Physiochemical assay helps identify virus lethal to Chesapeake blue crabs

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→ Introduction

The Chesapeake blue crab (*Callinectes sapidus*) is a crustacean found in the western Atlantic Ocean, the Gulf of Mexico and in the Pacific Region of Central America (see Figure 2). The Chesapeake Bay, located between Maryland and Virginia, has long been famed for its blue crabs. Their cultivation and sale is regarded as an important economic contributor to the region. Recently however, the population of blue crabs in this region has been falling - there has been a 70% fall in blue crab numbers between 1993-2008 due to overfishing and environmental degradation.

Figure 1: *Callinectes sapidus (taken from (1))*

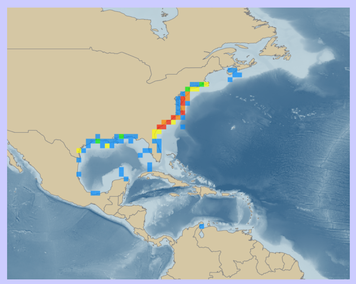

Figure 2: Geographical Location (4)

Although environmental safeguards have subsequently been put in place, such as harvesting restrictions which boosted blue crab numbers in 2009, high year to year population variability suggests that current explanations for the changes in crab population size are inadequate. Also, the practice of harvesting pre-molt, or soft shell crabs to offset yearly fluctuations carries a high intrinsic rate of crab mortality (around 25%), thought to be the result of:

- Poor water quality in harvest spaces
- Stress from handling and transportation
- Physiological stress of molting

These factors result in crabs being more susceptible to infection. Although many diseases are known to exist for blue crabs, little is known about their quantitative effect on the overall population size.

→ Investigating blue crab disease by assaying

Advances in molecular biology techniques have made it scientifically and economically feasible to develop assays to genotype and test for diseases. Bowers et al. designed an assay to detect virus genomes by enrichment of dsRNA from putatively infected crabs. This technique was then used to develop a PCR array to allow widespread viral testing in the blue crab population.

→ Methods

Bowers et al. collected diseased and healthy pre-molt and mid-molt crabs from a commercial soft shell crab operation near Baltimore and performed the following analyses:

- Separated 150mg of tissue and enriched for dsRNA versus ssRNA by exploiting the relative hydrophobicity between the RNA structures.
- Developed a virus detection assay to detect a specific 483 bp region of the 1.2 kb viral genomic segment.
- Used a pool of dsRNA from an infected crab to sequence a portion of the viral genome and use it for homology analysis using BLAST (2).
- Performed a virus transmission study by infecting healthy crabs with a viral preparation and analysing the crabs continually post inoculation.

→ Results: Detecting and genotyping the virus

The dsRNA assay suggested a virus was present in diseased crabs which was genotypically similar to a known crab reo-virus.

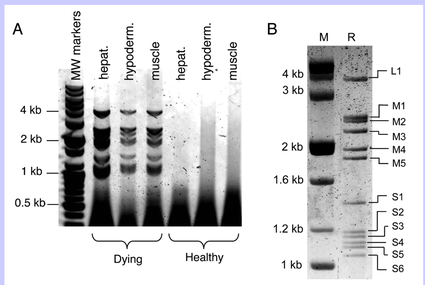

Figure 3: Visualisation of an unknown viral genome (3)

Figure 3A shows the differentiation of viral dsRNA between a diseased and healthy crab. The diseased crab shows 1-4kb size bands of distinct dsRNA after precipitation; in the healthy crab this banding is absent.

Figure 3B shows the dsRNA banding similarity of the putative virus and a known reo-like blue crab virus.

Additional supporting evidence was also found to suggest the presence of a new virus:

1. The dsRNA banding was also seen in high proportion of diseased crabs. The virus was not detected in crabs freshly harvested in the wild.
2. Homology modelling revealed that the putative virus shared some similarities in its RNA reverse transcriptase protein with a reo-virus taken from the mud crab *Scylla serrata*.

→ How deadly is the virus?

Bowers et al. developed a virus preparation and injected five healthy hatchery-reared crabs and compared their mortality over the following 16 days. All crabs injected with the preparation died within 13 days. None of the uninfected crabs monitored over the same period suffered any mortality.

The authors also investigated the passage of infection by sampling tissue from injected crabs over the time course of the study.

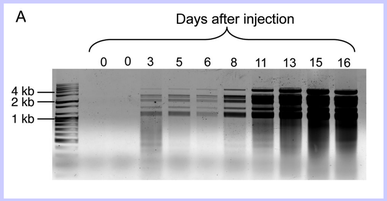

Figure 4: Time course of viral infection (3)

Figure 4 shows the progression of the virus after healthy crabs were injected with the virus preparation. After three days, crabs displayed detectible levels of viral dsRNA. Histological investigation was consistent with infection of a reo-like virus.

→ Conclusions

Bowers et al. used methods to discover and test for a putative reo-like virus infecting blue crabs in Chesapeake Bay. The main implications of this research are:

1. Improved testing of pre-molt crabs in farmed conditions can help inspire healthier hatchery conditions and help reduce crab mortality.
2. Lower mortality in farmed crabs would deliver economic benefits to crab farmers through reduced wastage.
3. This approach for enriching dsRNA can be adapted to test for a variety of viral genomes and therefore facilitates the discovery and monitoring of aquatic infections.

→ References

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