

Dredging Research

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DNA technology to impact dredged material projects through faster, more accurate testing methods

By Allison McDonald, ERDC-WES, Environmental Laboratory, contract support

DNA technology is growing in leaps and bounds, with scientists discovering new ways to use the technology every day. Most people associate DNA with criminal cases and paternity testing, but thanks to research projects such as the Human Genome Project, which has isolated and identified thousands of genes, many people are becoming aware of more beneficial uses for DNA technology.

Scientists are finding genes they believe could be responsible for major illnesses. In the future, it may be possible to test a person for such genes and begin preventative treatment for a disease if necessary. Also, scientists are working to determine exactly how pollutants affect living organisms. According to scientists at the Engineer Research and Development Center - Waterways Experiment Station (ERDC-WES), such work may have a great impact on understanding the effects of pollution on living organisms.

Researchers at ERDC-WES are using a new technology called

cDNA (sidebar, page 2) microarrays to test low to moderately contaminated sediments to see if placement in a confined disposal facility (CDF) is necessary. Existing CDFs are reaching capacity, and locations for new sites are hard to find. According to Laura S. Inouye, Ph.D., a research biologist at ERDC-WES, showing that some contaminated sediment levels are not harmful would help solve these problems in three ways. First, low to moderately contaminated dredged sediment could be used beneficially for beach and wetland restoration, landfills, etc. Second, dredged material that is proven not to be harmful could be disposed of in open water. Third, slightly contaminated sediment that has already been placed in CDFs could be cleared to make room for more highly contaminated material.

Continuous dredging is necessary to keep waterways open for use. National security and commerce will both benefit by finding a solution to the problem of what to do with the tons of material dredged annually. Testing of this material is necessary in order to determine if the material should be placed in a CDF or if it can be used beneficially. Traditional testing methods, however, take time and money. These tests are also unable to distinguish exactly what chemical is causing the sediment to be toxic. The new cDNA technology, and all of the benefits it entails, can assist scientists in addressing these problems.

DNA

With cDNA microarrays, scientists can test genes to determine how they are affected by known and unknown toxicants. Exposing tissue to a toxicant allows a scientist to see a change in DNA expression long before it would be evident in the organism. In the past, tests such as these took months to complete because scientists had to look at



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Explanation of Terms

Gene The basic unit of genetic information.

mRNA Messenger ribonucleic acid. Derived from transcription of expressed genes.

cDNA Copy deoxyribonucleic acid. DNA copy of mRNA from expressed genes made by RT-PCR.

RT-PCR Reverse transcriptase-polymerase chain reaction. A technique that is used to transcribe mRNA into cDNA.

Microarray A solid support to which 100s or 1000s of cDNAs have been attached. Used to determine patterns of gene expression.

one gene at a time. With cDNA microarrays, researchers can test hundreds or thousands of genes at a time. Scientists at ERDC-WES agree that use of the arrays results in significant time and cost savings. "Although initial costs are high for method development, once everything is in place, the cost savings can be significant," said Inouye.

Scientists create cDNA arrays on a membrane, chip, or glass slide. Onto each of these, thousands of sections of DNA, 600 to 2400 base pairs, are placed. Each section of DNA represents all or part of the messenger ribonucleic acid (mRNA) of an expressed gene. The arrays are used to measure changes in gene expression by extracting and comparing mRNA from control and exposed cells or organisms.

Scientists today are developing methods to use cDNA array technology to rapidly screen dredged sediment to determine if it should be confined or if it can be put to other uses. Using cDNA arrays to determine how toxicants in sediment affect organisms at the genetic level is one way to provide a rapid screen for toxicity. These tests will help scientists determine what

types of contaminants are in the sediment, how much of each contaminant is there, and what effects can be expected because of their presence. With the results of such tests, scientists are better able to determine which type of management or beneficial use is most appropriate for the dredged sediment.

