Environmental Scientists, Biologically Active Compounds, and Sustainability: The Vital Role for Small-Scale Science

ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · JANUARY 2011

Impact Factor: 5.33 · DOI: 10.1021/es100455d · Source: PubMed

2 AUTHORS:

Alan S Kolok
University of Nebraska at Omaha

Heiko Schoenfuss
St. Cloud State University

Environ. Sci. Technol. **XXXX,** *xxx,* 000–000

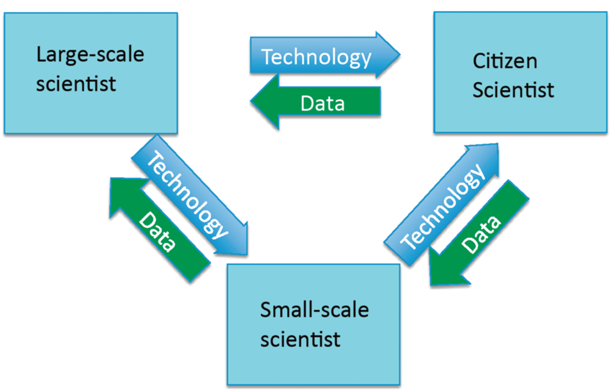
Environmental Science & Technology

Feature

Environmental Scientists, Biologically Active Compounds, and Sustainability: The Vital Role for Small-Scale Science†

ALAN S. KOLOK*
Departments of Biology & Environmental, Agricultural, And Occupational Health, University of Nebraska at Omaha & Medical Center, Nebraska

HEIKO L. SCHOENFUSS
Aquatic Toxicology Laboratory, St. Cloud State University, Minnesota



ALAN S. KOLOK

Employing the scientific method evokes the use of an inquiry-based process that emphasizes gathering observable, empirical, measurable, and repeatable evidence. Historically, the purview of observable evidence was limited by the powers of the human sensory systems. As science has become more sophisticated, the discriminatory power of scientific equipment has, in some circumstances, superseded human senses, so that we can now perceive beyond the range of our sight, and detect the presence of compounds well below the range of our taste or olfaction.

Extending the range of scientific observation is literally costly, and a direct relationship can be drawn between the acuity of scientific equipment and its cost. Nowhere is this more apparent than in the field of ecotoxicology, particularly as it pertains to biologically active compounds, such as human and veterinary pharmaceuticals and endocrine-disrupting compounds. Biologically active compounds, by definition, elicit their effects at low concentrations. Furthermore, these compounds have human health effects, as is evident from the persistent health issues that have been reoccurring in daughters and granddaughters of mothers exposed to diethylstilbestrol from 1938 until 1971 (*1*). While the diethylstilbestrol exposures were deliberate, the potential for incidental exposure to biologically active compounds, via drinking water or other sources, places high demands on their detection at trace concentrations in natural surface water and groundwater (*2*). Of course, as detection limits for these compounds have improved from parts per thousand (‰) to parts per trillion (ppt), the cost of chemical analyses has risen accordingly. For example, the cost for analyzing trace concentrations of steroids in water is on the order of hundreds of dollars per sample.

This is not to say that the need for monitoring these chemicals should be constrained, or that the need for environmental monitoring is waning, quite the contrary. Population growth and migration, coupled with the exportation of our chemically intensive lifestyles throughout the world may increase the frequency and diversity of biologically active compounds in aquatic environments. We anticipate that the problems associated with the release of these compounds into the environment will be substantial enough to warrant environmental regulations with respect to their monitoring and remediation in the near future. Beyond regulation, only sustained assessments can ensure the long-term health of aquatic environments experiencing anthropogenic inputs of biologically active compounds. The ability of local water resource managers to sustain assessment programs in the face of prohibitively expensive chemical and tissue analysis is a vexing issue that will need to be resolved as we move into an era of scientific sustainability.

Environmental Stewardship and Sustainability: A Matter of Scale

Environmental science practitioners can be characterized into three categories: large-scale, small-scale, and citizen scientists. In this context, large- versus small-scale science is solely in reference to the infrastructure available to the scientists conducting the research. Scientists practicing large-scale science, for example, work at well-endowed national or university laboratories, and have access to the most sophisticated analytical equipment. These scientists have the greatest number of resources, produce the bulk of the published scientific literature, and are responsible for paradigm-shifting changes in methodology and approach. In the sense that the research conducted by these scientists may focus on concepts that transcend geographic boundaries, the research is global. In stark contrast to these professional scientists are the citizen scientists. Citizen scientists are sparsely funded if at all, focus on issues ranging from personal to local, and are driven primarily by personal motivators, such as a civic ethic, or a concern for their own health and well-being. While these individuals may not directly contribute to the scientific dialogue, in the sense of publications or presentation, they are important, highly visible, community

† This manuscript is part of the Environmental Policy: Past, Present, and Future Special Issue.

