

United States Environmental Protection Agency

- Office of Research and Development
- National Health and Environmental Effects Research Laboratory
- Mid-Continent Ecology Division, Duluth, Minnesota

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Research Events

ECOTOX WEBINAR, JUNE 11

MED Scientist **Chris Russom** was invited by the National Atmospheric and Oceanographic Administration (NOAA), National Ocean Service, Hazardous Materials Response Division in Seattle to present a Webinar, *ECOTOX database: Historical overview*. NOAA and National Marine Fisheries Service staff across the country participated. ECOTOX is a comprehensive database which provides information on adverse effects of single chemical stressors to ecologically relevant aquatic and terrestrial species. ECOTOX as a system began in 1995, but was available as separate databases (AQUIRE, PHYTOX, TERRETOX) as early as the mid 1980s. ECOTOX is available on EPA's public web page at <http://www.epa.gov/ecotox/>.

Contact: Chris Russom (218) 529-5218



SP2 AND OFFICE OF PESTICIDE PROGRAMS MEET WITH MED, JULY 15-17



In July the Division hosted Elaine Francis (National Program Director for Safe Pesticides/Safe Products [SP2] and Endocrine Disrupting Chemicals [EDC]), Vicki Dellarco (OPP Science Advisor to the Director's Office), and Keith Sappington and Ed Odenkirchen (Senior Scientists from OPP Environmental Fate & Effects Division). Also visiting were Steve Hedtke, NHEERL Associate Director for Ecology; Doug Wolf, NHEERL Assistant Lab Director for SP2 and EDC; and Jennifer Orme-Zavaleta, NHEERL Research Planning & Coordination. Researchers from the other NHEERL ecology divisions (AED/Atlantic, GED/Gulf, and WED/Western) also attended and participated in discussion of SP2 research and the Wildlife Strategy.

The purpose of the meeting was to inform OPP and Dr. Francis of the extensive research the NHEERL ecology divisions are conducting on ecological effects modeling, the application of these models, and future directions for this research. A day was devoted to presentations and discussion of MED-specific SP2 and EDC research, and included a tour of the research facilities. MED Director **Carl Richards** gave an introduction and overview, and MED researchers **Gary Ankley, Rick Bennett, Larry Burkhard, Sig Degitz, Mike Hornung, Rodney Johnson, Dave Mount, John Nichols, Chris Russom, and Pat Schmieder** presented summaries of their work, as well as real-time demonstrations of software that MED developed for ecological effects modeling. The Division received feedback from OPP on the usefulness of the information it produces and technical support it provides, and had valuable discussions on future research directions that will help OPP meet its mission. Division staff also provided National Exposure Research Laboratory (NERL) colleagues with an overview of the eco-component of NHEERL's SP2 program, which will greatly influence the development of NERL's SP2 implementation plan. **Contact:** Pat Schmieder (218) 529-5161

MED SCIENTIST INVITED TO WORKSHOP ON POPULATION-LEVEL ERA, JUNE 16-18

EPA's Risk Assessment Forum (RAF) organized a technical workshop in Crystal City, VA, to consider the possible development of guidance based on the current state of the knowledge and science underlying population-level ecological risk assessment (ERA). Several NHEERL scientists were invited to participate, including Diane Nacci, Jason Grear, and Glen Thursby of AED; Sandy Raimondo of GED; and **Rick Bennett** of MED. Other experts in population ecology, ecological risk assessment, and risk management participated from federal, academic, and private sector organizations with stakes in population risk. Workshop products will inform the RAF about the maturity of population ecology theory, and models and tools cogent to population-level ecological risk assessment, and will influence the RAF's actions for developing guidelines supplemental to the 1998 ERA Guidelines. **MED Contact:** Rick Bennett (218) 529-5212



MED "-OMICS" WEBINAR, JULY 28

Jose Serrano presented a Web seminar to ORD researchers titled *Assessing the potential of newer omic and bioinformatic tools to provide mechanistic data needed to populate molecular and cellular levels of biological organization in system toxicology models*. The presentation focused on the latest high-throughput "-omic" and informatic tools being used and/or developed to address human and environmental health research after chemical exposure. Jose advised planning group members on the best type of proteomics and metabolomics instrumentation and informatics tools needed to create a proteomic/metabolomic core at Research Triangle Park (RTP). Open forum-style discussion followed the presentation. About 50 people attended, including members of MED's Molecular and Cellular Mechanisms Research and Toxic Effects Characterization Research Branches, members of all NHEERL branches, the Genomics Core, and the Genomics and Proteomics Development Group at RTP and Cincinnati-EPA. **Contact:** Jose Serrano (218) 529-5118



- courtesy Nature Publishing Group

AUGUST 21 ORD NEWS RELEASE PUBLICIZES MED'S SUSTAINABLE LANDSCAPING

In the fall of 2003, local EPA staff members developed a plan to replace approximately two acres of mowed lawn with a sustainable landscape of native grasses and flowering plants. Now the meadow is firmly established and is providing habitat for local wildlife and insects, savings in lawn upkeep, and an educational opportunity for visitors. The sustainable landscape is consistent with and supports many of the land use ideals that EPA promotes. It also complies with a 2000 Executive Order mandating such landscapes at federal facilities. The ORD news release led to articles in the local press on our "northern meadow."

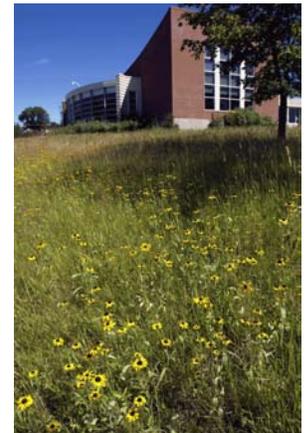
In March of 2007, the Duluth Landscaping Team won the Agency's first annual Green Thumb Award for its onsite sustainable landscaping. The Division also has created programs to reduce energy consumption and purchase renewable energy, and most of our waste products are recycled.

