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AN INEXPENSIVE, TEMPORALLY INTEGRATED SYSTEM FOR MONITORING OCCURRENCE AND BIOLOGICAL EFFECTS OF AQUATIC CONTAMINANTS IN THE FIELD

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Abstract: Assessment of potential risks of complex contaminant mixtures in the environment requires integrated chemical and biological approaches. In support of the US Great Lakes Restoration Initiative, the US Environmental Protection Agency lab in Duluth, MN, is developing these types of methods for assessing possible risks of aquatic contaminants in near-shore Great Lakes (USA) sites. One component involves an exposure system for caged fathead minnow (*Pimephales promelas*) adults suitable for the wide range of habitat and deployment situations encountered in and around the Great Lakes. To complement the fish exposure system, the authors developed an automated device for collection of composite water samples that could be simultaneously deployed with the cages and reflect a temporally integrated exposure of the animals. The present study describes methodological details of the design, construction, and deployment of a flexible yet comparatively inexpensive (<600 USD) caged-fish/autosampler system. The utility and performance of the system were demonstrated with data collected from deployments at several Great Lakes sites. For example, over 3 field seasons, only 2 of 130 deployed cages were lost, and approximately 99% of successfully deployed adult fish were recovered after exposures of 4 d or longer. A number of molecular, biochemical, and apical endpoints were successfully measured in recovered animals, changes in which reflected known characteristics of the study sites (e.g., upregulation of hepatic genes involved in xenobiotic metabolism in fish held in the vicinity of wastewater treatment plants). The automated composite samplers proved robust with regard to successful water collection (>95% of deployed units in the latest field season), and low within- and among-unit variations were found relative to programmed collection volumes. Overall, the test system has excellent potential for integrated chemical–biological monitoring of contaminants in a variety of field settings. *Environ Toxicol Chem* 2014;33:1584–1595. © 2014 SETAC

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INTRODUCTION

Assessing potential biological effects of complex mixtures of contaminants in aquatic environments is an ongoing challenge for ecotoxicologists. Instrumental analysis of contaminant occurrence or concentrations in environmental samples is an important tool for these assessments in terms of diagnosing causes of observed impairments, source identification, and evaluation of the success of remediation or mitigation; however, monitoring chemical presence alone does not ensure adequate environmental protection. There are many reasons for this, including a lack of knowledge (and/or analytical techniques) for all contaminants present, inadequate analyte detection limits relative to known (or unknown) biological effects of specific chemicals, limited capacity to account for bioavailability of contaminants, and inability to account for chemical interaction (mixture) effects. Recognition of these shortcomings has resulted in the adoption of effects-based approaches to complement instrumental analysis of chemicals by a number of regulatory programs. The National Pollution Discharge Elimination System program for effluents in the US, for

example, discharge permits can be based both on concentrations of specific chemicals in wastewaters and on toxicity of the effluents to different invertebrate and vertebrate species in standardized assays [1]. Another notable monitoring program that uses effects-based testing in the United States is assessment of dredged materials, whereby the toxicity of complex mixtures (elutriates, sediments) to invertebrates and fish is used to complement chemical analyses for evaluating risk [2].

Monitoring programs that utilize biological testing to assess water samples typically expose animals in the laboratory to samples collected from the field. Although this approach has positive attributes in terms of logistics and control of potentially confounding environmental variables, there are instances in which effects of fluctuating chemical exposures—associated with either point or nonpoint sources—would not be captured through the use of grab samples collected at discrete time points. Furthermore, some contaminants in samples may degrade relatively rapidly such that laboratory exposures with collected samples might not reflect field conditions. To address these types of issues, many researchers have employed in situ (caged) exposure systems using either invertebrates or fish. Animals for these caging studies are occasionally collected from the field; but to ensure deployment of healthy animals with a well-defined (chemical) exposure history, test organisms are more typically from laboratory cultures. A variety of different fish species have been employed for in situ testing. Among them, the fathead minnow (*Pimephales promelas*)

All Supplemental Data may be found in the online version of this article.

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probably has been the most commonly used freshwater species for caged fish studies in North America [3–13]. Widespread use of the fathead minnow for this type of work stems from a number of considerations, including its long history as a standard model for regulatory ecotoxicology [14].

The Great Lakes Restoration Initiative (GLRI), started in 2010 through the Office of the President of the United States, represents a significant effort focused on assessing and improving the health of the Great Lakes ecosystem (<http://www.epa.gov/glnpo/glri/>). Our laboratory is contributing to a large-scale, multiagency effort in 2 urgent focus areas associated with the GLRI: occurrence of toxic chemicals in areas of concern (AOCs) and near-shore effects of pollution runoff. To address aspects of these contaminant-oriented issues, we are evaluating the utility of effects-based monitoring studies with caged fathead minnows at a number of different locations and types of sites throughout the Great Lakes [15]. Previous caging studies with the fathead minnow have used a wide variety of test systems, depending on variables such as study objectives, water body characteristics, available materials, etc. [3–13]. For the GLRI work, we wanted to develop a relatively simple, standardized test system for *in situ* exposures suitable for the wide range of habitat or deployment situations that might be encountered in the Great Lakes. A critical aspect of this effort involved development of an automated device for collection of composite water samples, which could be simultaneously deployed with the caged fish, thereby aiding characterization of the temporally integrated exposure experienced by the animals. Specifically, these water samples could be used both for targeted analysis of specific chemicals of interest, and for determination of biological activities of concern (e.g., estrogenicity) using *in vitro* systems.

In the present study, we describe a system for caging fathead minnows (or other small pelagic species) for biological effects studies, which includes a time-integrated water autosampler. Our goal was to develop a relatively simple and inexpensive system that can be deployed easily and can function under a wide range of site conditions. In addition to describing the system, we present several examples of biological and analytical data to illustrate its performance.

MATERIALS AND METHODS

Overview

The present study explores 2 discrete facets of the work associated with the GLRI effort. We first describe assembly of the fish caging apparatus and the simultaneously deployable system for collecting composite water samples. Included in this description, as Supplemental Data, is a complete parts list, as well as photographs and figures detailing the construction of the autosampler, including a circuit diagram for programming water collection. We then describe the components of a number of studies conducted at Great Lakes AOCs to demonstrate performance of the caged fish/autosampler system from the perspectives of both biology and chemistry. This latter component includes discussion of improvements made to the system over the course of 3 field seasons (2010–2012), as well as experimental design considerations germane to deployment of this type of apparatus (e.g., appropriate biological controls, chemical blanks).

Construction of the test apparatus

Caging system. The overall design of the caged fish apparatus is quite similar to that described by Miller et al. [10], and consists of readily available commercial components (Figure 1; Supplemental Data Table S1). The actual exposure chamber is a

minnow trap constructed of vinyl-coated galvanized steel mesh (6.4 mm) approximately 79 cm in length and 23 cm in diameter, with an interior volume of approximately 20 L. The traps are comprised of 2 halves, closed at the ends, which are fastened together with plastic zip-ties once fish have been placed in the unit. Based on initial studies in which some smaller adult females escaped over the course of deployment, traps are currently deployed within a secondary containment barrier, comprised of a 40-cm × 80-cm bag with a 2.7-mm polyethylene mesh (Supplemental Data, Table S1). On deployment in the field, the bags containing traps are clipped to loops on vinyl-coated steel cable (~5 mm outer diameter) affixed to a cement block (~25 kg), and then marked with a buoy (Figure 1). For most of our work to date, cages have been set approximately 1 m to 1.5 m from the bottom. However, cage depth can be readily adjusted by creating attachment loops at different points along the cable. To facilitate experimental replication and/or time-course sampling, several of the modified minnow traps can be attached to each buoy/anchor configuration (Figure 1).

Water autosampler. To collect water samples for characterization of exposure of the caged fathead minnows, we developed an autosampler that could be deployed concurrently with the fish. For this application, we wanted a system that was structurally robust, relatively compact, completely submersible (for protection from physical disturbance), accurate in terms of water volume collection, easily programmable in terms of timing of sampling, and comparatively inexpensive. Components (as well as potential sources and approximate prices) for the deployable automated water sampler are listed in Supplemental Data, Table S1. The basic system consists of a pump, solenoid, battery, and controller encased in a waterproof container, with tubing connecting through the case that transfers water from the ambient environment to a sample collection vessel (Figure 1). The primary container is a commercially available case constructed of a hard polypropylene material that is durable, light-weight, and resistant to chemical or ultraviolet light degradation (Supplemental Data, Table S1). Exterior dimensions of the case are 33.9 cm × 29.5 cm × 15.2 cm. The cover of the container has an ethylene propylene diene monomer O-ring for waterproofing. Three holes are drilled through the body of the case and fitted with stainless steel bulkhead unions (6.4 mm outer diameter). These fittings, which have very precise tolerances, are inserted and tightened firmly to form a water-tight seal. The 3 fittings are used for sample water intake, presample purge (i.e., back to the environment), and final sample collection lines, which consist of clear, moderately flexible, polyethylene tubing (6.4 mm outer diameter; Supplemental Data, Table S1). The sample collection line is connected to a container for sample holding, which for most of our studies was a collapsible 20-L low-density polyethylene container fitted with bulkhead unions to allow for expansion as sample volumes increased over time in the vessel. Miniature check valves (6.4 mm inner diameter) are placed inside the sample collection line to prevent backflow from the container. To avoid plugging of the intake line with debris, a macrofiltering apparatus comprised of a 5.7-cm × 6.4-cm cone constructed of stainless steel screen (20 mesh) is attached to the end of the line. On deployment, to help control positioning, the container is placed in the same type of mesh bag as used for the cages and fastened top and bottom to the anchor cable and autosampler primary container, respectively, at a depth close to that of the caged fish.

