MARYLAND

Research, Education, Outreach March-April 1997

MARINE NOTES

SPOTLIGHT ON RESEARCH

TREASURE *from* TRASH

Is There Profit in Crab Waste?

BY MERRILL LEFFLER

Skip Brown

April marks the beginning of blue crab season in the Chesapeake. Crabs are already moving out of the deep channels and trekking north from winter grounds near the Bay's mouth and, in response, watermen start setting their traps. By May, processing plants on Maryland's Eastern Shore are in full swing, more than 30 of them, picking crab meat and discarding the leftovers.

Those leftovers are not insubstantial. For every pound of picked crabmeat, some six pounds of shell and runny chum are left behind, says Andrew Tolley, who owns Toddville Seafood, near Cambridge. In an average year on Maryland's Eastern Shore, he estimates that waste can add up to eight million pounds. Four thousand tons — a small smelly mountain of potential problems.

Decaying crab wastes release ammonia and nitrates that evaporate in air and seep through soil; in large enough concentrations, they can pollute the slowing moving ground waters below and, with them, freshwater wells that provide drinking water and shallow aquifers that eventually feed into the Bay. It is for such reasons that Dorchester and other Eastern Shore counties have been closing down landfills to the burial of crab waste.

And yet within that discarded scrap heap lies a potential mine of profit. The ore in this case is chitin, a substance that makes up some 20 percent of the shell (another 60 percent is calcium carbonate and 20 percent is protein). Chitin is a polymer — a large natural molecule composed of repeating units of simple sugar molecules; next to cellulose, it is the most abundant polymer on earth. The shells of crustaceans — like crabs, lobsters and

Please turn page

Crab Waste, continued

shrimp — of zooplankton, of insects, and the cell walls of fungi such as mushrooms, all are composed of chitin.

Two U.S. companies — one on the west coast and one in New Jersey — are already extracting chitin from crab waste; so are companies in Asia and Europe. They modify chitin to produce chitosan, a compound that offers applications ranging from medical sutures and seed coatings to dietary supplements and coagulants for waste treatment. Chitosan is hailed by many for its extraordinary versatility.

So what is the problem in the Bay region? With the largest crab harvests on the east coast, why is there no chitin-processing industry on the Eastern Shore? Such an industry would seem to be a win-win situation: it would create new jobs and it would convert crab waste from trash to treasure.

Raising the Bottom Line

"The reason is simple," says Pat Condon — costs. "To build a full-scale facility to extract chitin and process chitosan," he says, "you're talking millions." That's because stripping chitin from tightly-bound protein in shells requires heavy-duty treatment with acids and bases — not only are these consumables expensive, but they present potential environmental hazards and require careful treatment. "Because of their corrosive nature," says Condon, "they also eat up equipment," all of this driving up costs.

Condon heads New Earth Services, a company that composts poultry and vegetable wastes, as well as crab waste, marketing it to gardeners and others as Chesapeake Blue. But crab compost is a low-value product and cannot be manufactured profitably, says Condon. For several years, Dorchester County helped subsidize that composting as a way of meeting its recycling requirements, says Andrew Tolley; but the county no longer does so.

The bottom line, says Tolley, is that processors would have to subsidize that operation, in addition to paying transportation costs of the waste to New Earth Services. That is something many either cannot afford or are unwilling to do, especially if there is still land to receive waste.

Skip Brown

Researchers believe that if chitosan can be produced economically from the chitin in crab shell, its chemical properties could be tailored to create a diverse range of new products which are naturally degradable, from coagulants for use in waste treatment plants, like the one shown at left, to new kinds of wound dressings and casings for the controlled release of drugs.

Chitosan is hailed by many...so what is the problem in the Bay region?

While the prospects of such heavy investment in chitosan processing are daunting, says Condon, he has nevertheless been exploring markets for different chitosan products to determine if the prospects of return can justify such investment.

Though there are numerous applications in which chitosan outperforms competitive products, the margins of profit appear to be too low, or product costs are not competitive. Chitosan's use as a coagulant in waste treatment processes is a case in point, says Susan Murcott, a research environmental engineer at the Massachusetts Institute of Technology. Chitosan performs more effectively than synthetic petroleum-based polymers and is a naturally biodegradable substance. That is the upside. Unfortunately, the cost for chitosan is twice as much as the synthetics, she says. If those production costs could be brought down, or if new products could be developed, then this natural polymer could fare better in the marketplace.

