



U.S. Fish and Wildlife Service

Assessment of general health of fishes collected at selected sites within the Great Lakes Basin in 2012

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**ASSESSMENT OF GENERAL HEALTH OF FISHES COLLECTED AT
SELECTED SITES WITHIN THE GREAT LAKES BASIN IN 2012**

***U.S. GEOLOGICAL SURVEY'S CONTRIBUTION TO THE
FWS GLRI EARLY WARNING PROGRAM***

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INTRODUCTION

During the past decade, there has been a substantive increase in the detection of “emerging contaminants”, defined as a new substance, chemical, or metabolite in the environment; or a legacy substance with a newly expanded distribution, altered release, or a newly recognized effect (such as endocrine disruption). Emerging contaminants include substances such as biogenic hormones (human and animal), brominated flame retardants, pharmaceuticals, personal care products, plasticizers, current use pesticides, detergents, and nanoparticles. These contaminants are frequently not regulated or inadequately regulated by state or Federal water quality programs. Information about the toxicity of these substances to fish and wildlife resources is generally limited, compared to more highly regulated contaminants, and some classes have been shown to cause effects (for example feminization of male fish, immunomodulation) that are not evaluated via traditional toxicity testing protocols. As a result, these compounds may pose a substantial, but currently poorly documented threat to aquatic ecosystems. Failure to identify and understand the impacts of these emerging contaminants on fish and wildlife resources may result in deleterious impacts to Great Lakes resources that can result in adverse ecological, economic and recreational consequences.

The U. S. Fish and Wildlife Service received funding through the Great Lakes Restoration Initiative (GLRI) for an Early Warning Program to detect and identify emerging contaminants and to evaluate the effects of these contaminants on fish and wildlife. The U.S. Geological Survey (WV Cooperative Fish and Wildlife Research Unit and National Fish Health Research Laboratory, Leetown Science Center) developed and implemented a biological effects monitoring protocol to assist in this program. Fish collections and measurements of biomarkers of exposure in Fall 2010 and Spring 2011 occurred at individual sites within select Areas of Concern (AOCs). They provided an assessment of the utility of the suite of biomarkers and also identified sites for more in-depth analyses. Selected areas are characterized as areas with known emerging contaminants, sensitive or listed species, areas downstream from municipal wastewater discharges or receiving waters for industrial facilities, and/or areas susceptible to agricultural or urban contamination, or harbors or ports. The results of the 2010-2011 studies were summarized in Blazer et al. 2014 a, b, c; Braham et al. in review and Blazer et al. in review.

In 2012, the following locations were selected (Table 1):

Maumee River (Maumee River AOC) – multiple sites
Detroit River (Detroit River AOC)
Irondequoit Creek and Long Pond (Rochester Embayment AOC)
Fox River (Lower Fox River/Green Bay AOC) – upstream and downstream sites
St. Louis River (St Louis River and Bay AOC) – multiple sites
River Raisin (River Raisin AOC) – upstream and downstream sites

Table 1. Summary of 2012 sampling sites including latitude and longitude (decimal degrees, NAD 83 GCS)

SiteID	Area Of Concern	<u>Stream Name</u>	<u>Longitude</u>	<u>Latitude</u>
GL14A	Rochester Embayment	Irondequoit Embayment	-77.525	43.178
GL14B	Rochester Embayment	Long Pond Embayment	-77.696	43.289
GL16A	St. Louis River	St. Louis River	-92.115	46.759
GL16B	St. Louis River	Superior Bay	-92.020	46.705
GL2D	St. Louis River	St. Louis upper estuary	-92.143	46.727
GL2E	St. Louis River	St. Louis, upper AOC	-92.293	46.665
GL2F	Reference			
GL3	Maumee River	Swan Creek	-83.532	41.648
GL13A	Maumee River	Maumee River	-83.475	41.689
GL13A1F	Maumee River	Maumee River		
GL13A2F	Maumee River	Maumee River		
GL13A3F	Maumee River	Maumee River		
GL13B	Reference	Maumee River	-83.849	41.423
GL4	Detroit River	Trenton Channel (Detroit River)	-83.159	42.177
GL15A	Fox River/Lower Green Bay	Fox River	-88.005	44.539
GL15B	Reference	Chain O' Lakes (Crystal River)	-89.170	44.314
GL17	River Raisin	River Raisin	-83.381	41.912
GL17A	River Raisin	River Raisin	-83.366	41.902
GL18	Reference	River Raisin	-83.432	41.924