* Corresponding author e-mail: akolok@unomaha.edu.

10.1021/es100455d

ambassadors of the scientific enterprise. Furthermore, when bundled together, their efforts can lead to meta-data sets that can be vitally important.

Between these two extremes are the bulk of the world's scientists and resource managers, working on issues that are primarily of interest to specific communities or stakeholders. In the sense that this research focuses on a geographic area rather than a scientific concept, it can be considered local. These scientists are conducting small-scale science in that the resources available are often limited. Nevertheless, these scientists are the boots-on-the-ground workforce that deals with most of the day-to-day environmental stewardship, such as monitoring the efficacy of a wastewater treatment plant or the disinfectant system at a local water supply. They are also the local resource managers in developing countries monitoring environmental contamination even when their home country may not have the resources to fully address the potential contamination.

It is our contention that sustainable environmental monitoring is under the purview of each of these different subpopulations. The difference between small-scale and large-scale science is only in the resources and motivation of the scientists, as local scientists may be more interested in a nearby resource, whereas global scientists may be more concerned with professional dissemination of scientific ideas and concepts. Environmental stewardship related to biologically active compounds is going to increasingly fall to small-scale scientists, as the scope and the frequency of the issue increases. In the same manner that the current consumption patterns of individuals in developed countries cannot be sustainably expanded to the world's population, it is also true that the expense and approach of large-scale science, as related to the detection of biologically active compounds, cannot be sustainably applied to all of the scientists throughout the world conducting small-scale science. The rest of this paper documents how each of these subpopulations of scientists can contribute to the enterprise of small-scale science, and by doing so contribute to the sustainable monitoring of biologically active compounds.

Contributions to Small-Scale Science by Local Water Resource Managers. Local water resource managers must balance monitoring activities with other job aspects. Furthermore, they are also likely to encounter questions and concerns from local stakeholders regarding the presence and biological effects of biologically active compounds within a watershed. While familiar with the environmental impact of legacy chemicals, nutrient loads, and geomorphological alterations, these resource managers may be much less familiar with issues associated with biologically active contaminants. Furthermore, the methodologies available to researchers conducting large-scale science may not be available on the local watershed level due to cost and analytical complexity. Despite these drawbacks, the ubiquitous presence (*2*) of biologically active compounds across the developed world clearly warrants a response on the local level.

Here we are describing a tiered assessment approach, utilizing well-established tools and resources readily available to a local water resources manager to conduct a coarse survey to assess the threat of biologically active compounds to local water resources. The example specifically applies to endocrine-disrupting compounds, but could be equally applicable to other biologically active compounds. This approach is not exclusive of other assessments, nor is it applicable to all circumstances faced by water resource managers. However, it will provide the opportunity to monitor the presence and effects of biologically active compounds at the local level and, when necessary, can guide decisions to develop more in-depth assessment strategies for impaired waters.

A Tiered Approach to Sustainable Water Resource Assessment

The key to the successful implementation of sustainable, long-term monitoring systems is the reliance on well-established and readily available resources familiar to many water resource managers. Processes developed in the biodiversity community, including predictive niche modeling to assess the likelihood of invasive species to invade a watershed (*3*, *4*), may serve as a template to be applied to sustainable assessment of biologically active compounds at the scale of local watersheds.

Tier 1. Identification of Likely Pathways of Biologically Active Compounds Entering a Watershed. Inferences that identify likely pathways by which biologically active compounds enter watersheds have been, and continue to be, widely done. The recent development of geographic information systems (GIS), however, has provided water resource managers with an important tool to elucidate these pathways. Geospatial layers for watersheds are available from many sources, including departments of natural resources, environmental agencies, agricultural departments, extension services, and health departments. When combined, these layers will provide information about the watershed: location of point sources of effluents entering, e.g., municipal wastewater treatment plants, industrial effluents; land use indicative of nonpoint discharge, e.g., row crop density or animal feedlot operations; and other environmental features that may indicate a greater likelihood of the presence of emerging contaminants, e.g., urban stormwater drains. Similarly, GIS layers may also provide information about segments of the watershed free of obvious sources of contamination.

