LONG ISLAND SOUND
Lobster Health News

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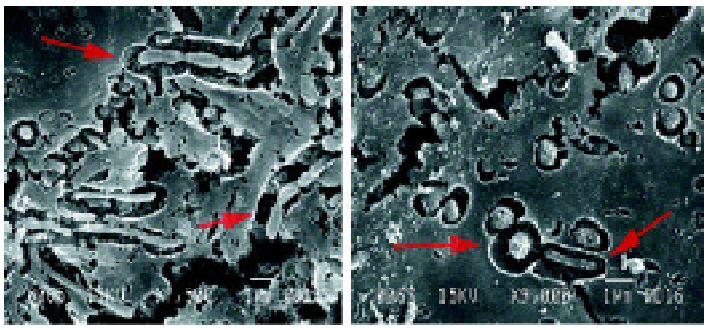
Highlights

Shell Disease: What We Know and What We'd Like to Learn

Lobsters from Buzzards Bay to Long Island Sound are suffering from a severe type of shell disease that is epidemic in nature, according to researchers Andrei Chistoserdov and Roxanna Smolowitz. Both the extent and severity are much greater than previously observed. This disease is different from "impoundment shell disease", which is contagious among caged lobsters. Each disease displays a unique gross pathology.

This epizootic shell disease is not being widely reported in other fishing areas, although a few reports have come from the Kittery, Maine area. Researchers still don't know how the disease is being transmitted, but are learning more about how epizootic shell disease is caused by bacteria.

Bacteria are microscopic, simple, cellular organisms lacking a nucleus as well as many other characteristics of a typical eukaryotic cell (such as a cell of a human or lobster). Shape varies significantly among species of bacteria and may be used in identification. Two major shape-classes are *coccus* (pl. cocci) referring to spherical cells, and *bacillus* (pl. bacilli), which are cells that are more or less rectangular in shape, similar to a rod. Some bacilli appear so short that they resemble cocci, and are called *cocco-bacilli*.



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Above and right: Scanning electron micrograph of bacteria found in lesions. Arrows indicate three typical morphologies of bacteria observed in lesions (cocco-bacilli, chains of rods, and filaments).

Bacteria taken from the lesions on shell-diseased lobsters come in three shapes— cocco-bacilli, chains of rods, and filaments. Believed to be the main cause of shell disease, bacteria form large colonies on the shell's surface.

Bacterial cells aggregate to form colonies comprising millions of organisms and are part of the natural environment, occurring on healthy and diseased lobsters alike. The number of bacteria on diseased animals is much higher than on healthy animals–as much as 10,000 times greater.

Closer investigation shows that the microbial communities associated with lobsters in various areas of Long Island Sound are all similar. Specific combinations of three to eight different species of bacteria occur in every location investigated, although the exact composition of these bacterial colonies varies.

Two species of bacteria, *Pseudoalteromonas gracilis* and *Cytophaga* sp., are of particular interest because both were isolated from shell lesions in each infected lobster sampled to date. The researchers believe both bacteria may be included in an exclusive group of organisms that settle on a

cont'd page 3

Long Island Sound Lobster Research Initiative is a collaboration funded by National Oceanic and Atmospheric Administration's (NOAA) National Marine Fisheries Service, Connecticut Department of Environmental Protection, and Sea Grant College Programs — Connecticut, New York and the National Office.

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LONG ISLAND SOUND
Lobster Research Initiative

The *ad hoc* Steering Committee was established by the Atlantic States Marine Fisheries Commission to oversee research into the causes of the Long Island Sound lobster fishery disaster.

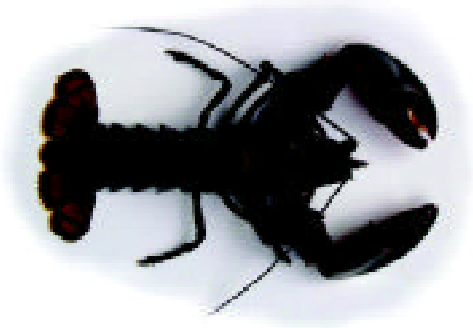
Letter from the Chair

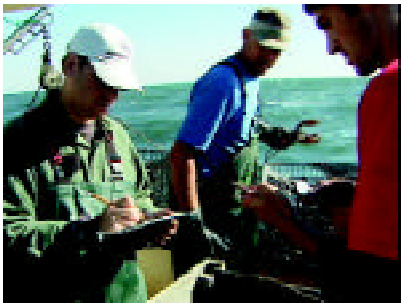
This second issue of *Lobster Health News* provides technical background on some of the research undertaken in the Lobster Research Initiative (LRI). These research efforts are "terminology-laden", and these articles are meant to serve as a primer of sorts on pesticides, paramoeba genetics, and shell disease. An update on the status of the resource monitoring efforts is also provided. As a special feature, we are pleased to enclose a poster insert with a description of the biology of the American lobster in Long Island Sound.

As the Lobster research initiative nears completion, the states are summing up the pesticide loads applied to control West Nile virus in 1999. Plans are underway to host a final symposium focused on these research initiatives (date TBA). The committee is asking several individuals to synthesize the research results and present their findings at that time. There will be integrated presentations from each of the research topic areas in a format similar to the third symposium held in March 2003, and an overall synthesis report. A special edition of papers from this comprehensive research effort will be published in a scientific journal.

I have served as the Chair of the Lobster Research Initiative (LRI) Steering Committee since its inception in 2000. However, as I have retired from the NOAA National Marine Fisheries Service in January 2004, I have also stepped down as Chair of the LRI Steering Committee. Dr. Emory Anderson, of the NOAA National Sea Grant College Program, has assumed the role as Chair, and will see the project through to its completion later in 2004.