METHODS

Field Methods

Attempts were made by FWS to collect 20 mature fish (10 males and 10 females) of each species at each site by electrofishing, minnow traps or fyke nets. While the 2010-2011 study focused on species comparison, the 2012 study focused more on comparing one species at multiple sites within a river system. The species of choice were either brown bullhead *Ameiurus nebulosus* or white sucker *Catostomus commersoni* (benthic species) and either largemouth bass *Micropterus salmoides* or smallmouth bass *M. dolomieu* (pelagic species). Spring sampling was conducted during the prespawn period. Fish were euthanized with a lethal dose of tricane methanesulfonate (MS-222), weighed and measured. A blood sample was taken from each fish using heparinized syringes, as subsequently described. Blood smears (two for each fish) were made, air-dried and fixed in methanol to be used for micronuclei and other nuclear abnormalities (genotoxic analyses). The remaining blood was stored on wet ice until centrifuged and plasma aliquoted into two cryovials. Plasma samples were stored at -80°C until analyzed. A comprehensive necropsy-based assessment was completed on all fish collected (Goede and Barton 1990; Smith et al. 2002; Rafferty and Grazio 2007). The liver and gonads were

removed, any abnormalities noted, and then weighed in order to calculate hepatosomatic and gonadosomatic indices. Liver weights were not recorded for white suckers as the liver is intertwined with the intestine. These necropsy-based and morphometric observations have been used in numerous monitoring programs and, while not as sensitive as histopathological or molecular analyses, do provide important information at the organism level (Goede and Barton 1990; Fournie et al. 1996; Schmitt and Dethloff, eds 2000). Small pieces of liver, spleen, anterior kidney, and gonad were placed into RNAlater for subsequent molecular analyses. Pieces of gill, liver, anterior and posterior kidney, spleen, gonad, thyroid and any lesions or abnormalities were placed in Z-fix for subsequent histological analyses. Otoliths were removed for aging.

Laboratory Analyses

Plasma Analyses

Plasma samples were analyzed for vitellogenin, reproductive and thyroid hormones. Vitellogenin, an egg-yolk precursor protein produced in response to estrogen and estrogen agonists, is normally found only in the serum of adult female oviparous vertebrates, but it can be induced in males and immature females by estrogenic compounds or estrogen mimics. The presence of plasma vitellogenin in male fishes is a well accepted bioindicator of exposure to estrogenic EDCs (Tyler et al. 1996; Denslow et al. 1999; Kime et al. 1999; Cheek et al. 2001). Conversely, decreased levels of circulating vitellogenin in female fish may result in lowered egg quality (Wheeler et al. 2005; Miller et al. 2007). Vitellogenin is quantified using an enzyme-linked immunosorbent assay (ELISA). The assay will use monoclonal antibodies developed for the specific species and will follow the methodology described by Denslow et al. (1996, 1997). The ELISA assay used can detect between 10 and 100 ng of VTG per well, resulting in a sensitivity of about 0.001 mg/ml.