This is the catch-22, says Greg Payne, a chemical engineer at the University of Maryland Baltimore County and the Center for Agricultural Biotechnology. "Chitosan's high production costs," he says, "have limited the development of new products," while "the low margins of existing products have not justified the investment for industry to develop more innovative methods for extracting chitin and processing chitosan."

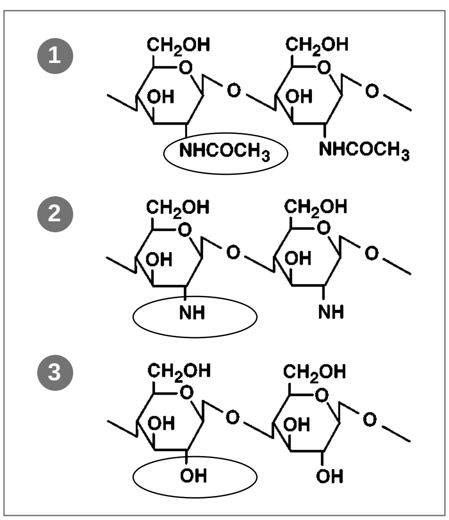
Payne believes that because of its unique chemical properties, chitosan has untapped commercial potential. If we can demonstrate how chitosan's potential can be released, he says, that could stimulate industrial innovation, so that even manufacturing costs for products such as coagulants could come down considerably and better compete with synthetic polymers.

Towards such ends, Payne, working with support from Maryland Sea Grant, has been studying how the structure of chitosan can be controllably altered so that manufacturers can tailor its chemical, mechanical and biological properties for a number of uses, uses that might include biocompatible materials for medical implants, wound dressings and casings for controlled drug release.

Tailoring the Chitosan Polymer

Payne's interest in chitosan grew out of innovative studies in which he had shown that enzymes could be used to remove phenols — a broad class of natural and synthetic compounds — from liquid mixtures. The problems he focused on involved industrial operations in which phenols appear as undesired contaminants in process streams. Although present at low levels, these contaminants had to be removed if manufacturers were to recover and reuse the desirable materials in the fluid. According to Payne, there are a surprising number of cases in which the success of an industrial waste minimization program requires the removal of a phenolic contaminant.

The innovative two-step approach of Payne's group was the use of the enzyme tyrosinase to "recognize" and react with the phenolic contaminant, while the product of this reaction underwent a subsequent reaction with the chitosan polymer. The product of the second step is a solid which can readily be filtered from the process stream. Based on these studies, his group was awarded a U.S. patent which is currently licensed by the University of Maryland to a local company.



Only one functional group's difference (circled above) separates the formulas of these chemical cousins — chitin (1), chitosan (2) and cellulose (3).

More recently, Payne's group has been examining how tyrosinase can be used to systematically modify the chitosan polymer. Although their function in nature is not fully understood, tyrosinase enzymes are ubiquitous — they are found in bacteria, fungi, plants and animals — and have the ability to react with phenol-type compounds. Since the products of these tyrosinase-catalyzed reactions undergo further reaction with chitosan, it is possible to use this approach, says Payne, to anchor various chemical groups to the chitosan surface, an approach that has the possibility for tailoring the physical, chemical and biological properties of chitosan.

There are numerous possibilities, says Payne, of how such control could then be used to tailor chitosan for specific commercial products, products as diverse as pharmaceuticals and paper. For instance, an oral drug designed to treat the intestine must be able to withstand stomach acids that could prematurely release the drug. Because the surface chemistry of chitosan can be designed to resist acid conditions but hydrolyze under neutral pH, the drug can be coated in the altered chitosan. In this way, it would pass through the stomach, then once in the intestine's neutral pH, the coating would become hydrophilic, allowing the drug to be released.

Already, small quantities of chitosan are being used in paper making to increase the mechanical strength of fibers. The chemical methods for strengthening the chitosan itself — a process called cross-linking — employ somewhat harsh chemical processes, says Payne. The proposed use of tyrosinase could provide a "cleaner" alternative for such cross-linking.