Contact: Corlis West (218) 529-5125

[Link to news release.](#)



2003



2006

MED SCIENTISTS ATTEND AQUATIC TOXICITY WORKSHOP, OCTOBER 5-8



Drs. Dan Villeneuve and Michael Hornung attended the 35th Annual Aquatic Toxicity Workshop held in Saskatoon, Saskatchewan. The meeting was attended by a few hundred participants from academia, industry, and government agencies with most of the participants from throughout Canada. Dr. Villeneuve delivered two invited platform presentations and chaired technical sessions on *Endocrine Modulating Substances and Genomics, Proteomics, and Metabolomics*, which included a series of talks focused on compensatory responses to chemical stressors. Dr. Hornung gave an invited presentation in the Endocrine Modulating Substances session on the use of in vitro and ex vivo assays for investigating thyroid hormone disruptors. This meeting provided an opportunity to exchange ideas, coordinate on-going research efforts, and develop additional international partnerships. **Contact:** Dan Villeneuve (218) 529-5217

MED SCIENTIST SPEAKS AT REGIONAL RISK ASSESSORS CONFERENCE, OCTOBER 6-9

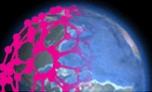
Lawrence Burkhard was invited to speak at the EPA-sponsored 23rd Annual Regional Risk Assessors Conference in Seattle. Conference attendees were state and federal human health and ecological risk assessors who work on hazardous waste sites. The focus of Larry's presentation was empirical and mechanistic methods to predict residues in fish and shellfish for sites with sediments contaminated with nonpolar organic chemicals, e.g., PCBs, PCDD/Fs, and DDTs. The presentation included introductory materials on the predictive methods, and then discussed the nuts and bolts of using them. **Dale Hoff**, Ecotoxicology Analysis Research Branch Chief, also attended the conference. **Contact:** Lawrence Burkhard (218) 529-5164



MED RESEARCHER ON ORGANIZING COMMITTEE FOR NANO CONFERENCE, OCTOBER 7-9

Steve Diamond, along with other ORD staff including Jon Josephs (OSP, R2), Barbara Karn (NCER), Eric Weber (NERL), Nora Savage (NCER), Chuck Maurice (OSP, R5), Barbara Walton (NHEERL), and Mike Gill (OSP, R9) served on the organizing committee for the International Environmental Nanotechnology Conference: Applications and Implications, held in Chicago. Dr. Diamond also served on the organizing committee for the two-day Toxicity session, chaired a portion of that session, and sat on a discussion panel with seven international scientists on the last day of the meeting. The meeting was attended by approximately 200 scientists from five continents and covered a full range of nanotechnology-related environmental issues including the use of nanomaterials in remediation, environmentally-responsible development of nanotechnology, and the fate and potential toxicity of nanomaterials. **Contact:** Steve Diamond (218) 529-5229

International Environmental
Nanotechnology Conference:
Applications and Implications



ARCHIVING OUR DIVISION'S HISTORY AND LINKING IT TO TODAY'S RESEARCH

The MED research facility pre-dates the EPA. Research began in Duluth in 1965 under the Department of Interior, Federal Water Pollution Control Administration, when the facility became the National Water Quality Laboratory. It became one of the first EPA research labs when the Agency was established by Congress in 1970, and was renamed the Environmental Research Laboratory in 1972. Materials documenting the history of the facility, staff, and research have been stored in various paper and electronic forms over the years, often without descriptive information. These include photographs, promotional and research films, video tapes of local TV news stories, and newspaper articles. Allan Batterman, Barbara Sheedy, Bob Spehar, and other interested staff, too numerous to list here, have created an archive of annotated material, converting video tapes and movies to DVD, with copies in the MED library. This group is also scanning, identifying, and labeling the historical photographic records. The electronic location of these materials will be advertised to staff and hard copies will be maintained in archival storage.

The movies and video tapes converted to DVD include:

- A Mandate for Clean Water, approx. 1975
- Water Quality: Our Challenge, 1972
- Laboratory Effects of Lead on Brook Trout
- History of Aquatic Toxicology: the Making of a Profession, 1990
- News reels by local and regional TV stations: Pesticides, Dioxin, Acid Rain, Conference Center Ground Breaking, and Confocal Microscope
- Adventure North (local PBS): The Fathead Minnow – White Mouse of Aquatic Research



Architect's drawing of the National Water Quality Laboratory

Staff also created a series of informational posters displayed in the Duluth facility, demonstrating the history of research and development and linking this rich legacy to today's research directions and accomplishments. These materials remind staff that their work is building on a significant body of science, and inform guests of the long and productive history of the organization.

OW-ORD TEAM INCLUDES MED RESEARCHERS TO CONSIDER CHEMICALS OF EMERGING CONCERN

In 1985, EPA published *Guidelines for Deriving Numerical National Water Quality Criteria for the Protection of Aquatic Organisms and Their Uses*. While these *Guidelines* remain the primary instrument the Agency uses to meet its broad objectives for the development of Ambient Water Quality Criteria (AWQC), there have been many advances in aquatic sciences relevant to deriving

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OW-ORD TEAM – CONTINUED

these criteria. Recently, considerable attention has been generated by a widely ranging group of chemicals collectively termed contaminants of emerging concern (CECs). A CEC of particular Congressional interest is 17 α -ethinylestradiol (EE2), a synthetic estrogenic steroid. EE2 is the active pharmacological component of most oral contraceptives, and acts as a potent estrogen receptor agonist in vertebrates. After use and excretion of the contraceptive, domestic sewage treatment plant effluents become the primary source of EE2 entering the aquatic environment. CECs like EE2 pose challenges to the traditional methodology for deriving AWQCs.