Microscopic Pathology

The fixed tissue samples were trimmed in, routinely processed and embedded into paraffin. Blocks were sectioned at 6 μ m and routinely stained with hematoxylin and eosin (H&E). One slide, containing pieces of liver, spleen and anterior kidney, was also stained using the Perl' Prussian blue method for iron to differentiate pigments within macrophage aggregates and hepatocytes. The method stains hemosiderin, an iron-containing protein, while melanin remains black and ceroid/lipofuscin remains yellowish-brown. Slides were examined for any abnormalities at the microscopic level. Sections of gonads were used to confirm sex, stage of development, percent atretic eggs, and any other abnormalities such as intersex, fibrosis, abnormal yolk accumulation, ceroid/lipofuscin accumulations, Sertoli cell proliferation and neoplasia as described by Blazer (2002), Leino et al. (2005) and Dietrich and Krieger (2009). Numerous histopathological changes in other tissues have been used as biomarkers of exposure and/or environmental stress (Myers and Fournie 2002; Stentiford et al. 2003; Au 2004; Lyons et al 2004). These include proliferative, preneoplastic and neoplastic changes in the skin and liver (Blazer et al. 2007; Blazer et al. 2009a,b), accumulation of ceroid/lipofuscin and/or macrophage aggregates (Fournie et al. 2001; Raldùa et al. 2008), gill lesions (Costa et al. 2009) and parasite infections, both type and severity (Lafferty 1997; Schmidt et al. 2003; Marcogliese et al. 2009).

Molecular endpoints

The genetic information of all living organisms is blueprinted in the form of DNA. Within the DNA of all organisms are the genes that contain the information necessary to build proteins. These proteins are ultimately the functional units of life and are necessary for enzymatic activity and are the structural components of tissues. In order for the information encoded in genes to be used to produce proteins they must first be turned on (activated). All genes are not active all of the time. When a gene is turned on messenger RNA (mRNA) molecules are produced (transcription) and are used as an intermediate, and transient blueprint of the DNA from which proteins are directly made (translation). A transcriptome is essentially the population of mRNA molecules present in a cell or tissue and is a direct reflection of what genes are turned on at the time of fixation and reflect certain conditions (contaminant exposure, physiological stress conditions, life-stage, etc). Measuring gene expression can be used to determine the physiological condition/health of an organism. In order to do so, however, one must know the gene sequences or barcode of the genes of interest. The sequences for most genes of interest in the species used as sentinel fish in this project (smallmouth bass and white suckers) were unknown at the initiation of the studies. We therefore developed transcriptome databases using new gene sequencing technologies. Once the gene sequences were identified, markers could be developed to quantify mRNA molecules in liver tissue of the individual fish. Genes may be turned off and on in response to normal changes in season and water temperature, but also to factors such as environmental stressors and contaminant exposure. Certain genes are only turned on under certain specific circumstances and can be used as an indication of exposure to contaminants. Thus the measurement of mRNA (gene expression) can be used as a biological indicator of contaminant exposure or other stressors.

By definition, endocrine disrupters modulate aspects of endocrine homeostasis. These effects, in general, occur at the level of gene expression given that the *modus operandi* of many EDCs is that of a functional agonist or antagonist of nuclear hormone receptors. Quantitative PCR has demonstrated that mRNA expression for nuclear hormone receptors such as estrogen, androgen, thyroid and glucocorticoid receptors, zona radiata protein, proteins involved in steroid synthesis such as CYP17, CYP19, 11 β -HSD, detoxification enzymes and factors important in immune regulation and disease resistance such as TGF- β and hepcidin are modulated by exposure to emerging contaminants (Arukwe and Goksøyr 2003; Filby et al. 2007; Ankley et al. 2009; Swedenborg et al. 2009). Quantitative measurement of multiple genes is advantageous in survey studies particularly when the presence of individual chemicals and complex mixtures are unknown. Measurement of a suite of genes also adds a level of rigor to the analysis. The Nanostring approach to evaluating gene expression is similar to qPCR in regards to sensitivity and specificity; however, multiple genes can be examined per sample thus reducing the cost per gene. This approach in some respects is a hybrid of qPCR and microarray. We are currently assessing this methodology in terms of the qPCR methods with smallmouth bass tissues.

Given the number of genes activated by endocrine disruptors and the uncertainty of which chemicals may be present at each site, a Nanostring multiplex assay was developed to assess 25-50 genes of interest per sample. Prior to the execution of this assay a transcriptome database was generated for brown bullheads and smallmouth bass. Fish were exposed to EDCs that affect the estrogen and thyroid axes in addition to aryl hydrocarbon receptor agonist benzo(A)pyrene. Liver, gonad, anterior kidney, and spleen tissue from all fish and treatments were pooled by species. Total RNA was extracted and shipped to Cofactor Genomic for 50 million sequence reads, BLASTX Gene

annotation and non-mapping reads assembly. Genes diagnostic of EDC exposure, carcinogenesis and other affects were identified and selected for Nanostring probe construction. Development of this suite of gene expression endpoint will further allow the evaluation of diagnostic genes on a seasonal and multi-species basis. Tables 2, 3 and 4 illustrate the genes chosen for analysis in bass, white sucker and brown bullhead livers.

