The Newsletter of the
Center for Coastal Environmental Health
& Biomolecular Research

National Ocean Service
Charleston, S.C. & Oxford, MD.
CCEHBR Home Page

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Welcome to CCEHBR Quarterly

Welcome to the first issue of CCEHBR Quarterly. The Center for Coastal Environmental Health & Biomolecular Research (CCEHBR) is one of four research centers (a fifth is proposed) of The National Centers for Coastal Ocean Science under the National Ocean Service, NOAA. The CCEHBR Quarterly will be published every three months (January, April, July, October) to highlight selected activities of the Center. The Center at Charleston, South Carolina, provides scientific information required to resolve important issues related to the health of coastal ecosystems. The Oxford Cooperative Lab, affiliated with Charleston, specializes in shellfish pathology and habitat restoration research.

Marine Forensics Branch Occupies New Facility

In April of 2000, Marine Forensics Branch staff occupied their newly renovated 2500 sq. ft. facility within CCEHBR. The new laboratory and office suite provides much needed space for a program that doubled it's staff and case load over the past couple of years. The new facility will allow continued expansion of Forensic investigations involving marine animals and provides a modern, secure base of operations. A major impetus for the construction of a new Marine Forensics facility was our goal of becoming accredited by the ASCLD/LAB (American Society of Crime Laboratory Directors/Laboratory Accreditation Board). The standards and criteria for accreditation encompass 1.)

Laboratory Management & Operations, 2.) Personnel Qualifications, and 3.) The Physical Plant. Prior to construction of the new facility, it would not have been possible to meet the physical plant standards and criteria. The U.S. Department of Justice, in publication NCJ168106, "Forensic Laboratories: Handbook for Facility Planning, Design, Construction, and Moving" details recommendations for an adequate and safe work environment in a forensic laboratory. In part, they state that "the design should maximize laboratory functions and activities, safeguard the physical evidence, protect the confidential nature of the laboratory operation, and provide a safe and healthy working environment". We are confident that our new space will allow us to meet or exceed the physical plant requirements. Some features of the new facility include:

- State-of-the-art security with a computerized card access system on each door in the area. Access to the area and to each room can be individually controlled as needs change.

- A secure Evidence Room includes space for dry, refrigerated, and frozen (-80°C) evidence and forensic comparison standards. A separate locked walk-in 4°C cooler and two walk-in -30°C freezers provide dedicated storage for larger items of evidence.

- Extensive photographic and graphics capability. A Polaroid MP-4+ Copy Camera, 35mm SLR Film Camera (Canon EOS A2E), and a high resolution digital camera (Kodak DSC-520) are available to document evidence, comparison standards, and forensic examinations. A high resolution scanner is available to scan photographs and other materials and a Tektronix Phaser 780 Color Laser Printer can output full bleed, continuous tone, 1200 dpi images at up to Tabloid Plus (13" X 19") size.

Graphics Workstation

Polaroid MP4+ for Gel Documentation

- Dedicated and secure lab space for protein and DNA analyses

IEF and PCR Work Area

Sample Prep Work Area

- Necropsy facilities to investigate unusual mortalities in marine anmals.

Necropsy Table

X-ray showing hooks

- Secure office space where confidential case materials can be worked on and discussed in private.

- Secure computer facilities. The Marine Forensics Branch operates its own network servers, separate from the main CCEHBR network.

- State-of-the-Art LIMS (Laboratory Information Management System) system with bar-coding capability to track evidence chain-of-custody, automate data collection, and prepare both case and management reports.

As we continue to expand our forensic capabilities, we will enhance our facility further with such items as:

- Enhanced capability for microscopic examinations. In the near future we plan to purchase a Stereo microscope with photomicrographic capabilities to document examination of forensic evidence too small for our existing macro photographic gear.

- X-ray machine with digital, filmless imaging capability to add to our necropsy capabilities.