The pump used is a relatively inexpensive but highly reliable low-flow miniature gear pump (Supplemental Data, Table S1). The pump is powered by a 12-V DC sealed lead-ion

Figure 1. Field photo and illustrated depiction of deployed fish cages and water autosampler (adapted from Ekman et al. [15]). [Color figure can be viewed in the online issue which is available at wileyonlinelibrary.com]

battery and equipped with a 3-port solenoid that allows timed collection of water first to the purge and then to the sample collection lines (Supplemental Data, Table S1). A depiction of assembly and placement of the different components of the autosampler within the primary container, and the water collection vessel is presented in Supplemental Data, Figure S1a–e. The battery, pump, solenoid, and controller box are held in place within the container using precut 6-cm hard Styrofoam®.

A secondary containment unit within the primary container is used to house the sample collection controller. This unit consists of a hard plastic box (120 cm × 65 cm × 40 cm), sealed with an O-ring, which contains a circuit board (Supplemental Data, Table S1) with space for the microcontroller and transistors that activate the pump and solenoid. A programmable microcontroller, which utilizes an internal clock to reduce the circuit board component count (Supplemental Data, Table S1), is used to control the solenoid and pump (see Supplemental Data, Figures S1e and S2 for the physical circuit board layout and wiring diagram, respectively). Wires (18 gauge) from the controller to the solenoid and pump pass through drilled holes in the secondary container, which are sealed with epoxy.

The final weight of a fully assembled autosampler is approximately 8.6 kg, with a buoyancy rating for the primary container alone of 9.1 kg. This slight positive buoyancy is important to ensure easy deployment of the autosampler in conjunction with the fish cages, and also means the unit would float if it became disengaged from the anchor cable.

The autosampler is very flexible in terms of programming options for timing and frequency of sample collection. MicroCode Studio Plus (microEngineering Laboratories) is employed as the integrated development environment, and the compiler used is PicBasic Pro, version 3 (microEngineering

Laboratories). The microEngineering Laboratory U2 programmer is utilized to program the compiled hex code into the microcontroller via a programmer header located on the circuit board (Supplemental Data, Figure S1e). Any changes to the collection parameters would first be edited in MicroCode Studio Plus, and then compiled and programmed into the microcontroller.

For our 4-d fish deployments, the pump/solenoid system was typically programmed to collect water samples periodically for a total of 5 d (the extra day was added to ensure that water sampling would continue if, for example, fish were collected at 98 h instead of 96 h). For these studies, samples were collected for 1 s every 15 min, which resulted in approximately 26 mL to 27 mL of water per sampling event, and a total sample volume of approximately 10 L for a 4-d deployment. Immediately prior to the periodic 1-s collections to the storage vessel, the solenoid and pump were activated such that sample lines were purged for 1.5 s, with the resultant water returned to the environment. After the programmed sample collection time, the solenoid is deactivated but the pump continues to run for 200 ms to minimize voltage spikes that might reset the microprocessor. Once the target number of samples has been reached, the program enters a sleep state.

Evaluation of the test apparatus

Field deployment and sample collection. The present study was part of a large, ongoing, multiagency project focused on the development of effects-based monitoring approaches both for legacy chemicals (e.g., organochlorines) and for contaminants of emerging concern (e.g., pharmaceuticals) in the Great Lakes [15]. Between 2010 and 2012, several different types of studies, including caged-fish exposures, were conducted at a number of sites distributed across 5 different Great Lakes AOCs,

including the Duluth Harbor/St. Louis River, MN; Milwaukee Estuary, WI; Lower Fox River/Green Bay, WI; Maumee River, OH; and Detroit River, MI. These AOCs are characterized by a diversity of biological use impairments related to the occurrence of point and nonpoint contaminant inputs (see <http://www.epa.gov/grtlakes/aoc/> for additional details). Many of the study sites were located downstream of municipal wastewater treatment plants (WWTPs). Study sites within the AOCs also exhibited a wide range of physicochemical characteristics in terms of variables such as depth, flow, substrate type, and basic water quality (e.g., pH, hardness, conductivity). Experimental designs varied at different sites and dates; for example, although 4 d was the most common exposure time utilized for the caged fish, durations in some instances ranged from 2 d to 8 d. However, the basic methods used for deployment, recovery, and sampling were common across all the studies.

Fish came from an on-site culture unit at the Duluth (MN) US Environmental Protection Agency (USEPA) laboratory that has continuously generated fathead minnows for research and regulatory activities for approximately 40 yr [14]. Prior to use in the *in situ* exposures, fish were held in mass cultures at 20 °C with a 16:8-h light:dark photoperiod and fed a mixture of brine shrimp and ground trout chow twice daily. Animals used for the deployments were mature adults, typically 5 mo to 6 mo old, that exhibited a sufficient degree of sexual dimorphism to allow accurate phenotypic sexing. Because many of the studies were conducted at Great Lakes sites some distance from the Duluth laboratory, it was necessary to ship the fish to research teams working at these locales. A standardized protocol that included multiple types of control animals was utilized for transport and deployment of the fish. Six fathead minnows of each sex were removed from the mass culture and placed together in plastic bags containing approximately 4 L of oxygenated culture water (from Lake Superior), and subsequently put in coolers on ice. The coolers were shipped overnight to the remote field sites, and the fish were deployed the day after receipt (i.e., the animals were in the coolers for approximately 2 d). Automatic temperature loggers (Onset HOBO Data Loggers) were placed in at least 1 bag per cooler so that we could ascertain the temperature regime to which the fish were exposed during shipping/handling. For studies conducted in the Duluth Harbor/St. Louis River, fish were still transported in the presorted bags, but they were typically deployed within 1 h of leaving the laboratory. Once at a site, fish were acclimated to the local water temperature (± 3 °C) by immersing the shipping bags in a bucket of ambient water prior to placement in the cages. One bag of fish (i.e., 6 males and 6 females) was used for each cage. The animals were not artificially fed during transit or while they were in the field.

A number of different molecular, biochemical, and apical endpoints were measured in the caged fish [15]; because of uncertainties as to how variables such as shipping and temperature changes might affect some of these endpoints, several different types of controls were typically used for the remote studies. These included fish sampled under the following scenarios: from the culture when bagging first occurred (culture controls), from bags held for 2 d on ice in coolers on site (shipping controls), from extra shipping bags at the time the test fish were deployed in the field (day 0 controls), and after holding in on-site aquaria (in Lake Superior water) for a duration corresponding to the length of the field deployment (time-matched laboratory controls).

Fish collected from cages or the various controls were anesthetized with MS-222 and weighed. They were evaluated for any obvious external lesions and status of secondary sexual

characteristics (e.g., dorsal nuptial tubercles). Biofluids (urine from males, blood from both sexes) and tissues (e.g., liver, gonads, brain) were collected and stored in a manner appropriate to the various types of measurements discussed by Ekman et al. [15]. For example, for the gene expression measurements in the present study, liver and ovary samples were flash-frozen in liquid nitrogen, shipped on dry ice (from remote sites), and stored at -80 °C until RNA extraction.

In conjunction with placement of fish in the cages, autosamplers were simultaneously deployed at several of the study sites in 2011 and 2012. The autosampler apparatus was placed at the same depth (~ 1 m from the bottom) as the caged fish. To help evaluate performance of the autosamplers in terms of chemicals and chemical activities detected in the composite, at a subset of the test sites grab water samples were also collected when fish were deployed or when they were sampled after 4 d. Grab samples were obtained using a peristaltic pump (model 410; Solinst) using site water-rinsed silicone tubing, from a depth of approximately 1 m from the bottom into a precleaned, muffled amber glass 1-L bottle, placed on ice, and shipped overnight to the US Geological Survey National Water Quality Laboratory in Denver, Colorado, USA. The composite samples were stored and shipped in a similar manner. Samples were processed immediately after receipt at the National Water Quality Laboratory.

In addition to the grab and composite field samples described above, a laboratory experiment was conducted in which Lake Superior water was pumped through an autosampler over the course of 4 d into either a low-density polyethylene container or a glass bottle similar to that used for collecting/shipping the grab samples from the field. The purpose of this experiment was to assess the occurrence of background contaminants that might leach from components of the autosampler or the collection vessels.

Analytical and biological measurements. Water samples were analyzed by the National Water Quality Laboratory for more than 130 organic compounds indicative of industrial, domestic, or agricultural wastewaters, including several chemicals known to be endocrine-active (e.g., steroids, alkylphenols, bisphenol A), and a suite of 48 human pharmaceuticals. The entire list of analytes, the specific procedures used (including quality assurance/quality control measures), and complete chemical results for several Great Lakes AOC studies, including those described in the present study, are detailed elsewhere [16].