Table 2. Genes Used for Nanostring Analysis of Smallmouth and Largemouth Bass Liver Samples.

Gene Name	
Ribosomal Protein L8	Glucocorticoid Receptor
β -actin	Catalase
Elongation Factor 1 α	Insulin-like Growth Factor 1
Hypoxanthine Phosphoribosyltransferase 1	CYP19A1A (Aromatase)
Tata Box Binding Protein	CYP1A
Eukaryotic Translation Initiation Factor 3D	CYP3A
RBMX2	CYP17
Estrogen Receptor β 1	Transforming Growth Factor Receptor 1
Estrogen Receptor β 2	Transforming Growth Factor β
Estrogen Receptor α	Type II Deiodinase
Vitellogenin	Type I Deiodinase
Androgen Receptor	Follicle Stimulating Hormone Receptor
Choriogenin	Epidermal Growth Factor Receptor
Thyroid Hormone Receptor β	β Catenin
Thyroid Hormone Receptor α	Ferritin
Aryl Hydrocarbon Receptor	Apolipoprotein A1
Warm Temperature Acclimation Protein 65	Phosphoenolpyruvate Carboxykinase
Heat Shock Protein 90 α	Metallothionein
Heat Shock Protein 71	3 β -Hydroxysteroid Dehydrogenase
Heat Shock Protein 70	17- β Hydroxysteroid Dehydrogenase
Glutathione Peroxidase 1	Epoxide Hydrolase 1
Glutathione S-Transferase	Arginase
Glucokinase	Cystenin-Rich Protein
Hepcidin 1	Fibroblast Growth Factor
Hepcidin 2	Superoxide Dismutase

Table 3. Genes Used for Nanostring Analysis of White Sucker Liver Samples.

Gene Name	
11 β Hydroxysteroid Dehydrogenase	Hepatitis B PreC Antigen
17 β Hydroxysteroid Dehydrogenase	Hepcidin
Androgen Receptor	Hypoxia-inducible factor
Apolipoprotein A1	Insulin-Like Growth Factor
Aryl Hydrocarbon Receptor	Keratin 8
Catalase	Metallothionein
CTNNB1	MUS81
CYP11C1	Peroxisome Proliferator-activated Receptor
CYP1B2	Phosphoenolpyruvate Carboxykinase
CYP3A	Proliferating Cell Nuclear Antigen
Elongation Factor 1 α	RBMX
Epidermal Growth Factor	Ribosomal Protein L8
Epoxide Hydrolase	Steroidogenic Acute Regulatory Protein
Estrogen Receptor α	Superoxide Dismutase
Estrogen Receptor β	TGF β 1A
Eukaryotic Translation Initiation Factor 3D	TGF β Receptor 2
Ferritin	Thyroid Hormone Receptor α
Fibroblast Growth Factor	Thyroid Hormone Receptor β
Follicle Stimulating Hormone Receptor	Trypsin
Glucocorticoid Receptor	Tumor Necrosis Factor
Glutathione Peroxidase 1	Tumor Protein p53
Glutathione-S-Transferase	Type II Deiodinase
Granulin	Vitellogenin
Heat Shock Proteins 70	V-Ki-Ras2
Heat Shock Protein 90	ZSH

Table 4. Genes Used for Nanostring Analysis of Brown Bullhead Liver Samples.