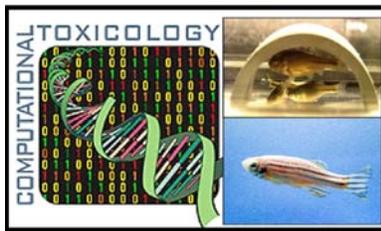
Since March of 2007, ORD scientists from NHEERL and NERL have been assisting the Office of Water's (OW), Office of Science and Technology, Health and Ecological Criteria Division to evolve EPA's current AWQC Guidelines into a scientifically defensible method for CECs. ORD scientists hosted a workshop in July of 2007 to outline a white paper with specific recommendations. Workshop participants identified several toxicological characteristics of CECs that make criteria development difficult. These characteristics stem primarily from the fact that many CECs (particularly pharmaceuticals) have a very specific mode of action and are extremely potent at low concentrations. From July of 2007 through May of 2008, weekly (and occasionally, biweekly) teleconferences were held with OW as ORD scientists authored with OW staff a two-part white paper. Part I identifies the problematic toxicological characteristics of CECs, and makes specific recommendations on how to address them. Part II illustrates the recommendations by using EE2 as a model CEC. Finally, ORD scientists participated in a Scientific Advisory Board review of the white paper at the end of June. Since then, ORD has continued to engage OW while responses to the review are being prepared. MED staff involved in this effort are **Gary Ankley, Russ Erickson, Dale Hoff, Dave Mount, Rick Bennett, Chris Russom, and Joe Tietge**.

Contact: Dale Hoff (218)529-5386

Featured Research

SMALL FISH COMPUTATIONAL TOXICOLOGY

The Division hosted the fourth annual face-to-face meeting of EPA's Small Fish Computational Toxicology research team in June 2008. This team is involved in a multi-



disciplinary, cross-ORD project, initiated in January 2005, that has focused on the use of global gene expression, protein, and metabolite analyses (i.e., transcriptomics, proteomics, metabolomics), and molecular systems biology-based approaches to: 1) identify new molecular markers of effects of, and/or exposure to, chemicals acting on the hypothalamic-pituitary-gonadal (HPG) axis of small fish through different modes of action; 2) link molecular markers to higher level outcomes relevant to ecological risk assessments (e.g., reproductive success); and 3) generate integrated modeling approaches that utilize mode/mechanism of action as a basis for predicting adverse outcomes. The meeting was attended by scientists from four different EPA divisions and centers, three academic institutions, and the US Army Engineering Research and Development Center (ERDC). This event was an opportunity for the participating research partners to share results, get an update regarding the overall project status, and coordinate ongoing research efforts.

MED scientists have completed over two dozen experiments, including seven 21 d fathead minnow (*Pimephales promelas*) reproduction assays, ten 96 h time-course experiments with zebrafish (*Danio rerio*), and seven 16 d time-course experiments with fathead minnow, that provide the core set of samples for the

research effort. Results of five of the seven fathead minnow reproduction assays have been published in the peer-reviewed scientific literature. Additionally, four papers focused on various modeling aspects of the research were published. This includes two papers that describe a mechanistically-based modeling framework for using measurements of steroids or the egg yolk precursor protein vitellogenin in the plasma of female fish as a basis for forecasting fish population trajectories (Miller et al. 2006; Ankley et al. 2007); a paper that provides the first dynamic, mechanistically-based, mathematical model of vertebrate steroid biosynthesis (Breen et al. 2007); and a paper that describes and applies a generalized graphical model the fish reproductive axis and the genes, proteins, and metabolites that regulate it, as a foundation for hypothesis-driven, systems-oriented, ecotoxicological research (Villeneuve et al. 2007). The latter paper was selected as one of *Environmental Science & Technology's* top four environmental science papers of 2007. Several other publications have laid out the technical foundation for the experimental designs and analyses used for DNA microarray experiments with zebrafish (Wang et al. 2008a,b) and provided proof-of-concept that nuclear magnetic resonance-based metabolomics could be successfully used to characterize stressor-induced alterations in metabolite profiles in tissues and biofluids (e.g., urine) collected from small fish (Ekman et al. 2007, 2008).

With the completion of the core set of experiments and the generation of large amounts of global gene expression data, much of the project emphasis has shifted away from sample and

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SMALL FISH COMPUTATIONAL TOXICOLOGY – CONTINUED

data generation to intensive analysis. For example, functional analysis of global gene expression changes observed in the zebrafish microarray experiments is being used to formulate novel hypotheses regarding the effects of the selected test chemicals on the fish reproductive axis as well as other toxicologically-relevant pathways. Additionally, meta-analyses that compare and contrast the gene expression data from all ten zebrafish microarray experiments, are being used to identify potential molecular markers of exposure and/or effect and gene regulatory elements that modulate biological response to the various endocrine disrupting chemicals (EDCs) tested. Through partnership with ERDC, microarray-based analyses of hundreds of fathead minnow tissue samples generated by MED are also being used to reverse-engineer the regulatory network of the fish ovary in an effort to understand organizing principles underlying the robustness and fragility of complex biological networks. Responses of the zebrafish and fathead minnow are also being compared to help evaluate how well the responses and regulatory networks are conserved among different fish species. As these analyses proceed, the data are being integrated into a framework of complementary graphical and dynamic (computational) biological systems models designed to aid predictive toxicology.

Thus far, results of the project have led to delineation of adverse outcome pathways through which EDCs acting through any one of four distinct modes of action can cause reproductive dysfunction in female fish. The studies have also yielded significant insights into compensatory responses to stressors acting on the reproductive axis. Over the initial four years, the research conducted by the Small Fish Computational Toxicology team yielded nearly 20 peer-reviewed publications, and various aspects of the project have been presented in over 40 technical presentations and seminars. Ongoing efforts by this multi-disciplinary research team, which includes several MED scientists, post-docs, and student services contractors, contribute to Agency interests in using cutting edge technology and systems biology approaches to develop the scientific foundation for greater use of cost-effective molecular assays and predictive models as a basis for ecological risk assessment and environmental protection.

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Gerald Ankley (218) 529-5147, ankley.gerald@epa.gov.

DNA-BASED GENETIC SEXING OF *XENOPUS*

Certain endocrine disrupting chemicals (EDCs) have the ability to override genetic sex determination in animals, resulting in sex reversal. It has long been known that exposure to estrogens during larval development results in sex reversal of male African frogs (*Xenopus* spp.). Recently, researchers at the Division demonstrated that female tropical clawed frogs (*Xenopus tropicalis*) could be sex-reversed by exposure to an anti-estrogen (Olmstead et al., 2008). The chemical used in this study, fadrozole, elicits its effects by inhibiting the enzyme aromatase which catalyzes the production of the endogenous estrogen, estradiol. These sex-reversed females were found to be male-like in every measured endpoint and were even capable of mating with normal females. Sex reversal of males by the environmental estrogen, ethynylestradiol, has also been produced by larval exposure here at MED and these animals were also capable of producing offspring.