Cell bioassays can be very useful in detecting pathway-specific biological activity of undefined chemicals, or mixtures of chemicals with a similar mechanism of action in complex samples. We have frequently used the T47D-KBluc assay, which employs a cell line stably transfected with a human estrogen receptor- α -luciferase reporter gene construct, to detect estrogenic activity in environmental samples [17]. In the current study, we present an example of bioassay data for water samples collected from 3 sites in the Duluth Harbor, along a discharge gradient for an effluent with established estrogenic activity [17–19]. Water tested in the T47D-KBluc assay included both grab samples collected when fathead minnow cages were deployed, and composite samples collected by the autosampler during the 4-d field exposure. The assay was conducted using a method in which culture media was directly prepared using site water, as described in detail elsewhere [17,20]. Samples were tested at both 100% and 25% of the original field sample, with the dilution achieved through use of an appropriate volume of media prepared in high-quality deionized water. Estrogenic activity in the samples was considered significant when relative luminescence units (i.e., luciferase activity) exceeded baseline values

(generated using only media prepared in deionized water) by more than 3 standard deviations.

In conjunction with the various Great Lakes caged-fish studies, we measured a variety of biochemical and molecular endpoints such as plasma sex steroid and vitellogenin concentrations, ex vivo (gonadal) production of steroids, tissue-specific alterations in metabolite profiles, and changes in gene expression using both real-time quantitative polymerase chain reaction (qPCR) and microarrays [15,19]. For illustrative purposes, we present qPCR data for hepatic cytochrome P4501a1 (CYP1a1) in males caged for 2 d, 4 d, or 8 d at 4 different sites in the St. Louis River/Duluth Harbor (September 2010), or for 4 d at 2 different sites in the Detroit River (April–May 2011). Cytochrome P4501a1 is a xenobiotic-metabolizing enzyme whose expression can be induced by exposure to a variety of arylhydrocarbon (Ah) receptor agonists such as some polychlorinated biphenyls and polycyclic aromatic hydrocarbons [21]. We also present qPCR data for expression of 3 genes (follicle-stimulating hormone receptor, *fshr*; cyp-cholesterol side chain cleavage, *cyp11a*; and cyp-aromatase, *cyp19a1a*) related to reproductive endocrine function, including sex steroid synthesis, in ovary tissue of females caged for 4 d at 3 sites along an effluent gradient in Duluth Harbor (August 2011). Changes in expression of these genes in fathead minnows can result from exposure to a variety of endocrine-active chemicals [22]. Finally, we present qPCR data for hepatic expression of estrogen receptor- α (*esr1*) in males caged for 4 d at sites in the Detroit River in the 2011 study; increases in expression of *esr1* can be indicative of exposure to exogenous estrogens [17].

Methods and all primer and probe sequences used for the qPCR analyses have been described in detail elsewhere [23–26]. Briefly, total RNA was extracted from the appropriate tissues (gonad or liver) from individual fish using Qiagen RNEasy mini kits. Total RNA quantity and quality was evaluated using a Nanodrop ND-1000 spectrophotometer and diluted to a consistent concentration of 10 ng/ μ L for use as template in the qPCR reactions. Relative abundance of *cyp11a*, *cyp19a1a*, and *fshr* transcripts were measured using Taqman RNA-to- C_T 1-Step kits (Applied Biosystems) and a gene-specific RNA standard curve using thermocycler conditions identical to those described previously [26]. Relative abundance of *esr1* transcripts was measured using Power SYBR Green RNA-to- C_T 1-step kits (Applied Biosystems) as described elsewhere [27]. Statistical analysis of the qPCR data was performed using Statistica 12 (Statsoft). Data were tested for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Levene's test) and, in most cases, log transformed to meet parametric assumptions. Data were then analyzed using one-way analysis

of variance followed by Duncan's multiple range or Dunnett's tests. Differences were considered significant at $p \leq 0.05$.

RESULTS

Performance of the caging system

For an experienced crew, deployment of fish cages (including addition of fish to the cages) and an autosampler at 1 field site (Figure 1) can be achieved within approximately 15 min to 20 min. During field seasons from 2010 to 2012, we deployed approximately 1500 caged fish at over 30 sites in multiple locations in the Great Lakes (Table 1). Most of these exposures were for 4 d, but some of the fish were caged for up to 8 d in 2010. Over the 3-y period, only 2 of 130 total cages were lost, 1 in 2010 and 1 in 2012. During the 2010 pilot season, almost 10% of the fish from the 23 successfully retrieved cages had escaped the primary enclosure and were in the secondary mesh bag. However, in subsequent years, the number of fish that escaped from the primary cage was substantially lower, likely because of the slightly larger size of the animals used in 2011 and 2012 (Table 1). The number of dead or missing fish was uniformly low, at 3.2%, 0.8%, and 0.7% in 2010, 2011, and 2012, respectively. Importantly, survival of deployed animals that had been shipped to the remote study sites was 100% in both 2011 and 2012 (Table 1).

The caging system was successful under a variety of conditions (Table 2). Water depths for the deployments ranged from approximately 1 m to greater than 9 m, with the cages being placed at sites ranging from immediately offshore to approximately 200 m from the shore. Qualitative assessment of bottom sediments ranged from gravel to soft or packed mud and, when flow data were available from adjacent US Geological Survey monitoring stations, system discharge spanned almost 2 orders of magnitude (from ~ 1300 m³/s to greater than 55 000 m³/s; Table 2). Water temperature on placement of the cages ranged from approximately 10 °C to 25 °C (Table 2). Other water quality parameters were similarly variable, with hardness, for example, ranging from less than 100 mg/L (St. Louis River) to approximately 300 mg/L (Milwaukee Estuary) as CaCO₃/L.

A large number of endpoints have been measured in caged fish samples from the various studies we have conducted. For the purposes of the present study, we report only a selected subset of data from these studies to illustrate the performance of the system and the type of data that can be obtained. Comprehensive reporting of the data from our different Great Lakes studies and a discussion of the biological implications of the results will be described elsewhere. Results for hepatic *cyp1a1* expression in fish caged in the Duluth Harbor/St. Louis

Table 1. Mean ($\pm$ standard error) fish weights and deployment success for caged-fish exposures^a

	Female body weight (g)	Male body weight (g)	Cages deployed	Cages retrieved	Fish deployed ^b	Fish retrieved	Fish in secondary bag	Fish dead/missing
2010								
Duluth	1.24 $\pm$ 0.03	2.51 $\pm$ 0.06	24	23	276	267	28	9
2011								
Duluth	1.32 $\pm$ 0.04	2.95 $\pm$ 0.07	18	18	216	211	2	5
Shipped	1.30 $\pm$ 0.07	2.71 $\pm$ 0.10	30	30	360	360	6	0
2012								
Duluth	1.51 $\pm$ 0.07	2.98 $\pm$ 0.09	42	41	492	487	5	5
Shipped	1.79 $\pm$ 0.07	3.03 $\pm$ 0.11	16	16	192	192	3	0

^aSix adult fathead minnows of each sex were used in the cages. Most exposures were for 4 d, but some of the 2010 exposures were up to 8 d long.

^bTotal excludes animals from cages that were entirely lost.

Table 2. Summary of study sites and characteristics for caged fish/autosampler studies

Location	Water depth (m)	Shore distance (m)	Mean water temperature (°C) ^a	Mean discharge (m ³ /s) ^b	Substrate type	Mean pH	Mean conductivity (μS/cm)	Mean alkalinity (mg/L CaCO ₃)	Mean hardness (mg/L CaCO ₃)
Detroit River				55 354					
Pt. Hennepin	1.1	75	9.4	—	Gravel	8.01	188	90	106
Wyandotte	1.8	5	10.2	—	Gravel	7.76	305	113	138
Grosse Ile	1.2	5	10.3	—	Gravel	8.01	236	94	111
Trenton	1.8	5	10.6	—	Gravel	7.68	339	115	154
Maumee River				9021					
Near Clark Oil	1.9	85	13.2	—	Fine sediment	7.85	301	101	135
Swan Creek mouth	1.2	10	12.7	—	Mud	7.89	464	127	191
Near Toledo	1.2	20	13.3	—	Fine sediment	7.85	472	120	166
Fox River				1391					
De Pere	1.2	50	19.8	—	Solid mud	8.13	479	163	180
East River	1.5	5	20.2	—	Fine sediment	8.24	616	216	237
Proctor & Gamble discharge	2.4	15	20.5	—	Fine sediment	8.30	323	137	165
Green Bay	4.6	15	21.1	—	Solid mud	8.21	313	146	158
Milwaukee Harbor									
Menomonee River	4.6	0 ^c	24.9 ^b /24.4	—	Fine sediment	8.26	943	255	302
Milwaukee River	2.4	0 ^c	22.8	—	Fine sediment	8.61	784	259	300
Jones Island	9.1	0 ^c	15.9	—	Fine sediment	7.81	607	146	195
Kinnickinnic River	5.5	0 ^c	18.3	—	Fine sediment/tar	8.00	637	153	215
St. Louis River									
Proximal	1.2	5	24.6	—	Fine sediment	7.60	837	158	159
Distal	1.7	100	22.4	—	Fine sediment	7.68	367	93	99
Far Distal	1.8	200	19.8	—	Fine sediment	7.68	269	81	90

^aAverage water temperature recorded by data logger attached to sampling unit for duration of exposure.

^bData were from nearby US Geological Survey water resources monitoring location collected during the period of deployment.

^cCaged fish were tied off to shipping channel break wall/steel pilings.