Gene Name	
Elongation Factor 1 α	Glutathione Peroxidase
3 β Hydroxysteroid Dehydrogenase	Granulin 1
17 β Hydroxysteroid Dehydrogenase	Granulin Precursor b
Androgen Receptor	Heat Shock Protein 70
Apolipoprotein A1	Heat Shock Protein 90
Arginase	Hepcidin
Aryl Hydrocarbon Receptor	Hypoxanthine Phosphoribosyltransferase 1

Catalase	Hypoxia-inducible Factor
Catenin β	Insulin-like Growth Factor
Cytochrome C Oxidase	Interferon
CYP17	Interleukin
CYP19A1A (Aromatase)	MYXO Parasite
CYP1A	Peroxisome Proliferator-activated Receptor
CYP3A	Proliferation Cell Nuclear Antigen
Epidermal Growth Factor Receptor	RBMX2
Epoxide Hydrolase	Ribosomal Protein L8
Estrogen Receptor α	Superoxide Dismutase
Estrogen Receptor β	Thyroid Hormone Receptor α
Eukaryotic Translation Initiation Factor 3D	Thyroid Hormone Receptor β
Ferritin	Transforming Growth Factor β 1
Fibroblast Growth Factor	Tumor Protein p53
Follicle Stimulating Hormone Receptor	Tumor Protein p73
Gluathione S-Transferase	Vitellogenin
Glucocorticoid Receptor	V-K-Ras
Glucokinase	Warm Temperature Acclimation Protein 65

Erythrocyte Analysis

Blood smears were prepared for all individuals collected by methods described by Belpaeme et al. (1996). Briefly, peripheral blood was collected from the caudal vein into a heparinized syringe. One drop was then smeared onto a pre-cleaned glass slide using a spreader slide at an angle and allowed to dry. The smear was fixed with absolute methanol for 10 minutes. Slides were stained with Giemsa solution (Fluka Analytical, Sigma-Aldrich, St. Louis, Mo. 1:12:2 (w/w/w) in glycerol/methanol 5:24 (v/v)) for 45 minutes, followed by two 45-minute distilled water baths. Stained slides were cover-slipped and evaluated under light microscopy at 600x magnification. Scoring is consistent with methods described in Carrasco et al. (1990). A minimum of 200 erythrocytes were scored at 5 stratified random locations on the slide such that a minimum of 1000 erythrocytes were evaluated for the presence of micronuclei (defined as a round cytoplasmic intrusion having a diameter one-tenth to one-third of the primary nucleus), as well as additional nuclear abnormalities of notched, lobed, blebbed and binucleated cells.

RESULTS

Collection Summary

Four species of fish, white sucker, brown bullhead, largemouth bass and smallmouth bass were collected in the spring and fall of 2012. Fish were collected at 11 sites in Spring 2012 between 14-April and 12-June. Only nine brown bullhead and 13 smallmouth bass were collected at two of the sites within the Maumee AOC. In Fall 2012 smallmouth bass were collected at three sites within the

St. Louis River AOC between 25 and 26-September and at four sites within the Maumee River AOC between 17 and 18-September (Table 5, Appendix 1).

Table 5. Fish Collected for Fish Health Assessments

<i>Site</i>	<i>Date</i>	<i>Brown Bullhead</i>	<i>White Sucker</i>	<i>Largemouth Bass</i>	<i>Smallmouth Bass</i>
Rochester Embayment AOC					
GL14A Irondequoit Bay	Spring 2012	11 F 9 M			
GL14B Long Pond	Spring 2012	5 F 15 M		11 F 9 M	
St. Louis River AOC					
GL16 A (same as GL2A and B)	Spring 2012		14 F 6 M		3 F 4 M
GL16B (same as GL2C)	Spring 2012		11 F 2 M		
GL2D Middle Estuary	Fall 2012				13 F 7 M
GL2E Upper St. Louis	Fall 2012				10 F 10 M
GL2F Above St. Louis AOC	Fall 2012				12 F 8 M
Maumee AOC					
GL3 Swan Creek	Fall 2012			11 F 9 M	
GL13A Maumee River	Spring 2012	5 F 4 M			
GL13A-1F	Fall 2012			15 F 5 M	
GL13A -2F	Fall 2012			11 F 9 M	
GL13A-3F	Fall 2012			6 F 14 M	
GL13B Maumee River	Spring 2012				4 F 9 M
Detroit River AOC					
GL4 Trenton Channel	Spring 2012	7 F 13 M		13 F 7 M	
River Raisin AOC					
GL17	Spring 2012			6 F 14 M	19 F 1 M
GL18 Waterloo	Spring 2012				12 F 8 M
Lower Fox River and Green Bay AOC					
GL15A	Spring 2012				10 F 10 M
GL15B	Spring 2012				10 F 8 M
Totals		69	33	140	158