Chair Emeritus
Anthony Calabrese





Researcher interviews lobsterman aboard boat.

Homarus americanus up close.

All photos this page: ©2003 Matt Sclafani and Rory Mc Nish, CCE Marine Program

Special Supplement in this issue!

As noted by our Chair, this issue of *Lobster Health News* includes a special bonus - a pullout supplement featuring a poster depicting the life cycle and habitat of Long Island Sound lobsters on one side, and a feature on lobster biology by Jan Factor and Antoinette Clemetson on the other. The artwork is an original watercolor by Jan Porinchek, created especially for this issue. To request additional copies, while supplies last, contact one of the Outreach Coordinators. The supplement makes a marvelous visual aid for classrooms.

Shell Disease

cont'd from page 2

lobster to start these bacterial colonies. Called "pioneer species," these types of organisms can be used to monitor the health status of a stock. Someday this knowledge could help to develop "early warning" detection for imminent disease outbreaks.

It is possible that both bacteria may be responsible for triggering the onset of shell disease. But, for the moment, it is uncertain exactly how they work together. Healthy lobsters can coexist with infected lobsters without contracting the disease.

At least one of the species of bacteria was isolated from the microbial communities on Long Island Sound lobster lesions for genetic analyses. One method used to verify its identity is *Denaturing Gradient Gel Electrophoresis* (DGGE), which uses the 16S rRNA gene that is amplified (replicated many times) by *polymerase chain reaction* (PCR) (see also "Using Forensics in the Hunt to Identify Paramoeba", page 8).

The PCR products, fragments of DNA, separate into their components as they travel through an electrical field. Fragments derived from each individual bacterium appear as distinct bands following the separation. Each band consists of minute quantities of a denatured PCR product derived from only one species. The DNA comprising the bands can be further analyzed to determine the identity of the bacterium from which the bands originate. Preliminary results from DNA isolated from lobster lesions show three prominent bands on DGGE gels.

Researchers believe that these bacteria work together to cause epizootic shell disease. More research is underway to understand *how* they cause the disease. Since the disease-causing bacteria are present on both healthy and infected lobsters, it is an as-yet-unsolved mystery how healthy lobsters can live in the same environment without contracting the disease.

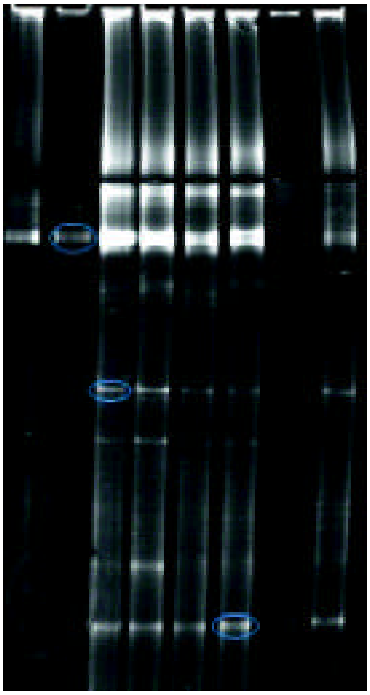
The next step is to infect a healthy lobster so that it develops shell disease (see *Koch's Postulates and Lobster Diseases,* page 7). This step is the way to verify that both bacteria are the causative agents for shell disease. However, infection studies are complicated because the disease occurs under specific environmental conditions that are difficult to replicate in the laboratory. Healthy lobsters were held at 16°C in tanks containing both species of bacteria, but this experiment did not succeed in transmitting shell disease.

Shell disease appears to be worse where waters are warmer. It is not clear whether warm temperatures may be changing the composition of organisms that consume bacteria, such as amoebae, or simply increasing the number of bacteria.

Researchers are now studying whether the long-term exposure of healthy lobsters to shell-diseased lobsters makes the disease transmissible, and what role, if any, stress and a weakened immune system play in the development of shell disease.

Faculty members Andrei Chistoserdov (University of Louisiana, LaFayette) and Roxanna Smolowitz (Marine Biology Laboratory, Woods Hole) are investigating the bacterial assemblages involved in the development and progression of shell disease in American lobsters.

Above: Lobster showing symptoms of severe shell disease.



DGGE of microbial communities from Buzzard's Bay and Eastern Long Island Sound lobster lesions. Encircled bands indicate denatured PCR products from the same bacterial species, which were sequenced and are being analyzed.

Types of Pesticides Used for Mosquito Control

Joseph Conlon

Editors' note: The pesticides being investigated for their effects on lobsters as part of this lobster mortality research effort are all used for mosquito control to curtail the spread of West Nile virus. Mosquito control involves a variety of techniques not discussed here, besides pesticides.

People are becoming increasingly aware of mosquito control programs using insecticides to prevent outbreaks of diseases to humans, and they may be wondering about these chemicals and how they are engineered to target specific organisms. This article provides information about several of the pesticides currently being used, and their toxicity to humans. The Environmental Protection Agency (EPA), which enforces the stringent standards mandated by the Federal Insecticide, Fungicide, and Rodenticide Act, is charged with regulating all chemicals being used to control pests and disease-carrying organisms. As a condition of their registration, products are tested extensively–up to 12 years–to ensure that risks to humans and the environment are minimal when the chemicals are used as directed.

Pest management control programs involve ongoing research, field surveillance and control operations, and public education. Control operations are based upon a thorough knowledge of the target. Only when surveys indicate a specific need, are public health insecticides considered for use.