Effects on sex ratios in tadpoles exposed to EDCs were among the most sensitive endpoints measured, are likely to be deleterious to any exposed natural population, and are highly relevant for the risk assessment of these types of compounds. Evaluations of changes in sex ratios, however, are hampered by the lack of statistical power inherent in comparing treatment ratios to controls which have sex ratios around 50% female. In the study cited above, a shift in sex ratio of 23% was the smallest sex ratio difference that would have been statistically significant ($\alpha=0.05$). This would mean that roughly half of the female frogs (>46%) in this experiment would need to become sex reversed before a statistically significant effect would be observed.

Recently, researchers at MED have identified sex-linked DNA markers that allow for the genetic sexing of *Xenopus tropicalis*. Application of this method allows for the separation of genetic males and females for data analysis. Instead of comparing sex ratios to a control level of 50%, comparisons can be made to a control level of 0%. This method results in a greater statistical power for



A male (left) and female (right) tropical clawed frog (*Xenopus tropicalis*). Exposure to EDCs can result in sex reversal in which the affected frogs are fully reproductively capable and are identifiable only through genetic testing.

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GENETIC SEXING OF *XENOPUS* – CONTINUED

comparisons made in EDC studies as well as a reduction in the number of animals needed in a bioassay. This method is expected to be included in the Amphibian Growth, Reproduction, and Development assay being developed as a tier II assay in EPA's Endocrine Disruptor Screening Program. This work was initiated by the analysis of amplified fragment length polymorphisms which identified sex-linked stretches of DNA. PCR methods were then developed using the sequences of these sex-linked polymorphisms yielding a way to identify the sex genotype of frogs used in bioassays. While the gonad phenotypes in amphibian toxicity studies have been historically evaluated by histopathology, MED researchers are investigating the usefulness of gene expression markers as an alternative to histopathology. Genes that were most sex-biased in gonad expression as determined by gene array experiments were evaluated in EDC-exposed frogs. Results demonstrated that the expression of these genes varied in a concentration-dependent fashion indicating intersexuality and sex-reversal. The combination of genotyping sex and evaluating phenotype by gene expression has the potential to be a powerful tool in evaluating the ability of chemicals to alter gonad differentiation in larval amphibians.

A prospective strategy for establishing breeding populations that contain the necessary genetic make-up to utilize these genotyping methods has been developed and is in the process of being implemented. The results of this work are also being applied to samples from other research groups that investigate endocrine disruption using *Xenopus tropicalis*. These groups include those at the US Army Center for Environmental Health Research and Uppsala University in Sweden. The methods developed for the identification of sex-linked amplified fragment length polymorphisms (AFLPs) is also being applied to the fathead minnow (*Pimephales promelas*), another important environmental toxicology model species currently being used for endocrine disruptor bioassays. **Contact:** Allen Olmstead (218) 529-5122



Exposure to the pharmaceutical fadrozole during larval development resulted in some frogs developing an intersex phenotype as adults. Genetic sexing of the affected individuals during EDC testing help to determine if these are females that have become masculinized or males that have become feminized.

Reference

Olmstead A.W., Kosian P.A., Korte J.J., Holcombe G., Woodis K.K., and Degitz, S.J. 2008. Sex reversal of the amphibian, *Xenopus tropicalis*, following larval exposure to an aromatase inhibitor. *Aquat Toxicol* (in press), DOI:10.1016/j.aquatox.2008.07.018.

BIOASSESSMENT OF THE GREAT RIVER ECOSYSTEMS OF THE MID-CONTINENT

The objectives of the EMAP Great River Ecosystems Program (EMAP-GRE) were to develop and demonstrate, in collaboration with states and EPA regions and other ORD labs, an assessment approach to estimate the condition of the Upper Mississippi, Missouri, and Ohio Rivers. The scope was audacious because at the mouth of the Ohio River, the Upper Mississippi River system drains about 2.3 million km² or about 29% of the contiguous US. The sampling design and field methods were required to deliver consistently high-quality data from 1400 km, 2900km, and 1560 km of those rivers, respectively. Sampling for multiple biotic assemblages, water quality, and physical habitat was conducted in 2004-2006. We presumed that ecological conditions in the main channel reflected, at least in part, condition of off-channel habitats. Main-channel sampling was considered most amenable to routine monitoring by states and tribes, an important consideration for the program. Parallel with the field effort, landscape and hydrological data were assembled for each of the 447 sites. Results are being used to characterize the magnitude and extent of biotic and abiotic conditions at the scale of the river and states. The successes of the design process and field campaign can be directly attributable to partnerships formed among EPA scientists at ORD's national labs of Health and Environmental Effects, and Environmental Exposure, and at EPA Regions 3, 5, 7, and 8.

The EMAP-GRE team is now developing indicators of water quality and biological conditions with which to assess the environmental conditions of the rivers. Indicators include biotic integrity of fish, macroinvertebrates, phytoplankton, zooplankton, and periphyton assemblages; water chemistry; aquatic and riparian habitat; fish and sediment contaminants; and sediment microbial enzyme activity. Preliminary results have focused on determining reference condition. Because these rivers are highly managed for navigation, flood control, wildlife, hydropower, and recreation, definitions of reference condition vary widely. Fundamental characteristics of the rivers, such as connectivity to floodplains and hydrograph variability, have been systematically reduced or eliminated. This means that river conditions reminiscent of pre-European settlement or otherwise unaltered by humans may not be relevant. We acknowledge that the rivers are permanently disturbed, but some reaches are less disturbed than others. Because the probability design yielded data

