Researcher Anne McElroy (right) and doctoral student Lourdes Mena in New York Harbor aboard the *Seawolf*, the research vessel of the Marine Sciences Research Center, Stony Brook University.
Photo by Mark Wiggins.

Estrogenic Compounds in Urban Waterways:

An Interview with Anne McElroy

Dr. Anne McElroy, Associate Professor at Marine Sciences Research Center, Stony Brook University, and a former New York Sea Grant director, sat down with Coastlines editor Barbara Branca for a Q & A about some of her work on winter flounder. She and her research team have used fish as a model animal to examine the potential effects of toxic chemicals in New York City's waterways.

You've done a lot of research in various areas of toxicology. Two of your most recent NY Sea Grant projects related to a specific group of pollutants in urban waterways. You and your team looked at pollutants that mimic the female hormone estrogen. What exactly is an estrogen mimic and why look at these compounds?
Many everyday compounds–from pharmaceuticals, plastics and personal care products to industrial strength paints, detergents and pesticides–contain compounds that can act as hormone mimics. We've been looking at a group of compounds called nonylphenol ethoxylates or NPEOs. These are surfactants, chemicals that have both water soluble and non-water soluble parts that make them good cleaning agents among other things. Some NPEOs can mimic the hormone estrogen, which is important for female reproduction. We want to look for any effects these chemicals may have on animal populations—and set out to see if their presence causes males to exhibit female characteristics.

What got you so interested in this research area and why is it so important to study?
Toxicologists are looking closely at hormone mimics in many urban waterways, particularly in Europe. It has become a global concern.

In one NYSG-funded study, you sampled at New York City municipal sewage treatment plants. What were you looking for?
Our team assessed the estrogenic potential of sewage effluent from three plants in Brooklyn, NY: Newtown Creek, Red Hook, and 26th Ward. We looked for the egg-yolk precursor protein vitellogenin (Vtg) in three live stages of sunshine bass—newly hatched larvae, and two- and five-month old juveniles. We chose Vtg because estrogen turns on its production. Female fish need Vtg to make egg yolk protein. But all fish, including males, have the genes to make Vtg, it's just a question of whether or not the gene gets turned on. Estrogen mimics in the environment can cause immature and male fish to make Vtg. That's why Vtg makes a good biomarker for exposure to these chemicals. We also measured levels of NPEOs and natural estrogens in the plant effluents, and expression of the estrogen receptor or ER in larval fish.

And were the results in keeping with your hypothesis?
Yes. All three plants elicited some kind of estrogenic response in larval bass. However, juveniles only responded to effluent from the largest plant that also provided the least level of treatment, Newtown Creek. There, both Vtg and ER were elevated by a factor of 3-5 in larvae that were exposed to sewage effluent. Older fish exposed to effluent from Newtown Creek showed 20 to 200 fold elevations in Vtg expression, with the older fish showing the largest response. Levels of NPEOs were also much higher at Newtown Creek than at the other plants studied (more than 500 ug/L or parts per billion). Based on laboratory studies conducted on other species, the levels of NPEOs found in Newtown Creek sewage effluent are likely to be

high enough to be responsible for the estrogenic effects observed. At the time of the sampling, the Newtown Creek plant did not provide full secondary treatment. Even though it has been recently upgraded, it is my understanding that it still does not provide full secondary treatment.

Those numbers sound off the charts. Did you tell people about it?
Yes. The results were presented at numerous scientific meetings including the *11th Symposium on Pollutant Response in Marine Organisms* in Plymouth, England, annual meetings of the *Society of Environmental Toxicology* and I was invited to talk in a special session on endocrine disruptors to the *Society of Environmental Journalists*. Our colleagues on this project, **Bruce Brownawell** at Marine Sciences and his former student **Lee Fergusson**, now a faculty member at the University of South Carolina, also presented the data on developing methods to detect ultra trace levels of NPEOs and hormones at meetings of the Society of Mass Spectroscopy. The American Society for Testing Materials has expressed interest about developing their approach into a standard method of analysis.

Your work over the years has also gained you some national, as well as some very local, notoriety. You were interviewed, along with your husband and collaborator, Bruce Brownawell, by the local newspaper, *The Three Village Times*, about the Environmental Leadership award you both received from Southampton College.
It was very nice to be recognized for our environmental work, but my real interest is in answering applied science questions. It's an opportunity to address questions that really matter to people and our world, but it's very messy at times.

Who were your collaborators on this project?
This project was a collaborative effort between many people and places, including two departments at Stony Brook University (Marine Sciences and Pharmacology with **Dr. Charles Iden**), and scientists at other universities, including **Adria Elskus** who used to work with me at Stony Brook, but moved on to the University of Kentucky, **Daniel Schlenk** who used to be at Ole Miss but is now at the University of California at Riverside, and **Nancy Denslow** at the University of Florida. **Phil Heckler** of the NY State Department of Environmental Protection generously gave us permission to sample effluent. Part of this work was supported by related projects funded by the Hudson River Foundation to Schlenk and Brownawell.

