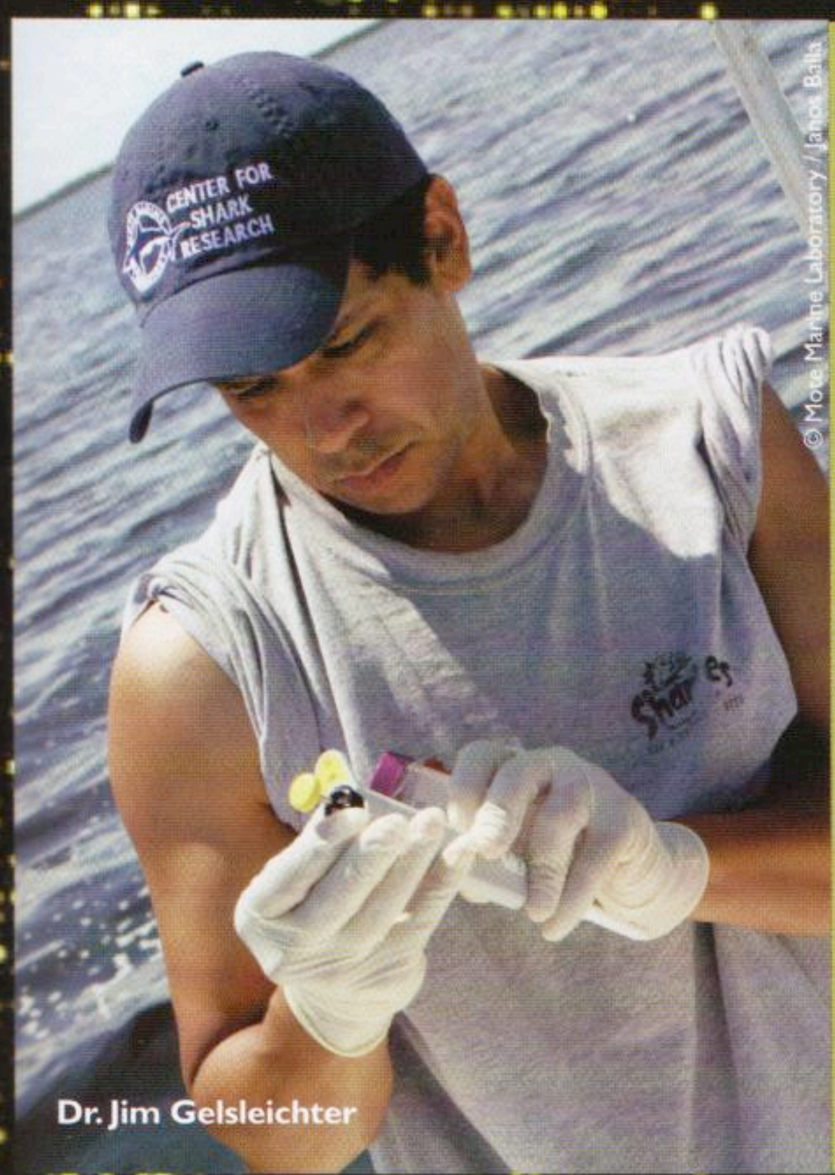
ILLUMINATING SHARK EXPOSURE TO

ECOESTROGENS

BY ADAM VOILAND



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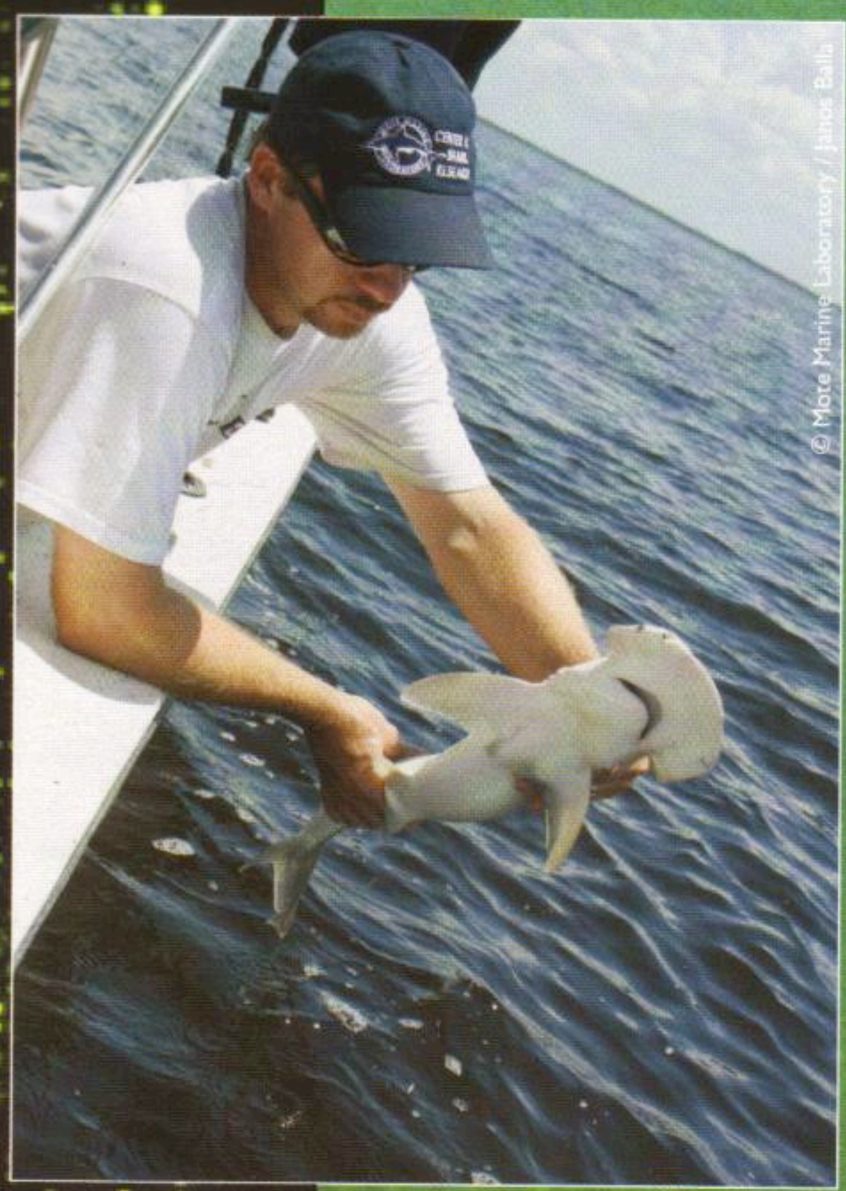
Dr. Jim Gelsleichter

At Lake Apopka in Central Florida, the alligators have tiny penises. They run a third the size of normal alligator phalli and have significantly diminished sperm counts. In England, on the Lee River, feminized male fish have eggs as well as sperm in their gonads. And in the Great Lakes region, birds — including male herring gulls, terns and bald eagles — start exhibiting hermaphroditic changes after eating feminized fish.



Mote staff scientist John Tyminski returns a bonnethead shark to Charlotte Harbor after taking a small blood sample.

Like those of cats, bonnethead shark eyes shine because of the presence of *tapetum lucidum*, a mirror-like layer of cells that amplifies light and allows sharks to see well in dim light.

Some scientists think the culprit in all of these gender-bending events might be exposure to ecoestrogens — a broad class of chemicals that mimic the hormone estrogen and disrupt normal functioning of the endocrine system, a body-wide network of hormone-producing glands that control things like growth and reproduction.

Wastewater treatment plants constantly release ecoestrogen-containing effluent into the wild. "There's little doubt that wastewater-related pollutants represent significant risks to human and wildlife populations," said Dr. James Gelsleichter, who manages Mote's elasmobranch physiology and environmental biology program in the Center for Shark Research. "They do clear out the effluents pretty efficiently — usually about 80 to 90 percent of contaminants are removed — but the problem is the level of human usage and development."