Case Studies

Environmental Cleanup: Herbert L. Fredrickson, Ph.D., a research microbiologist at ERDC-WES, is using cDNA arrays to assess the hazard of contaminated sediments. In select cases, such as in contaminated wetlands, it may cost billions of dollars to dredge and dispose of the sediment. Fredrickson's research focuses on how to determine if such extreme measures

are necessary. Current methods call for destruction of a wetland if it shows contamination. With cDNA arrays, scientists may be able to prove that low to moderate contamination levels are not harmful. Thus, the harm to the environment would be in destroying the wetland.

Fredrickson's work tests the genes of organisms that have been exposed to a toxicant, and compares them to genes of organisms that have not been exposed, using cDNA microarrays. The arrays measure stress response genes to determine how they respond to toxicants in the sediment. Fredrickson also looks for responses at the DNA level that may cause problems for the whole organism, for example lack of reproduction or death. According to

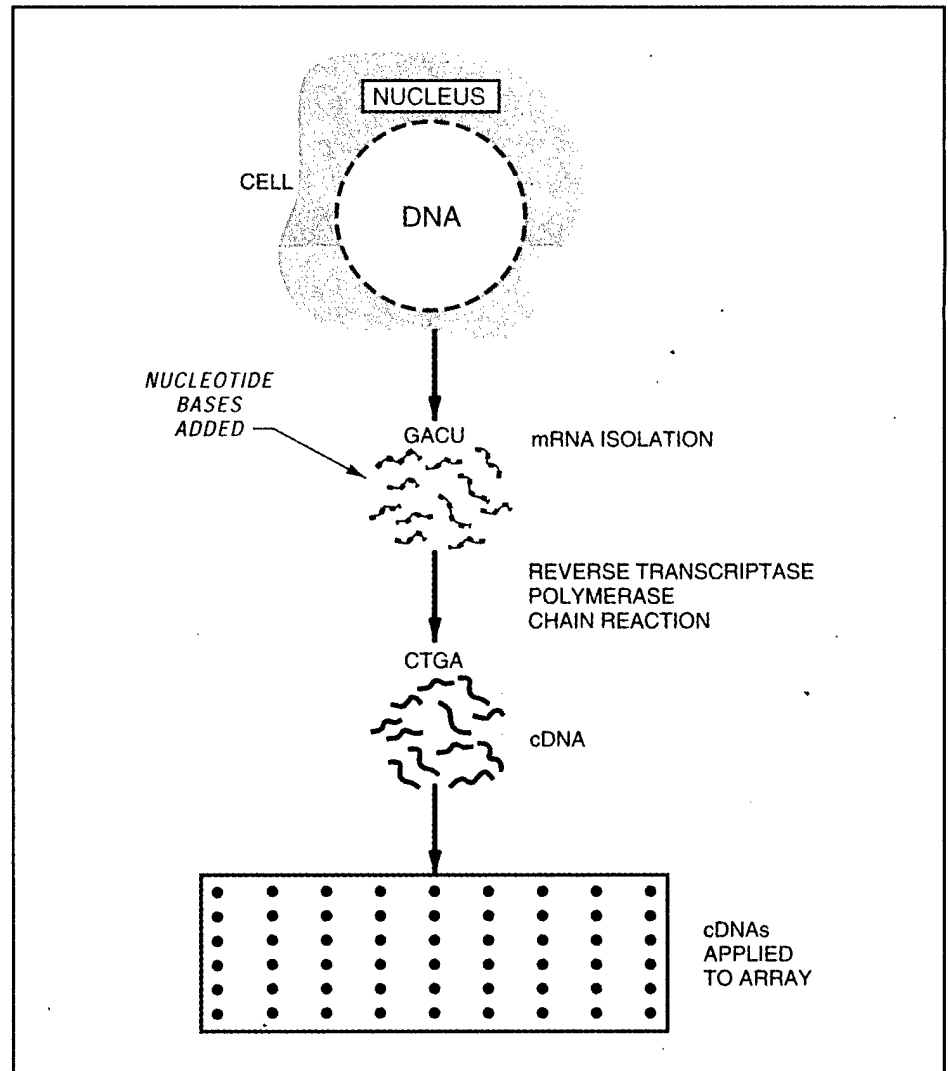


Figure 1. Extraction of mRNA

Fredrickson, if researchers can prove that slightly contaminated sediment has little or no effect, then much unwarranted cleanup and dredging could be stopped, saving thousands of acres of wetlands and billions of dollars.

