



Ninth Annual

# RONALD C. BAIRD SEA GRANT SCIENCE SYMPOSIUM

## NEW INFORMATION ON SHELL DISEASE

- Epizootic shell disease (ESD) is a newly described (since 1996) form of shell disease found in lobsters in Southern New England. Several other diseases/syndromes have also been discovered during this same time period (limp lobster, calcinosis, and paramoeba-induced mortality).
- The composition of the bacterial community is not radically different between healthy and diseased shells, although a select subset of bacteria that occur on healthy shells are more abundant on diseased shells.
- Bacterially produced protein and cellulose-degrading enzymes are much more active in diseased shells than on healthy shells, and although chitin-degrading enzymes are much more active than other enzymes tested, their activity does not differ with health status.
- A novel bacterium, *Aquimarina homaria*, initiating formation of the lesions was shown to be a prominent member of these communities. In the environment, this potential shell disease pathogen most likely associated with various marine Arthropod species.
- Develop temperature model for shell disease occurrence.
- Observe lobsters over multiple molts for re-occurrence after the molt process.
- In addition to serving as a physical barrier, lobster shell has antimicrobial activities of its own likely associated with small heat stable peptides. The defense properties of lobster shell are poorly understood and need further evaluation.
- Calcium efflux from cuticle is paralleled by an apparent  $\text{OH}^-$  efflux (measured as  $\text{H}^+$  influx). The  $\text{OH}^-$  comes from water during  $\text{HCO}_3^-$  formation when  $\text{CO}_3^{2-}$  dissolves. This becomes exaggerated in lesions both natural and artificial. We are able to measure the loss of  $\text{CaCO}_3$  from the lobster cuticle. Oxygen influx into normal intermolt cuticle is close to zero but is substantial into artificial lesions but only when the calcite layer has been penetrated.
- Lobsters with ESD have compromised immune systems relative to those who do not have shell disease. In Long Island Sound lobsters from the eastern portion of the sound where ESD is prevalent have compromised immune systems relative to lobsters from the western sound or from Maine where ESD is uncommon. These data indicate immune status may predispose lobsters to developing ESD.
- Low arginine kinase expression in muscle, indicates the symptomatic lobsters may be energetically compromised.
- Altered expression of ecdysteroid receptor and cytochrome P450 enzymes indicates that shell disease is associated with disruption of chemical metabolism and hormonal signaling.
- Statistical differences in trace metals concentration in the hepatopancreas between shell diseased and non-diseased lobsters, particularly for chromium, copper and mercury - where the shell diseased are also higher than the reference lobsters (mercury concentrations were compared using muscle tissue). Concentrations for these metals were generally 2-3 times higher in shell diseased organisms compared to non-diseased organisms.
- Alkylphenols are incorporated into the lobster shell, increasing time to harden after molting and cause a delay in molting.
- Ecdysone concentrations are higher in shell diseased lobsters, especially in berried females.
- Alkylphenols are toxic to lobster larvae, interfere with molting in adults and larvae and mimic juvenile hormone activity (JH).
- Diseased males lost in paired fights with non-diseased males of equal size.
- Despite larvae dispersing for weeks in ocean currents and despite migratory adults, lobsters manage to form and maintain local populations. In choice experiments, female lobsters preferred males from their own population, but did not discern if the lobster had shell disease.



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## **NEW TOOLS DEVELOPED / USED DURING PROJECTS**

- The NE Lobster Shell Disease Initiative was a new approach using investigators from various disciplines and expertise to investigate a complex issue.
- The 100 lobster project is a new approach to looking at whole systems and disease.
- Development of a laboratory model to test various factors for the initiation of shell disease including abrasion.
- Plate reader based microspectrophotometric assays for phenoloxidase and antimicrobial activity, and phagocytosis in lobster plasma, hemocyte lysates, and shell extracts.
- Quantitative real time polymerase chain reaction (q-RT-PCR) for analysis of gene expression.
- Use of fluorogenic substrates to determine ectohydrolase activities.
- Terminal Restriction Fragment Length Polymorphism (TRFLP) of diagnostic gene (16S rRNA) to produce community fingerprints.
- Three dimensional confocal imaging of bacteria colonizing lesions using Fluorescent In Situ Hybridization (FISH) of genetic probes.
- Denaturing gradient gel electrophoresis of PCR amplified 16S rRNA gene fragments was applied to DNA isolated from shell lesion communities.
- A real-time PCR assay was developed for quantification of *A. homaria*, a potential shell disease pathogen.
- Created and sequenced gene libraries from symptomatic and asymptomatic lobsters.
- Developed qPCR assays to measure expression of 28 genes indicating lobster physiological condition.
- Multi-residue method to look at a large number of organic contaminant classes at once.
- Developed new genetic markers to study migration and differences in lobster populations. The genetic markers – simple sequence repeats (SSR) linked to expressed sequence tags (ESTs) – are genetic tags linked to genes that regulate reproduction and growth. These results show greater resolution of population structure than microsatellites alone.