

Coordinated Assessment of Shell Disease In lobsters from Narragansett Bay, RI

Jeff Shields, Kersten Wheeler, Jessica Moss and Christopher Magel

Objectives of the Research:

Lobsters were collected by a fisherman participating in the Ventless Trap survey in coordination with the Rhode Island Department of Environmental Management. All lobsters were collected from the same area approximately ½ mile north of the Newport Bridge which spans the waters of the East Passage of Narragansett Bay. Lobsters were shipped on ice live to VIMS via FedEx by URI personnel Barbara Somers. Upon arrival, lobsters were housed in one of three cold-water systems: a 260 gallon commercial chilled system or two 150 gallon static troughs in a cold room.

Work flow for the “100 lobsters”:

Shields: Various morphometric and meristic measurements were taken from each animal including: size (CL), sex, reproductive condition, disease condition, obvious pathology, estimated percentage of body covered by shell disease, missing limbs, and molt stage (intermolt, premolt or postmolt). Each animal was photographed (Olympus E5000) on the dorsal aspect to record the severity of the disease in that lobster. Animals were bled for hemolymph samples (see Allam, Laufer and Tarrant & Verslycke below). Lobsters were then killed by injection of potassium chloride into the ventral ganglia. Several tissues were taken for histological analysis including shell (affected and unaffected), hepatopancreas, gill, eyestalk and eye, antennal gland, heart, hindgut and epidermis. (see photo under Gillevet). Tissues were later processed through standard paraffin histology.

Jeff Shields is an Associate Professor at the Virginia Institute of Marine Science.

Atema: Pleopod samples were preserved in 100% ethanol. Samples were shipped FedEx.

Lobster

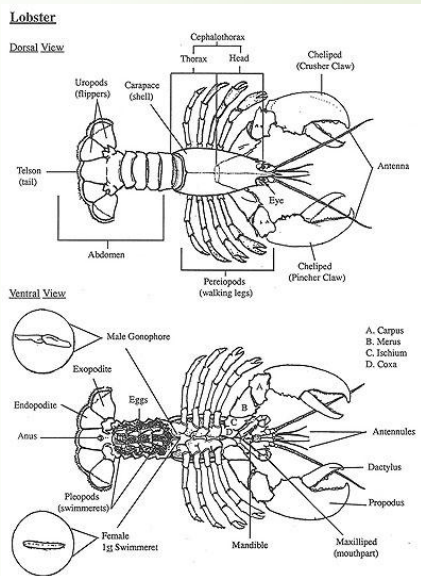


Pleopods

Jelle Atema is a Professor of Biology at Boston University Marine Program, and an Adjunct Senior Scientist at Marine Biological and Woods Hole Oceanographic Institution.

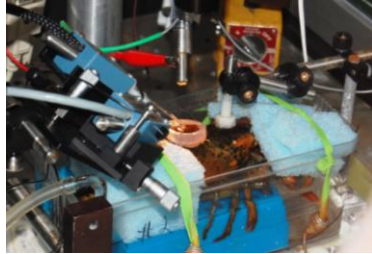
Why “100 Lobsters”?

Having teams of researchers studying the same 100 lobsters provides a coordinated assessment of lobster health. This holistic approach allowed researchers to replicate their assessments using numerous animals, providing a statistically significant sample size. This approach also allows research results to be correlated with each other to provide a more comprehensive picture of lobster health. These studies provide a “bank” of samples for baseline and follow-up studies.

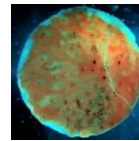
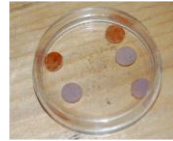


One lab processed all the lobsters, collected various tissues and performed initial isolations. The lab coordinated the shipping of samples to participants for study. This process optimized the use of lobsters, saving time, money and resources.

Kunkel: Two medallions of cuticle from affected and unaffected areas of the carapace will be removed and fixed in absolute or 95% ethanol. Adjacent pieces will be taken for Laufer's component (see below). Samples will be shipped FedEx.