WHITE SUCKER

White sucker were only collected at two sites within the St. Louis River AOC in Spring 2012. Twenty fish (14 F and 6 M) were collected in the St. Louis Bay (GL16A) and 13 (11 F and 2 M) in Superior Bay (GL16B). Suckers collected in Superior Bay were younger and smaller than those in St. Louis Bay (Table 6).

Table 6. Morphometric Parameters of White Sucker Collected in 2012¹

Site	Season/Year	n	Length (mm)	Weight (gm)	Age (yr)	Condition Factor ²
St. Louis GL16A	Spring 2012	20	434 ± 9.8	963 ± 62.9	9.0 ± 0.61	1.15 ± 0.04
GL16B	Spring 2012	13	401 ± 13.2	778 ± 78.8	7.4 ± 0.65	1.16 ± 0.02

¹Data are presented as mean ± standard error.

The most commonly observed gross lesions on white sucker were the slightly raised, pale mucoid lesions, melanistic areas and raised papillomatous lesions on the body surface, fins and lips. Slightly raised mucoid lesions and melanistic areas were more common in sucker captured in St. Louis Bay, while raised papillomatous lesions were more common in those captured in Superior Bay, although not significantly different (Table 7).

Table 7. Percentage of White Sucker with Eye or Skin Lesions

Site	n	Percentage of Individuals with Abnormality					
		Eye ¹	Red Eroded ²	Parasites ³	Melanistic ⁴	Raised ⁵	Mucoid ⁶
St. Louis Bay	20	5	0	15	25	0	25
Superior Bay	13	0	15	15	15	15	0

¹Eye abnormalities include opaque, missing or otherwise abnormal eyes.

²Includes eroded, reddened or raised reddened areas and wounds.

³Parasites included leeches, copepods and trematodes (black spot, grubs).

⁴Melanistic areas are non-raised black areas on body surface, lips or fins.

⁵Raised lesions on body surface, fins and lips that grossly appeared as tumors.

⁶Slightly raised pale lesions on body surface or fins.

BROWN BULLHEAD

Brown bullhead were collected at four sites in Spring 2012 (Table 8). These included two sites within the Rochester Embayment AOC, Irondequoit Bay (GL14A) and Long Pond (GL14B), the Detroit River, Trenton Channel (GL4) and Maumee River (GL13A).

Table 8. Morphometric Parameters of Brown Bullhead Collected in 2012¹.

Site	Season/Year	N	Length (mm)	Weight (gm)	Age (yr)	Condition Factor ²
Detroit GL4	Spring 2012	20	306 ± 6.9	452 ± 41.5	6.6 ± 0.6	1.50 ± 0.04
Maumee GL13A	Spring 2012	9	281 ± 15.2	362 ± 65.9	3.9 ± 0.6	1.50 ± 0.04
Irondequoit Bay GL14A	Spring 2012	20	306 ± 15.6	402 ± 31.1	8.0 ± 0.6	1.35 ± 0.05
Long Pond GL14B	Spring 2012	20	316 ± 7.0	379 ± 26.7	7.5 ± 0.4	1.16 ± 0.02

¹Data are presented as mean ± standard error.

Lesions observed on brown bullhead included melanistic areas, raised dark lesions on the fins and body surface, slightly raised to large raised lesions on lips and body surface. Barbel abnormalities were also evaluated in brown bullhead. These included missing, shortened, deformed and knobbed barbels (with small raised areas). The Detroit River had a higher number of fish with raised lesions and melanistic areas, while the Maumee had a higher prevalence of eye abnormalities and red or eroded skin lesions (Table 9).