Tier 2. Biological Integration at Key Locales in the Watershed. Once locations of interest have been established through GIS applications, a first, cursory assessment for the presence of emerging contaminants in the watershed can be initiated. A simple tool for this approach is the caging of aquatic organsms, such as fish within the watershed. Commonly this has been accomplished by placing mature fathead minnows, or other locally appropriate fish species in small cages (often modified fish traps), into the stream (*5*−*7*). Although this approach has some drawbacks (see below), it provides a simple mechanism for integrating exposures to emerging contaminants over time. Once retrieved (usually after 7−14 days), fish need to be assessed for physiological responses frequently associated with exposure to emerging contaminants. Water resource managers are likely familiar with basic ichthyological techniques and are able to draw blood, excise organs from the retrieved fish, and calculate body condition factor, gonadosomatic index, and hepatosomatic index. Environment Canada has used data gathered from these simple procedures to establish a survey program for pulp and paper mill effluents that has proven remarkably robust over several survey cycles (for a review see ref *8*). An additional analysis particularly appropriate for the assessment of exposure of an environmental estrogen would be the quantification of plasma vitellogenin in the blood of the exposed fish through enzyme-linked immunosorbant assays (ELISAs). Although this technique may not be familiar to many water resource managers, the availability of commercial kits with simple instructions will allow many resource managers to add this tool to their assessment repertoire without much difficulty. Furthermore, local resources such as academic institutions and medical facilities use ELISAs routinely and may provide initial help with the setup and operation of this assay. In some cases, especially if body indices and plasma vitellogenin analysis provide strong indication for effects consistent with exposure to biologically active compounds, follow-up histopathological analysis of a selected subset of the caged fish may be appropriate. Again, local resources may provide a ready route for these analyses as many academic institutions and hospitals maintain histological facilities.

Tier 3. Follow-up and Expanded Analysis. Once hotspots in the watershed have been identified as likely pathways for emerging contaminants, results from the initial caging should be confirmed in additional rounds of caging fish, or even collection of resident fish populations. Temporal and spatial variability in occurrence and concentration of emerging contaminants warrants caution when interpreting single time point results. If follow-up and/or expanded study of a site consistently indicates the presence of biologically active compounds, limited chemical analysis may be warranted. Commercial water quality laboratories and some state agency laboratories have the capacity to analyze water samples for some of these compounds at environmentally relevant concentrations. The information gleaned from the GIS analysis and the caged fish will provide guidelines in selecting which chemical classes to quantify in the samples. For example, strong vitellogenin induction downstream from a wastewater effluent source may point toward natural and synthetic steroidal hormones such as 17-β estradiol, estrone, or ethynylestradiol.

Chemical analysis may or may not have a great deal of utility in the tiered approached discussed above. If the inputs are few and well-defined, chemical analysis for the "usual suspect" molecules, such as 17-β estradiol, estrone, or ethynylestradiol may be particularly informative. If, however, the inputs are diverse, the interaction of chemical mixtures may minimize the value of quantification of specific biologically active compounds. In these cases, in vitro (cell culture) assays that demonstrate biological mode of action (e.g., estrogenicity) may prove to be more valuable than direct measurement of specific chemical compounds.

Flexibility in the Tiered Approach. An example of the tiered approach described above can be modified to preexisting assessment programs as described below. This assessment of a Northern Plains (U.S.) watershed, was initiated with a 2-week deployment of caged fathead minnows at nine sites (Figure 1). These deployments revealed physiological responses consistent with exposure to biologically active compounds (vitellogenin induction in male fish or reduced vitellogenin concentrations in female fish when compared to baseline fish sampled prior to deployment) at five sites (solid red dots) and no response at four sites (solid green dots). Feedback from these results into the GIS database allowed for the identification of seven additional locations (hollow red circles) predicted to exhibit estrogenic activity. These locations become study sites in subsequent field seasons leading to a gradual refinement of the watershed assessment for biologically active compounds.