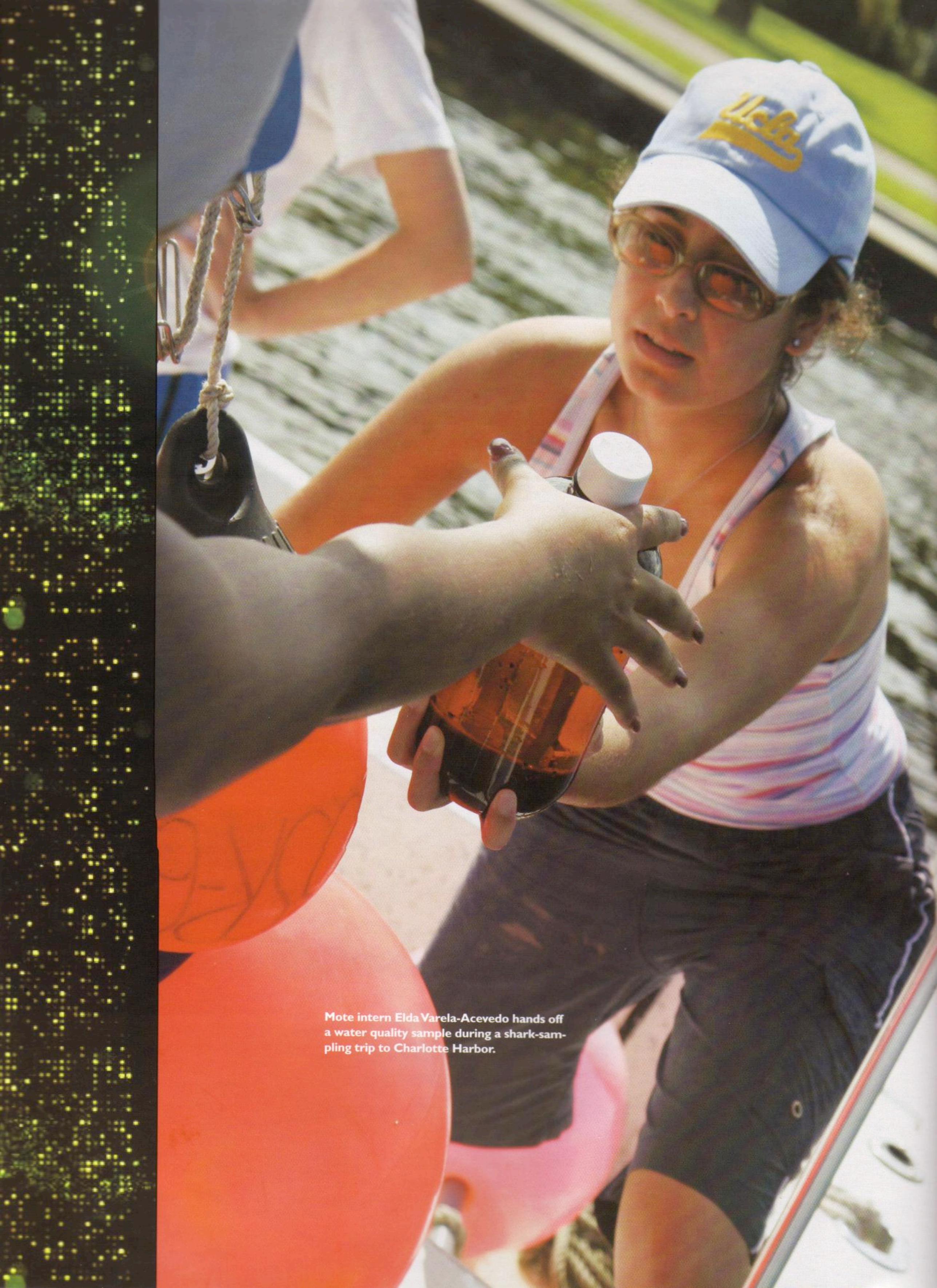
Gelsleichter wants to know exactly what endocrine disruption looks like at the molecular level. To do this he's in the process of developing an innovative microarray in conjunction with EcoArray, a Florida-based biotech company.