River (September 2010; Figure 2) provide an example of a biological endpoint that was impacted as a function of both site and duration of exposure in the field. Hepatic *cyp1a1* expression was assessed in male fathead minnows caged for up to 8 d at 4 sites in the Duluth Harbor/St. Louis River: 2 sites were located proximal (~5–10 m) and distal (~200–250 m) to discharge from a WWTP located in Duluth that processes a mix of municipal and industrial waste; 1 site was located near a municipal WWTP discharge from Superior, Wisconsin (USA); and the 4th site (Fond du Lac, intended as a reference) was upstream of major point source inputs on the St. Louis River. Based on a comparison with time-matched laboratory controls, induction of *cyp1a1* occurred relatively quickly (within 2 d) at the 3 WWTP sites, with the greatest magnitude of response observed at the site proximal to the Duluth WWTP discharge. Across-site separation (e.g., the gradient-type response by the Duluth WWTP outflow;

Figure 2. Expression of hepatic transcripts for a cytochrome P450 isozyme, *cyp1a1*, in adult male fathead minnows (*Pimephales promelas*) caged for 8 d at 4 sites in the Duluth Harbor/St. Louis River (MN, USA). Data are presented as mean (standard error of the mean, $n = 11-13$). Different letters denote significant differences from time-matched laboratory control animals. WWTP = wastewater treatment plant.

no significant effect at Fond du Lac) appeared to be maintained through 4 d of exposure; however, by 8 d the magnitude of *cyp1a1* expression was relatively similar in caged fish from all the effluent sites and was slightly elevated even at the upstream reference site (Figure 2).

Data for expression of 3 genes (*fshr*, *cyp11a*, and *cyp19a1a*) in ovary tissue collected from female fathead minnows caged for 4 d at the proximal and distal sites mentioned above, as well as at a far-distal site located 400 m to 450 m from the Duluth WWTP discharge (August, 2011), are shown in Figure 3. These data illustrate the robustness of some of the biological endpoints measured relative to potential differences in environmental conditions between the laboratory and field, and among different sites in the field. Expression of the 3 genes, which are all related to reproductive status of the fish, was comparable across the study sites, and also comparable to time-matched laboratory controls held in Lake Superior water (Figure 3).

Results from males held for 4 d adjacent to 2 WWTP discharges into the Detroit River, (Trenton, Wyandotte) demonstrate the potential importance of different types of controls relative to interpreting site-related effects in caged fish. In the first instance (Figure 4a), hepatic expression of *cyp1a1* was elevated in fish from the 2 field sites, but was uniformly low in the 4 different types of controls discussed in the *Materials and Methods* section (i.e., culture controls, shipping controls, day 0 controls, and time-matched laboratory controls). However, for a second hepatic gene, *esr1*, there was a significant difference between the different types of controls, with the day 0 remote shipping control exhibiting expression levels that were lower than the other 3 controls and comparable to those observed in caged fish from the 2 field sites (Figure 4b).

Assessment of autosampler performance

Different metrics can be used to evaluate the performance of the autosampler. One involves maintenance of physical and

Figure 3. Expression of 3 ovarian genes in adult female fathead minnows (*Pimephales promelas*) caged for 4 d at 3 sites adjacent to a wastewater treatment plant in Duluth Harbor (MN, USA). (a) Follicle-stimulating hormone receptor (*fshr*); (b) cytochrome P450 cholesterol side-chain cleavage (*cyp11a*); (c) cytochrome P450 aromatase (*cyp19a1a*). Data are presented as mean (standard error of the mean, $n = 11$ –12).

functional integrity during field deployment. Another basis for evaluation is consideration of observed versus expected (programmed) water volumes collected in different settings. Finally, assessment of qualities of the samples from a chemistry or biology perspective can be used to help evaluate utility of the technology. Each of these 4 points is addressed separately below.

Physical and functional integrity. Autosampler designs conceptually similar to or the same as those described in the present study were utilized, respectively, during the 2011 and 2012 field seasons. The final 2012 version differed from that used in 2011 in that it had a battery upgrade (from a series of D batteries to a single 12-V DC battery), employed a more rugged primary container, and included a secondary container to protect components of the collection controller. In 2011, prototypes of the autosampler were deployed at a total of 15 sites in the Milwaukee Estuary, Lower Fox River/Green Bay, Maumee River, and Detroit River in conjunction with 4-d caged-fish studies. These deployments occurred under a variety of site conditions and water quality characteristics (including temperature; Table 2). Water samples were successfully collected by all 15 of the autosamplers; however, in approximately one-half of the cases, collection volumes were less than intended because of battery failure and/or the occurrence of partially crimped collection lines. In 2012, the autosamplers described in the present study (Supplemental Data, Table S1 and Figures S1 and S2), were deployed at 22 sites in the Duluth Harbor/St. Louis River and Maumee River. One system failed to collect a sample

Figure 4. Expression of hepatic transcripts for (a) a cytochrome P450 isozyme, *cyp1a1*, and (b) estrogen receptor- α (*esr1*) in adult male fathead minnows (*Pimephales promelas*) caged for 4 d at 2 sites in the Detroit River (MI, USA) and initial time-matched control animals from the laboratory and field. Data are presented as mean (standard error of the mean, $n = 11$ –12). Significant differences among different types of controls and the field-exposed animals are denoted by different letters.

because of operator error. The other 21 autosamplers, including 1 in which water had entered the primary container, successfully collected consistent volumes of water, resulting in a >95% success rate.

Accuracy of water volume collection. A more quantitative way to assess performance of the autosamplers is through measuring collected sample volumes over time. This was done under both laboratory and field conditions. In a laboratory study, 3 of the autosamplers were used to collect Lake Superior water from a large stainless steel tank, and daily measurements were made of the volume of sample collected. The samplers were programmed to collect water for 1 s every 15 min, a regime similar to that used in the field (Table 3). Because sample volumes were determined at slightly different times during the workday, collection data were normalized to the elapsed time (min) between measurements. Although there were slight differences between the samplers (e.g., the volume collected by sampler 1 was ~6% lower than that collected by sampler 3), variations for a given sampler over time were small (coefficients of variation of 1% or less). Output voltage of the batteries also remained quite stable over the 4 d, indicating good functionality of the power source for this period (Table 3).

A field trial of the autosamplers conducted at several sites in the Maumee River in 2012 produced results similar to those of the laboratory study. At each of 8 sites, an autosampler was

Table 3. Performance of three replicate autosamplers (water volume collected, battery voltage) over a 4-d period in a controlled laboratory setting

Autosampler	Day					Total volume (mL)	Mean (standard deviation) (mL/min)
	0	1	2	3	4		
1							
Voltage	13.22	13.07	13.01	12.97	12.94		
Total volume collected (mL)	0	2520	2660	2395	2645	10 220	
Collection time	14:15	14:08	15:15	13:55	15:10		
Minutes elapsed (between d)	0	1433	1507	1360	1515		
Volume (mL/min)	—	1.76	1.76	1.76	1.75		1.76 (0.008)
2							
Voltage	13.19	12.99	12.98	12.95	12.88		
Total volume collected (mL)	0	2635	2805	2475	2820	10 735	
Collection time	14:15	13:48	15:11	13:50	15:05		
Minutes elapsed (between d)	0	1413	1523	1359	1515		
Volume (mL/min)	—	1.86	1.84	1.82	1.86		1.85 (0.020)
3							
Voltage	13.33	12.99	12.92	12.88	12.8		
Total volume collected (mL)	0	2690	2820	2540	2845	10 895	
Collection time	14:15	14:00	15:03	13:44	15:01		
Minutes elapsed (between d)	0	1425	1503	1361	1517		
Volume (mL/min)	—	1.89	1.88	1.87	1.87		1.88 (0.009)

deployed with caged fish for 4 d, with both the mechanical unit and the sampling vessel fully submerged and arranged so that the intake tube was at the same depth (~0.5–2 m) as the caged-fish, and well above the sediment (to minimize potential clogs). On retrieval, collection vessels were detached from the autosampler, capped, and weighed. One sampler failed to collect water because of an operator error; the seal on the primary container was obstructed by an electrical line and the unit filled with water, causing the pump to fail. The average weight of water collected in the other 7 vessels was 10.96 ± 0.19 kg. When adjustments were made for actual time in the field (i.e., 94–96 h) the autosamplers collected at a mean rate of 1.93 ± 0.035 mL/min, with a 1.82% coefficient of variation among the 7 successful units. There was a 4.8% difference (0.09 mL/min) between the maximum and minimum collection rates for the 7 autosamplers, which tracked very closely with the laboratory experiment.

Comparative chemistry profiles. Another approach used for assessing performance of the autosamplers was evaluation of chemical composition of the composite samples versus that of water samples collected at different times during the autosampler deployment. This is somewhat subjective relative to interpretation because, unless target analytes are continually monitored over time, it is impossible to know what the true incidence of occurrence or concentrations are in a fluctuating environment (and, hence, what might be expected in a fully representative composite or a grab sample at any given time). However, this type of information can offer insights as to whether the composite sample seems to be yielding reasonable results, as indicated by data obtained via multiple grab samples.