Higher Level Organism Approach:

Todd S. Bridges, Ph.D., a research biologist at ERDC-WES, is using the cDNA arrays to test for responses in sediment-dwelling invertebrates after performing bioassays on the whole organism. Traditional methods for measuring toxicity using whole organisms can be time-consuming and expensive. The cDNA arrays can be applied as screening tests to determine which sediments require additional testing. However, just because there is a response at the lower levels of organization, i.e., genes and cells, does not mean that the whole organism will be affected. The research challenge is trying to determine exactly what the cDNA data imply in terms of survival and growth of organisms or populations. By experimenting with whole organisms, Bridges hopes to provide a link between the results of experiments at lower levels and what this means in terms of the whole organism. In order to determine how contaminated sediment affects an organism, Bridges' research design involves placing organisms with short life cycles into the sediment. Then he looks for the effects of the toxicants on the organisms. Possible outcomes are death, stunted growth, lack of reproduction, or the toxicant may have no obvious effect on the organism. Once the tests are complete, usually in about a month, the organisms' cells are tested on cDNA arrays to look for genetic stress responses. The results of the cDNA array tests are then compared to what happened to the whole organism in order to provide a link between genetic stress responses and whole organism effects.

In order to test the effect of a toxicant on a particular cell or organism, the scientist first has to extract the mRNA (Figure 1). After it is taken from the cell, the genetic code of the mRNA is transcribed into copy deoxyribonucleic acid (cDNA) by a process known as reverse transcriptase-polymerase chain reaction (RT-PCR). This process pairs the nucleotide bases of the mRNA with their complementary nucleotide bases, resulting in the new strand of cDNA. For example, if the base code of the mRNA is GACUACUG, then the base code for the cDNA will be CTGATGAC. Once this process is complete, the cDNA can be identified and quantified using cDNA arrays. The cDNA, which has been tagged with a fluorescent or radiolabeled nucleotide during the RT-PCR process, is placed onto the array. The cDNA in solution will bind specifically to the cDNA on the array that is its complement. This process is completed for the control population and the treated population, and then the two arrays are compared.

One benefit of these new arrays that Bridges pointed out is the ability of scientists to determine exactly which contaminant in a sediment sample is responsible for its toxicity. This can be achieved by using cDNA arrays to look at the chain of events associated with particular toxicant responses. It is common for sediment to be contaminated with multiple contaminants. If scientists can determine which contaminant is making the sediment toxic, they can focus resources on management of that contaminant.

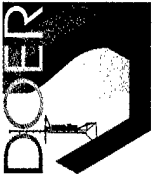
Contaminant/Toxicity Level

Research: Inouye is testing slightly contaminated sediments to determine how much contaminant is too much. Rather than focusing on the prevention of unnecessary cleanup like Fredrickson, Inouye's research looks at ways to prevent confined disposal of contaminated sediments. By proving that slightly contaminated dredged sediment is not harmful, it can be used

beneficially. Inouye uses cDNA arrays to test human cells that have been directly exposed to chemicals extracted from contaminated sediments. The arrays allow for rapid determination of if and how the cell will be affected by the contaminants. Because the human cells are unprotected and are exposed directly to the extracted chemicals rather than immersed into the sediment sample, the effect can be seen much faster. Inouye said, "The test will not necessarily show what will happen to the organism, but is a very sensitive rapid screen that can give you an idea of what type of effect you should be looking for." The screen can also show the types and amounts of toxicant present. If the cells are relatively unaffected by the extracted chemicals, then the sediment should be safe to use for beach restoration, landfills, open-water disposal, and other beneficial uses, according to Inouye.

Additional information is available from Dr. Todd S. Bridges, e-mail bridget@wes.army.mil, Dr. Herbert L. Fredrickson, e-mail fredrih@wes.army.mil, and Dr. Laura S. Inouye, e-mail inouyel@wes.army.mil (Note: Allison McDonald is a contract student performing DOER technology transfer when not attending law school.)