The microarray, called the SharkChip, will use cutting-edge genomics technology to produce a "genetic fingerprint" that will enable Gelsleichter to determine if ecoestrogens are affecting bonnethead sharks.

A microarray, sometimes called a gene chip or a biochip, is a small, nylon or glass wafer-like chip capable of holding thousands of genes. Such arrays represent a quantum leap forward for scientists who study pollution impacts because they allow the comprehensive study of how pollution affects gene expression — when genes are turned on or off.

Before microarrays, toxicologists could only study how individual, isolated genes responded to pollution. Now they can look at thousands of genes at once.

The SharkChip's creation is part of a long-term study of 10 species that Gelsleichter hopes will reveal how much danger ecoestrogens pose to wildlife and people. "We know ecoestrogens are all over in the environment," Gelsleichter said. "We just don't know exactly how they impact wildlife."



Mote intern Elda Varela-Acevedo hands off a water quality sample during a shark-sampling trip to Charlotte Harbor.

TECHNO-GUIDE

17a-ethnylestradiol: The synthetic form of estrogen found in birth control pills.

Alkylphenol Ethoxylates: Surfactants that are used widely in household cleaning products and a number of industrial applications.

Biomarkers: A biological response to pollutant exposure.

Bisphenol A: A weak ecoestrogen used primarily to make polycarbonate plastic and epoxy resins and found in everyday objects such as plastic food and drink containers.

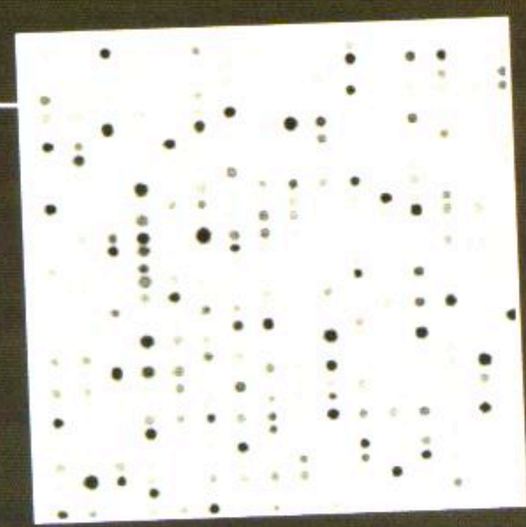
Ecoestrogen: Environmental chemicals that mimic the natural female hormone estrogen.

Gene: A unit of hereditary information. A gene is a piece of a DNA molecule that specifies the production of a particular protein.

Gene expression: The biochemical process through which a gene's information is converted into the structures and functions of a cell.

Genomics: An emerging field of science that studies the structure and function of large numbers of genes simultaneously.

Microarray: Genomics technology capable of storing information about gene expression for thousands of genes on the space the size of a postage stamp.



mRNA: A chemical cousin of DNA responsible for transferring and translating the genetic code of DNA into proteins.

Organismal markers: An organ-level effect of pollutant exposure, such as a reduction in sperm production in males or poor egg production in females.

Protein: Large molecules required for the structure, function, and regulation of cells, tissues and organs.

Vitellogenin: A protein produced in the liver of fish, amphibians, reptiles and birds that gives rise to egg yolk. Vitellogenin production is regulated by estrogen and normally found only in female fish. Males exposed to high concentrations of ecoestrogens also produce vitellogenin. That makes it a good indicator of pollutant exposure.

Irksome Ecoestrogens

Ecoestrogens are common byproducts of the industrial world and exist in a slew of everyday products such as plastics, pesticides and birth control pills. Since they are so pervasive, ecoestrogens quickly find their way into the nation's waterways and can wreak havoc on the reproductive systems of organisms exposed to them in high doses, particularly fish and other marine animals. In the wild, ecoestrogens cause feminization of males and tend to become concentrated in large predators such as sharks. "They're a potentially huge problem," Gelsleichter said.

Of all the different ecoestrogens out there, Gelsleichter said he believes synthetic estrogen from the birth control pill is one of the most concerning. More than 100 million women worldwide take the pill. Together they excrete massive amounts of the synthetic estrogen into wastewater systems each day.