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THE BIOASSESSMENT OF THE GREAT RIVER ECOSYSTEMS – CONTINUED

representative of all extant conditions, we can empirically derive least disturbed conditions. Ordination of fish assemblage data and examination of spatial variations in water quality revealed that the impounded Mississippi (St. Paul, MN to St. Louis, MO), the unimpounded Mississippi (St. Louis to Cairo, IL), the Upper Missouri (Ft. Peck, MT to Sioux City, IA), the Lower Missouri (below Sioux City, IA), and the entire Ohio, were sufficiently different from each other to warrant individual definitions of reference condition. Disturbance gradients were constructed using combinations of water quality, habitat, sediment toxicity, and landscape metrics. Metrics were selected to represent anthropogenic (rather than natural) stresses on fish but it is presumed they would be applicable to other biota. Metrics were screened and scored to ensure they made additive contributions to the gradient. Completed disturbance gradients included between 3 (Ohio) and 21 (impounded Mississippi) metrics. Metrics common to most gradients were presence of human disturbances in the riparian zone and on the floodplain, and the concentrations of total nitrogen and dissolved metals. The disturbance gradient for the impounded Mississippi is presented in Figure 1. Areas of highest disturbance were located near the urban areas of Minneapolis-St. Paul (Locks and Dams 1-3) and the Quad cities (Lock and Dams 14-16). Areas of the lowest stress were more widely dispersed and included parts of Pools 10, 11, and 25. Pool 25 is situated just above the confluence of the Illinois River, a major tributary that drains the Chicago metro area and areas of intensive row-crop agriculture.

A similar process of screening and scoring metrics was used to construct indices of biotic integrity (IBI) for fish for each reference strata. Combinations of 8-10 metrics were found to be consistently responsive to the disturbance gradients. Metrics of fish species richness, abundance, biomass, and health were included. The highest IBIs at the lowest disturbance score defined the least disturbed condition for fish. By convention, the range between least disturbed conditions for fish and the most disturbed condition for fish was trisected to define least, intermediate, and most disturbed condition classes. Figure 2 shows the distribution of fish condition classes in the impounded Mississippi River. Consistent with the areas of high stress, the metro areas of St. Paul and Quad Cities had the most disturbed fish assemblages. Pool 10 (including the confluence of the Wisconsin River) was an area of relatively low anthropogenic stress and least disturbed fish assemblage. In contrast, pool 25 had a most disturbed fish assemblage but had least anthropogenic disturbance. This may demonstrate the difficulty fish have in accessing potentially high quality habitat because of the dams. It could also indicate the difficulty of accurately estimating disturbance and fish assemblages as the river transitions from impounded (a series of shallow lakes) to un-impounded (a single constrained channel). Invertivore, detritivore, darter, sucker, and non-indigenous species were characteristic of fish in the impounded Mississippi River. Piscivores, great river obligates, and centrarchid species characterized the un-impounded river.

These preliminary results demonstrate that our approach using explicitly defined resources and reference condition, probability designs, and universally prescribed field methods can be used to assess great river ecosystems. The EMAP-GRE team continues to work with our partners to develop and refine indices of habitat, landscape, and water quality and biotic integrity. These indicators blur the lines between research and management. The value of some indicators will be immediately recognized. The value of other indicators may not be apparent without further corroboration and application. In any case, the amassed data alone guarantee a lasting legacy in the science and practice of the assessment of great rivers.

Contact: Dave Bolgrien (218) 529-5216



Figure 1: Map of the disturbance gradient for the impounded Mississippi River. Thresholds for the low disturbance class (<0.21; green) and high disturbance class (>0.42; red) were set at the 25th and 75th percentiles, respectively. The intermediate condition is yellow.



Figure 2: Map of the Great Rivers Fish Index (GRFin) condition classes for the impounded Mississippi River. Thresholds for the most disturbed class (<5.2; red) and least disturbed class (>7.5; green) were set at the 5th and y-intercept of the 95th quantile regression results, respectively. The intermediate condition is yellow.

Current Events

NHEERL SCIENTISTS CHAIR NALMS SYMPOSIUM, NOVEMBER 11-14

The North American Lake Management Society (NALMS) held its 28th International Symposium in Lake Louise, Alberta, with the theme *Lake Management in a Changing Environment*. NALMS is devoted to forging partnerships among citizens, scientists, and professionals to manage and protect lakes and reservoirs. Information on NALMS and the scientific program can be found at: <http://www.nalms.org/>



David Miller of MED/Grosse Ile (Large Lakes & Rivers Forecasting Research Branch) organized and chaired a session on *Ecological Impacts of Invasive Species*. He also presented a platform paper, *Application of a lower food web ecosystem productivity model to investigate population dynamics of invasive species in Lake Michigan*. **Jack Kelly**, Chief of Duluth's Ecosystem Assessment Research Branch, presented a platform paper in the session entitled *Early detection monitoring approaches developed from a case study on a vulnerable Great Lakes embayment*.

MED research represents results from the ORD Great Lakes Invasive Species Initiative, in cooperation with the National Center for Environmental Assessment, National Exposure Research Laboratory/NHEERL, and the Great Lakes National Program Office, regarding detection, monitoring, prioritization, and modeling of invasive species. This symposium was an opportunity for ORD to showcase multi-disciplinary research which supports the National Invasive Species Act, Executive Order for Great Lakes Regional Collaboration, Clean Water Act, US-Canada Great Lakes Water Quality Agreement, and the Critical Programs Act.

Contact: David Miller (734) 692-7613

ANKLEY RECEIVES SETAC FOUNDERS AWARD



Gerald Ankley received the 2008 Founders Award from the Society of Environmental Toxicology and Chemistry (SETAC) in a ceremony November 16 at the 29th Annual SETAC North America meeting in Tampa. SETAC is an international professional society with thousands of members involved in the study, analysis, and solution of environmental problems; management and regulation of natural resources; and environmental education. The Founders Award is a lifetime achievement recognition that has been presented annually since 1980. Previous EPA winners are William Ruckelshaus (1986); Donald Mount, former MED Director (1987); Samuel Karickhoff (1991); and Glenn Suter (2004).