Payne's research is ongoing with support from the National Science Foundation and Sea Grant. In addition, he is collaborating with Pat Condon and New Earth Services — with funding from the University's Maryland Industrial Partnerships program — in first-phase studies that will explore whether chitin processing from Eastern Shore crab waste is practicable. That means, says Condon, whether it can be profitable.

There are many technical, economic and social questions.

Chitin extraction and chitosan processing, for example, can produce different grades of purity depending on the treatment with acids and bases. What are the costs and benefits of different levels of purity, with regard to marketable products? Furthermore, is it even possible to produce chitin profitably from crab waste when it is only available on a seasonal basis? And is it cost effective to work with a wet crab chum from processors or will a dry, dehydrated product be required?

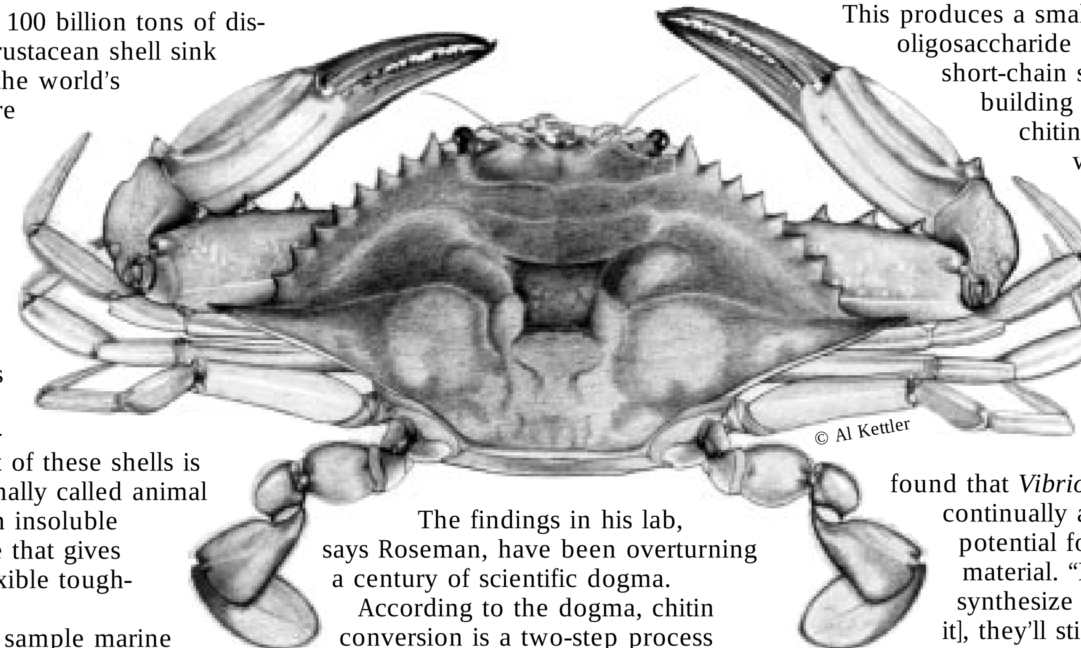
Will processors, or an independent operator, be willing to shoulder the costs for transporting and drying out crab scrap? Again, says Condon, that will depend on whether it is profitable to the processors: if a chitin processing plant is set up on the Eastern Shore, then it could be; on the other hand, unless there is a ready supply of dehydrated chitin, it may not be profitable enough for industry to invest in a chitosan processing operation.

Do environmental and public health concerns over land application of crab waste justify public investment for chitosan processing if it *appears* to be commercially viable? With nutrient runoff from farm animal manure a major threat to Chesapeake Bay water quality, government currently provides subsidies to poultry and dairy farmers to construct waste containment facilities. Does crab scrap deserve the same?

While there may be a promise of chitosan treasure in crab waste, turning that promise into reality will take even more than lowering extraction and processing costs. Only the demand for products derived from chitin — based on the availability and costs of alternatives — will determine whether its extraction and processing can be profitable. Until then, mountains of crab waste may remain an untapped treasure.

Chitin Breakdown: The Bacterial Way

BY MERRILL LEFFLER



Each year, 100 billion tons of discarded crustacean shell sink through the world's oceans — more than a billion tons of this have been molted by copepods alone, the rest from shrimp, crabs and multitudes of other crustaceans. A major component of these shells is chitin — originally called animal cellulose — an insoluble polysaccharide that gives shells their flexible toughness.