- Expanded evidence warehousing capabilities to allow efficient evidence handling and storage of evidence and standards. We will be gradually implementing the use of cryogenic freezers to maximize the storage life of difficult to obtain standards

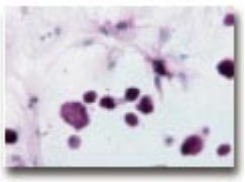
from endangered and threatened marine animals.

Additional information on CCEHBR's Marine Forensic Branch, including a FAQ page covering Frequently Asked Questions can be found at www.chbr.noaa.gov/mfpage.html. Also, if you have an interest in Wildlife and Fisheries Forensics, visit or subscribe to our Wildlife Forensics Listserv here.
http://www.chbr.noaa.gov
/cgi-bin/lyris.pl?site=ccehbr&page=topic&topic=wildlife+forensics&text_mode=0

A Snapshot of the NOAA Cooperative Oxford Laboratory By CCEHBR - Pathology Branch

by Fred Kern

The Oxford Laboratory opened its doors in 1960 as an expansion of the Annapolis, Maryland US Fish and Wildlife Service, Bureau of Commercial Fisheries program. Research focused on Chesapeake Bay Ecology, Oyster Culture, and a newly expanded focus on Shellfish Pathology.



Early research focused on two disease agents that caused severe losses to the oyster industry of the mid-Atlantic region. The first, referred to as MSX, Multi- nucleated Sphere X and later identified as *Haplosporidium nelsoni* , and the second DERMO or *Perkinsus marinus* . Both parasites have expanded their ranges and continue to cause major losses to shellfish resources.

In 1980, the laboratory became part of NOAA's National Marine Fisheries Service and continued to expand research programs to include health of a variety of marine and estuarine species. In 1987, NOAA entered into a Cooperative Agreement with the Maryland Department of Natural Resources to share the facility and conduct research on the health of living marine resources. In 1998, new construction was completed to expand the facility as well as completely refurbish the old building. More recently the Laboratory programs were transferred to the National Ocean Service, National Centers for Coastal Ocean Science, Center for Coastal Environmental Health and Biomolecular Research at Charleston, South Carolina.

CURRENT RESEARCH

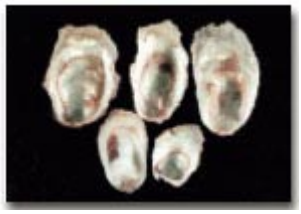
Oyster Diseases:
Juvenile Oyster Disease (JOD)

This is a disease of cultured oysters that was first observed in 1984 in oysters imported

into New Hampshire from Maine. By 1990, the disease was documented from Maine south to New York. The cause of JOD remains unknown, but mortalities of 60-100% are common. NOAA Oxford scientists in collaboration with industry and university colleagues found the disease to be infectious, documented the incubation period, and found a relationship of the disease to water temperature and salinity. The greatest accomplishment was working with industry colleagues to implement management techniques and develop a disease-resistant strain of oyster that allowed a return to a record production of oysters. In the last several years problems from the disease have diminished greatly.

Cryptosporidium in shellfish and shellfish growing waters

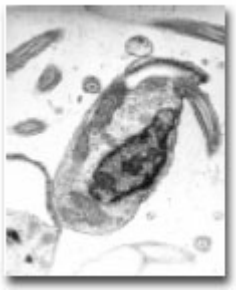
Cryptosporidium parvum is a protozoan parasite which causes human disease. The source of the pathogen is fecal material, thus the parasite is not only an environmental contaminant, but its presence in shellfish consumed raw potentially compromises seafood safety. NOAA, USDA, and Johns Hopkins University scientists collaborated in studies to determine if molluscan shellfish can serve as biological indicators for waterborne fecal contamination with *Cryptosporidium*, and if oysters harvested from the



Maryland portion of Chesapeake Bay contain oocysts of *C. parvum*. These are the first such studies of *C. parvum* in shellfish and their growing waters. The team has authored ten peer-reviewed publications; the most recent publication documents the recovery of infectious *C. parvum* from oysters in state-approved growing waters where shellfish are routinely harvested by commercial watermen. The research team is looking to extend their initial research to determine the prevalence and source of *C. parvum* in coastal waters beyond Maryland and to conduct epidemiological studies.