Table 9. Percentage of Brown Bullhead with Eye, Barbel or Skin Lesions

Site	N	Barbel Abnormalities ¹		Skin/Fin Lesions			
		Raised Areas	Other	Eye ²	Red Eroded ³	Melanistic ⁴	Raised ⁵
Irondequoit	20	30	30	0	5	5	20
Long Point	20	20	15	0	0	20	20
Detroit	20	25	35	5	20	25	40
Maumee	9	0	11	22	30	11	20

¹Barbel abnormalities identified as other included missing, shortened and deformed.

²Eye abnormalities include opaque, missing or otherwise abnormal eyes.

³Includes eroded, reddened or raised reddened areas and wounds.

⁴Melanistic areas are non-raised black areas on body surface, lips or fins.

⁵Raised lesions on body surface, fins and lips that grossly appeared as tumors.

Largemouth and Smallmouth Bass

In 2012, the majority of fish collected were bass (see Table 5). In the spring largemouth bass were collected at one site on the Detroit, Maumee and Raisin rivers as well as in Long Pond in the Rochester Embayment AOC. In the fall largemouth bass were collected at four sites within the Maumee River drainage (Table 10).

Smallmouth bass were collected at upstream and downstream sites on the Fox and Raisin rivers in the spring and at three sites along the St. Louis River in the fall (Table 10).

Table 10. Morphometric Parameters of Largemouth and Smallmouth Bass Collected in 2012¹

Site	Season/Year	N	Length (mm)	Weight (gm)	Age (yr)	Condition Factor ²
<i>Largemouth Bass</i>						
Detroit GL4	Spring 2012	20	363 ± 11.9	865 ± 82.7	5.9 ± 0.4	1.68 ± 0.03
Maumee GL13A	Spring 2012	20	303 ± 7.6	481 ± 44.3	4.5 ± 0.1	1.63 ± 0.03
Long Pond GL14B	Spring 2012	20	312 ± 9.6	551 ± 67.1	5.4 ± 0.3	1.67 ± 0.04
River Raisin GL17	Spring 2012	20	389 ± 7.0	913 ± 49.6	6.5 ± 0.2	1.52 ± 0.04
Swan GL3	Fall 2012	20	291 ± 8.3	392 ± 33.8	3.0 ± 0.3	1.55 ± 0.05
Maumee GL13A1F	Fall 2012	20	329 ± 11.9	614 ± 68.1	3.9 ± 0.3	1.59 ± 0.03
Maumee GL13A2F	Fall 2012	20	318 ± 10.6	556 ± 61.3	3.2 ± 0.3	1.59 ± 0.03
Maumee GL13A3F	Fall 2012	20	280 ± 4.0	335 ± 15.9	2.4 ± 0.1	1.50 ± 0.03
<i>Smallmouth Bass</i>						
Fox GL15A	Spring 2012	20	396 ± 15.2	1123 ± 123.8	6.2 ± 0.3	1.64 ± 0.04
Fox GL15B	Spring 2012	18	308 ± 11.9	413 ± 69.9	6.0 ± 0.4	1.27 ± 0.02
Maumee GL13B	Spring 2012	13	416 ± 12.2	1168 ± 99.7	6.5 ± 0.2	1.58 ± 0.02
River Raisin GL17	Spring 2012	20	347 ± 11.4	641 ± 61.1	6.0 ± 0.4	1.45 ± 0.03
Waterloo GL18	Spring 2012	20	348 ± 12.1	559 ± 57.0	8.5 ± 0.6	1.27 ± 0.03
St. Louis GL2D	Fall 2012	20	416 ± 17.2	1234 ± 132.5	7.6 ± 0.5	1.56 ± 0.06
St. Louis GL2E	Fall 2012	20	346 ± 11.7	613 ± 77.2	7.4 ± 0.3	1.34 ± 0.03
St. Louis GL2F	Fall 2012	20	341 ± 7.1	623 ± 40.2	6.0 ± 0.2	1.50 ± 0.02

¹Data are presented as mean ± standard error.