"But if you sample marine sediments," says Saul Roseman, "you will find only traces of chitin — it's just not there." The reason: bacterial recycling. Bacteria chemically grind the shell away with a tool box full of proteins and enzymes, breaking down chitin's fibrous structure to simple sugars, which the microbes use as food.

Were it not for this recycling, says Roseman, a biochemist in the Department of Biology at The Johns Hopkins University, carbon and nitrogen in the chitin would simply accumulate, depleting the oceans in less than 50 years. "If chitin deposits weren't recycled," he adds, "the White Cliffs of Dover would consist of chitin instead of diatoms."

Most of these chitin-hungry microbes are from the genus *Vibrio,* ubiquitous motile aquatic forms which are the most abundant of all marine bacteria. For several years now, Roseman has been detailing the biochemical processes that bacteria employ in breaking down chitin — from first detection, to attachment, to the compounds they release and the enzymatic processes involved.

The findings in his lab, says Roseman, have been overturning a century of scientific dogma.

According to the dogma, chitin conversion is a two-step process that involves the production of a monosaccharide and a disaccharide. Roseman and the graduate and postgraduates in his lab — they originally included Charley Yu and Bonnie Basler and now include Nemat Keyhani, Alexi Fomenkov and Xibing Li — have found that that is not the case at all and have been uncovering what Roseman calls completely unexpected bacterial processes at every step — a virtual cascade.

The first thing bacteria have to do is "find," or sense, the chitin. When a crustacean molts, it releases an enzyme, chitinase, to loosen the shell.

Saul Roseman's lab is overturning a century of scientific dogma on the processes of bacterial recycling of chitin.

This produces a small amount of oligosaccharide (a class of short-chain sugars, the building blocks of the chitin polymer) which the *Vibrios* can detect. Once the bacteria arrive, they attach to the surface of the shell.

Charley Yu found that *Vibrio furnisii* are continually assaying the potential food value of material. "If they can synthesize protein [from it], they'll stick; if not, they'll go away," says Roseman. Then the bacteria begin to produce the enzymes needed to break down and digest the chitin.

His group has discovered that bacteria produce suites of these enzymes — outside the cell, between the cell walls and plasma membranes, and within the cell itself — and that those enzymes are involved in releasing a cascade of oligosaccharides of different molecular weights. So far they have identified a series of nine steps leading from chitin to a simple sugar (fructose-6-phosphate) and ammonia.

By identifying the genes that express each protein and enzyme, he intends to clone each of those genes in order to overexpress proteins in greater volume. In this way, each protein can be completely characterized with respect to its catalytic and other functions.

"While the scientific goal," Roseman says, "is to completely delineate the steps involved and how each step is genetically and metabolically regulated," his work could lead to major changes in the commercial production of oligosaccharides. Chitin oligosaccharides, for example, are known to play an important role in plant disease resistance by "triggering" a plant's defense mechanisms against invasion by fungi (which have chitin in their cell walls). Also, symbiotic bacteria release chitin oligosaccharides to signal the formation of root nodules, sites for nitrogen-fixation in plants such as beans and clover. Chitin oligosaccharides may have potential use in human medicine as well. Recent studies have revealed that animals and humans produce chitinase-like proteins — though just what their role is remains open to question.

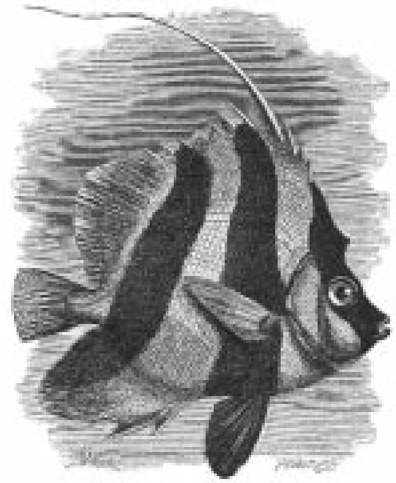
Exploring such possibilities, however, has been nearly impossible because the costs of obtaining pure oligosaccharides that are suitable for research are so prohibitive.