"Synthetic estrogen is far more biologically potent than naturally occurring estrogen," Gelsleichter said. "As a result, it is more stable in the blood and has been shown to have a much greater impact on wildlife than natural estrogen."

Some industry insiders and regulators say the synthetic estrogen that women secrete is inactive. But scientists have demonstrated that bacteria in the sewage treatment process may reactivate synthetic estrogens. By the time water is discharged from a treatment plant, it is often teeming with estrogen capable of affecting marine life.

Nobody knows exactly what synthetic estrogen exposure does to people, but studies have linked ecoestrogens to decreased sperm counts in men, breast cancer in women and early puberty in girls. "There's still some controversy over these findings, especially with the accelerated puberty because that might also be connected to the epidemic of obesity," Gelsleichter said.

Since ecoestrogens accumulate in many species of fish that people eat, a good understanding of how they impact marine environments and travel through the aquatic food chain is one question Gelsleichter hopes his study will answer.

First he must determine the levels of ecoestrogens that are actually in Florida waters , and he also needs to devise a reliable monitoring system that could tease out pollutant impacts at a molecular level.

Fingerprints of change

One technique Gelsleichter is using to monitor ecoestrogen exposure is to test for the presence of vitellogenin in sharks and other marine animals. Vitellogenin is an egg yolk precursor protein expressed only in female fish that is normally dormant in male fish. However, when male fish are exposed to ecoestrogens, the vitellogenin gene turns on, causing organisms to produce the substance.

Vitellogenin indicates exposure to ecoestrogens in a broad sense, but it doesn't tell scientists the precise chemical causing the vitellogenin reaction. That's where the SharkChip comes in. It will allow Gelsleichter to associate specific changes in gene expression with exposure to specific types of ecoestrogens.

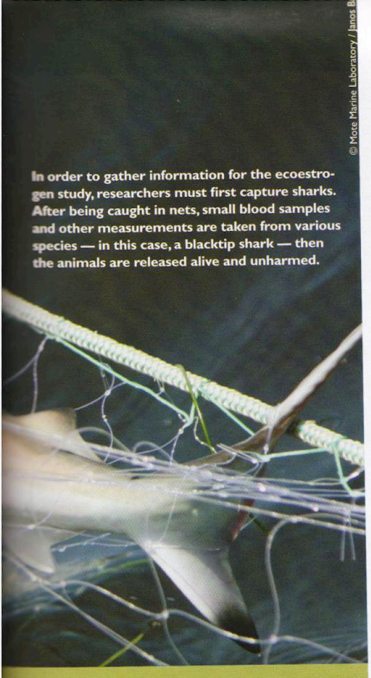
Gelsleichter, for example, plans to look at the molecular fingerprints of *17a-ethnylestradiol*, synthetic estrogen from birth control pills, *Bisphenol A*, a common component of certain plastics, and *Alkylphenol Ethoxylates*, a class of chemicals found in home and industrial cleaning products. Scientists suspect that all of these chemicals may disrupt the reproductive activities of fish and other marine animals at environmentally relevant levels.

"We feel these tests are the wave of the future," said Dr. Patrick Larkin, the director of research at EcoArray, the company partnering with Gelsleichter to make the chip. "They really allow us to get a snapshot of what's going on in a cell."

In some situations, as in the case of the alligators of Lake Apopka, the impact is drastic and quite apparent. Scientists call these large-scale changes "organismal markers." But often the impact of ecoestrogens is much less obvious, revealing itself only years later in the form of high rates of animal cancers and other reproductive disorders. Scientists call these more subtle changes in gene expression "biomarkers."

"The problem with organismal markers is that they are relatively insensitive to pollutants. Biomarkers can tell you exactly what's going on in an organism when it is exposed to even low levels of contaminants," Gelsleichter said.

Eventually Gelsleichter expects the SharkChip to highlight biomarkers that uncover the molecular underpinnings of chronic problems long before they actually appear as a change in an organism. EcoArray's Larkin agrees: "What we're really doing here is using the technology like a canary in a coal mine."