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DOER nearshore-placement research project creates partnering opportunities

Research on sand transport processes on the North Carolina coast suggests that strong, sequential storms can move sand offshore beyond the 5-m depth. Then, over a period of years, that sand slowly migrates back toward the shallower portions of the profile. Research in North Carolina has also shown that the trend in sediment grain size is for coarse sediments to exist near the beach and finer sediments to exist beyond the 6-m depth. These results suggest that placing sediment with a mixture of sands and silt/clay on the nearshore profile will result in the sand component moving gradually shoreward while the fine component remains in deeper water. This sediment dispersal hypothesis is important to validate in the field, as nearshore placement of dredged material may become one of several environmentally preferable methods of managing coastal sediment.

As part of the Corps' Dredging Operations and Environmental Research (DOER) Program, research is being conducted by the the U.S. Army Engineer Research and Development Center-Waterways Experiment Station (ERDC-WES) in cooperation with the U.S. Army Engineer District, Wilmington, to validate the sediment transport hypothesis and to study the ecological effects of a nearshore placement of mixed sediments. For the study, a small mound of mixed sand and fine-grained sediments will be constructed near Duck, North Carolina. The mound will be monitored for at least a year after construction. While the Corps' research activities will focus on the morphologic change of the mound, the dispersal mechanisms of the sediment, and the potential ecological effects of dispersion, other Corps and

non-Corps researchers are encouraged to participate in exploring these and other research topics.

Mound design and monitoring

The experimental dredged sediment mound will be built during the winter or spring of 2001 offshore from the ERDC Coastal and Hydraulics Laboratory, Field Research Facility (FRF) at Duck, North Carolina. The mound will contain up to 45,000 m³ of sediment and cover a 150-m² area. The mound will be centered on the 8-m depth contour and be approximately 2 m high. The mound contains sufficient sediment to monitor well, but is not so large as to create concern about possible detrimental conditions for the environment. Ideally, the mound should be placed as close to shore as possible to get the sediment in the most active region of the nearshore profile. However, the draft of the ocean-going hopper dredge will limit placement to about the 8-m depth.

The mound will be constructed from clean (uncontaminated) sediment dredged from an area south of the FRF and about a mile offshore of the Kitty Hawk pier. Only the first half meter of the bed will be dredged. The sediment appears to contain a percentage of fines greater than 10 percent, though additional sampling is forthcoming to verify the observation.

Before construction of the mound, sediments and bathymetry in and around the placement area will be measured. After construction, monitoring will include measurements of:

- ↳ Bathymetry.
- ↳ Waves and currents.
- ↳ Bottom sediment grain-size distributions and geotechnical properties.

- ↳ Turbidity and suspended sediment.
- ↳ Temperature, salinity, and dissolved oxygen.

Environmental effects of nearshore placement

Environmental researchers want to be sure that nearshore placement of sediment that contains silts or clays will not create pulses or persistent, long-term elevations of suspended sediment concentration as a result of the finer sediments being winnowed from the placed sediment. This is important because most future nearshore-placement projects would likely occur at or near coastal inlets, which are viewed as critical corridors for the movement of organisms into and out of estuaries. Of particular concern is the exposure of fish and shellfish during their early life-history stages to elevated suspended-sediment concentrations. Such exposure might cause detrimental effects both:

- ↳ In a physiological sense (e.g., tissue abrasion, reduced survival, impaired development, starvation).
- ↳ In a behavioral sense (e.g., turbidity plumes may elicit avoidance responses or mask sensory cues used for orientation).

The gaps in our understanding of the risk to biological resources through nearshore placement of mixed sediment can be addressed by properly designed field and laboratory studies such as the one planned at the FRF.

The temporal and spatial scales of fine-sediment resuspension will be measured by instrumentation deployed at the study site. These data will be used to design laboratory investigations of the effects of exposure of organisms to suspended sediment concentrations. This approach will employ standard

bioassay methods in a manner similar to sediment toxicity testing, although in this case for clean sediments.