Contact: Gerald Ankley (218) 529-5147

NANOTECHNOLOGY NEWS

SETAC Session, November 16-20

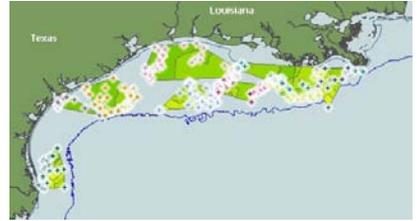
Steve Diamond organized and co-chaired a full-day session entitled *Environmental Toxicity of Nanomaterials* at the 29th annual meeting in Tampa. Dr. Diamond co-chaired the session with Linda Ziccardi of Exponent, Inc., and also presented a paper entitled *An evaluation of regulatory ecotoxicity test guidelines: Their adequacy for nanomaterials*. Steve's presentation summarized the work of nine international scientists who comprised an Organization for Economic and Cooperative Development's (OECD) review panel that evaluated 30 toxicity test guidelines. Steve chaired this review panel for the Working Party on Manufactured Nanomaterials. The findings of the review panel suggest that current test guidelines do not provide adequate guidance for the preparation and quantification of nanomaterial media for toxicity testing.

Grantees Workshop, November 19-21

Steve Diamond represented the NHEERL Ecology Divisions' research in nanomaterials ecotoxicity at the Interagency SETAC Nanomaterials Grantees Workshop, also held in Tampa. He discussed ongoing and planned research at the Atlantic, Mid-Continent, and Western Ecology Divisions within NHEERL. This workshop provided an opportunity for STAR-funded researchers from academia to interact with scientists and representatives from as many as 25 government agencies currently involved in nanotechnology research. Steve discussed how NHEERL's Ecology Divisions are addressing EPA's regulatory needs in their research, and how they are contributing to international efforts within the OECD Working Party on Manufactured Nanomaterials. **Contact:** Steve Diamond (218) 529-5229

MED SCIENTIST TO PARTICIPATE IN REGIONAL HYPOXIA STRATEGY DISCUSSION, DECEMBER 16-18

This workshop in New Orleans was organized by EPA Region 7 and the Louisiana Universities Marine Consortium to discuss approaches to the Gulf hypoxia problem that may be useful for the Regions with basins draining to the Gulf of Mexico. Attendees will include the Administrators, Deputy Administrators, and Water Division Directors for EPA Regions 4, 5, 6, 7, and 8; and representatives from the EPA Gulf of Mexico Program Office and the states of Iowa and Louisiana. **Brian Hill**, Chief of the Watershed Diagnostics Research Branch, was invited to present results on nutrient export from small streams and large rivers of the Upper Mississippi, Missouri, and Ohio River basins. These export estimates are based on data collected during the Environmental Monitoring and Assessment Program for Great Rivers Ecosystems, and the Wadeable Streams Assessment. **Contact:** Brian Hill (218) 529-5224



New Publications since June 2008

- Angradi, T.R., D.W. Bolgrien, T.M. Jicha, M.S. Pearson, B.H. Hill, D.L. Taylor, E.W. Schweiger, L. Shepard, A.R. Batterman, M.F. Moffett, C.M. Elonen, and L.E. Anderson. 2008. A bioassessment approach for mid-continent great rivers: the Upper Mississippi, Missouri, and Ohio (USA). *Environmental Monitoring and Assessment*, <http://www.springerlink.com/content/xw510xp123562r87/fulltext.pdf>. DOI 10.1007/s10661-008-0327-1.
- Ankley, G.T., D.H. Miller, K.M. Jensen, D.L. Villeneuve, and D. Martinovic. 2008. Relationship of plasma sex steroid concentrations in female fathead minnows to reproductive success and population status. *Aquatic Toxicology* 88:69-74.
- Barron, M.G., S. Raimondo, C.L. Russom, D. Vivian, and S.H. Yee. 2008. Accuracy of chronic aquatic toxicity estimates determined from acute toxicity data and two time-response models. *Environmental Toxicology and Chemistry* 27:2196-2205.
- Barron, M.G., D. Vivian, S.H. Yee, S.A. Diamond, and T. Parkin. 2008. Temporal and spatial variation in solar radiation and photoenhanced toxicity risks of spilled oil in Prince William Sound, Alaska, USA. *Environmental Toxicology and Chemistry* 27:727-736.
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- Brooks, B.W., G.T. Ankley, J.F. Hobson, J.M. Lazorchak, R.D. Meyerhoff, and K.R. Solomon. 2008. Chapter 5. Assessing the aquatic hazards of veterinary medicines. *Veterinary Medicines in the Environment*, M. Crane, A. Boxall, and K. Barrett, Eds., SETAC Press, Pensacola, FL, pp. 97-128.
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- Ekman, D.R., Q. Teng, D.L. Villeneuve, M.D. Kahl, K.M. Jensen, E.J. Durhan, G.T. Ankley, and T.W. Collette. 2008. Investigating compensation and recovery of fathead minnow (*Pimephales promelas*) exposed to 17 α -ethynylestradiol with metabolite profiling. *Environmental Science & Technology* 42:4188-4194.
- Etterson, M.A. and T.R. Stanley. 2008. Incorporating classification uncertainty in competing- risks nest-failure analysis. *The Auk* 125:687-699.
- Greenberg, M., S. Eells, and L. Burkhard. 2008. SAMS #1: Using fish tissue data to monitor remedy effectiveness. USEPA, OSRTI/ORD, EPA/600/X-07/014.
- Grigorovich, I.A., T.R. Angradi, and C.A. Stepien. 2008. Occurrence of the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*Dreissena polymorpha*) in the upper Mississippi River System. *Journal of Freshwater Ecology* 23:429-435.
- Grigorovich, I.A., J.R. Kelly, J. Darling, and C.W. West. 2008. The quagga mussel invades the Lake Superior basin. *Journal of Great Lakes Research* 34:342-350.

