. 2007. J Fish Dis. 30:133-9

Partial sequencing of the viral genome Part II



1.3 kb segment
-full sequence
-basis of Rt-PCR virus detection



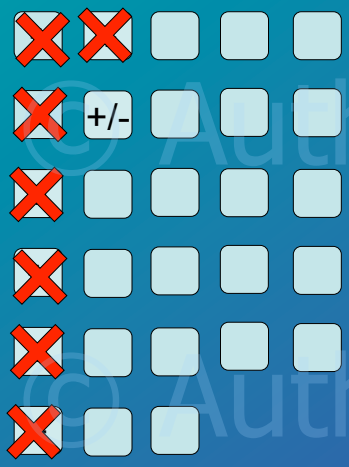
Application of the RT-PCR assay to hatchery broodstock

Test 28 crabs by PCR: 8 / 28 positive

+	+			
+	+/-			
+				
+				
+				
+				

Application of the RT-PCR assay to hatchery broodstock

5 Weeks later: 7/28 died, all REO+



Apply the RT-PCR assay to virus transmission studies

Cannibalism: Feed infected crab meat to naïve crabs for 8 days

--> 1/6 become + by Rt-PCR

Cohabitation: Place naïve crabs in a soft-shell facility for 10 days

--> 3/9 become + by Rt-PCR

Use the RT-PCR assay to assess virus prevalence in Chesapeake Bay

River	Season	RLV/ total
Fort Howard	June 2009	0/16
Magothy	Fall 2008	2/30
Magothy	July 2009	2/30
Corsica	Fall 2008	1/30
Corsica	July 2009	0/30
Rhode	Fall 2008	22/30
Rhode	July 2009	1/30
Tilghman	June 2009	1/40
Overall prevalence (w/out Rhode 2008)		3.4%

Reovirus summary

- Highly prevalent in dying captive crabs
- Related to reoviruses of other portunids
- Rt-PCR assay developed
- Passage by injection, feeding, cohabitation
- Prevalence in healthy crabs <5% but outbreaks possible

Does RLV play a role in wild blue crab mortality?

Ongoing and future studies

- Wider prevalence studies:
  - Geography
  - Age/development
  - Seasonality
- Transmission studies:
  - Environmental conditions and stress
  - Virus shedding
- Full genome sequence

Acknowledgements

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