The field data collection will characterize the ambient and placement-induced turbidity and suspended-sediment concentrations. Periodic water sampling will also determine water temperature, salinity, and dissolved oxygen parameters. These will be reproduced in the laboratory. Based on input from resource agency personnel, appropriate species and life-history stages will be selected. Depending on specific requirements for maintaining these fishes in the laboratory, an apparatus for keeping sediments in suspension under constant temperature, salinity, and dissolved oxygen conditions will be developed.

Retention of valuable sand resources through nearshore placement

In many areas around the country, a rule-of-thumb for placing dredged sediments in the littoral zone is that the sediments should have less than 10 percent fine sediment by weight. Sediments that contain greater than 10 percent fine sediment are placed in other locations, often offshore in deep water. Yet, this sediment that contains more than the desired amount of fine sediment also contains a substantial volume of sand. Issues surrounding the nearshore placement of dredged

sediment are whether the sand in the mixed sediment will become part of the littoral system without creating any adverse environmental conditions. By placing the sediment within the littoral system, say between the 6- and 8-m depths, the sand has an opportunity to migrate toward the beach, essentially creating an indirect nourishment of the beach and nearshore profile, or at the very least, remaining in the littoral system. The fine fraction of the sediment, on the other hand, would disperse and remain in water depths greater than 6 m where fine sediment is normally encountered.

Predictive capability

The Corps has developed several numerical models that can be used to predict the fate of dredged sediment in aquatic environments. The models have been shown to be reasonable predictors for the fate of sandy, non-cohesive sediment. However, their ability to predict the fate of sediment that contains a large fraction of fine sediment, particularly cohesive sediment, has not been evaluated. The erodability of sediment with a large fine or cohesive fraction may decrease by orders of magnitude given only modest increases in the bulk density of the sediment (say, due to consolidation). The predictive models must

account for that relationship, but data with which to develop the relationship are limited.

Call for Partnering

For this project, the Corps is inviting partnering opportunities. The study team presently includes representatives from ERDC-WES, U.S. Army Engineer Districts, Wilmington and Jacksonville, U.S. Environmental Protection Agency, U.S. Fish and Wildlife Service, National Marine Fisheries Service, Beaufort Marine Fisheries Laboratory, University of North Carolina -Wilmington Department Marine Sciences, and the North Carolina Department of Coastal Management. Collaborative participation in the study design and execution, as well as full sharing of data with all interested parties, is necessary to ensure that all significant questions are addressed. In the end, the study will not answer all questions regarding the effect of nearshore placements on the ecosystem, but the experiment will advance our understanding of the magnitude of impacts on coastal ecology and guide future studies.

Interested researchers may contact the following for additional information:

Coastal and modeling issues:

Dr. Jack Davis, davisj@mail.wes.army.mil

Environmental issues:

Dr. Douglas Clarke, clarked@wes.army.mil

Articles for Dredging Research requested:

Dredging Research is an information exchange bulletin for publication of ERDC-generated dredging research results. Included are articles about applied research projects. The bulletin serves all audiences and is accessible on the World Wide Web in addition to a paper circulation of 2,800.

Articles from non-ERDC authors are solicited for publication, especially if the work described is tied to the use of ERDC-generated research results. Research articles that complement ERDC research or cover wide field applications are also accepted for consideration. Manuscripts should use a nontechnical writing style and should include suggestions for visuals and an author point of contact. Point of contact is Elke Briuer, APR, at briuer@wes.army.mil.

UV spectrometry for characterization of harbor sediment used in New England project

One of the essential missions of the U.S. Army Corps of Engineers is to monitor and maintain navigable waterways. Particulates originating from rivers, land runoff, and atmospheric deposition accumulate in harbors and channels. Dredging is required to restore the depth and width of these channels and harbors. A variety of chemicals of concern are associated with these particulates which may pose a problem for aquatic biota depending upon their concentration. One major class of such chemicals is polycyclic aromatic hydrocarbons (PAHs). PAHs enter the environment in raw (i.e., petrogenic) form as oil and fuel products or in a combusted (i.e., pyrogenic) form such as stack ash and automobile exhaust.