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- Hoffman, J.C., D.A. Bronk, and J.E. Olney. 2008. Organic matter sources supporting lower food web production in the tidal freshwater portion of the York River estuary, Virginia. *Estuaries and Coasts* 31:898-911.
- Kelly, J.R. 2008. Nitrogen effects on coastal marine ecosystems. *In: Nitrogen in the Environment: Sources, Problems, and Management*, Second Edition, J.L. Hatfield and R.F. Follett, Eds., Elsevier, Amsterdam, the Netherlands, pp. 271-332.
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- Martinovic, D., J.S. Denny, P.K. Schmieder, G.T. Ankley, and P.W. Sorensen. 2008. Temporal variation in the estrogenicity of a sewage treatment plant effluent and its biological significance. *Environmental Science & Technology* 42:3421-3427.
- Nawrocki, T., M.A. Starry, M.F. Moffett, and D.W. Bolgrien. Landscape metrics arranged by hydrological proximity to sites on the Mississippi, Missouri, and Ohio Rivers. *In: GIS and Water Resources V, AWRA Spring Specialty Conference, San Mateo, CA, March 17-19, 2008*, D.R. Maidment, Ed., American Water Resources Association, Middleburg, VA, TPS-08-1, CD-ROM / TPS-08-1. ISBN 1-882132-76-9.
- Nichols, J.W., A.D. Hoffman, P.N. Fitzsimmons, and G.J. Lien. 2008. Quantification of phenol, phenyl glucuronide, and phenyl sulfate in blood of unanesthetized rainbow trout by online microdialysis sampling. *Toxicology Mechanisms and Methods* 18:405-412.
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- Pauer, J.J., A.M. Anstead, W. Melendez, R. Rossmann, K.W. Taunt, and R.G. Kreis, Jr. 2008. The Lake Michigan eutrophication model, LM3-Eutro: Model development and calibration. *Water Environment Research* 80(9): 853-861.
- Perkins, E.J., N. Garcia-Reyero, D.L. Villeneuve, D. Martinovic, S.M. Brasfield, L.S. Blake, J.D. Brodin, N.D. Denslow, and G.T. Ankley. 2008. Perturbation of gene expression and steroidogenesis with *in vitro* exposure of fathead minnow ovaries to ketoconazole. *Marine Environmental Research* 66:113-115.
- Russom, C.L., L.P. Burkhard, and D.J. Hoff. 2008. Availability of biota-sediment accumulation factor data set and PCB residue effects database. *SETAC Globe* 9:5.
- Rygwelski, K.R. 2008. Results of the Lake Michigan Mass Balance Project: Atrazine modeling report, USEPA, EPA/600/R-08/111, 140 pp.
- Stevenson, R.J., B.H. Hill, A.T. Herlihy, L.L. Yuan, and S.B. Norton. 2008. Algae-P relationships, thresholds, and frequency distributions guide nutrient criteria development. *Journal of the North American Benthological Society* 27:783-799.
- Villeneuve, D.L., L.S. Blake, J.D. Brodin, J.E. Cavallin, E.J. Durhan, K.M. Jensen, M.D. Kahl, E.A. Makynen, D. Martinovic, N.D. Mueller, and G.T. Ankley. 2008. Effects of a 3 α -hydroxysteroid dehydrogenase inhibitor, trilostane, on the fathead minnow reproductive axis. *Toxicological Sciences* 104:113-123.
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- Zhang, X., K.R. Rygwelski, R. Rossmann, J. Pauer, and R.G. Kreis, Jr. 2008. Model construct and calibration of an integrated water quality model (LM2-Toxic for the Lake Michigan Mass Balance Project. *Ecological Modelling* 219(1-2):92-106.

MED Seminars

RECENT AND UPCOMING SEMINARS

June 25

Dr. Kathryn Saterson, Assistant Lab Director, NHEERL

- NHEERL's roles in ORD's Global Change and Ecological Research Program

Aug. 26

Chuck Nace, Region 2, Regional Risk Assessor

- A day in the life of a Regional Risk Assessor

Sept. 24

Dr. Jennifer Liang, UM-Duluth

- Nodal signaling is required for closure of the zebrafish neural tube

Oct. 8

Dr. Jerry Hembd, UW-Superior

- Sustainable community development and ecosystem services: A recovering economist's view

Oct. 15

Dr. Elizabeth Minor, UM-Duluth/Large Lakes Observatory

- How important is "old" carbon in Lake Superior: Preliminary results from radio-carbon analyses

Oct. 29

Dr. Dalma Martinovic, NRC Associate, MED

- Embracing complexity: Elucidating effects of environmental and social stress with omic technologies

Nov. 12

Dr. Michael Hornung, MED

- Thyroid toxicity pathways

Dec. 3

MED Poster Session

- This all day poster session will highlight MED research presented at scientific meetings throughout 2008. Last year's session included over 20 posters and we expect a similar level of participation this year. Authors will be in attendance at their posters in the afternoon.

Dec. 10

Dr. Steve Diamond, MED

- Update on nanotechnology research

Dec. 17

Dr. Phil Cook, MED

- Fine particles/asbestos research

Awards

ORD HONOR AWARDS

Division recipients who worked with staff from other ORD offices to support ORD and the EPA mission:

Office of Resources Management & Administration

ORD Principles Writing Team:

Janet Keough – developing the final ORD Guiding Principles, which articulate a set of core beliefs supporting scientific, organizational, and individual excellence for all of ORD.

ORD Financial System Modernization Project Team:

Mary Hautajarvi – extraordinary efforts made to integrate ORD's budget, performance, and financial system requirements into EPA's new financial system.

Office of the Science Advisor

Metals Framework Guidance Team:

Dave Mount – developing the Framework for Metals Risk Assessment with key guiding principles to assess the hazards and risks of metals to human health and the environment.

NHEERL

EMAP Great Rivers Team:

Ted Angradi
Allan Batterman
Dave Bolgrien
Colleen Elonen
Brian Hill
Terri Jicha
Jack Kelly
Janet Keough
Mary Moffett
Mark Pearson
Debra Taylor

(with other ORD and Regions 5 and 7 staff)

– successful planning and implementation of the Environmental Monitoring and Assessment Program for Great River Ecosystems.

Office of Science Policy

ORD Detection and Quantitation Workgroup:

Chuck Stephan – assisting the Office of Water in developing new procedures for determining detection and quantitation levels for use in Clean Water Act programs.