Control of mosquito larvae is accomplished through proper water and land use management, in conjunction with the EPA-approved larvicide program, when required. Larvicides come in four basic types, each possessing a different mode of action. Stomach poisons must be ingested; *Bacillius thuringiensis israelensis* (or Bti) is a live bacterial spore that produces a toxin when it comes in contact with the chemicals in the mosquitoes' gut. Growth regulators include methoprene, which is a hormone that prevents the larvae from eventually molting to an adult. Surface films inhibitors include Agnique MMF®, which reduces the surface tension of the water, making it impossible for the larvae to attach their breathing tubes at the surface, thus drowning them. Others may physically obstruct the breathing apparatus of the larvae, in effect suffocating them. Contact poisons such as temephos, a nerve poison, are rarely used; however, it is labeled for use in potable water.

Because these larvicides are to be used in sensitive aquatic environments, they are specifically designed to minimize their impact on nontarget organisms. They must be applied, by law, only to a predefined target site following guidelines that are specified on the label. To ensure its effectiveness, the application rate for each larvicide is calculated on the basis of its toxicity profile and degradation characteristics. For example, the application rate for methoprene is calculated to achieve a final concentration in water of between 0.22 to 1.1 parts of product per billion (ppb). This would be equivalent to an initial dose of roughly one drop of the chemical in an Olympic-sized swimming pool.

Modern pest management strategies endorsed by the EPA and the Centers for Disease Control and Prevention include application of adulticides when surveillance indicates that larval control measures have proven inadequate to prevent imminent disease outbreaks. Certified operators, trained in the special handling requirements for adulticides, apply them after dusk when

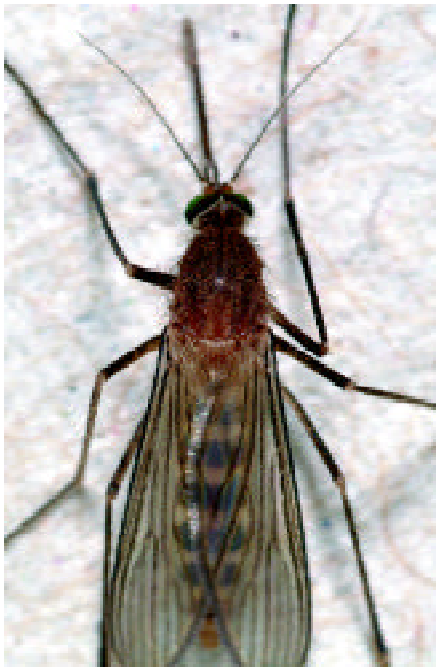
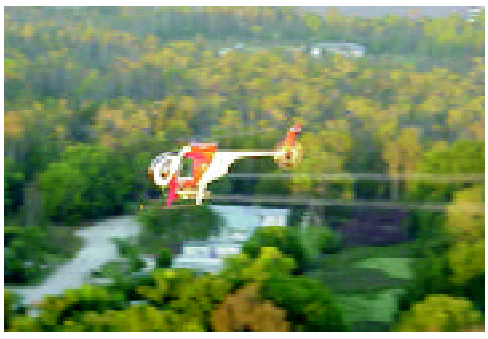


Photo: © 2003 Renee Anderson, Cornell Univ.

Culex pipiens, the mosquito species that carries the West Nile virus.

Pre-dawn aerial spraying of pesticide.



Aerial spraying of adulticide on a target site.

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Use of Pesticides... *cont'd from page 4*

mosquitoes are most active and nontarget species are generally at lower risk. Larvicides, such as Bti, are applied in tablet form or as briquettes. Adulticides are usually applied in aerosol form composed of extremely small droplets (74 million droplets could fit inside a pellet from a "BB" gun) so that they remain airborne to impinge upon mosquitoes in flight at the time of application. The minute droplet size also ensures that products dissipate and degrade quickly, to minimize deposition of active ingredient on the ground or other surfaces. The low application rates of these aerosols—generally less than half an ounce of insecticide per acre treated–further minimizes environmental risk.

Adulticides used in the United States fall into two general chemical categories, organophosphates and pyrethroids. Only two organophosphates, malathion (Fyfanon®) and naled (Dibrom®, Trumpet®), are in general use for adult mosquito control. Malathion is a popular choice because of its low price, proven efficacy and relatively low level of toxicity. Pyrethroids, considered natural insecticides because they use a highly potent extract from chrysanthemum plants, constitute the other class of adulticides. Three pyrethroid products currently on the market, resmethrin (Scourge®), sumethrin (Anvil®), and permethrin (Aqua-Reslin®) are synthetically derived. These have a longer shelf life and are as much as 50 times less toxic than the natural insecticide, while performing the same function. The pyrethroids and organophosphates are rotated at specified intervals in mosquito management programs to prevent the mosquitoes from becoming resistant after long-term exposure to a single group of pesticides.

Toxicity profiles of adulticides (to humans) are well-known and form the basis for the label recommendations mandated by EPA. Table 1 lists toxicity categories applicable to insecticide labels defined by EPA. Malathion and all pyrethroids are designated Category III (Slightly Toxic), and all larvicides that are applied fall under Category IV. A comparison of mosquito larvicide oral toxicities along with common household products is provided in Table 2. However, ingestion by humans of adulticides during their normal usage is unlikely, and lethal amounts for an average adult are more likely to result from inhalation and skin absorption (Table 3). Note that lethal toxicities to humans in this context range from 250 to 10,000,000 times the standard label rate.

Joseph Conlon is the technical advisor for the American Mosquito Control Association (http://www.mosquito.org).