As part of the regulatory requirements for dredging, the Corps must characterize the quantity and type of sediments that require dredging and determine their suitability for land or aquatic disposal. Although the ultimate determination of suitability rests on biological effects testing, the measurable levels of chemicals of concern in surface sediments provide critical guidance for project managers for sample location.

Traditional analyses for PAHs in bottom sediments for purposes of contaminant characterization typically involve sample collection, chemical extraction, and instrumental analysis using ultraviolet (UV) spectrometry, gas chromatography/mass spectrometer, and/or high performance liquid chromatography. These traditional methods have the advantage of low analytical detection limits but are laborious, expensive, and time-consuming. The sheer number of sediments requiring characterization as well as the long analytical time-frame and high cost per

sample of chemical analysis present an increasingly formidable challenge to the Corps when characterizing harbor sediments per regulatory requirements. As a result, project managers have expressed a need for field instrumentation that can be rapidly and inexpensively deployed to characterize and quantify PAH concentrations in sediment.

A commercial vendor has developed a prototype field instrument called UV-REMOTSO (UV-R), which shows great promise for the rapid evaluation of PAH composition and concentration in sediment. The basic format for the technology is based on the original REMOTSO sediment profile camera, which produces a photographic image of the sediment-water interface to the 20-cm depth while displaying various habitat attributes such as sediment, grain size, fabric, depth of mixture, and community structure. In UV-R, the analog visible (white light) camera system of REMOTSO was replaced with a digital camera and UV-light source. This system allows detection of chemicals that fluoresce under UV light, which can then be quantified.

The U.S. Army Engineer District, New England, under the DAMOS Program partnered with ERDC scientists and the vendor in a project that fielded the UV-R. The results of this first substantial field deployment in the Providence River of Narragansett Bay were published in a report dated February 2000, *Application of In Situ UV Spectrometry for Characterization of Harbor Sediment*. The objectives of the study were to:

- ↳ Assess the operational performance characteristics of the instrument under field conditions.
- ↳ Evaluate the fluorescence properties of field sediments in relation to

expected (laboratory-based) PAH distribution/concentration measurements.

- ↳ Evaluate the spatial patchiness of PAHs in undisturbed (i.e., in situ) material sediments.
- ↳ Evaluate the ability of UV-R to distinguish differences in PAH distribution within the sediment column.

Results of the laboratory tests with spiked sediments indicate the UV-REMOTSO system can detect differences in PAH concentration and composition in the range of 10 to 100 ppm ($\mu\text{g/g}$ dry weight) and above. Field results suggest measurable differences in fluorescence between sampling locations as well as small-scale variation in fluorescence within the image of a single sample. The report is available from the DAMOS Program Manager, Regulatory Branch, USACE-NAE, 696 Virginia Rd., Concord, MA 01742-2751.

Additional information is available from Dr. Thomas J. Fredette, thomas.j.fredette@usace.army.mil, Regulatory Branch, U.S. Army Engineer District, New England.





Dredging Products

Recently published technical notes for the DOER Program are listed below. These technical notes can be found in .pdf format at <http://www.wes.army.mil/el/dots/doer/technote.html>.

