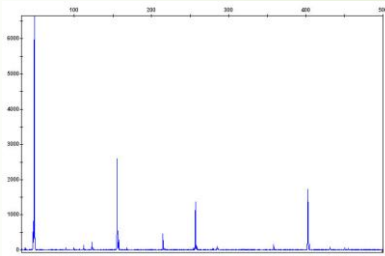


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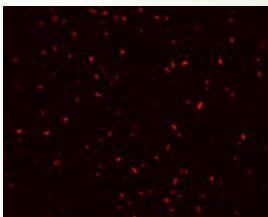
*Terminal Restriction Fragment Length Polymorphism (T-RFLP) and Fluorescent In Situ Hybridization (FISH) Analyses*

*T-RFLP is a molecular technique to characterize bacterial communities in mixed species samples. The technique works by PCR amplification of DNA using primer pairs that have been labeled with fluorescent tags. The PCR products are then digested using restriction enzymes and the resulting patterns visualized using a DNA sequencer.*



*TRFLP fingerprint from ESD lesion generated with 16S rDNA primers 63F-FAM and 778R digested with the restriction enzyme MspI.*

*FISH is a cytogenetic technique that can be used to detect and localize the presence of specific RNA sequences on ribosomal RNA. It uses fluorescent probes that bind only to those parts of 16S rRNA which show a high degree of sequence complementarity. Microscopy is then used to enumerate cells that hybridized with the fluorescent probe.*



*Bacteria labeled using probe EUB338, specific for domain Eubacteria.*

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## Characterization of the exoskeletal microbial communities and host immune response associated with epizootic shell disease in lobsters

Bassem Allam, Alistair Dove, Gordon Taylor and Anne McElroy

### *Objectives of the Research:*

We are investigating the pool of potential pathogens residing on the carapace of wild healthy and ESD-affected lobsters, its composition, seasonal dynamics and activity. We are examining lobsters collected from locations in Long Island Sound with varying degrees of ESD prevalence using culture-independent molecular as well as classic cultivation techniques. We are comparing phylogenetic “fingerprints” of bacteria on the carapace of asymptomatic specimens with those of lesions and lesion-free areas from symptomatic specimens in varying stages of infection as well as specimens from a remote “shell disease-free” reference site (Maine). Terminal Restriction Fragment Length Polymorphism (T-RFLP) and Fluorescent In Situ Hybridization (FISH) analyses are the primary tools for comparison. We are also comparing bacteria cultures isolated from diseased and healthy shell. To assess pathological activity of these bacterial communities, we are profiling ectohydrolytic activity using fluorogenic tracers on fresh biopsies from diseased and healthy carapace.

Simultaneously, we are exploring several specific immune parameters in lobsters with and without ESD, characterizing levels, seasonal dynamics, and scope for response.

### *Results to Date:*

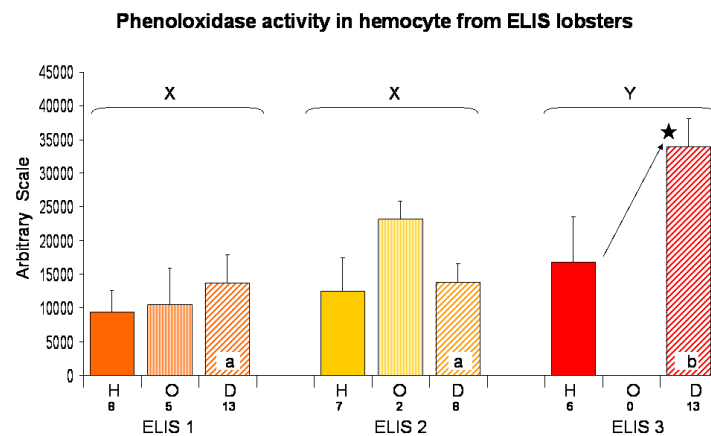
Sixty one lobsters have been collected from eastern (E) and western (W) LIS and from Maine for assessment during early summer when prevalence of ESD is highest. Thirty one additional specimens were collected from ELIS in mid summer and in the fall to evaluate the seasonal responses.

**Bacterial Assemblages:** Carapace biopsies were incubated in sterile water with fluorogenic substrates for ectohydrolase activities. Significant differences in chitinase,  $\beta$ -glucosidase and leucine-aminopeptidase activities were seen between diseased and healthy lobsters.

Our sampling has generated a large culture collection (hundreds of isolates) and currently 5 colonies have been isolated in pure cultures that appear to have chitinoclastic activity. DNA from carapace samples has been extracted; PCR amplified with 16S rRNA primers, and digested with restriction enzymes. These samples have been submitted to TRFLP analysis and data interpretation is underway. FISH probes have been ordered to target organisms cultured by another investigator and protocols are being refined.

Immune System Responses: Phagocytosis, reactive oxygen species production, and phenoloxidase production assays have been completed on all samples collected. Methods for measuring antibacterial activity in lobster plasma, and for evaluation of phenoloxidase-like activity and reactive oxygen species production in lobster carapace have also been designed and validated.

Although not fully analyzed, preliminary results show spatial and temporal differences in defense related factors among lobsters. For example, Maine lobsters displayed a better response toward the infection than lobsters collected from ELIS as demonstrated by increased hemocyte counts in affected lobsters when compared to healthy ones. Among lobsters collected from ELIS, those collected in October displayed significantly better immune performances than lobsters collected in late June or August. Results also indicated negative correlations between lobster activity and the presence of bacteria in the blood suggesting that bacteraemia might be at the origin of morbidity of diseased lobsters.



### Summary:

Our approach focuses on characterizing the composition of the exoskeletal microbial community of healthy and ESD lobsters while measuring the response of the lobster immune system. Through this approach we expect to gain significant insights into the etiology, the development and host responses toward ESD, as well as optimizing tools for the study of crustacean diseases.

*Bassam, Gordon and Anne are all researchers at the Marine Sciences Research Center at Stony Brook University in New York. Alistair is a researcher at the Georgia Aquarium.*