While the approach discussed above focused on the in situ exposure of caged fish to different regions of the watershed, other technical approaches could easily fit into the overall tiered approach. For example, Hillwalker et al. (*9*) advocate the use of extracts from passive contaminant samplers to expose embryonic zebrafish to trace concentrations of biologically active contaminants. They further advocate the use of nonspecific biological end points (i.e., abnormal tissue development) particularly in environments in which complex mixtures of biologically active compounds would be present. Providing that the methodologies associated with this approach are transferrable to the environment of a local resource manager, it could substitute for in situ, caged fish studies in the tiered approach discussed above.

Tier 4. Source Identification and Remediation. The dual role of water resource managers to monitor and develop water resources includes the need to remediate environmental impact. Thus, identification of hotspots in the watershed is not the end of the water resource manager's responsibility, but rather moves management into the next phase. Identification of sources for biologically active compounds can be a relatively simple task if a discrete point source with state-mandated documentation of users can be located or may be impossible if a diffuse nonpoint source is being suspected. Although remediation efforts for biologically active compounds are still in their infancy and lack regulatory stature or definition, the tiered approach outlined above may be applicable to future remediation efforts, and to the assessment of their success. The process for sustainable watershed assessment for biologically active compounds outlined above does not provide a panacea for solving all challenges facing water resource managers. In watersheds dominated by chemical mixtures, the quantification of signature compounds, such as 17-β estradiol, may add very little to the overall understanding of the biological activity of the chemical milieu within the waterway. Furthermore, the co-occurrence of biologically active compounds with multiple other stressors can alter the effect of biologically active compounds on fish in various sites within the watershed. For example, the availability of additional nutrients may provide additional resources for fish synthesizing vitellogenin. Previous studies have reported increased body weights and relative organ size for fish caged or captured downstream of wastewater treatment plant effluents known to serve as pathways for estrogenic endocrine-active compounds (*10*). Similarly, alterations in the physicochemical environment (for example thermal effluent outfalls from power plants) may affect the expression of biomarkers of biologically active compounds exposure. In addition to the site-specific conditions of the physicochemical environment, responses to emerging contaminants exposure may differ among different indicator species. Although not yet fully explored in the relevant literature, a picture is emerging suggesting that some species of fish may be more sensitive in their response to estrogenic biologically active compounds than others (*11*).

Interactions between Large-Scale and Small-Scale Scientists. The relationship between large-scale and small-scale scientists is straightforward. The local resource manager is likely to have some laboratory space, as well as some level of scientific training. Interactions between the two groups will, at least initially, focus on expertise and analytical techniques being transferred from university or government laboratories to local resource managers. As outlined in the tiered approach, there are abundant opportunities for these interactions to reverse so that information can flow from the small-scale back to the large-scale scientist.

The Citizen Scientist: Challenges and Opportunities

There are obvious trade-offs in any technologic enterprise between the sophistication of the data collected, the funding available to collect those data, and the rate at which the data can be amassed. Nowhere is this more apparent than for the citizen scientist. It would be easy to dismiss the role of the citizen scientist in the scientific enterprise as this subpopulation has the least sophisticated equipment at their disposal, and also the least amount of funding to expend on the scientific enterprise. Furthermore, even if the equipment and funding were available to outfit the citizen scientist, quality assurance and control issues might prove so problematic as to completely negate all of their efforts.

There are at least two approaches that would allow citizen scientists to become involved in assessment programs. The first would be for these stakeholders to play a direct role in the tiered approach discussed above. For example, citizen scientists living near deployment sites may volunteer to maintain caged fish, take daily water readings for long periods