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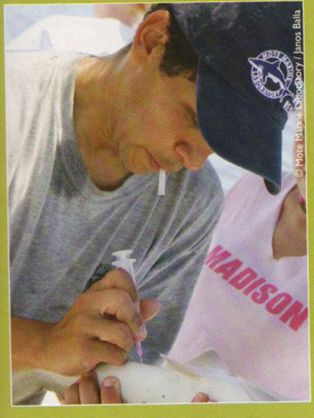
In order to gather information for the ecoestrogen study, researchers must first capture sharks. After being caught in nets, small blood samples and other measurements are taken from various species — in this case, a blacktip shark — then the animals are released alive and unharmed.



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Dr. Jim Gelsleichter draws a small sample of blood from a bonnethead before releasing it back to the sea unharmed. In the lab, Gelsleichter's team will study how ecoestrogen exposure affects gene expression.

The Rise of Microarrays

Microarrays are a relatively new technology spawned by the Human Genome Project that are capable of storing thousands of genes on tiny spaces. To develop them, scientists and engineers borrowed miniaturization, integration and parallel-processing techniques from the computer industry and applied them to biology.

Their creation has led to a new field of science called genomics, which is distinguished from traditional genetics because of its broad-brushed approach to genetic problems. Rather than studying and sequencing individual genes, genomic inquiries involve the study of multiple genes as well as the interactions between genes. This provides a more complete, real-world understanding of gene expression.

Microarrays, or gene arrays, allow scientists to collect and organize massive amounts of information about gene expression onto a tiny chip. Ultimately, biotech firms have created wafer-sized chips capable of storing 30,000 to 60,000 genes on a space the size of a postage stamp.

Microarrays augment traditional gene expression tests that could look at only one gene at a time. Gelsleichter emphasized that microarrays don't necessarily provide new or different information, but simply that they provide such vast quantities of it so researchers can see the metaphorical forest through the trees.

Although microarrays first emerged as biomedical tools for humans about 10 years ago, scientists have just started applying the technology to the marine environment in the last few years. EcoArray is one of the first and only companies to specialize in developing gene chips for marine animals.

Recently the company constructed similar gene chips for bass and sheepshead minnows. According to Larkin, they will use a similar process to create the SharkChip.

EcoArray will begin constructing the chip by taking a few hundred chunks of DNA, called probes, which will be placed onto a small nylon membrane with a robotic hand. Eventually, Larkin hopes to refine the chip by replacing the nylon base with glass, which can hold thousands of genes instead of just a few hundred.

When exposed to specially prepared blood, liver or gonad samples from sharks, the probes will detect and bind to a product of DNA, called mRNA, which is a cell's template for producing proteins. The more mRNA detected by the probes, the greater the level of gene expression and the more likely it is that a gene is expressed, or turned on.

Once the chip is built, Gelsleichter will inject one-time, non-lethal doses of common ecoestrogens into bonnethead sharks captured from the wild. By looking at what genes turn on and what genes turn off, he'll be able to create a pollution profile that shows how shark genes change in response to specific ecoestrogens.

With the pollution profile created, he can compare the lab results with samples from wild sharks to test for ecoestrogen impact. If the pattern of gene expression looks similar to the pollution profile from the experimentally injected sharks, then he'll know ecoestrogens are also affecting wild sharks.

Defining the future

Both the biomedical and environmental monitoring communities are buzzing about the promise of biochips. In 2002, Google turned up 95,700 hits for the word "microarray." Today, the number of hits is more than 2.8 million.

"Microarrays are very significant to environmental monitoring," said David Lattier, a molecular biologist who works in the EPA's National Exposure Research Laboratory in Ohio. "However, they're not a panacea. We still have a lot of challenges to face."

One problem is organizing and sorting through the mountains of data that microarrays produce. Another is separating chemically-induced effects from naturally occurring changes in gene expression. Small environmental changes such as the weather, can influence gene expression, as can other difficult-to-discern factors such as diet, or even mood.

None of this daunts Gelsleichter, however. "Ultimately, the more genes you look at, the more accurate your diagnosis is going to be," he said. ■