Analytical data for the autosampler (composite) versus grab samples collected when fish were deployed (day 0) and at the end of the exposure (day 4) from 2 caged-fish sites in the Maumee River AOC (Swan Creek and adjacent to the Toledo WWTP) were compared (Supplemental Data, Tables S2 and S3). Of 133 target analytes, 34 were detected at the Swan Creek site in at least 1 of the 3 samples; 19 of these were detected in the composite and in 1 or both of the grab samples (Figure 5a; Supplemental Data, Table S2). Certain analytes were detected in only 1 of the 3 sample types. Chemicals that were detected in the composite but not the grabs included diethyl phthalate, 4-nonylphenol, piperonyl butoxide, and tris(2-chloroethyl)phosphate. Chemicals detected in both the grab samples, but not the composite,

included 9,10-anthroquinone, hexahydrohexamethylcyclopentabenzopyran, and *p*-cresol.

Approximately one-half (67) of the 133 target analytes were detected in 1 or more of the 3 sample types at the site adjacent to the Toledo WWTP site, with 47 of the 67 detected in the composite and in 1 or both of the grab samples (Figure 5b; Supplemental Data, Table S3). Again, certain analytes were detected in only 1 of the 3 sample types. Chemicals detected in the composite but in neither grab sample included 17 β -estradiol, 2,6-dimethylnaphthalene, 2-methylnaphthalene, diethyl phthalate, and venlafaxine. Analytes occurring in both the grab samples but not the composite were 4-nonylphenol diethoxylate, 4-nonylphenol monoethoxylate, 9,10-anthroquinone, acetylhexamethyltetrahydronaphthalene, and oxycodone.

As noted above, it is difficult to make valid quantitative comparisons between the grab and composite samples based on analyte concentrations; however, potentially important insights might be achieved based on the absence/presence of specific chemicals in the different types of samples. For example, the occurrence of chemicals in the composite but not grab samples could indicate 2 possible scenarios: compounds whose environmental occurrence might be highly variable over a 4-d period, or chemicals present as artifacts associated with the sampling system. In our studies it appears that both situations likely occurred. Supplemental Data, Table S4, presents data from a laboratory study in which Lake Superior water was collected over 4 d using the autosampler into either a low-density polyethylene container or a glass bottle. None of the 133 target analytes were detected in the laboratory-source Lake Superior water or Lake Superior water that had been pumped into glass (Supplemental Data, Table S4). The large majority of analytes (~125) also were not detectable in the polyethylene container. However, some were detected, including 3 chemicals that occurred in the composite but not grab samples from the field: diethyl phthalate, 2,6-dimethylnaphthalene, and 2-methylnaphthalene. Other chemicals that appeared to leach from the polyethylene container in the laboratory study included several that likely were present in the Maumee River water (i.e., they were detected in both grabs and composites), such as bisphenol A and benzophenone (Supplemental Data, Table S4).

Comparative biological profiles. Water collected by the autosampler can also be used for purposes other than chemical

Figure 5. Venn diagram depicting comparative occurrence of chemical analytes in 2 grab samples (day 0 deployment and day 4 retrieval) and a composite sample corresponding with 4-d caged fish exposures at 2 sites in the Maumee River in 2011: (a) Swan Creek and (b) adjacent to the Toledo wastewater treatment plant. Data are summarized from Supplemental Data, Tables S2 and S3, respectively.

analyses. As for the instrumental analysis described above, estrogenic activity measured in the T47D-KBluc cell bioassay was compared for 4-d composite versus grab samples (collected only when fish were sampled at the end of the exposure in this instance; Figure 6). Water samples were from the proximal, distal, and far-distal sites near the Duluth WWTP described above (August 2011). Both grab and composite water samples exhibited significant estrogenicity that reflected the spatial gradient of the effluent (Figure 6), indicating that estrogenicity of the discharge was relatively consistent for this sampling period.

DISCUSSION

In situ exposures can be an important tool along the continuum of controlled laboratory assays with field-collected samples to evaluate chemical impacts on extant fish populations [15]. Although caged-fish experiments can be more resource-intensive than laboratory testing, they offer realistic exposure conditions reflective of a fluctuating environment, which cannot be easily replicated in the laboratory. Evaluations of feral fish, although important relative to direct assessment of relevant impacts, can be both expensive and uncertain in terms of the ability to consistently collect necessary numbers of animals for robust analysis. Furthermore, when one is collecting or evaluating animals from the field, it can be difficult to ascertain their chemical exposure history, thus complicating interpretation of cause-and-effect relationships. Confidence in terms of exposure history can be substantially enhanced through the use of caged fish.

Figure 6. In vitro estrogenic activity (determined using the T47D-KBluc assay) in grab versus composite water samples (100% and a 25% dilution) associated with 4-d caged fish exposures at 3 sites adjacent to a wastewater treatment plant in Duluth Harbor (MN, USA). Data are expressed as relative luminescence units and are presented as mean (standard error of the mean, $n=3$). The dashed line denotes a 3-standard deviation difference from baseline (deionized water) control values.

Many different species have been used for caging studies, but the fathead minnow has been especially prominent for these types of experiments in North America. It is widely used because of a number of attributes, including broad natural distribution, ready availability of animals for testing from laboratory cultures, ability to tolerate a wide range of water types (and temperatures), comparatively small body size, and relevance to regulatory ecotoxicology [14]. Caged fathead minnows have been used for a variety of purposes, including determining bioaccumulation of metals or organic chemicals [6,12,13]; assessment of the effects of ambient conditions on apical responses such as growth and reproduction [3,8,10]; and determination of histological, biochemical, or molecular alterations indicative of perturbation of different biological pathways by, primarily, chemical stressors [4,5,7,9,11,13]. Because of varying objectives and endpoints, previous fathead minnow caging studies have used a wide range of physical test systems and experimental conditions such as, age or size of animals, exposure durations (from a few days to several weeks), and feeding regimes.

The objective of the present study was to develop a standardized, caged-fish system and associated experimental protocol that would be useful under the different types of physicochemical conditions that might be encountered in rivers, bays, and near-shore sites in the Great Lakes or similar settings. To ensure adequate tissue mass for the various biological analyses of interest, and to minimize the need for feeding, we focused on adult rather than larval or juvenile fish. Given the complexity of the contaminant mixture present at most locations of this type, our emphasis was on endpoints, such as changes in gene and protein expression or metabolite profiles, potentially indicative of early perturbation of any of a number of different biological pathways of possible concern [15]. An important attribute of the overall test system and deployment strategy from a logistic perspective was that it be simple enough that multiple sites could be assessed within a given location (e.g., an AOC) by

a single team of personnel over a relatively short period (e.g., within the course of 1 wk). To support this, for example, we wanted to assess the feasibility of shipping test organisms in a uniform manner so that exposures could be routinely conducted at locations without access to a primary laboratory facility/animal culture.

Based on results to date, the caging system/deployment approach described in the present study meets the stated objectives and requirements. The basic exposure system is inexpensive, and is reliable in terms of recovery of adult fathead minnows from 4-d exposures (typically >99%). Furthermore, using this technology, during the 2011 field season a single team of researchers was able to simultaneously conduct successful studies on 2 different occasions at multiple sites within 2 different AOCs (the Detroit River and Maumee Rivers; Lower Fox River/Green Bay and Milwaukee Estuary), with animals shipped from the Duluth USEPA laboratory. Biological data collected from the caged fish appear robust; for example, in the present study we describe changes in *cyp1a1*, a hepatic gene whose expression is known to be induced by a variety of environmental contaminants [21], which reflected reasonable site-specific and temporal dynamics based on a priori expectations. Similarly, Davis et al. [19] describe a 2012 caged-fish study we conducted near the Duluth WWTP, in which it was possible to discern changes in the hepatic metabolome of fathead minnows associated with shutdown of a pulp and paper mill processor that provided influent to the WWTP, again demonstrating the utility of the basic test system and design for effects-based contaminant monitoring.

Although the present study was successful overall in terms of biological performance, our data do show the need for careful selection of appropriate controls for caged-fish studies, particularly when animals are being shipped to study sites. This was illustrated by data from the Detroit River, in which expression of hepatic *cyp1a1* was seemingly not affected by the transport conditions or process; that is, the various types of shipping and laboratory controls were all similar but were different from those of animals exposed to the river water/effluent. Conversely, hepatic expression of *esr1* in the same fish was affected by aspects of handling or transport. Specifically, expression of the gene in the various types of laboratory controls was higher than in shipped animals, irrespective of whether they were exposed in the field. Because this gene is associated with endocrine function, perhaps it is not surprising that an extended period (48 h) at a cool temperature in the dark affected expression. However, this observation highlights the fact that, without proper controls, impacts of shipping and handling conditions on gene expression could not be distinguished from the potential impacts of the field exposures.

Although effects-based testing and monitoring are important for assessing possible effects of complex contaminant mixtures in a field setting, biology data alone cannot be used to identify a specific chemical or chemicals responsible for impacts or, subsequently, necessary mitigation or remediation options [15]. This requires instrumental chemical analyses of samples that, ideally, capture exposure of the biological model(s) of concern in an accurate manner. This can be extremely challenging in a fluctuating environment. Hence, a complementary goal of our work was to develop a method that would help identify, in a rigorous, temporally integrated manner, chemicals to which the caged fish were exposed. We considered different approaches through which to achieve this, including collection of multiple grab samples of water over

time, evaluation of tissue residues in the caged fish, and use of various passive samplers (e.g., solid-phase microextraction resins) deployed in conjunction with the fish; however, all had potentially important drawbacks. For example, collection and analysis of multiple grab samples can be quite costly and may not adequately capture temporal variability in a poorly characterized system [28]. Analysis of tissue residues in exposed fish certainly could help address or integrate variations in exposure over time, but there is no assurance that the chemical(s) responsible for biological effects would accumulate to any significant degree in the animals. For example, some biologically active chemicals, including many pesticides and pharmaceuticals, are readily metabolized and/or excreted. Different types of passive sampling devices are conceptually attractive in terms of extracting or concentrating chemicals from water over time; however, there are important uncertainties associated with their specificity for different classes of contaminants and ability to generate time-weighted average concentration estimates [29–31].