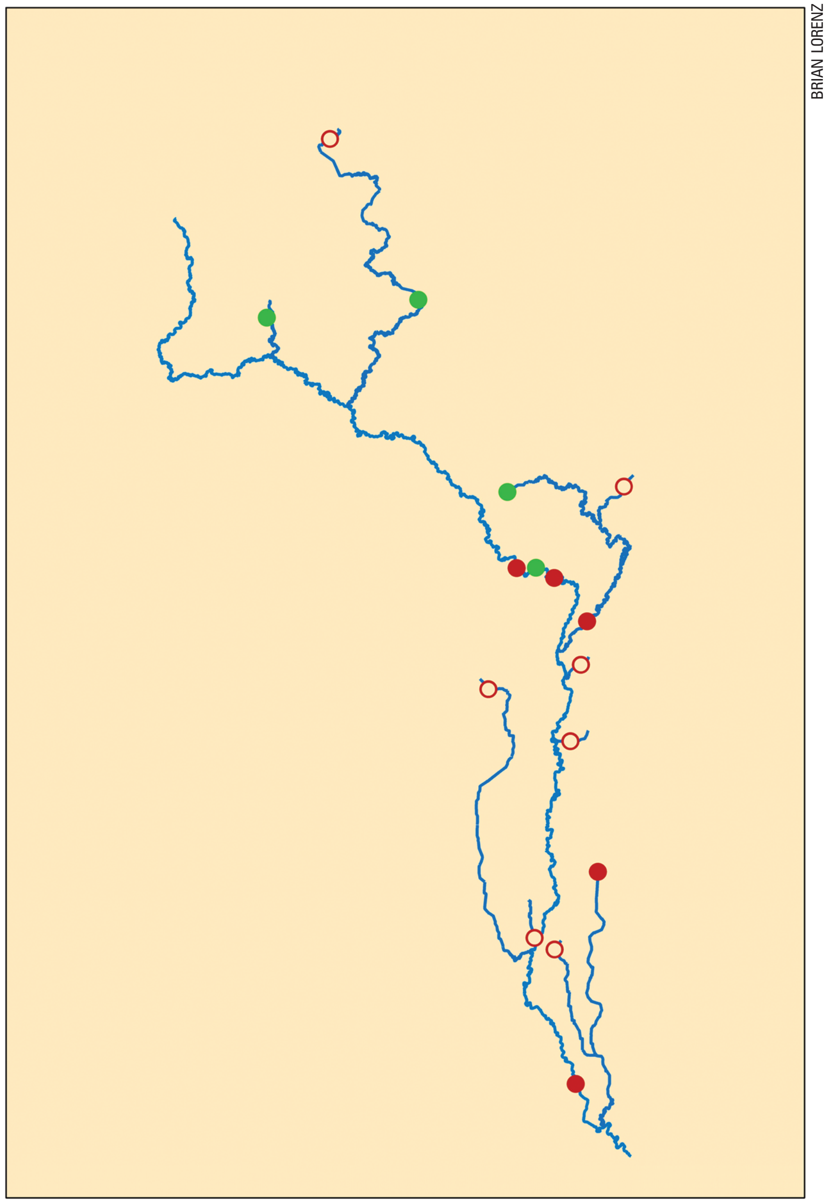


FIGURE 1. Assessment of a Northern Plains (U.S.) watershed using locally available tools. Nine sites (solid dots) were selected within the watershed using GIS data from local (county or state) agencies. Selection criteria included land use (i.e., row-crops, concentrated animal feedlot operations), point sources (i.e., municipal and industrial effluents), and spatial diversity (upstream, midriver, downstream). Based on the observed effects at some of the nine sites (red solid dots), seven additional sites were identified for further analysis (hollow red circles).

of time (i.e., to develop temporal patterns of discharge), or in some cases may actively contribute to the setup or breakdown of the study. Creating ownership in the monitoring effort can be critical in establishing long-term relationships among citizen scientists, small-scale scientists, and large scale scientists and will ultimately facilitate the scientific process.

An alternative, but by no means mutually exclusive, approach toward citizen scientists would be an indirect interaction between them and the tiered approach discussed above. In this approach, the citizen scientist would provide low-level observational information to small-scale or large-scale scientists regarding point source water quality information at sites within the watershed. Alterations in watershed flow patterns, odor, or stream coloration are not necessarily trivial, and can help to define where sampling stations may be placed within a watershed. Furthermore, as the watershed of interest increases in size, the familiarity of the watershed to the large-scale or small-scale scientist diminishes, increasing the value of the observationally minded citizen scientist.

This indirect approach is already taking place on an informal basis, although it may not be recognized as such. In areas of privately owned agricultural landscapes, community groups can be invaluable sources of information related to pesticide or manure application, and the probability of its subsequent runoff into local waterways. This information can be invaluable to the local resource manager, and can be fed directly into the tiered approach discussed previously.

The “Christmas Bird Count” as a Model For Citizen Scientists In Research

While independent citizen scientists can act as environmental whistle blowers, can they do more? Almost certainly they can, and a classic example of the power inherent in large groups of citizen scientists is the "Christmas bird count". For the 110th year, between December 14, 2009 and January 5, 2010, tens of thousands of bird watchers across North America spent time in the field, cataloging local bird diversity and abundance. What began in 1900 as a replacement for the annual Christmas "side hunt" in which participants would go out on Christmas Day and shoot as many birds as possible, the Christmas bird count has provided extensive information regarding changes in the geographic range of birds in North America over time. When information from these disparate groups of birders is entered into a common database, the collective enterprise becomes a decentralized reconnaissance network capable of following large-scale spatial and temporal trends in avian abundance and distribution. It has been proposed that these alterations in species abundance and range can be used as environmental bioindicators of environmental degradation (*12*). For example, the analyses of the winter distribution of 305 bird species over the last 40 years has documented that over 170 of those species showed a significant shift northward, presumably due to trends in overall global warming (*13*).