Table 1. Human Toxicity Rating and Labeling Requirements for Pesticide

Category (Human toxicity)	Signal Word Required on Label	Probable Oral Lethal Dose (Human)
I Highly Toxic	DANGER-POISON (skull and crossbones)	a few drops to 1 teaspoon
II Moderately Toxic	WARNING	> 1 teaspoon to 1 ounce
III Slightly Toxic	CAUTION	> 1 ounce
IV Practically Non-Toxic	None Required	-

Table 2. Comparative Toxicity (to humans) of Larvicides Used in Mosquito Control Compared to Common Household Products

Common Name	Trade Name®	Oral LD_{50} (Rat)
Aspirin	Various	1,900
Table Salt	Various	4,500
Monomolecular Film	Agrique MMF Golden Bear	> 20,000
Table Sugar	Various	27,000
Methoprene	Altosid	> 34,600
Bti	Various	Non-toxic

LD_{50} is the dose, expressed in milligrams of active ingredient per kilogram of body weight needed to kill 50% of the subject (rat) population. Higher numbers indicate lower toxicity.

Table 3. Comparison of lethal levels* of insecticide for an adult human (165 lbs male) exposed to adulticide.

Insecticide	Dermal (lethal amount)	Actual Dermal Exposure	Actual Inhalation Exposure	Inhalation (lethal amount)
Naled	29.25	0.0084	0.193	0.0007
Phenothrin	>150	0.00156	2.1	0.00013
Malathion	>150	0.0504	4	0.0042
Resmethrin	187.5	0.00312	> 9.5	0.00026
Permethrin	300	0.00312	2,350	0.00026

(*grams of insecticide per cubic meter of air)

Ongoing Efforts to Determine the Effects of Pesticides in Long Island Sound

A research team headed by Anne McElroy and Bruce Brownawell from the Marine Sciences Research Center (MSRC) is using a two-pronged approach to 1) evaluate the sensitivity of lobster larvae and juvenile lobsters to pesticides, and 2) develop methods to measure pesticide levels in the aquatic environment. The chemicals of interest include methoprene, malathion, sumethrin, and resmethrin, all used to control mosquito populations within close proximity of Long Island Sound (see page 4), and piperonyl butoxide, a chemical that is added to make pyrethroids work more efficiently.

McElroy and Sea Grant Scholar Ann Zulkosky are evaluating the toxic effects of specific pesticides on lobster Stage II (2-3 day old) larvae. When exposed to a constant dose of resmethrin, toxicity increased from 24-hours to 96 hours. Most experiments were conducted at 16°C, a non-stressful temperature for lobsters. When the experimental temperature was raised to 24°C, significant mortality was observed, whether or not lobsters were exposed to resmethrin. Resmethrin was found to be about ten times more toxic to lobster larvae than malathion, and methoprene was not toxic to larvae at the highest concentration tested, 10μm/L (parts per billion). Zulkosky and McElroy are now evaluating the effect of pesticide exposure and elevated temperature on immune response in juvenile lobsters.

In order to assess risk, toxicity data must be compared with concentrations likely to be found in the environment. To evaluate pesticide levels in the aquatic environment, where fresh water runoff is a major source, it is necessary to accurately measure these compounds at extremely low levels. When the project began, means to detect trace amounts did not exist, so Brownawell, assisted by technician Joe Ruggieri, developed a new technique for the simultaneous detection of a broad group of pesticides in coastal waters. Detection levels can be as low as 0.1-0.2 parts per trillion for each compound, which would be equivalent to one fluid ounce of chemical in about 75 billion gallons of water (or approximately 3,000 swimming pools).

Samples were collected from surface waters of the East River and far-western Long Island Sound during the summers of 2002 and 2003 following spraying events and rainfall. While nearly a third of the samples analyzed contained detectable levels of pesticides, the synergist, piperonyl butoxide, was detected in almost all water samples in locations where it was used during spraying events. Piperonyl butoxide appears to be more soluble in water and more persistent in the environment than pyrethroids. Possible explanations include more rapid transformation, mediated by chemicals or microbes, or rapid removal by sediments. In future work, Brownawell plans to develop methods to quantify breakdown products (whose toxicological consequences are largely unknown) from these pesticides in the environments, to further understand the environmental chemistry of these compounds, and to potentially use them as a tool to better characterize potential inputs and exposures of nonpersistent pesticides in receiving waters.

Anne McElroy and Bruce Brownawell are faculty members of the Marine Sciences Research Center at Stony Brook University, New York. Ann Zulkosky is a graduate student, and Joe Ruggieri is a laboratory assistant.

Sea Grant Scholar Ann Zulkosky is working with Dr. Anne McElroy to investigate the toxic effects that pesticides have on select lobster development stages.

Flow-through dosing system at the Flax Pond Laboratory that is used to expose larval stages to varying concentrations of pesticides.

Stage II lobster larva

Micromass high pressure liquid chromatography-time-of-flight-mass spectrometry (HPLC-TOF-MS) equipment that is used to measure ultra trace pesticide levels.

Koch's Postulates and Lobster Disease

Nancy Balcom

Over the past few years, the phrase "Koch's Postulates" has come up during various presentations or articles on shell disease and paramoebiasis. This seems like a good opportunity to describe exactly what Koch's Postulates are, and how they fit into the research efforts investigating these lobster diseases.