People

DOUG KUEHL RETIRES

Doug Kuehl, Research Chemist in the Ecotoxicology Analysis Research Branch, has retired after nearly 40 years at the Division. When he started here, it was the National Water Quality Lab! In the 80s and early 90s, Doug was a major contributor to the National Dioxin Survey, which put the Division on the map as a center for trace chemical analysis. He worked in many areas of analytical biochemistry, including the development of novel ultra-sensitive analytical and mass spectrometric methods for analysis in fish species of biomarkers of environmental exposure, such as DNA and glutathione adducts.



Highlights of Doug's career:

- Branch Chief for three years
- 1993 EPA Scientific & Technological Achievement awardee, with Jose Serrano
- 1995 Innovative Research Grant awardee, with Jose Serrano
- Research collaborator with UMD's Chemistry and Biochemistry Departments
- Adviser to many fellows in specialized areas of mass spectrometry such as analysis of PFOS
- National expert on analytical methods for assessment of dioxin-induced dolphin mortality
- Organizer and Chairman of MED's Journal Club
- Voluntary mentor to many undergraduate and graduate college students during the "stay-in -school" EPA internship program.

Doug's publication record includes efforts to quantify DNA adducts for reactive compounds, analysis of trace compounds (PCBs and perfluorinated compounds) in marine mammals, and general support (methods development and identification of "unknowns") for research conducted by a host of others.

Doug was always very forward looking, always interested in the next "big thing," both in terms of methods and toxicology problems. Some folks slow down toward the end of their career; Doug didn't. We wish him the best in his retirement and trust that he will continue to create exquisite wood carvings.

SHARON BATTERMAN RETIRES

Sharon Batterman retired in June 2008. Sharon (Sherry) joined the EPA in 1993 as a summer intern in 1993 and joined the permanent staff as a Research Biologist in 1995. Prior to joining the EPA, Sherry conducted EPA research as a chemist for a series of technical support contracts at the Division. Sherry is a recognized GIS expert and toxicologist, and also served as a Project Officer and Contracting Officer Representative. She authored, with others, a number of publications, leading to five STAA awards in 2002, 2005, 2006 (2 awards), and 2007. We wish Sherry all the best in retirement!



GOODBYE, RON

Ron Vormwald, our IT Coordinator, is leaving Duluth to assume new responsibilities as the user-provisioning Coordinator for the western half of ORD based out of Las Vegas. Ron's new duties will cover MED (among many other places). Part of his responsibilities will be the oversight of the new workstations that will replace our current computers early in 2009. Ron has been an invaluable member of the Program Operations Staff and has done his utmost to provide us with the best IT services possible over his 12 years with us. We wish him the best. We will miss his informative and colorful emails announcing IT news!



FAREWELL, TRUDY

Trudy Brown, Senior Environmental Employee (SEE), is returning with her cat Bo to Minneapolis after 36 years away, to be closer to her children and family. For 11 years Trudy has worked with HR, IT, and Health & Safety, always with great energy and a wonderful attitude. We wish Trudy well and wonder if/how we can ever replace her!

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WELCOME, TOM



Tom Hollenhorst, Landscape Ecologist, joined the Watershed Diagnostics Branch in September. He comes to MED from the Center for Water and the Environment at the Natural Resources Research Institute (NRRI), University of Minnesota Duluth, where he has worked for the last ten years. Tom has been applying GIS and Remote Sensing technologies to ecologic research projects since 1991, first working at Southern Illinois University, Carbondale, in the Cooperative Wildlife Research Unit. Since then Tom has worked in British Columbia and the Yukon Territories of Canada, applying GIS in support of contemporary native land claims, and various watershed restoration efforts and wildlife research projects throughout the area. At NRRI Tom coordinated the GIS efforts for many different projects including the development of ecological indicators for streams and the evaluation of amphibians as ecological indicators. More recently Tom coordinated the GIS efforts and watershed delineation work in support of the Great Lakes Environmental Indicators Project. Tom received his MS degree in Wildlife Management from West Virginia University, Morgantown, but grew up in Minneapolis. Having lived in some beautiful places, including Vancouver Island, BC, Tom still likes northern Minnesota, especially the north shore of Lake Superior, the best. Tom is in Room A102, x5220.

WELCOME, RUSTY

Royanne (Rusty) Pedersen joined the Program Operations Branch in June as a SEE, working in Tech Info Management with Mary Ann Starus. She was born and raised in Duluth, is a twin, and has two children and two grandchildren. After 20-some years in the coffee and vending business, a definite change was in order. She knew this job was for her when she finally got "carded" after all these years, in order to get "in"!!! Rusty says she loves being around people of all ages and talents. She is in Room 140, x5077.



WELCOME, KARIS

Karis Boerner joined the Division in September as our new Administrative Officer – a brand new position covering administrative support to Grosse Ile, timekeeping, office management to the IO, and liaison responsibilities with the upcoming AEP lines of business – among other things.



Karis grew up in Southern Minnesota. She is a Navy veteran, with experience as a Hospital Corpsman in Florida, deployed with the USS John F. Kennedy to the Mediterranean and Persian Gulf, and serving in Kuwait. She also has experience in law and clinical research. Karis is located in Room 142, x5035.

NEW FEDERAL EMPLOYEES



Matt Etterson, a Division NRC research associate for the past two years, is now an Ecologist and Population Biologist. Matt received his PhD in Conservation Biology from the University of Minnesota in 2000, after finishing his undergraduate degree in mathematics and philosophy at Yale University. For the past eight years, Matt has worked in post-doctoral positions at the Smithsonian Institute in Washington DC, and at MED. Matt's mathematical skills, coupled with his knowledge of ecology, bring advanced expertise in translating toxicity test endpoints into

demographic endpoints for population modeling. Matt has been assigned to the Ecotoxicology Analysis Research Branch, Room A210, x5158.

Dan Villeneuve, former NRC and then NHEERL post-doc for a total of almost five years, has accepted a position as Toxicologist. Dan received his PhD in Zoology and Environmental Toxicology at Michigan State University in 2000. Before coming to MED, Dan worked as a post-doc at Oregon State University. His extensive experience in reproductive toxicology of fishes, coupled with recent experience and training in systems biology brings important expertise to the Division. Dan has been assigned to the Toxic Effects Characterization Research Branch, Room 257, x5217.