But how can the Christmas bird count model be translated into the monitoring of biologically active compounds in local waterways? The community networks are already being established for this purpose, as Adopt-A-Stream and Adopt-A-Lake programs are becoming widely available in many states throughout the U.S. These programs focus on straightforward tasks such as the physical cleaning-up of riverbanks and the acquisition of basic water quality data, such as temperature, pH, or turbidity, or the assessment of the diversity of aquatic invertebrate communities. These groups also represent the eyes and ears of the local citizenry that can provide information on point-source degradations in water quality, as mentioned previously. As valuable as these current programs are, the collection of information regarding biologically active compounds is beyond the analytic capability of most of these citizen scientist groups. What is currently lacking is the technology necessary so that these citizen scientists can perform simple assays in the field that will provide information related to the presence of trace organic compounds.

As far-fetched as a rapid and inexpensive detector for the presence of trace organics may at first appear, the technology, with the necessary sensitivity, already exists for other applications. There are commercially available test strips that can determine whether house paint is contaminated with environmentally relevant concentrations of lead, or whether or not a woman is pregnant. As with these test strips, environmental biomonitoring tools for citizen scientists would not have to be quantitatively continuous data, but rather only data at the level of presence or absence beyond some predetermined threshold value. The value of the data set would not be in the sophistication of the analysis, but rather, in its sample size. Consider the power of a data set, if every advanced placement high school science student on a state-wide basis were to test a natural surface water source for environmentally relevant concentrations of caffeine (as a proxy for the presence of human wastewater) all on the same day! The students could couple their presence or absence data with GIS coordinates, then enter those data onto a Internet-based Web site, much in the same way that Christmas bird count data can currently be registered. While the data would not provide information on local conditions, it could give a snapshot of environmental exposure to wastewater on a very large geographic scale. Simultaneously collected data across broad landscapes is virtually unheard of in environmental science.

Toward a Sustainable Monitoring Network for Biologically Active Compounds. It is becoming more and more apparent that minute concentrations of biologically active compounds elicit adverse impacts on environmental and human health. Chemical and biological monitoring of these compounds and their effects is expensive and requires a sophisticated expertise in the resource managers. At the same time, there is a wealth of information suggesting that these compounds are widespread and that small-scale scientists and citizen scientists are concerned about these compounds on a local level. Clearly, the dissemination of expensive and highly sophisticated biomonitoring systems to small-scale and citizen scientists is not a sustainable (or even advisible) solution to this burgeoning problem. We therefore propose that an integrated feedback system be developed so that technological advancements can flow from large-scale to small-scale and citizen scientists, while locally generated data can return from these scientists back to university and government laboratories.

As discussed above, small-scale scientists do not have the local resources to design and operate comprehensive monitoring programs for biologically active compounds. As such, they will have to rely on data sets (e.g., GIS maps) that are currently available through state or federal agencies. Furthermore, they will also have to rely upon simplified monitoring tools (e.g., caged fish or fish embryo bioassays) to acquire locally produced data that can be used for appropriate management of their water resources. One role for large-scale scientist will be to provide their small-scale colleagues with the tools (both conceptual and methodological) to inexpensively and routinely monitor for these biologically active compounds.

As the number of small-scale scientists generating data on the presence and effects of biologically active compounds increases, it will become increasingly important to integrate these data into a large-scale scientific framework. The dissemination of these data to large-scale, small-scale, and citizen scientists can be managed through the Internet, much the same way that the Audobon Society has disseminated their Christmas bird count data (National Audubon Society 2002). In fact, the data feedback may represent a continuum in which small-scale scientists conduct a relatively small number of sophisticated bioassays, while citizen scientists contribute very large data sets of single compound presence/absence data that can be used on a much larger and more coarse time and geographical scale. Together the large-scale, small-scale, and citizen scientists can intellectually interact in meaningful ways that all contribute to sustain a monitoring program focusing on biologically active compounds.