In 1890, German microbiologist Robert Koch developed some postulates or criteria to help researchers determine if a specific bacterium was the cause of a particular disease. Koch argued that only after "yes" is answered to each of his four postulates could it be definitively said that agent "X" causes disease "Y". Scientists now commonly accept Koch's Postulates, which state:

- The bacterium must be present in every case of the disease, but should not be found in healthy animals.
- The bacterium must be isolated from the diseased host and grown in pure culture in a lab dish (so that you know that's all there is in the culture).
- This freshly cultured microorganism should cause the same disease and symptoms seen in the original animal when inoculated into a healthy susceptible host.
- The bacterium must be recovered from the experimentally-infected host and cultured again in a lab dish.

(While the postulates were developed for bacteria, the concepts were later extended to other pathogens.) "Control" animals are used in experiments to guard against the chance that the "experimental" animals get sick due to reasons unrelated to the bacterium under investigation. The only difference between the control and experimental animals is that the experimental animals are deliberately infected with the bacterium. Everything else is held constant.

Koch's Postulates do have their limitations. For example, some disease agents will grow only in living cells, and cannot be grown in lab dishes. In this case, scientists have to try to prove a sort of "modified" Koch's Postulates, by coming at the infectivity problem more obliquely. Enter American lobster epizootic shell disease and paramoebiasis. Since 1999, there have been several unsuccessful attempts to isolate and culture the paramoeba parasite, and failed attempts to infect healthy lobsters from lobsters afflicted with shell disease. Earlier this year, Richard Robohm, a scientist with the National Marine Fisheries Service in Milford, Connecticut, initiated another effort to try to prove a modified Koch's Postulates for paramoeba that does not involve actually culturing the organism in a lab dish.

"We are trying a two-pronged approach," says Robohm. "Unfortunately, it's a very time-consuming process to undertake. First we must identify lobsters that do not have the parasitic paramoeba by removing one antenna and examining the nervous tissue for the parasite. Those paramoeba-free lobsters are then held in tanks to be used in the experiments as healthy lobsters that we will try to infect." Robohm further explains, "If we find lobsters that do have the paramoeba present in their nervous tissue, then we will try to remove the paramoeba, partially purify it, and then inject it into healthy lobsters to see if we can infect them. This is a modified approach to proving Koch's Postulates, but one we must try, since no one has been able to grow the paramoeba in a lab culture as of yet."

The second "prong" of Robohm's approach is to obtain cultures of *Neoparamoeba* species that cause paramoebiasis in blue crabs ("Grey Crab Disease") and green sea urchins. He is now injecting healthy lobsters with those organisms to see if they will induce symptoms similar to those observed in "limp lobster syndrome," which afflicted thousands of lobsters in 1999.

While researchers and lobstermen alike would prefer to know definitively whether Koch's Postulates for either paramoebiasis or epizootic shell disease can truly be met, modified procedures such as those Robohm is taking will certainly provide additional insight into these diseases. "When someone does figure out how to culture these organisms, next year or in ten years, it will suddenly be pretty obvious to everyone that we could have done it now," says Robohm. "That's science for you."

In the meantime, we will wait and see what results come from these modified approaches to meeting Koch's Postulates.

Nancy Balcom is an associate extension educator with Connecticut Sea Grant, based at the University of Connecticut in Groton.

Using Forensics in the Hunt to Identify Paramoeba

Patrick Gillevet

Deoxyribonucleic acid (DNA) is being used to identify parents of human offspring, and has wide application in forensic science. Researchers use the same principles and techniques to target and analyze the structure of microbial communities. DNA base components are different for various organisms, and they can be analyzed quantitatively to work out how organisms may be related on an evolutionary scale. It is necessary, or at least helpful in the analysis, for organisms to share at least one common feature at the genetic level to make quantitative comparisons. One such genetic marker is the gene that codes for *small subunit ribosomal RNA* (SSU rRNA), an important standard used to define all species on the planet.

The success of these techniques relies on the capability to copy the genetic marker and identify it. This involves the use of *polymerase chain reaction* (PCR), a quick and easy method for generating unlimited copies of a DNA fragment encoding various genetic markers. PCR has been used in a wide range of applications over the past few years, including medical diagnosis, courts of law, and studies of animal behavior. The identification of organisms is based on the theory that genetic material of each living organism (plant or animal, bacterium or virus) possesses sequences of DNA and RNA that are unique. Variations in the genetic composition of these sequences make it possible to trace an organism back to its evolutionary origin, and scientists can identify which ancestral species a particular organism came from. *Phylogeny*, the evolutionary relationship of organisms, makes it possible to draw an evolutionary "tree" to illustrate this relatedness, such as the family tree that people reconstruct to trace their ancestry (Figure 1).

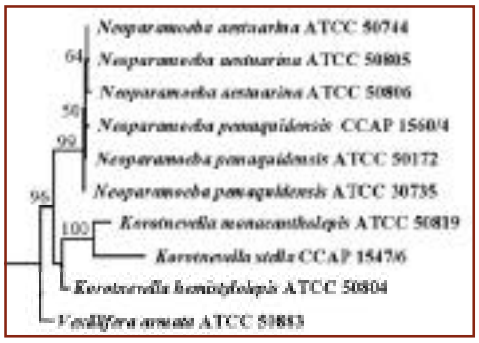

Figure 1. A phylogeny, or "family tree" of *amoeboids related to Neoparamoeba. Note close relationship of N. aestuarina and N. pemaquidensis.*

Researchers now have the ability to amplify DNA fragments from minute samples and they can even use degraded or partial DNA molecules. This means researchers can generate a more complete picture of the evolutionary tree, and include a much larger number of species, even rare ones. The basic PCR process uses a certain type of enzyme known as *DNA polymerases* which is present in all living things, and whose sole purpose is to copy genetic material. These enzymes can duplicate genetic material taken from cells, blood, hair, water or sediment samples. They work equally well on microbes, animals, or plants. PCR uses the organism's genomic DNA or cellular RNA as a *template-molecule* and two primer molecules to get the copying process started. Primers are short chains of the four different chemical components that make up the strands of all genetic material. These four components are the building blocks that are used to construct genetic material and are called *nucleotides* or bases.