While bacteria produce the oligosaccharides with ease, thanks to their natural enzymes, replicating this process in the laboratory is environmentally problematic since those techniques require heavy use of acids and bases. The process is also time consuming and, as a consequence, very expensive. Roseman should know. More than 30 years ago, he developed chemical techniques for isolating saccharide compounds, a process that requires treatment after treatment to obtain high quality compounds. Those same techniques are still in commercial use today for manufacturing oligosaccharides from chitin.

Pure oligosaccharides can cost from $5 to $15 a milligram — one small experiment can cost many thousands of dollars.

As an example of the potential of the methods under development in Roseman's lab, Nemat Keyhani has already produced 25 gram quantities of the pure, crystalline disaccharide from chitin, which he is using in his own research. If the laboratory's progress continues, the group could provide opportunities for uses that medical and agricultural research have not even begun to think about. "Equally satisfying," says Roseman, "is that the source of these opportunities is a product of the seafood industry that is now going to waste."

Marine Ornamentals and Aquaculture



Marine ornamentals are not as innocuous as the name might imply. Fish and shellfish used mainly in aquarium tanks are big business. Although it is difficult to obtain reliable dollar values, a 1992 estimate put the wholesale value of trade at greater than $400 million in the U.S., with a retail value at some $7 billion globally. While this is good news for the ornamental industry, it can be a problem for the ecosystems that provide these species.

The reason, says Chris D'Elia, Director of the Maryland Sea Grant Program, is that a majority of marine ornamentals are taken from the wild. "Much of the collection pressure focuses on coral reefs in developing nations where harvesting practices are often destructive." Harvesting, says D'Elia, often involves the use of poisons (e.g., cyanide) or explosives, and such practices can result in high mortalities to the catch and by-catch alike — they may also damage reef superstructures as well as other members of the reef community. The economic implications alone are of concern since many reef organisms are sources of important compounds such as biomedical materials and pharmaceuticals.

D'Elia has been instrumental in coordinating support for an initiative in the conservation and culture of marine ornamental fishes and invertebrates. The objectives are two-fold, he says: "to ease harvest pressure on natural stocks of reef organisms that are now in serious jeopardy, and to develop the economic potential of the marine ornamental aquaculture industry." This initiative was the focus of a meeting at the World Aquaculture Society in Seattle, Washington that brought together representatives from the National Sea Grant Office, the USDA Agricultural Research Service, Sea Grant Directors from across the country, the Cooperative Extension Service, the New England and National Aquariums, private institutions, the USDA Northeast Regional Aquaculture Center, and the National Coastal Resources Research Institute.

A steering committee from this group identified several immediate goals:

- Raise the awareness of the importance of the industry and the challenges and/or opportunities for the development of a renewable resource through culture and husbandry activities.
- Assemble data and information on the environmental impacts of the industry as it is now practiced.
- Identify areas where new information is needed and where education could enhance sustainability.
- Develop a network of those active in marine ornamental issues, current partnerships among industry users, scientists and conservation.

The plan of action calls for (1) developing of a brief outline for a national Sea Grant initiative; (2) convening a steering committee meeting in the fall in conjunction with the Florida Aquaculture Association to interact with the Florida ornamental industry; and (3) develop formal marine tropical ornamental interest groups.

Those who are interested should contact Dr. Reggie Harrell of the Sea Grant Extension Program at (410) 221-8466 for further information.

Open House at Marine Laboratory

The public can explore the wonders of marine science at an open house at the CEES Horn Point Environmental Laboratory, located on the banks of the Choptank River near Cambridge, Maryland. From 10:00 am to 2:30 pm on Saturday, May 17, the lab will offer visitors the chance to take self-guided tours of its facilities. The open house is free.

As part of the day's activities, CEES scientists, students and staff will offer presentations, special exhibits, videos and tours highlighting recent research efforts in Chesapeake Bay ecology, environmental education and oceanography. Visitors can learn how land use affects water quality at a display featuring Chester and Choptank river studies, how scientists measure the health of the Bay with special, high tech sensing instruments, why fish and oyster farming are important to Maryland's future and how to become better caretakers of Maryland's environment. Children can enjoy hands-on activities and projects at various locations throughout the Horn Point campus.