Dr. Kolok holds a joint appointment in the University of Nebraska system as a Professor of Biology and a Professor of Environmental, Agricultural and Occupational Health at the Medical Center. He has published widely related to the response of fish to environmental stressors, including anthropogenic contaminants. Dr. Schoenfuss is Professor of Anatomy and Director of the Aquatic Toxicology Laboratory at St. Cloud State University. He has published approximately 40 manuscripts detailing the effect of environmental changes on anatomical structure focusing on the effects of contaminants of emerging concern on aquatic ecosystems.

Acknowledgments

We gratefully acknowledge the assistance of Brian Lorenz for providing the GIS mapping associated with Figure 1. Mark Ferrey and L. LaReesa Wolfenbarger provided helpful comments to an earlier version of this paper.

Literature Cited

(1) McLachlan, J. A. Prenatal exposure to diethylstilbestrol (DES): a continuing story. *Int. J. Epidemiol.* **2006**, *35*, 868–870.

(2) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999−2000: A national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202–1211.

(3) Chen, P.; Wiley, E. O.; McNyset, K. M. Ecological niche modeling as a predictive tool: silver and bighead carps in North America. *Biol. Invasions* **2007**, *9*, 43–51.
(4) DeVaney, S. C.; McNyset, K. M.; Williams, J. B.; Peterson, A. T.; Wiley, E. O. A tale of four "carp": invasion potential and ecological niche modeling. *PLoS One* **2009**, *4* (5), e5451.
(5) Nichols, K. M.; Miles-Richardson, S. R.; Snyder, E. M.; Giesy, J. P. Effects of exposure to municipal wastewater *in situ* on the reproductive physiology of the fathead minnow (*Pimephales promelas*). *Environ. Toxicol. Chem.* **1999**, *18*, 2001–2012.
(6) Pyle, G.; Swanson, S. M.; Lehmkuhl, D. M. Toxicity of uranium mine-receiving waters to caged fathead minnows *Pimephales promelas* G. *Ecotoxicol. Environ. Saf.* **2001**, *48*, 202–214.
(7) Sellin, M. K.; Snow, D. D.; Akerly, D. L.; Kolok, A. S. Estrogenic compounds downstream of three small cities in Eastern Nebraska: occurrence and biological effects. *J. Am. Water Resour. Assoc.* **2009**, *45*, 1–8.
(8) McMaster, M.; Hewlett, M. L.; Parrot, J. A decade of research on the environmental impacts of pulp and paper mill effluents in Canada: field studies and mechanistic research. *J. Toxicol. Environ. Health* **2006**, *B 9*, 319–339.
(9) Hillwalker, W. E.; Allan, S. E.; Tanguay, R. L.; Anderson, K. A. Exploiting lipid-free tubing passive samplers and embryonic zebrafish to link site specific contaminant mixtures to biological responses. *Chemosphere* **2010**, *79*, 1–7.
(10) Barber, L. B.; Lee, K. E.; Swackhamer, D.; Schoenfuss, H. L. Response of Male Fathead Minnows Exposed to Wastewater Treatment Plant Effluent, Effluent Treated with XAD8 Resin, and an Environmentally Relevant Mixture of Alkylphenol Compounds. *Aquat. Toxicol.* **2007**, *82*, 36–46.
(11) Palace, V. P.; Evans, R. E.; Wautier, K. G.; Mills, K. H.; Blanchfield, P. J.; Park, B. J.; Baron, C. L.; Kidd, K. A. Ethynylestradiol (EE2) added to a whole lake induced different biochemical, histopathological and population level responses in four species of wild fish. *Can. J. Fish. Aquat. Sci.* **2009**, *66*, 1920–1935.
(12) Temple, S. A.; Wiens, J. A. Bird population and environmental changes: can birds be bio-indicators? *Am. Birds* **1989**, 260–270.
(13) National Audobon Society. Birds and climate change; ecological disruption in motion. A briefing for policymakers and concerned citizens on Audobon's analyses of North American bird movements in the face of global warming. 2009. Available at birdsandclimate.audubon.org/cbcanalysis.html.
(14) Arnold, S. F.; Robinson, M. K.; Notides, A. C.; Guillette, L. J.; McLachlan, J. A. A yeast estrogen screen for examining the relative exposure of cells to natural and xenoestrogens. *Environ. Health Perspect.* **1996**, *104*, 544–548.

ES100455D