DNA is a chain of molecules, which, under most conditions, exists as a double stranded helix consisting of two nucleotide chains that wrap around each other. A primer is a single strand of nucleotides arranged in a specific order that, under the right conditions, binds to a specific complementary sequence of nucleotides in another piece of single strand RNA or DNA.

The PCR process includes three basic steps. First, the target genetic material must be *denatured*; that is, heat is used to unwind the two strands of the DNA helix. The second step is *hybridization,* where primers are mixed in, and if they find their complementary sequences in the DNA, bind to them. The third step is the synthesis of new DNA by polymerase. Starting with the primer, the polymerase reads the original template strand and matches it with complementary nucleotides that are then linked together into a newly-synthesized strand. The result is that two new helixes are generated; each composed of an original strand plus its newly assembled complementary strand. These steps are completed in a test tube by adding reagents and a heat source. Different temperatures are required for each of the steps described, and machines control these temperature variations automatically.

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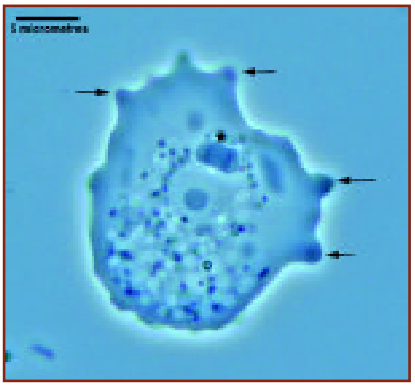
Investigators have been using PCR-based approaches to identify the organism found in high numbers of lobsters during the mass mortality in Long Island Sound. It is now evident that the organism is not a plant, a bacterium, or an animal, but is a particular *amoeboid protist* known as *paramoeba*. Amoebae are single-celled eukaryotic organisms; the cells in eukaryotes possess a clearly defined nucleus containing chromosomes, bounded by a membrane. (Paramoebae are distinguished from other amoebae by having a nucleus plus a lesser, secondary nuclear body.) Many species of amoebae can cause animal disease, such as amoeboid dysentery in humans. Richard French, a UConn pathologist, first isolated this organism in 1999. Although there were reports of paramoeba about twenty years ago, it is uncertain if they are the same species affecting lobsters today.

Researchers have successfully extracted SSU rRNA from cells in lobster tissue, and used PCR to determine the exact order of the bases that make up this genetic marker, a process known as *sequencing*. The results have been compared to the SSU rRNA marker in other species, and used to construct a phylogenetic tree. From the tree, the researchers have determined that the organism found in 1999 likely belongs to the genus *Neoparamoeba* (meaning "new amoeba lookalike"); the species is probably either *pemaquidensis* or the very closely-related *N. aestuarina* (Figure 2).

Researchers Patrick Gillevet and Tom Nerad are continuing the research to identify *Neoparamoeba* in the environment, using a modified PCR technique. They have identified a specific genetic marker for *Neoparamoebae* and will amplify it to create a "fingerprint" of the organism. This information will be used to determine its distribution in the natural environment. This new technique, allows the researchers to monitor a community of organisms; each organism produces different amplification products. Figure 3 depicts an example of such a fingerprint, in which different size amplification products are separated and identified as peaks in a profile.

These researchers are taking the genetic analyses a step further, to determine whether *Neoparamoeba* was the primary, or sole, cause of the lobster mortality in LIS in 1999, or a secondary cause. It is unclear whether other factors, including other organisms, were involved in the mortality. To find out whether other organisms were involved, researchers must simultaneously identify all potential infectious organisms present. As many as 1000 different organisms can be identified simultaneously in a single environmental sample from water or sediment, using *microarray analysis*. A microarray consists of a small glass microscope slide where DNA primers specific for the SSU rRNA of the 1000 different organisms are synthesized directly onto the support. The microarray is then hybridized with fluorescent labeled environmental samples and the labeled genomic DNA then sticks to the corresponding primers on the array. Figure 4 is an example of a microarray experiment. The location of the signal and its intensity indicates whether an organism is present and how abundant it is. This technique allows the researchers to survey a large number of organisms quickly to determine if there is a correlation between lobster mortality and the presence of an infectious agent.

Patrick Gillevet and Thomas Nerad are members of the biology faculty, George Mason University; They are working with Charles O'Kelly, a researcher with Bigelow Laboratory for Ocean Sciences, to develop a tool to conduct genetic "fingerprinting" of the paramoeba organisms.



C. O'Kelly, Bigelow

Figure 2. View of a settled, flattened cell of a strain of *Neoparamoeba pemaquidensis,* with short dactylopodia (fingerlike pseudopodia; arrows), parasome (*), and nucleus evident.

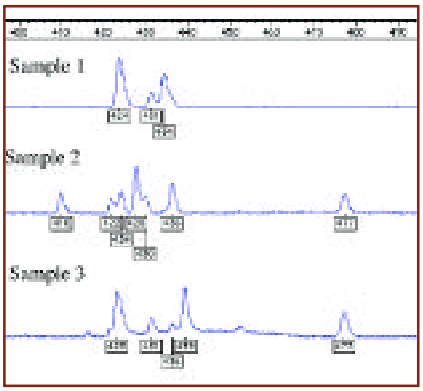


Figure 3. "Fingerprints" or specific genetic markers from a sample of *Neoparamoeba*. Different size amplification products are separated and identified as peaks in a profile.