In considering different options for sample collection in conjunction with the caged-fish exposures, we felt that the least biased approach would be a composite sample comprised of uniform volumes of water collected at relatively frequent time intervals. There are a number of commercially available autosamplers through which this could be achieved; however, those best suited to our purposes (i.e., relatively small, submersible units with flexible programming options) were too expensive to contemplate routine use in large-scale deployments in field settings where either unpredictable weather events or vandalism could result in damage or loss of the units. Therefore, we sought to design and characterize a programmable autosampler for the collection of composite water samples that was relatively compact and reliable, while being comparatively inexpensive.

Early prototypes of the current autosampler had some degree of success but were problematic in areas such as adequate battery life, sensitivity to impacts (e.g., jostling at the deployment sites), and being consistently waterproof, particularly in terms of maintaining controller function. Thus, although usable chemistry data could be derived from the initial models employed, we did not feel that they were reliable enough to recommend to others for routine use in the field. However, the present study's design (described in the *Materials and Methods* section and depicted in the Supplemental Data), has performed very well with regard to reliability in terms of both function in the field under different conditions, and collection of uniform water sample volumes over time. Moreover, the price of materials for this particular model was less than 600 USD, making it a very cost-effective testing/sampling option for routine applications.

Although the autosampler used in our studies appears to be a robust system for collecting samples that correspond directly to water exposures experienced by the caged fish, improvements can be made to the system. For example, use of polyethylene containers as collection vessels, although attractive in terms of cost, durability, and ability to collect samples underwater (i.e., easily vented), appears to be problematic in terms of the occurrence of trace organic chemicals which can leach from plastics, such as alkylated naphthalenes, phthalates, camphor, benzophenone, p-cresol and bisphenol A (Supplemental Data, Table S4). Unfortunately, some of these chemicals do occur as contaminants in aquatic environments (e.g., see grab sample data in Supplemental Data, Tables S2 and S3), and could be toxicologically relevant (e.g., bisphenol A is an estrogen

receptor agonist in vertebrates, including fish [32]). As a consequence, despite many positive attributes, depending on the study conditions or objectives related to discharge type, target analytes, and biological pathways of concern, an alternative to polyethylene containers for sample collection may be necessary. One option we currently are investigating is custom-made, Teflon-coated collapsible bags that could be deployed within a container for structural support. Irrespective of the material used for the sampling unit, based on our experience, a prudent analytical/biological control (or reference) sample for this type of study would be a vessel containing clean water that is held either in the field, or under conditions (e.g., temperature) similar to the field, for the duration of the fish deployment and composite sample collection.

Also of concern were compounds detected in grab samples collected at the start and completion of the caged-fish exposures, but not in the composite sample. For example, at both study sites in the Maumee River, 9,10-anthroquinone was detected in all the grab samples but not the composites (Supplemental Data, Tables S2 and S3). This observation may be indicative of chemicals with relatively short aqueous half-lives under conditions in the storage vessel or field, because of either degradation or adsorption to materials in the sampling lines or collection vessel. Further studies are ongoing to ascertain those chemicals (or chemical classes) that may not be amenable to reliable sampling with the autosampler.

In conclusion, the utility of effects-based approaches for detecting pollutant impacts in freshwater and marine systems is increasingly being recognized [33]. For example, the Great Lakes International Joint Commission recently issued a report highlighting the need for effects-based tools to augment traditional chemistry-based approaches for monitoring the occurrence and effects of chemicals of emerging concern in the Great Lakes [34]. To this end, our laboratory, together with collaborators from several other US federal agencies, has worked via resources from the GLRI to develop biology-based approaches suitable for assessing and monitoring chemicals in the near-shore waters of the Great Lakes [15]. An important component of this effort, described in the present study, has involved the development of a simple and comparatively inexpensive caged-fish/water sampling system that could be widely deployed at a variety of different sites in an efficient manner. Although our efforts thus far have focused on the Great Lakes, we feel that the test system described has broad practical utility in many types of systems.

SUPPLEMENTAL DATA

Tables S1–S4.

Figures S1–S2. (963 KB PDF).

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Disclaimer—This article was reviewed and approved in accordance with USEPA guidelines. Mention of specific products or trade names does not indicate endorsement by the USEPA or the federal government, which provided all support for this work.

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Supplementary Information Figures and Tables

Supplementary Information Figure 1. Photographs of auto-sampler construction: (a) primary container with unassembled components, (b) primary container with line ports and battery placement, (c) fully assembled unit with battery, pump, solenoid, and controller placement (parts numbered in Fig. 1c: hard-sided container (1), pump (2), solenoid valve, (3), box (4), check valve (5), battery (6), collapsible container (7), suction strainer (8), compression fitting (9), bulkhead compression union (10), inlet screen fitting (11)), (d) sample collection vessel and coarse filter assembly, and (e) secondary containment unit with physical circuit board layout, including placement of microcontroller, transistors and programmer header (parts numbered in Fig. 1e: box (4), prototyping board (12), microcontroller (13)).

Supplementary Information Figure 2. Wiring diagram for auto-sampler collection system.

Supplementary Information Table 1. Parts list, potential sources and approximate prices (USD) for cage-auto-sampler system.

Supplementary Information Table 2. Contaminants detected in grab and/or composite water samples associated with a 4-d caged fathead minnow (*Pimephales promelas*) exposure at a site near Swan Creek on the Maumee River, OH (Kathy Lee, USGS, personal communication and Lee et al. [16]). An (E) indicates concentration estimate.

Supplementary Information Table 3. Contaminants detected in grab and/or composite water samples associated with a 4-d caged fathead minnow (*Pimephales promelas*) exposure at a site adjacent to the Toledo wastewater treatment plant on the Maumee River, OH (data from Lee et al. [16]). An (E) indicates concentration estimate.

Supplementary Information Table 4. Contaminants detected in Lake Superior water (LSW) from the Duluth EPA lab, in LSW collected by an auto-sampler over a 4-d period into a glass container, and in LSW collected by an auto-sampler over a 4-d period into a cubitainer (Kathy Lee, USGS, personal communication). An (E) indicates concentration estimate.

Supplementary Information Figure 1a.

Supplementary Information Figure 1b.

Supplementary Information Figure 1c.

Supplementary Information Figure 1d.

Supplementary Information Figure 1e.

Supplementary Information Figure 2.

Supplementary Information Table 1. Parts list, potential sources and approximate prices (USD) for cage-autosampler system.

Part-Generic	Part-Specific	Source ¹	Part #	Cost
Battery	Sealed lead-acid battery 12V, 18AH	Interstate Battery ²	BSL1116	\$52.95
	SLA FR Nut/Bolt			
Board spacer	Hex standoff 4-40 nylon 1/2"	Digi-Key ³	1902CK-ND	\$0.64 x 4
Bolts	Machine screw pan Phillips 4-40	Digi-Key	H542-ND	\$0.09 x 8
Box	Box abs 4.7X2.6X1.6" gray	Digi-Key	HM983-ND	\$8.77
Buoy	Heavy duty, orange vinyl, 12"	Aquatic Ecosystems ⁴	BY12	\$5.25
Cable tie	Standard cable tie, 11.8"	Grainger ⁵	3LP22	\$16.39/100
	Standard cable tie, 7.9"	Grainger	6X754	\$6.61/100
Capacitors	Cap film 0.1UF 100VDC axial	Digi-Key	495-3516-ND	Call
	Cap alum 1.0UF 50V 20% radial	Digi-Key	P1196-ND	\$0.30
	Cap alum 10UF 50V 20% radial	Digi-Key	P5178-ND	\$0.20
	Cap alum 470UF 25V 20% radial	Digi-Key	1189-1733-ND	\$0.45
Cement block	12" Standard concrete block	Menards ⁶	1794364	\$3.68
Check valve	PP miniature check valve, 3/16" hose barb, Viton diaphragm	Cole-Parmer ⁷	S-98553-11	\$5.75
Compression fittings	Bulkhead compression union, PP, 1/4" OD	Cole-Parmer	T-06390-10	\$1.90
	Compression fitting, bulkhead union, 316 SS, 1/4" OD	Cole-Parmer	T-31406-13	\$21.00
	Ferrule nut, PP, 1/4"	Cole-Parmer	T-06380-11	\$0.80
Connector header	Conn hdr dual 10POS .100 srt au	Digi-Key	WM50012-10-ND	\$2.53
Container, collapsible	Collapsible container, LDPE with PP Cap, 5 gal	Cole-Parmer	LG-06100-40	\$6.20
Container, hard-sided	Pelican case 1400, black, no foam	CPD Industries ⁸	APP-1400E	\$68.28
Field-effect transistor	MOSFET N-ch 100V 12A to-220AB	Digi-Key	RFP12N10L-ND	\$0.89 x 2
Fish trap	Minnow-Napper	Aquatic Eco-Systems	MT13	\$12.96