- ▶ ERDC TN-DOER-C13 Determining Recovery Potential of Dredged Material for Beneficial Use—Soil Separation Concepts, July 2000
- ▶ ERDC TN-DOER-C14 Determining Recovery Potential of Dredged Material for Beneficial Use - Site Characterization: Prescriptive Approach, August 2000
- ▶ ERDC TN-DOER-C15 Determining Recovery Potential of Dredged Material for Beneficial Use - Site Characterization: Statistical Approach, August 2000
- ▶ ERDC TN-DOER-C16 Leachate Screening Considerations, June 2000
- ▶ ERDC TN-DOER-C17 Equipment and Processes for Removing Debris and Trash from Dredged Material, August 2000
- ▶ ERDC TN-DOER-C18 Confined Disposal Facility (CDF) Containment Features: A Summary of Field Experience, August 2000
- ▶ ERDC TN-DOER-E8 Improved Methods for Correlating Turbidity & Suspended Solids for Monitoring, June 2000
- ▶ ERDC TN-DOER-E12 Demonstration of the SSFATE Modeling System, July 2000
- ▶ ERDC TN-DOER-I2 Initial Corps Experience with TDS Measurements, August 2000
- ▶ ERDC TN-DOER-I3 Application of Dredge Contract Administration to Dredge Monitoring System, August 2000
- ▶ ERDC TN-DOER-I4 Silent Inspector for Hydraulic Pipeline Dredges, August 2000
- ▶ ERDC TN-DOER-I5 Uncertainty Analysis Applied to Dredge Production Calculation, August 2000
- ▶ ERDC TN-DOER-N5 Geotechnical Design Guidance for Contained Aquatic Disposal, July 2000
- ▶ ERDC TN-DOER-N7 Underflow Spreading from an Open-Water Pipeline Disposal, August 2000

Recently published technical notes for the EEDP program are listed below. These technical notes can be found in .pdf format at <http://www.wes.army.mil/el/dots/eedptn.html>.

- ▶ ERDC TN-EEDP-01-45 Genotoxicity Testing in Sediments: Progress in Developing a Transgenic Polychaete Model, June 2000
- ▶ ERDC TN-EEDP-02-29 Simplified Laboratory Runoff Procedure (SLRP): Procedure and Application, June 2000

Dredging Calendar

September 7-9 - Annual Ohio Lake Erie Conference, sponsored by Ohio Lake Erie Commission, in Sandusky, OH.

POC: jill.woodyard@www.epa.state.oh.us

September 11-14 - Oceans 2000 in Providence, R. I.

POC: <http://www.OCEANS2000.com/>

September 18-20 - Coastal Environment 2000 in Las Palmas, Canary Islands.

POC: <http://www.wessex.ac.uk/conferences/2000/coastal2000/>

September 27-29 - National Waterways Conference Annual Meeting in St. Louis, MO.

POC: <http://www.waterways.org>

September 27-29 - Ports 2000; Second International Conference on Maritime Engineering and Ports in Barcelona, Spain.

POC: <http://www.wessex.ac.uk/conferences/2000/ports2000/>

October 11-13 - PIANC - Italian Days of Coastal Engineering. Reggio Calabria, Italy.

POC: cynthia.gianani@mbox.lpp.it

October 14-18 - Water Environment Federation Technology (WEFTEC) 2000 Exhibition; will provide the most up-to-date information on every wastewater treatment and water quality subject. Choose workshops and technical sessions with over 500 relevant presentations — not to mention almost 100 poster

presentations over the course of five days, in Anaheim, CA.

POC: <http://www.wef.org/Weftec/index.htm>

October 15-16 - Annual Meeting of Great Lakes Commission, in Hamilton, Ontario.

POC: mdonahue@glc.org

October 16-20 - AAPA - Veracruz, Mexico

October 17-19 - 4th State of the Lakes Ecosystem Conference (SOLEC), in Hamilton, Ontario.

POC: <http://www.epa.gov/glindicator> or paul.horvatin@epa.gov

November 11-16 - Lake Buena Vista, FL, The 7th International Conference on Wetland Systems for Water Pollution Control.

POC: Dr. K. R. Reddy, <http://www.ifas.ufl.edu/~conferweb/wpc/>

December 3-6 - Dredged Material Management: Options and Environmental Considerations. Massachusetts Institute of Technology, Cambridge, MA. Deadline for submission is September 1, 2000.

POC: <http://massbay.mit.edu/marinecenter/conference/>

July 17-19, 2001 - Coastal Zone 2001, Cleveland, OH.

POC: www.csc.noaa.gov/cz2001

September 23-27, 2002 - 30th International Navigation Congress. Sydney, Australia.

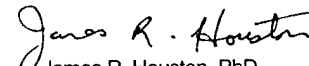
POC: <http://www.pianc-aipcn.org/pi233.html>



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