In another NYSG-funded project you collaborated with **Martin Schreibman** and **Lucia Magliulo-Cepriano** from City University of New York's Brooklyn campus to assess whether or not winter flounder in Jamaica Bay are showing evidence of endocrine disruption.
We chose this economically-important species as a model because it lives in sediments where these compounds accumulate and if endocrine disruption is a problem for local fish, we would expect to see effects in Jamaica Bay animals. We believe this study will provide information for the protection of ecosystem health and be of use to managers of toxic substances, wastewater treatment, and fisheries resources.

This project got off to a slow start because we had trouble finding male fish in Jamaica Bay during our first sampling season. So we decided to return to the field the next spring to collect additional adult fish from Jamaica Bay and our reference site, Shinnecock Bay on Long Island's east end. Because of problems we encountered

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**Lourdes Mena dissecting a winter flounder.
Photo courtesy of Anne McElroy**

Spotlight on Lourdes Mena

Lourdes Mena, a graduate of the University of Puerto Rico, has been working on the winter flounder project since 2002, first as a NY Sea Grant Scholar and later as a Turner Minority Fellow. She completed her MS thesis in 2004 and immediately entered the Ph.D. program at Marine Sciences Research Center where she is continuing on with this project.

Mena presented the results of this Jamaica Bay project at the Association for Limnology and Oceanography (ASLO) Annual Meeting in Salt Lake City, at the 12th Symposium on Pollutant Responses in Marine Organisms in Tampa Bay, and at the Annual meeting of the Society of Environmental Toxicology and Chemistry in Austin TX.

"For my PhD project, I am looking at the assessment of endocrine disruption in wild populations of winter flounder (*Pseudopleuronectes americanus*) by use of molecular biomarkers (Vitellogenin, E2, 11-ketotestosterone), enzyme activities and histopathology. I expected to find fish with high levels of the protein vitellogenin (egg-yolk precursor protein) in male fish and use this as a biomarker of endocrine disruption as it has been used before in many studies. However, our findings have surprised me and I am now looking at more complicated aspects. I am looking at enzyme activities, protein expression, steroid metabolism and histopathology to try to find what is really happening to the fish population in Jamaica Bay. We know there is endocrine disruption in female fish and young fish, but the pattern is less clear in adult male fish. What is driving the changes we are seeing? Is one of the pathways in the endocrine system being interrupted? Are the fish functioning well reproductively?"

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finding adult male fish, we expanded our project to include young (> 1 year old) fish which we obtained with help from staff at the New York Department of Environmental Conservation who regularly sample these fish as part of their annual surveys. We ended up sampling fish over almost a four year period from 2002 to 2005. Although this significantly increased our level of effort, we ended up with a much more robust field sample including adult fish from multiple sites over a two year period as well as young-of-the-year (YOY) fish from multiple sites within each area.

So serendipity applies to research, too?
Sure. You can't always predict what is going to happen in the field with real animals. As a researcher, you make the most out of the field experience. Sometimes this makes for very good collaborations among scientists from different fields. I'm a toxicologist, not a fish biologist or pathologist. But by working with others from those fields, we get to integrate our disciplines and broaden the scope of the work.

Who were some of those researchers who helped out on this project?
We worked closely with staff at the Gateway National Recreation Area, the NYS DEC Division of Fish and Wildlife and expanded collaborative efforts with Dr. Nancy Denslow at the University of Florida. **Dr. Augustine Awruke** from Norway provided antibodies required for protein analysis and **Dr. Alistair Dove** of the Stony Brook Marine Pathology Laboratory provided advice on histological analysis.

What kinds of results did your extended three-year sampling reveal?
There were some unexpected results. We did not see classical evidence of endocrine disruption we had hypothesized from adult male fish living in Jamaica Bay, despite the extremely high concentrations of the endocrine disruptor nonylphenol present in some sediments. The Vtg levels in adult male fish were not significantly elevated over those in reference fish and preliminary analysis of gonads from adult fish provided no evidence of abnormalities. But the YOY fish from all sites in Jamaica Bay showed significantly elevated Vtg as compared to YOY

Sea Grant Scholar, grad student Julia Todorov with samples of effluent from a municipal waste treatment plant in New York City. Photo courtesy of Anne McElroy

fish from Shinnecock Bay, and adult male fish had depressed levels of reproductive hormones. Some of the most compelling evidence we have is highly female biased sex ratios in both YOY and adult fish caught in some parts of Jamaica Bay. It can be as high as 10:1 females to males. We also have observed developmental delays in winter flounder embryos exposed to sediments from Jamaica Bay, and clean sediments dosed with NPEOs.

How about female fish in Grassy Bay? Isn't that the most polluted part of Jamaica Bay?
Yes. Grassy Bay, right off the runways to JFK Airport is the most sewage-impacted site in the Bay. There we saw significantly elevated levels of Vtg in female fish. We are not sure what the biological importance of this is yet, but it has been observed in other female fish exposed to estrogen mimics.

So where does your work lead you?
We have strong evidence that chemicals in Jamaica Bay are altering enzyme activities and expression in resident fish, and that chemicals in Jamaica Bay sediment can cause developmental delays in young fish. The sex ratio data also indicate that population level impacts may be likely. Doctoral student **Lourdes Mena** is continuing to work on all these questions. The next step would be to see if winter flounder in other parts of the New York metropolitan area are being affected and to estimate potential population impacts. There's a lot more work for us to do.

— Barbara A. Branca and Patrick Dooley