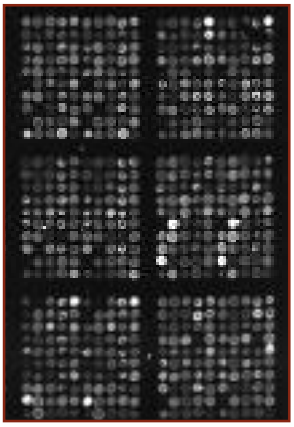


Figure 4. In a microarray such as this, the location and intensity of the fluorescent signals from labelled DNA markers indicate whether or not an organism is present and how abundant it is. 1000 organisms can be tested simultaneously.

Monitoring Long Island Sound Lobster Populations

The state agencies in Connecticut and New York continued to deploy staff in regional at-sea monitoring of the commercial lobster catch. CT DEP staff conducted over 150 sea-sampling trips since the enhanced monitoring program began (January 2001 to December 2002). "We have achieved a lot in the past two years. Additional sea-sampling effort allowed us to fill data gaps in our 25-year time series. The Department is in a better position now to provide information that's more helpful for lobstermen and researchers to use," says Eric Smith, Acting Director of DEP's Marine Fisheries Division.

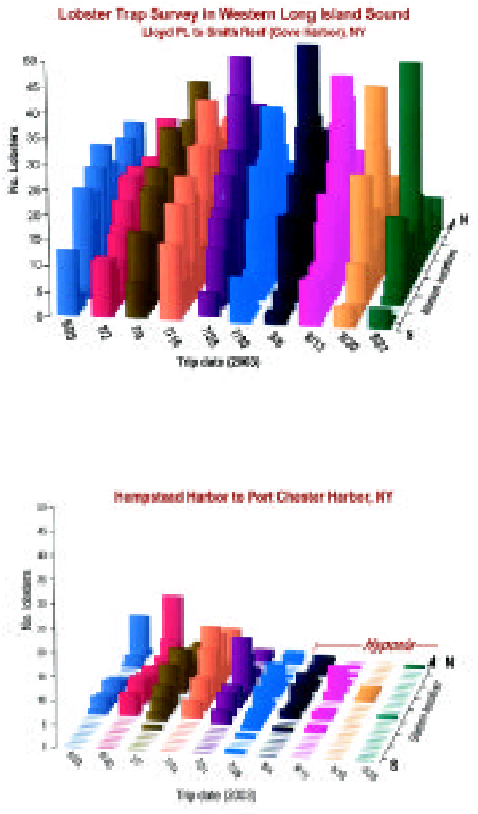
Dead lobsters are still being recorded in commercial catches especially in the fall, and this incidence has increased in recent years. Since these mortality events are localized and usually sporadic, it is difficult to have a complete description of what is happening in Long Island Sound. CT DEP developed a data logging system to archival reports of limp and dead lobsters, and other marine organisms. Commercial license holders can report incidents in their monthly logbook and everyone is encouraged to call-in unusual marine events. Such reports should provide a useful tool for researchers and resource managers to investigate the timing of marine disease outbreaks. The majority of the incidents that have been reported to date (71 per cent) concerned dead, limp, weak, or dying lobsters, while the others were related to blue crabs, blue mussels and menhaden.

The **Long Island Sound Trawl Survey** is designed to generate an index of lobster abundance, which can be used to assess the fishery. Some of the lobster research grant was used to boost CT DEP's program so that extra sampling in the Narrows can provide a means of comparing lobsters populations from areas that were hardest hit in the 1999 die-off with other areas that were less impacted. The spring mean catch rate in the Narrows more than doubled in 2002 (10.2 lobsters/tow in 2002 compared to 4.9 lobsters/ tow in 2001), but remained well below the level observed in 2000 (15.8 lobsters/tow). The index for the rest of the Sound remained essentially the same (6.3 lobsters/ tow in 2002 and 7.6 lobsters/tow in 2001).

Penny Howell, CT DEP Biologist, reported that the lobster tagging study has been a success so far. "This is an excellent case of the lobster industry working side by side with our staff to collect data, and everyone wins." About 13,000 lobsters in Long Island Sound have been released with tags by CT DEP staff, and NYS DEC staff recently joined this effort. The recapture rate for these lobsters is about 25 per cent, with an average release period of over 150 days. Lobsters that are 'at-large' for more than one month generally traveled less than 3 miles (5 km) from the point of release; however, the results indicate maximum distances can be greater than 12 miles. So far, the data show no difference in the movement pattern for males, females, and egg-bearing females.

A part of the research is investigating stock discrimination using genetic techniques, in a study being led by Joseph Crivello, a University of Connecticut faculty member. The genetic tests analyzed tissue samples taken from egg-bearing females and larval lobsters to determine if there are differences in lobsters at the genetic level in the three basins of the Sound,

Fisheries biologists collect data to provide better estimates of annual harvest and catch rates for lobsters within the three basins in Long Island Sound. Carapace measurements are being taken under the resource monitoring program being conducted by the state agencies.



