



**Title:** Integrated watershed-based molecular and hydrologic monitoring techniques to assess pathogen loading

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**Organization:** UNC Coastal Studies Institute

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**Theme:** Watershed Management

**Situation:** Thousands of acres of shellfish waters in NC and other coastal U.S. states are permanently or conditionally closed to harvesting due to elevated levels of bacteria. The source(s) of the bacteria is rarely known, and fecal coliform levels determined using traditional monitoring approaches do not differentiate relative contributions of human and animal sources. NOAA, Sea Grant and the ISSC hosted several workshops (Scott et al, 1996) and international symposiums (First Shellfish Restoration Symposium) on shellfish restoration issues. Two findings have clearly emerged from discussions with shellfish regulators, industry representatives and scientific researchers are: (1) water quality issues will drive shellfish research and policy in this region; and (2) there is a sore need for new analytical techniques which help differentiate human versus animal pollution sources. Those most affected by and interested in these issues are local leaders, land management and fishery professionals, academic colleagues, environmental groups, citizens, and the watershed communities will be involved in the project for both for feedback and input.

**Objectives:** The first objective of this project is to further the knowledge base with regards to sources and transport vectors of fecal coliform bacteria contamination in estuarine waters. Three watersheds in the coastal zone with various land uses were the original targets for the first study-residential, industrial, row-crop agriculture, and forestry. With the new award a hog farm will be added to these study sites. Secondly, this project will broaden the DNA and MAR source libraries and further refine the API, MAR and ribotyping techniques with regards to spatial effects and various land uses. This information will be used to improve the capacity of TMDL modeling and management for these and other watersheds on the coast. Third, the modeling output will be synthesized and applied to develop expanded management options for the Shellfish Sanitation Program; and fourth this research will supplement the curriculum and provide study opportunities for students enrolled in the Coastal Environmental Management Program at Duke Marine Lab in Beaufort, NC and the NC State Microbiology and Environmental Toxicology programs.

**Methods:** Methods to be employed and tested in this project are divided into three major areas of effort: first, bacteria source tracking using watershed assessment, hydrologic monitoring, MAR and ribotyping; second, an assessment and comparison of state's approach for determining sources and loads using the TMDL model development and evaluation approach; and third, review, comparison and assessment of shellfish program's hydrographic-based approach for management.

**Partnerships:** During the previous and ongoing projects cooperation between NC State and Duke University, NC Sea Grant scientists and NC-DENR Shellfish Sanitation Branch (SSB) and NOAA-CCEHBR personnel has been extensive. A group of representatives from DENR subgroups including DWQ, Division of Environmental Health, and Shellfish Sanitation met to provide guidance to the original bacteria source tracking project proposal with the personnel from the NOAA/NOS Center for Coastal Environmental Health and Bimolecular Research adding experience and expertise in bacteria source tracking to the team. Representative(s) from each entity serve on a steering committee for the overall effort. Collaboration between the SSB and NC State has been seamless thus far such that data is regularly shared and will continue. Duke and DEH agencies are formally listed as subcontractors on this proposal. A MOA has been executed with NOAA. Additional partners with the new award are the NC State College of Veterinary Medicine, College of Agriculture and Life Sciences Departments of Environmental Toxicology and Microbiology.

**Research:** NC State University, Shellfish Sanitation, and Duke University Marine Laboratory members will be jointly responsible for project management, technical assistance, data analysis, extension and curricular education, and project reporting. NC State, Duke Marine Lab and Shellfish Sanitation will organize and supervise field data collection. The NC State-CMAST lab and Shellfish Sanitation Branch Laboratory in Morehead City will be responsible for collecting and analyses of the bacterial samples. Split samples will be provided to NOAA/CCEHBR for analysis and Quality Control/Quality Assurance. UNC Coastal Studies Institute will conduct scanning, GIS and statistical analyses. All team members will review analytical procedures and results. Duke Marine Lab is coordinating educational output in conjunction with National Estuarine Reserve Program, NC Department of Environmental and Natural Resources, Division of Water Quality Coastal Nonpoint Source Program, NC State and the NC Cooperative Extension Service. All members of each facet of the project serve on the project team, and meetings are coordinated so that a representative from each area can attend, participate, and provide input. Meetings are held bi-annually.

**Resources:** NCSU and Shellfish partnered with NC State's Center for Marine Sciences and Technology and created a 42ft by 42ft lab space outfitted with equipment necessary to conduct the MAR, and DNA processes (Expenditures in excess of \$100,000.00). The majority of the cost for the equipment was covered by the grants already funded by NC-DENR-DWQ. USDA funds covered the costs for a refurbished autoclave and a new computer for the DNA and MAR scanning and spatial analyses. NCSU paid for all lab retrofit and equipment installation. Shellfish Sanitation's resources applicable to all their shellfish program management for these field sites have utilized to support this effort. NOAA-CCEHBR lab has dedicated their staff, space, and lab time to technical advising, training, and running split samples for this effort.

**Results:** Initial MAR results, as reported in Arizona (2003), were very general and ambiguous indicating that additional antibiotics and methods would be needed if that method was to add to the knowledge base beyond the information generated by watershed assessment methods. Partnering with NCSU-Vet med, Microbiology, and Env Tox, a new antibiotic panel and dose regimen was developed for the MAR component. The lab staff from NCSU and NOAA traveled to Florida to work with the BST Team there and developed a standard method to be used by all three labs for the DNA / ribotyping component. A review of statistical analysis methods and software available has left the team also feeling concerned that the approaches currently being reported in the literature are not adequately explaining the data. The new award will allow the team to hire an experienced biostatistician to work on improving these methods. Two more seasons of sampling have been conducted, and the analyses are being conducted. Split sample and comparison analyses with NOAA are nearing completion.



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