Displays will also feature current Bay restoration efforts led by Maryland's Department of Natural Resources. In addition to the open house, the following events will also take place in the vicinity of the lab:

Saturday, May 17

6:30-8:30 am — Bird watch and walk led by CEES Vice President Wayne Bell. Those interested should meet at the campus entrance off Horns Point Road by 6:30 am, wear comfortable walking shoes and bring binoculars and/or scopes. The bird walk will be cancelled if weather is inclement. Free and open to the public.

9:00 am and 2:00 pm — One-hour skipjack cruises aboard the *Nathan of Dorchester* leaving from the Horn Point marina. Tickets can be purchased by calling the Dorchester Tourism Office at (410) 228-1000 or at the boat on Saturday.

Saturday and Sunday, May 17 and 18

9:00 am-5:00 pm (both days) — Antique Aircraft Fly-In: a two-day event featuring 250 antique aircraft on display at the Horn Point airstrip adjacent to the laboratory. Free and open to the public.

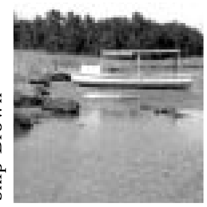
9:00 am-9:00 pm (Sat) and noon-6:00 pm (Sun) — Cambridge Watersports Show at Sailwinds Park, $5.00 per person. A special water taxi will be available to transport people between Sailwinds Park and the Horn Point marina during the open house on Saturday ($5.00 round-trip per person).

Taking part in the open house and other activities will require some walking, so comfortable shoes are recommended. Refreshments will be available and picnic areas are plentiful. For more information about the open house, call (410) 228-9250, ext. 614.

UMCEES

To get to the laboratory, take Route 50 to Cambridge, then take Route 343 west (Washington Street) for 3.5 miles to Horns Point Road and turn right. Proceed 1.5 miles to a stone "Ram" gate; follow parking attendant's instructions.

New Environmental Plan for Maryland



Skip Brown

The state of Maryland, working with the federal government, has issued a draft long-term plan for maintaining and improving the quality of Maryland's air, land and water. The plan calls for a ten-percent increase in the state's wetland acreage, and includes benchmarks for protecting "ecologically significant species." A ten-percent increase in wetlands would mean an additional 60,000 acres of wetland areas important for flood control, nutrient removal and habitat for a range of species.

Copies of the plan are available from the Maryland Department of the Environment (MDE) by calling (410) 631-4187.

Nitrogen Removal

As part of the cooperative effort to reduce nutrient loadings to the Chesapeake Bay, the Blue Plains regional wastewater treatment plant has been operating a pilot project for nitrogen removal since mid-1996. The jurisdictions involved in cleaning up the Bay — Maryland, Virginia, Pennsylvania and the District of Columbia — have set the goal of reducing nitrogen and phosphorus loadings to the Bay by 40 percent from the levels measured in 1985. These goals are considered the major attack on the Bay's decline.

The pilot plan to remove nitrogen from the plant's effluent currently treats half of the plant's flow. Its performance will be assessed for a year or longer. If successful, the entire flow of the plant will be treated. Blue Plain's effluent is the largest point-source of nitrogen in the Chesapeake watershed — about double the next largest source. Removal of the nitrogen would comprise 12 percent of the total amount of nitrogen needed to be removed to obtain the overall goal for the Chesapeake.

— *Potomac Basin Reporter*

End Notes

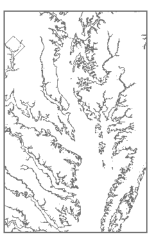
Video on Watersheds

Oregon State University Extension Service has a video called "We All Live Downstream," which will interest those concerned about healthy watersheds and clean water supplies. It explores how Oregon residents and government officials are trying to reduce nonpoint source pollution, but has implications for other watersheds in the country. To order the video, which costs $30.00 including shipping, write: Publications Orders, Extension and Experiment Station Communications, Oregon State University, 422 Kerr Administrative Services Building, Corvallis, Oregon 97331-2119.