Summary of catch taken in traps set in the western basin; the preliminary results indicate a general reduction in catch during periods of hypoxia.

and outside the Sound. Preliminary results support the current belief that lobsters don't migrate like other marine animals. There are quantifiable differences between the egg-bearing females within the three basins, which implies local breeding populations of lobsters occur within the Sound. A similar situation is observed when samples from lobsters in Long Island Sound and Hudson Canyon were compared. These first results of genetic analyses support the separation of lobsters in the Sound from offshore stocks for management purposes, as well as the hypothesis that western Long Island Sound supports a separate breeding population. However, larval lobsters don't appear to be as clearly separated as the adults. Analyses are ongoing to clarify possible differences in survival that may be contributing to the genetic make-up of the stock.

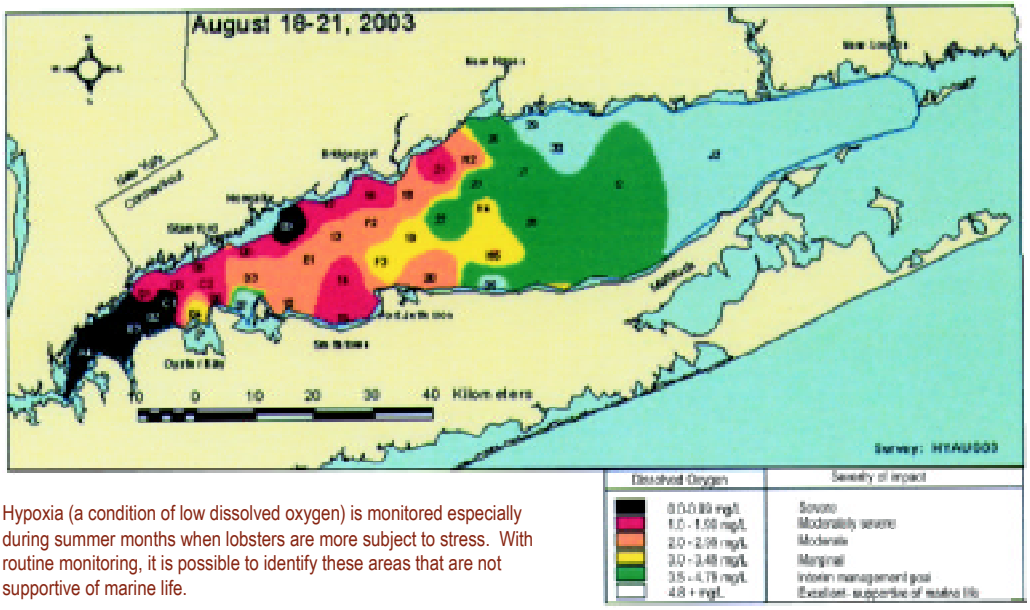
CT DEP is looking forward to getting results from a new study to analyze how lobsters select habitat throughout the Sound. This study is being headed by Roman Zajac, faculty member of the University of New Haven, who is developing a computer model using *Geographic Information System* (GIS) software. This software will allow users to look at all kinds of different variants at the same time, such as bathymetry, slope, sediment type, and multiple layers of environmental data.

The **Lobster Trap Survey** completed its first year in the western basin. This program was designed by NYS DEC as three series of trap-strings that are deployed between Hempstead Harbor and Oyster Bay (NY), from June to December. Gordon Colvin, Director of the Bureau of Marine Resources notes that "the mass mortality event changed the fishing operations and many lobstermen no longer work these areas, however, everyone recognizes the need to generate data from the area, so we had to develop a new program for this purpose. But, we will be able to provide information to correlate with the environmental research that is ongoing in this region." NYS DEC staff use special traps to capture sub-legal lobsters; these traps don't have escape vents and they have a smaller mesh gauge.

NYS DEC biologists began to analyze the data, and so far the results indicate that lowest catch rates were experienced in trap-strings set in the extremely western sites, but this generally improved when the easterly traps were examined. Catch rates also increased from June through early August.

The first year sample intercepted an important period when lobsters are known to be under stress in Long Island Sound. In late August 2003, staff caught lobsters and crabs that were lethargic and didn't look very healthy, especially when the dissolved oxygen measurements had fallen below 1 mg/l in the western basin. These observations are consistent with the initial reports that were associated with the 1999 lobster mass mortalities, and more detailed analyses of the data are necessary to assess the overall status of the fishery in this region of the Sound.

Source: Penny Howell and Kim McKown, biologists with CT Department of Environmental Protection, and NYS Department of Environmental Conservation, respectively.



Hypoxia (a condition of low dissolved oxygen) is monitored especially during summer months when lobsters are more subject to stress. With routine monitoring, it is possible to identify these areas that are not supportive of marine life.

Lobster Health Symposium

Seeing fishing through the eyes of a lobsterman

Matthew Sclafani

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Lobster vessel *Wendy J.* returning from sea.

Cornell Cooperative Extension of Suffolk County Marine Program developed an educational multimedia documentary to educate the public about the natural history of lobster fishing in Long Island Sound. This program is timely in wake of the lobster mass mortality, which caused many lobster fishermen to abandon their businesses. The show is entitled "*Long Island Sound Lobsters: A Fishery on the Brink,*" and was shown at the Vanderbilt Museum Planetarium Dome in Centerport, Long Island (NY). The EPA Long Island Sound Study supported the project. The audience experienced a unique perspective of this important natural resource, and learned about the humble beginnings of this fishery. The presentation immersed the audience in the underwater world of the American lobster. Viewers had an opportunity to be a lobsterman for a day, but didn't get to keep the catch!

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Matthew Sclafani is the Senior Extension Educator, Cornell Cooperative Extension of Suffolk County Marine Program,

Please check our web site: www.seagrant.sunysb.edu/LILobsters for the latest updates as well as archival information. Or, contact one of the Lobster Outreach Coordinators to place your name on the mailing list:

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