Inlet screen fitting	SS Swagelok tube fitting, ¼"Tube OD x ½" male NPT	Swagelok Company ⁹	SS-400-1-8BT	\$16.50
Low-flow pump	Low-flow miniature gear pump with finned-aluminum enclosed motor, 12VDC	Mc-Master Carr	8220K43	\$62.29
Mesh bag	Spat Bag	Aquatic Eco-Systems	SCB4	\$3.50
Microcontroller	IC mcu 8bit 3.5KB flash 18dip	Digi-Key	PIC16F628A-I/P-ND	\$2.08
Prototyping board	Prototyping circuit board for 18 pin PICmicro microcontrollers	microEngineering Labs ¹⁰	PICProto18	\$9.95
Rectifier	Diode gen purpose 1000V 1A DO41	Digi-Key	641-1312-1-ND	\$0.11 x 3
Resistor	Res 4.7K ohm 1/8W 5% cf axial	Digi-Key	CF18JT4K70CT-ND	\$0.09
Rigid tubing	Bev-A-Line V tubing 1/8"ID, ¼"OD, 50-ft roll	Cole-Parmer	T-06491-12	\$57.00
Solenoid valve	3-way solenoid pinch valve; 12VDC, 1/8" ID, ¼" OD	Cole-Parmer	T-98302-46	\$127.00
Steel cable	Vinyl coated cable, 3/16"	Grainger	1DLA9	\$153.25 250ft
Suction strainer	Miniature nylon suction strainer, ½" NPT female, 20 mesh	McMaster-Carr ¹¹	9877K514	\$10.48
Threaded connectors	Oval threaded connector, zinc-plated steel connector, 5/16"	Mc-Master Carr	8947T17	\$2.26
Toggle switch	Switch toggle spst 2A 120V	Digi-Key	CKN1549-ND	\$5.98
Transistor	IC socket .300 18 dip gold	Digi-Key	AE10037-ND	\$1.45
Voltage regulator	IC 5 volt regulator LDO 5V 0.1A TO-92-3	Digi-Key	MC78L05ACPFS-ND	\$0.45

¹ Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

² Interstate Battery, 426 John Ave, Superior, WI 54880

³ Digi-Key, 701 Brooks Avenue South, Thief River Falls, MN 56701

⁴ Aquatic Eco-Systems, 2395 Apopka Blvd., Apopka, FL 32703

⁵ Grainger, 101 N. 46th Ave W., Duluth, MN 55807-2763

⁶ Menards, 503 N 50th Ave W, Duluth, MN 55807

⁷ Cole-Parmer, 625 East Bunker Court, Vernon Hills, IL 60061-1844

⁸ CPD Industries, 4665 State Street, Montclair, CA 91763

⁹ Swagelok Company, Solon, OH 44139

¹⁰ microEngineering Labs, Inc., 2845 Ore Mill Road, STE 4, Colorado Springs CO 80904

¹¹ Mc-Master Carr, P.O. Box 4355, Chicago, IL 60680-4355

Supplementary Information Table 2. Contaminants detected in grab and/or composite water samples associated with a 4-d caged fathead minnow (*Pimephales promelas*) exposure at a site near the Swan Creek site on the Maumee River, OH (Kathy Lee, USGS, personal communication and Lee et al. [16]). An (E) indicates concentration estimate.

Chemical (unfiltered, recoverable, µg/L)	Grab (Deployment)	Grab (Retrieval)	Composite
1-Methylnaphthalene	0.007	<0.04	<0.04
2,6-Dimethylnaphthalene	<0.04	0.006	<0.04
3,4-Dichlorophenyl isocyanate	0.022 (E)	0.157 (E)	0.031 (E)
3-beta-Coprostanol	0.221	<0.2	<0.2
Anthracene	0.005	0.007	0.004
9,10-Anthraquinone	0.028 (E)	0.065 (E)	<0.04
Atrazine	0.072	0.083	0.083
Benzo[a]pyrene	0.01	0.018	0.01
Bisphenol A	<0.04	0.027	0.063
Bromacil	0.031	<0.16	<0.16
Caffeine	0.036	0.071	0.074
Carbazole	0.005	0.012	0.023
Cholesterol	2.79	1.55	1.88
Diethyl phthalate	<0.4	<0.4	0.41
Estrone	<0.0008	0.00029	<0.0008
Fluoranthene	0.036	0.067	0.025
Hexahydrohexamethylcyclopentabenzopyran	0.017	0.023	<0.04
Isophorone	0.009	0.01	0.007
Metolachlor	0.097	0.082	0.095
DEET	0.03	0.032	0.066
4-Nonylphenol	<1.6	<1.6	0.78(E)
<i>p</i> -Cresol	0.013	0.015	<0.08
Phenanthrene	0.021	0.031	0.023
Piperonyl butoxide	<0.08	<0.08	0.091
Prometon	0.013	0.042	0.071
Pyrene	0.024	0.045	0.02
Tetrachloroethylene	<0.16	0.005 (E)	<0.16
Tris(2-butoxyethyl) phosphate	0.135 (E)	0.185 (E)	0.318 (E)
Tris(2-chloroethyl) phosphate	<0.16	<0.16	0.037
Tris(dichloroisopropyl) phosphate	<0.32	0.038	0.038
Tributyl phosphate	0.051	0.021	0.067
Triclosan	<0.32	0.048	<0.32
Triethyl citrate	<0.04	0.506	<0.095
Triphenyl phosphate	<0.08	0.007	0.071

Supplementary Information Table 3. Contaminants detected in grab and/or composite water samples associated with a 4-d caged fathead minnow (*Pimephales promelas*) exposure at a site adjacent to the Toledo wastewater treatment plant on the Maumee River, OH (data from Lee et al. [16]). An (E) indicates concentration estimate

Chemical (unfiltered, recoverable, µg/L)	Grab (Deployment)	Grab (Retrieval)	Composite
1,4-Dichlorobenzene	0.0902	0.0177	0.0276
17-β-Estradiol	<0.0008	<0.0008	0.00116
1-Methylnaphthalene	0.0083	<0.04	0.0163
2,6-Dimethylnaphthalene	<0.04	<0.04	0.0121
2-Methylnaphthalene	<0.04	<0.04	0.0224
3,4-Dichlorophenyl isocyanate	0.1030 (E)	0.0717(E)	0.0581 (E)
3-beta-Coprostanol	2.0765	0.747	1.540
3-Methyl-1H-indole	<0.04	0.0037	<0.04
4-Nonylphenol	0.3030 (E)	0.1700 (E)	0.6170 (E)
4-Nonylphenol diethoxylate	1.4100 (E)	0.5860 (E)	<1.60
4-Nonylphenol monoethoxylate	0.2810 (E)	0.2000 (E)	<1.60
4-tert-Octylphenol	0.0335 (E)	<0.4	0.0318 (E)
4-tert-Octylphenol diethoxylate	0.1070 (E)	0.0386 (E)	0.0421 (E)
4-tert-Octylphenol monoethoxylate	0.0332 (E)	<0.60	0.0450 (E)
5-Methyl-1H-Benzotriazole	0.1720 (E)	0.1280 (E)	0.1470 (E)
9,10-Anthraquinone	0.0418 (E)	0.0251 (E)	<0.04
Acetylhexamethyltetrahydronaphthalene	0.0382	0.0156	<0.04
Anthracene	0.0079	<0.02	<0.02
Atrazine	0.0773	0.119	0.0675
Benzo[a]pyrene	0.0084	<0.02	<0.02
Benzophenone	0.0892	0.0352	0.0684
beta-Sitosterol	0.6100 (E)	<1.6	<1.6
beta-Stigmastanol	<1.6	0.1720 (E)	<1.6
Bisphenol A	0.175	0.0577	0.144
Bromacil	<0.16	0.0496	<0.16
Caffeine	0.22	0.0601	0.114
Carbamazepine	0.0195	0.0104	0.027
Celecoxib	<0.64	0.0342	<0.64
Chloroxylonol	0.0608	0.0154	0.0126
Cholesterol	6.30	4.58	5.85
cis-Androsterone	0.00122	<0.0008	<0.0008
Citalopram	0.0293	0.0088	0.038
Cotinine	0.0850 (E)	0.0364 (E)	0.0967 (E)
DEET	0.0893	0.0591	0.15
Diethyl phthalate	<0.4	<0.4	0.48
Diltiazem	0.0089 (E)	<0.04	0.0094 (E)

Diphenhydramine	0.0261	0.0145	0.0412
Estrone	0.00114	<0.0008	<0.0008
Fluconazole	<0.16	0.0614	0.109
Fluoranthene	0.0317	0.0174	0.0081
Hexahydrohexamethyl cyclopentabenzopyran	0.319	0.11	0.0186
Ibuprofen	0.5560 (E)	0.3020 (E)	0.3680 (E)
Iminostilbene	0.0222	<0.08	<0.08
Isophorone	0.0154	0.0113	0.0076
Lidocaine	0.0375	<0.08	0.0358
Metaxalone	0.0156	<0.08	0.0218
Metolachlor	0.101	0.122	0.0932
Oxycodone	0.0564 (E)	0.2130 (E)	<0.30
p-Cresol	0.0209	0.0198	0.014
Pentachlorophenol	0.0920 (E)	0.0497 (E)	0.0848 (E)
Phenanthrene	0.018	0.0105	0.0149
Phenobarbital	0.04	<0.16	<0.16
Phenytoin	0.0308	0.0146	0.0339
Piperonyl Butoxide	0.0479	0.0084	0.02
Prometon	0.0275	0.0411	0.131
Pyrene	0.0281	0.0139	0.0064
Tetrachloroethene	0.0876 (E)	0.0115 (E)	0.0147 (E)
Tramadol	0.0488	0.0233	0.0705
Tribromomethane	0.0347	<0.16	0.0315
Tributyl phosphate	0.0645	0.0243	0.0689
Triclosan	0.122	0.071	0.0733
Triethyl citrate	0.1320 (E)	<0.04	0.198
Triphenyl phosphate	0.0272	0.0128	0.0985
Tris(2-butoxyethyl) phosphate	2.0800 (E)	0.8370 (E)	1.4800 (E)
Tris(2-chloroethyl) phosphate	0.0711	0.0307	0.0869
Tris(dichloroisopropyl) phosphate	0.0761	0.0379	0.0849
Venlafaxine	<0.04	<0.04	0.0311

Supplementary Information Table 4. Contaminants detected in Lake Superior water (LSW) from the Duluth EPA lab, in LSW collected by an auto-sampler over a 4-d period into a glass container, and in LSW collected by an auto-sampler over a 4-d period into a cubitainer (Kathy Lee, USGS, personal communication). An (E) indicates concentration estimate.