Clam Diseases:
Soft Clam Dermo *Perkinsus chesapeaki*

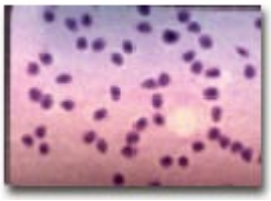
Despite the ecological importance and economic potentials of soft-shell clams, *Mya arenaria*, surprisingly little is known about their basic biology in general and diseases. Histopathology studies have provided some important new insights into diseases of soft-shell clams. Investigators were able to link severe mortalities of soft-shell clams in the Chesapeake Bay with a progressive and fatal proliferative disorder known as disseminated sarcoma. These sarcomas have been found at high prevalence and infection intensities in soft clams examined from a number of bay sites. Recently investigators discovered increased prevalence and intensities of *Perkinsus* spp. parasites in soft-shell clams of the Chesapeake Bay. Histopathology studies showed Perkinsus infections to be generally mild in soft-shell clams; however, large abscesses could be found in most any tissue in advanced cases of infections. Attempts to isolate the parasite *in vitro* were successful and resulted in the isolation of two distinct *Perkinsus* spp. Morphology, life cycle, biochemical, and genetic characterization studies resulted in the identification



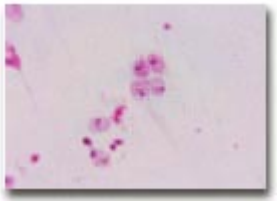
of the one isolate as the oyster pathogen, *P. marinus* , and in the description of the second isolate as a new species, *P. chesapeaki.*

Crustacean Diseases:
Blue Crab *Callinectes sapidus* diseases

Numerous diseases are found to cause mortalities in both wild and captive blue crabs which are an important economic and recreational resource on the Atlantic and Gulf coasts of USA. Research involves *Hematodinium* sp., a parasitic dinoflagellate which infects and kills blue crabs. Investigations show the parasite has a wide distribution along the Atlantic and Gulf coasts. Experiments indicate salinity and temperature influence the prevalence of the parasite.



The staff at Oxford first described *Paramoeba perniciosa* as a parasite found in blue crabs. It was also identified in lobsters and other crustaceans. This valuable information was presented at a recent workshop discussing elevated mortalities of lobsters from Long Island Sound.



Recent reports of copepods with tumors in the Great Lakes regions initiated collaboration with researchers at the Great Lakes Environmental Research Laboratory. A recent publication describes the sudden appearance of cysts and ellobiopsid parasites on zooplankton in a Michigan lake which gives a potential explanation of the tumor-like anomalies.

Disease Diagnostic Techniques:

In the forty years of studying the diseases of marine organisms a long list of histological procedures were developed by the personnel at Oxford. Many were adapted from the studies of other species from human to insects. These techniques were published in a manual of Histological Techniques for Marine Bivalve Mollusks that is recognized as a standard for processing marine animal tissues. A revised edition of the manual that expands on the techniques for mollusks and other marine species is soon to be published. Over the years the Oxford staffs have worked with and trained many researchers from states, universities, and foreign countries in the identification of disease agents, pathology, and the techniques we have developed to demonstrate their presence.

Non-Indigenous Species:

Staff members participate in the Chesapeake Bay Program Non-Indigenous Species Committee activities. Issues addressed include the grass carp, Pacific sturgeon, Asian oysters, and Rapa whelks.

Staff members also support activities of the National Aquatic Nuisance Species Task

Force. They are currently involved with the Task Force-Green Crab Committee that is supporting state and industry efforts to monitor and control this European species that is new to the west coast of the United States.

Marine Mammal and Turtle Stranding:

Staff support the regional Marine Mammal and Turtle Network by assisting State of Maryland researchers in responding to and recording information on animals reported stranded along the Delaware, Maryland, and Virginia coasts.