Call for Papers

The International Association on Water Quality (IAWQ) will hold its Nineteenth Biennial Conference, titled "Water Quality International 1998," on June 21-26, 1998 in Vancouver, BC. The mission of IAWQ is to promote internationally the professional advancement of the science and practice of water quality management. Technical papers and posters are currently being invited on all topics relating to water quality. Manuscripts for oral presentations must be received before July 1, 1997, while manuscripts for poster presentations are due February 15, 1998. For a description of the conference or details about submitting a paper, write or call: WQI '98 Conference Secretariat, 645-375 Water Street, Vancouver, BC V6B 5C6, Canada; phone (604) 681-5226; fax (604) 681-2503.

Request for Proposals



The Chesapeake Bay Program (CBP) is soliciting challenge grants for habitat restoration projects that will support CBP living resource and habitat commitments. Grants, which require a 1:1 nonfederal match at minimum, typically range from $10,000 to $75,000. This is the fifth year of the challenge grant offer. Among the projects encouraged this year are those involving riparian zones, in-stream habitat, emergent freshwater wetlands, forested wetlands, and similar freshwater habitat. Highest consideration will be given to projects addressing more than one of these priorities. The deadline for receipt of proposals is May 15, 1997. To find out more about submitting a proposal, contact the representative in your area: Frank Dawson, (410) 974-3016 (Maryland); David Norris, (804) 253-4180 (Virginia); Kelly Heffner, (717) 787-6827 (Pennsylvania); and Peter May, (202) 404-1120 (Washington, DC).

Technology Grants Available

The Maryland Industrial Partnerships (MIPS) Program is soliciting grants for the development of products or processes, providing market expansion opportunities or strengthening a company's position. MIPS provides an easy way for companies to access the specialists and facilities of the 13 UMS institutions. For more information about the MIPS program or applying for a grant, call the MIPS office on the College Park campus of the University of Maryland, (301) 405-3891. Applications are due May 1, 1997.

Sunken Ships of the Chesapeake

A new book by Donald Shomette entitled *The Ghost Fleet of Mallows Bay and Other Tidewater Treasures*, is an archaeological trilogy that provides a tour of some of the Bay's hidden treasures. Shomette, a maritime historian and underwater archaeologist, begins the book with a description of the exploration of the wreck of the steamship *New Jersey*, which sank in

the waters of the Chesapeake in 1870. He describes the cutting-edge technology, including remotely operated vehicles, ultrasonic mapping and imaging sonar that were used for the large-scale relic-hunting expedition.

The second part of the book describes the excavation of a site off Kent Island, where the remains and relics of the earliest permanent settlement in Maryland lie. Kent Island was also the site of the first armed conflict between English-speaking people in the New World.

The final section of the book chronicles a fascinating, yet little-known site off the Potomac River 30 miles below Washington, Mallows Bay, which holds the greatest concentration of sunken ships in America. The diverse collection of ships includes sailing vessels, steamships and even an 18th-century longboat, the oldest wreck in the cove. The largest group of boats in the graveyard is a fleet of huge wooden steamships, ranging from 265 to 285 feet in length, built under an emergency program during World War I, but never actually put into service.

Today many historians, conservationists and citizens are interested in designating Mallows Bay as a special shipwreck preserve to maintain it as a unique historical site.

Ghost Fleet of Mallows Bay costs $29.95 and is available from local booksellers or directly from Tidewater Publishers by calling (800) 638-7641.

Calendar

May 25-29 — Coastal Management

Genoa, Italy. The International Conference on Education and Training in Integrated Coastal Management: The Mediterranean Prospect will be of interest to those involved in graduate and postgraduate academic as well as extra-academic training courses in integrated coastal management. A contribution to the 1998 United Nations International Year of the Ocean, the conference is free. For more information or to register, write: Secretariat, ICCOPS, c/o The University of Genoa, Department Polis, Stradone di S. Agostino, 37-16123, Genoa, Italy; or visit the web: http://www. polis.unige.it/1998education.

June 2-13, 1997 — Biotechnology Workshop

Baltimore, Maryland. This ten-day workshop on Recombinant DNA Methodologies and Applications will take place from 9-5 daily on the campus of the University of Maryland Baltimore County (UMBC). The laboratory-intensive workshop is designed to introduce the strategies, techniques and applications of current recombinant DNA and molecular biology methodology. Fees are from $1,200 (for UMS students and faculty) to $1,400. Enrollment is limited to 14, so please register early. Registrations must be received by May 19, 1997. For additional information call (410) 455-2336 or e-mail: bartholo@umbc.edu.

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