

## Endocrine Disrupting Compounds

*In two related projects, Sea Grant researchers have found evidence that endocrine disrupting compounds in effluent from sewage treatment plants and in an urban estuary in the New York metro area have caused some level of feminization of resident winter flounder and striped bass.*



Sea Grant Scholar Lourdes Mena dissecting a winter flounder aboard *R/V Seawolf*.  
Photo courtesy of Anne McElroy

### Sources of endocrine disruption compounds (EDCs)

In the 1990s, the potential impact of contaminants altering hormonal status and reproductive success of wildlife species became a global environmental issue. Collectively these compounds are termed endocrine disruption compounds or EDCs. In some areas of Europe, widespread evidence of exposure to estrogenic EDCs has been documented. In the following decade increased availability of more sensitive analytical techniques demonstrated the presence of biologically significant concentrations of two groups of compounds with estrogenic activity.

Natural and synthetic estrogens and nonylphenol, a breakdown product belonging to a common class of surfactants, are used in many products including industrial detergents. Regulatory agencies in the U.S. and Europe scrambled to develop procedures for evaluating endocrine disruptive capabilities for new compounds as well as those already licensed for use. A major source of EDC exposure within aquatic and estuarine environments comes from sewage treatment plants (STPs). Although EDCs are metabolized during secondary (biological) sewage treatment, the resulting products can be more toxic and persistent in the environment than their parent compounds. At the time these projects started, little was known about the degree of EDC contamination or effects on native fish populations in the New York metropolitan region.

### Landmark research in NYC waste treatment plants and Jamaica Bay

The first of two EDC projects teamed Drs. Anne McElroy, Bruce Brownawell & Charles Iden of Stony Brook University with Dr. Adria Elskus from the University of Kentucky and Dr. Daniel Schlenk from the University of Mississippi. This team of researchers examined three New York City STPs looking at estrogenic effects on striped bass and the concentrations of compounds known to be estrogenic in sewage effluent. This provided the first chemical and toxicological data from NYC area STPs to managers and environmental groups.

The second project paired Dr. Anne McElroy from Stony Brook University with Dr. Martin Schreiber from Brooklyn College – CUNY. This effort expanded on the previous project to study the effects of chronic exposure to environmental estrogen mimics using a resident benthic fish, winter flounder, as a model species of exposure in the field. Specifically, the researchers looked to see if winter flounder were being feminized or impaired reproductively and to see if the metabolite nonylphenol could be implicated in any adverse effects observed in winter flounder in Jamaica Bay, NY.

## Results show endocrine disruption in wild fish

Application of newly developed, extremely sensitive, liquid chromatography- mass spectroscopy (LC-MS) methods indicated levels of the natural estrogens, estradiol and estrone to range from 5 to 18 parts per trillion (ng/L) in sewage from the three STPs. Levels of the estrogenic detergent breakdown products, nonylphenol and its 1,2, and 3 ethoxylate metabolites (collectively termed NPEOs) were thousands to tens of thousand times higher, ranging from 100 to 600 parts per billion ( $\mu\text{g/L}$ ). Effluent from the largest, oldest, and least effective STP, Newtown Creek, proved to be highly estrogenic to juvenile striped bass. The effluent increased levels of the egg yolk precursor protein vitellogenin, normally elevated only in ripe female fish, by up to 200 times over levels observed in fish not exposed to effluent.

Young flounder throughout Jamaica Bay showed biochemical signs of exposure to estrogenic compounds in their environment. High levels of vitellogenin were observed and the young winter flounder also showed signs of female reproductive tissues within the testes of male fish. Altered sex ratios were observed in Jamaica Bay winter flounder with many more females caught than males as compared to the reference site, Shinnecock Bay, NY. Preliminary evidence also indicated that healthy winter flounder embryos exposed to sediment from Jamaica Bay showed delayed development and reduced hatching success. Winter flounder exposed to sediments dosed with nonylphenol showed some of the same responses seen in fish collected from Jamaica Bay, indicating that this estrogenic contaminant could be responsible for the effects observed.

The high levels of NPEOs in the least effective sewage treatment plant demonstrates the importance of treatment level in the ability of sewage treatment plants to remove polar compounds such as the nonylphenol ethoxylates (NPEOs) from effluent. It also provides some of the first direct evidence of endocrine disruption in wild fish (striped bass and winter flounder)

exposed to sewage-derived endocrine disruptors, particularly nonylphenol, in New York waters. This information could support legislation to limit their use and release into the coastal environment.