For More Information:

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Oxford Laboratory
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Phone: 410 226 - 5193
Fax: 410 226 - 5925
URL: http://www.chbr.noaa.gov/colpage.html

The Development of Sediment Quality Test Protocols for Estuarine Organisms

By Michael H. Fulton

Chemical contamination of sediments has resulted in the need to develop an appropriate suite of toxicity tests to assess ecotoxicological impacts in estuarine ecosystems. Existing Environmental Protection Agency (EPA) protocols recommend a number of test organisms, including amphipods, polychaetes, mollusks, crustaceans and fish for use in sediment toxicity tests. While this suite of test animals represents a diverse group of fauna, many of the species recommended by the EPA are not indigenous to all geographic regions of the United States, particularly the Gulf of Mexico and South Atlantic. As a result, environmental risk assessment based on these organisms may not adequately protect ecosystem health in all regions of the country. Ideally, test organisms to evaluate sediment toxicity should include species that are indigenous to the various regions of the country and represent a variety of faunal classes and feeding types. Additionally, the toxicity test endpoints should include both lethal (mortality) and sublethal (reproduction, growth, physiological impairment) effects and be sensitive to either porewater and/or whole sediment exposures of all major classes of chemical contaminants (trace metals, polycyclic aromatic hydrocarbons [PAHs], pesticides). Finally, test species should be easy to collect and maintain in the laboratory.

CCEHBR's Marine Ecotoxicology Branch, in collaboration with SCDNR, the University of

South Carolina and EPA, conducted a series of experiments which evaluated the relative sensitivity of a variety of test organisms, broadly distributed throughout the southeastern United States and Gulf of Mexico, to several classes of chemical contaminants in both whole sediment and aqueous/porewater tests. Additionally, several rapid screening assays were compared with these more traditional toxicity evaluations. The three model contaminants selected for study were cadmium (an inorganic toxicant), DDT (a persistent organochlorine pesticide) and fluoranthene (a PAH). These compounds represent contaminants frequently measured in sediments throughout the southeastern United States and Gulf of Mexico. Species tested included the grass shrimp (*Palaemonetes pugio*), two amphipods (*Ampelisca abdita* and *Ampelisca verrili*), a copepod (*Amphiascus tenuiremis*) and a juvenile clam (*Mercenaria mercenaria*). In addition, several rapid toxicity-screening assays (Rotoxkit M™, Mysid IQ Test™, Microtox™ and Mutatox™) were evaluated.

Overall, the juvenile clam was the most sensitive species tested from an acute toxicity standpoint. The grass shrimp and two amphipod species were similar in sensitivity to each of the three compounds. The copepod assay, although relatively insensitive in terms of adult mortality, was capable of detecting sublethal reproductive effects at contaminant concentrations below those which caused mortality in the other more sensitive species. Both the juvenile clam assay and the copepod partial life-cycle test have the potential to serve as sensitive indicators of potential sediment-associated toxicity which might not be detected using standard acute toxicity bioassays.

The relative sensitivity of the species tested varied for the different chemical contaminant classes. This suggests that multiple species approach may be more appropriate for a holistic ecological risk assessment of sediment contamination. The "Crustacean Triad" (copepods, amphipods and grass shrimp) provides a battery of tests which predicts toxicity in epibenthic and benthic crustaceans. These organisms represent the base of the food chain for most recreationally and commercially important fish species that utilize estuarine nursery grounds. The addition of the juvenile clam assay provides a herbivorous filter feeder with the ability to bioconcentrate pollutants and which is extremely sensitive in the size range tested (>212<350 µm). Field assessments in South Carolina have indicated that sites with high sediment contaminant levels have degraded benthos, with significant effects observed in crustaceans and mollusks. These findings are in agreement with our laboratory results and suggest that an integrated battery of tests may be most appropriate for estimating field effects.