Lobster being prepared for medallions of cuticle to be removed.



Medallions of cuticle

Joe Kunkel is a Professor in the Biology Department at the University of Massachusetts Amherst.

Gillevet: Pieces of carapace were removed from six different areas, including the carapace, claws, abdomen, and legs. Samples were frozen at -80°C until shipped to George Mason University on dry ice. Additionally, a piece of epidermis, approximately 1 cm^2 was taken from the dorsal abdomen of each animal and frozen for later enzyme analyses. Samples were shipped via FedEx.



Samples are taken from these areas
(Ventral side)
upper abdomen
lower abdomen



(Dorsal side)
abdomen

tail

claws

Eye stalk

gills (under shell)

Patrick Gillevet is an Associate Professor in the Department of Environmental Sciences and Policy at George Mason University in Virginia.

Tarrant and Verslycke: Approximately 100 mg each of tail muscle, gonad, and hepatopancreas were saved in RNA and placed in the freezer for later analyses. An [aliquot](#) of hemolymph was also sampled and saved, but protocol for this sample was not yet determined. This group needed approximately 40 animals total: 10-15 healthy, 10-15 slightly diseased, 10-15 more seriously diseased. Samples were shipped via FedEx on dry ice.



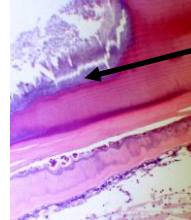
Tail muscle (left)

Lobster dissection for gonad and hepatopancreas samples (right)



Ann Tarrant is a Assistant Scientist of Biology at the Woods Hole Oceanographic Institution in Massachusetts and Tim Verslycke is an Environmental Consultant at Gradient Corporation in Cambridge, MA.

Smolowitz: A 2 x 2 cm piece of the dorsal carapace of abnormal tissue was excised and fixed in 10% NBF (neutral buffered formalin). The sample included a portion of “normal” shell as well. Caution was taken to ensure sterility of the carapace to be examined. Further precautions were taken to make sure the underlying epithelium/subdermal membrane was attached to the carapace along its length and width. Samples were shipped via FedEx to Woods Hole Marine Biological Laboratory.



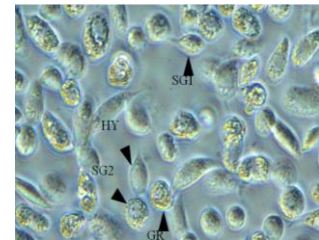
cross section of tissue under shell of diseased lobster p pillars are due to lack of chitin.

Roxanna Smolowitz is the Director of Animal Health at the New England Aquarium in Boston.

Allam, McElroy, Taylor and Dove: A 1-2cm² piece of the dorsal carapace was excised from healthy, diseased and asymptomatic areas of diseased animals. The samples were frozen at -80°C until shipped on dry ice to SUNY Stony Brook. The same approximate area was sampled each time when possible. A 1-ml aliquot of hemolymph was taken with a 23 gauge needle, transferred to a microcentrifuge tube and centrifuged (4°C, 600-800 g, 10-15 minutes). The plasma and cell fractions were individually frozen (flash freezing in liquid N₂ then transferred to -80°C). Note: this process can be done with anti-clotting solution (Smith & Soderhall 1983 is commonly used).



Collection of hemolymph (left)



Lobster hemocytes (right)

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.

Laufer, Jacobs and Chen: Aliquots of 4 ml of hemolymph were drawn with a 23 gauge syringe (Note: the syringes can be “rinsed” with anti-clotting agent prior to use), and the sample fixed in acetonitrile fixative. Samples of the dorsal carapace (1-2cm²), 4g samples of hepatopancreas, shell, gill and gonad, possibly nerve were collected and fixed in acetonitrile fixative.



Lobster dissection for the removal of gill, gonad and hepatopancreas samples. Samples of dorsal shell were also taken.



Hans is a Professor Emeritus, Molly is a Postdoctoral Fellow and Ming is a Research Assistant in the University of Connecticut Department of Molecular and Cell Biology.