Chemical (unfiltered, recoverable, µg/L)	LSW	Composite Glass	Composite Cubitainer
1,4-Dichlorobenzene	<0.08	<0.08	<0.08
Atrazine	<0.16	<0.16	<0.16
Bromacil	<0.16	<0.16	<.16
Camphor	<0.08	<0.08	0.02
Carbaryl	<0.06	<0.06	<0.30
Carbazole	<0.020	<0.020	<0.020
Chlorpyrifos	<0.12	<0.12	<0.12
DEET	<0.04	<0.04	0.3
Diazinon	<0.32	<0.32	<0.32
Dichlorvos	<0.32	<0.32	<0.32
Metaxyl	<0.16	<0.16	<2.77
Metolachlor	<0.04	<0.04	<0.04
<i>p</i> -Cresol	<0.08	<0.08	0.08
Pentachlorophenol	<1.6	<1.6	<1.6
Piperonyl butoxide	<0.080	<0.080	<0.080
Prometon	<0.16	<0.16	<0.16
11-Ketotestosterone	<0.002	<0.002	<0.002
17- α -Estradiol	<0.0008	<0.0008	<0.0008
17- α -Ethinyl estradiol	<0.0008	<0.0008	<0.0008
17- β -Estradiol	<0.0008	<0.0008	<0.0008
1-Methylnaphthalene	<0.04	<0.04	0.02
2,6-Dimethylnaphthalene	<0.04	<0.04	0.02
2-Ethyl-2-phenylmalonamide	<0.100	<0.100	<0.100
2-Methylnaphthalene	<0.04	<0.04	0.03
3,4-Dichlorophenyl isocyanate	<0.32	<0.32	<0.32
3- β -Coprostanol	<0.20	<0.20	<0.20
3-Methyl-1H-indole	<0.04	<0.04	<0.04
3- <i>tert</i> -Butyl-4-hydroxyanisole	<0.16	<0.16	<0.16
4-Androstene-3,17-dione	<0.0008	<0.0008	<0.0008
4-Cumylphenol	<0.04	<0.04	<0.04
4- <i>n</i> -Octylphenol	<0.02	<0.02	<0.02
4-Nonylphenol	<1.6	<1.6	2.1 (E)
4-Nonylphenol diethoxylate	<1.6	<1.6	<1.6
4-Nonylphenol	<1.6	<1.6	<1.6
4- <i>tert</i> -Octylphenol diethoxylate	<0.2	<0.2	<0.2
4- <i>tert</i> -Octylphenol monoethoxylate	<0.6	<0.6	<0.6

4-tert-Octylphenol	<0.4	<0.4	<0.4
5-Methyl-1H-benzotriazole	<0.32	<0.32	<0.32
9,10-Anthraquinone	<0.04	<0.04	<0.04
Acetophenone	<0.4	<0.4	<0.4
Acetyl hexamethyl tetrahydronaphthalene	<0.04	<0.04	<0.04
Amitriptyline	<0.160	<0.160	<0.160
Anthracene	<0.02	<0.02	<0.02
Antipyrine	<0.320	<0.320	<0.320
BDE congener 47	<0.04	<0.04	<0.04
Benzo[a]pyrene	<0.02	<0.02	<0.02
Benzophenone	<0.08	<0.08	0.17
beta-Sitosterol	<4.8	<4.8	<4.8
beta-Stigmastanol	<3.4	<3.4	<3.4
Bis(2-ethylhexyl) phthalate	<2	<2	<2
Bisphenol A	<0.04	<0.04	0.05
Butalbital	<0.160	<0.160	<0.160
Caffeine	<0.08	<0.08	<0.08
Carbamazepine	<0.160	<0.160	<0.160
Carisoprodol	<0.160	<0.160	<0.160
Celecoxib	<0.640	<0.640	<0.640
Chirald	<0.160	<0.160	<0.160
Chloroxylenol	<0.080	<0.080	<0.080
Chlorpheniramine	<.080	<0.080	<0.080
Cholesterol	<0.20	<0.20	<0.20
cis-Androsterone	<0.0008	<0.0008	<0.0008
Citalopram	<0.080	<0.080	<0.080
Codeine	<0.320	<0.320	<0.320
Cotinine	<0.08	<0.08	<0.08
Dextromethorphan	<0.160	<0.160	<0.160
Diazepam	<0.160	<0.160	<0.160
Diethyl phthalate	<0.4	<0.4	1.2
Dihydrotestosterone	<0.004	<0.004	<0.004
Diltiazem	<0.040	<0.040	<0.040
Diphenhydramine	<0.080	<0.080	<0.080
D-Limonene	<0.16	<0.16	<0.16
Efavirenz	<0.320	<0.320	<0.320
Epitestosterone	<0.002	<0.002	<0.002
Equilenin	<0.002	<0.002	<0.002
Equilin	<0.008	<0.008	<0.008
Estriol	<0.002	<0.002	<0.002
Estrone	<0.0008	<0.0008	<0.0008
Fluconazole	<0.160	<0.160	<0.160

Fluoranthene	<0.02	<0.02	<0.02
Griseofulvin	<0.320	<0.320	<0.320
Hexahydrohexamethyl cyclopentabenzopyran	<0.04	<0.04	<0.04
Hydrocodone	<0.320	<0.320	<0.320
Ibuprofen	<0.640	<0.640	<0.640
Iminostilbene	<0.080	<0.080	<0.080
Indole	<0.16	<0.16	<0.16
Isoborneol	<0.090	<0.090	<0.090
Isophorone	<0.050	<0.050	<0.050
Isopropylbenzene,	<0.04	<0.04	<0.04
Isoquinoline	<0.16	<0.16	<0.16
Lidocaine	<0.080	<0.080	<0.080
Menthol	<0.32	<0.32	<0.32
Meperidine	<0.080	<0.080	<0.080
Meprobamate	<0.320	<0.320	<0.320
Mestranol	<0.0008	<0.0008	<0.0008
Metaxalone	<0.080	<0.080	<0.080
Methadone	<0.080	<0.080	<0.080
Methyl salicylate	<0.08	<0.08	<0.08
Methylphenidate	<0.080	<0.080	<0.080
Naphthalene	<0.02	<0.02	0.03
Norethindrone	<0.0008	<0.0008	<0.0008
Norpropoxyphene	<0.320	<0.320	<0.320
Oxycodone	<0.320	<0.320	<0.320
Pentobarbital	<0.160	<0.160	<0.160
Pentoxifylline	<0.320	<0.320	<0.320
Phenanthrene	<0.02	<0.02	<0.02
Phendimetrazine	<0.040	<0.040	<0.040
Phenobarbital	<0.160	<.0160	<0.160
Phenol	<0.16	<0.16	<0.16
Phenytoin	<0.160	<0.160	<0.160
Primidone	<0.320	<0.320	<0.320
Progesterone	<0.008	<0.008	<0.008
Propofol	<0.040	<0.040	<0.040
Pyrene	<0.02	<0.02	<0.02
Temazepam	<0.320	<0.320	<0.320
Testosterone	<0.0016	<0.0016	<0.0016
Tetrachloroethene	<0.16	<0.16	<0.16
Ticlopidine	<0.080	<0.080	<0.080
Tramadol	<0.040	<0.040	<0.040
trans-Diethylstilbestrol	<0.0008	<0.0008	<0.0008
Tribromomethane	<0.16	<0.16	<0.16

Tributyl phosphate	<0.064	<0.064	0.122 (E)
Triclosan	<0.32	<0.32	<0.32
Triethyl citrate	<0.04	<0.04	0.05
Triphenyl phosphate	<0.08	<0.08	0.05
Tris(2-butoxyethyl) phosphate	<0.64	<0.64	0.30 (E)
Tris(2-chloroethyl) phosphate	<0.16	<0.16	<0.16
Tris(dichloroisopropyl) phosphate	<0.32	<0.32	<0.32
Venlafaxine	<0.040	<0.040	<0.040
Verapamil	<0.080	<0.080	<0.080