Two FAQ Links Added to CCEHBR's Website

Two new "Frequently Asked Questions" (FAQ) links have recently been added to the CCEHBR website. One group of FAQ provides answers to questions about the CCEHBR's

Forensics Branch. Most of what goes on in the Forensics Branch is behind locked doors, and active cases can't be discussed. So just what do they do? Here's your chance to find out! Learn about how and why the Charleston Lab got involved in forensics, who their clients are, what's a chain of custody or an expert witness, what type of analyses do they conduct, and more. There's even a place for you to ask a question about forensics. Just click here (http://www.chbr.noaa.gov/ForensicsFAQ.htm) to learn about CCEHBR's Forensics Branch.

The second FAQ link recently added to our website deals with our cooperative Shellfish Information Management System (SIMS). At a recent workshop in Atlanta with our state molluscan shellfish partners, it was agreed that a SIMS FAQ link would be added to CCEHBR's website. Questions are divided into two types - a general category for individuals interested in an overview of the SIMS Program and a technical category to assist cooperators and users of SIMS. To learn more about this integrated database of molluscan shellfish resources and water quality information with geographic information systems (GIS)functionality click here (http://www.chbr.noaa.gov/newraim/SIMSfaq.htm).

Scientists Identify New Clam Parasite

Shawn M. McLaughlin, a biologist in the Oxford-based Pathobiology Branch, lead a study that resulted in the identification of a new parasite, Perkinsus chesapeaki, in softshell clams of the Chesapeake Bay. The parasite may be a major factor in the sharp decline of the state's soft-shell clam population. It is closely related to the deadly oyster pathogen, *Perkinsus marinus*, commonly referred to as dermo.The study was a collaborative project of the National Oceanic Atmospheric Administration, Virginia Institute of Marine Science and the U.S. Department of Agriculture's Agricultural Research Service. The story was covered by Associated Press with articles in the Baltimore Sun and The Washington Post. An article was also published in the June issue of the Journal "Parasite".

Upcoming Events

Interstate Shellfish Sanitation Conference Meeting - July 15-20, 2000

Paul Comar and Geoff Scott will participate in the 18th annual meeting of the ISSC whose goal is to promote safety in the consumption of molluscan shellfish and encourage the restoration of shellfish habitat. Approximately 200 shellfish industry, federal and state government representatives will review and take action on 52 formally submitted issues and resolutions. One major effort will include decisions on a new long term risk management plan to reduce morbidity and mortality from pathogenic *Vibrio vulnificus* in

oysters.

Phycologoical Society of America Annual Meeting

Tod Leighfield and Michele Barbier will be attending the 54th annual meeting of the Phycological Society of America in San Diego, CA in mid-July. They will be presenting three posters entitled: Measurement of microalgal cell volume by flow cytometry, Identification of a cyclic AMP-dependent protein kinase in the dinoflagellate *Amphidinium operculatum*, and Cell cycle regulators in the Florida red tide dinoflagellate *Gymnodinium breve*. The Phycological Society of America is a scientific organization that promotes the study of algae and fosters phycological research and education. This year's meeting will be held in conjunction with the American Society of Plant Physiologists.

Coastal and Estuarine Risk Assessment Forum - July 20-21, 2000

Dr. Geoff Scott will attend the Coastal and Estuarine Risk Assessment Forum to be held at the College of William and Mary, VIMS at Williamsburg , VA on 20-21 July. Dr. Scott has been invited to speak on risk assessment techniques used to assess urban and agricultural nonpoint source runoff impacts.

July 26 - CCEHBR Seminar Series* - The use of teeth aging as a method to estimate the age of bottlenose dolphins and pygmy sperm whales presented by Wayne McFee.

American Chemical Society Meeting - August 22-25

Dr. Geoff Scott has been invited to make a presentation at the American Chemical Society meeting in Washington, DC August 22-25. The topic of his talk will focus on the agricultural pesticide nonpoint source runoff effects in South Florida Ecosystems.

August 23 - CCEHBR Seminar Series* - Coastal landcover classification using NASA's Airborne Terrestrial Applications Sensor (ATLAS) data presented by Chris Nichols.

*CCEHBR Seminars are held on the 4th Wednesday of the month at 12:00 a.m. in the Lois Winemiller Auditorium.