Aspects of this work have been picked up by the popular scientific press, appearing in the *Amicus Journal*: Rivlin, M.A. 2000, "The Next Big, Bad Thing." McElroy was invited to speak on the subject at a special session on emerging issues at the Society of Environmental Journalists. Additionally, multiple publications have resulted from applying methods developed as part of this project.

The results of this work have led to several additional major funded research projects, totaling over \$1.2 million, in McElroy and Brownawell's laboratory. These leveraged funds led to the development of an environmental mass spectrometry facility housed at the Marine Sciences Research Center, Stony Brook University that brings in between \$70,000 to \$100,000 per year. Orange County, California has used these methods to test out new treatment technologies for removing estrogen from sewage effluent.

## Students

Three students successfully completed their degree programs from these two projects. In 2002, Patrick Ferguson received his PhD from Stony Brook University for his dissertation entitled: Analysis and Fate of Sewage-Derived Polar Organic Contaminants in the Marine Environment. For his highly significant efforts, he received the President's Award for Distinguished Doctoral Student from Stony Brook University in 2002. He is currently an assistant professor in the Department of Chemistry and Biochemistry at the University of South Carolina.

Luke Roy received his Master's degree in 2002 for his thesis entitled: Assessing Sediment Quality Using Biomarkers in Various Flatfish Species Off the Coast of Southern California. He has since entered

the doctoral program at Auburn University in Alabama.

Lourdes Mena received her Master's from Stony Brook University in 2004 for her thesis entitled: Endocrine Disruption in Winter Flounder (*Pseudopleuronectes americanus*): In Jamaica Bay, NY.

## Publications

Ferguson, P.L., R.F. Bopp, S.N. Chillrud, R.C. Aller, and B.J. Brownawell. 2003. Biogeochemistry of Nonylphenol Ethoxylates in Urban Estuarine Sediments, *Environ. Sci. Technol.* 37:3499-3506.

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Ferguson, P.L., and B.J. Brownawell. 2003. Degradation of Nonylphenol Ethoxylates in Estuarine Sediment Under Aerobic and Anaerobic Conditions. *Environ. Toxicol. Chem.* 22(6):1189-1199.

Ferguson, P.L., C.R. Iden, and B.J. Brownawell. 2001. Analysis of Nonylphenol and Nonylphenol Ethoxylates in Environmental Samples by Mixed-Mode High-Performance Liquid Chromatography-Electrospray Mass Spectrometry. *J Chromatogr.* 938:79-91.

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Ferguson, P.L., C.R. Iden, and B.J. Brownawell. 2001. Distribution and Fate of Neutral Alkylphenol Ethoxylate Metabolites in a Sewage-Impacted Urban Estuary. *Environ. Sci. Technol.* 35:2428-2435.

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Roy, L.A. 2002. Assessing Sediment Quality using Biomarkers in Various Flatfish Species off the Coast of Southern California. Thesis 100 pp.

Schlenk, D., Y. Sapozhnikova, M.A. Irwin, L. Xie, W. Hwang, S. Reddy, B.J. Brownawell, J. Armstrong, M. Kelly, D.E. Montagne, E.P. Kolodzie, D. Sedlak, and S. Snyder. 2005. In Vivo Bioassay-Guided Fractionation of Marine Sediment Extracts from the Southern California Bight, USA, for Estrogenic Activity. *Environ. Toxicol. Chem.* 24(11):2820-2826.

Swartz, C.H., S. Reddy, M.J. Benotti, H. Yin, L.B. Barber, B.J. Brownawell, and R.A. Rudel. 2006. Steroid Estrogens, Nonylphenol Ethoxylate Metabolites, and other Wastewater Contaminants in Groundwater Affected by a Residential Septic System on Cape Cod, MA. *Environ. Sci. Technol.* 40:4894-4902.

Todorov, J.R. A.A. Elskus, D. Schlenk, P.L. Ferguson, B.J. Brownawell, and A.E. McElroy 2002. Estrogenic Responses of Larval Sunshine Bass (*Morone saxatilis* X *M. chrysops*) Exposed to New York City Sewage Effluent. *Mar. Environ. Research.* 54:691-695.

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R/CTP-25 Estrogenicity of Municipal Sewage Treatment Plant Effluents: Vitellogenic and Estrogen Receptor Responses in Striped Bass

R/CTP-28 Endocrine Disruption in Jamaica Bay: Are Winter Flounder Being Affected?

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