²Condition factor (KtL) is calculated as ((body weight – gonad weight)/length³) X 10

A variety of grossly observable abnormalities were noted externally and internally. The most common external lesions observed in bass were red raised or eroded areas, melanistic areas and raised areas which included fairly smooth, slightly raised, mucoid lesions. External parasites such as leeches, grubs (cysts containing trematode metacercariae) and black spots (melanocyte accumulation around trematode metacercariae) were also observed (Table 11). For smallmouth bass, upstream sites had higher observable external parasites than downstream sites, while the downstream sites had a higher prevalence of raised skin lesions.

Table 11. Percentage of Smallmouth or Largemouth Bass with Eye or Skin Lesions

Site	SiteID	n	Percentage of Individuals with Abnormality				
			Eye ¹	Red or Eroded ²	Parasites ³	Melanistic ⁴	Raised ⁵
Largemouth Bass Spring							
Detroit	GL 4	20	5	10	25	10	10
Maumee	GL13A	20	10	5	30	0	15
Long Pond	GL14B	20	5	5	20	5	5
River Raisin	GL17	20	0	15	5	35	15
Largemouth Bass Fall							
Swan	GL 3	20	55	0	35	0	5
Maumee	GL13F1	20	15	5	15	5	5
	GL13F2	20	80	10	5	0	20
	GL13F3	20	10	0	15	0	5
Smallmouth Bass Spring							
Maumee	GL13B	13	23	15	23	0	8
Fox AOC	GL15A	20	0	5	10	5	15
Fox upstream	GL15B	18	0	0	100	0	0
River Raisin	GL17	20	10	15	0	0	25
Raisin upstream	GL18	20	25	25	45	0	10
Smallmouth Bass Fall							
St. Louis	GL2D	20	15	15	35	0	25
St. Louis	GL2E	20	25	20	25	0	5
St. Louis	GL2F	20	10	5	65	0	0

¹Eye abnormalities include opaque, missing or otherwise abnormal eyes.

²Includes eroded, reddened or raised reddened areas and wounds.

³Parasites included leeches, copepods and trematodes (black spot, grubs).

⁴Melanistic areas are non-raised black areas on body surface, lips or fins.

⁵Raised lesions on body surface, fins and lips.

Molecular Endpoints

Gene Expression Analysis

Liver samples of smallmouth bass, largemouth bass, brown bullhead and white sucker collected in 2012 have been analyzed (Table 12). The gene expression analyses and interpretation is the research of a PhD student, Cassidy Hahn, and will be presented in her dissertation and resultant journal articles.

Table 12. Smallmouth bass, largemouth bass, brown bullhead, and white sucker liver samples processed with Nanostring analysis.

Site ID	Species	Area of Concern/Site Name	Date	N
GL16A (same as GL2A & B)	<i>M. dolomieu</i>	St. Louis River – WLSSD Bay	Spring 2012	7
GL2D	<i>M. dolomieu</i>	St. Louis River – Middle Estuary	Fall 2012	20
GL2E	<i>M. dolomieu</i>	St. Louis River – Upper St. Louis	Fall 2012	20
GL2F	<i>M. dolomieu</i>	St. Louis River – Above St. Louis AOC	Fall 2012	20
GL15A	<i>M. dolomieu</i>	Green Bay	Spring 2012	20
GL15B	<i>M. dolomieu</i>	Green Bay	Spring 2012	18
GL13B	<i>M. dolomieu</i>	Maumee – Maumee River	Spring 2012	13
GL17	<i>M. dolomieu</i>	Raisin River	Spring 2012	26
GL18	<i>M. dolomieu</i>	Raisin River - Waterlou	Spring 2012	20
GL14B	<i>M. salmoides</i>	Rochester - Irondequoit Bay	Spring 2012	20
GL3	<i>M. salmoides</i>	Maumee - Swan Creek	Fall 2012	20
GL13A	<i>M. salmoides</i>	Maumee - Maumee River	Spring 2012	20
GL13A1F	<i>M. salmoides</i>	Maumee - Maumee River	Fall 2012	20
GL13A2F	<i>M. salmoides</i>	Maumee - Maumee River	Fall 2012	20
GL13A3F	<i>M. salmoides</i>	Maumee - Maumee River	Fall 2012	20
GL4	<i>M. salmoides</i>	Detroit River – Trenton Channel	Spring 2012	20
GL17	<i>M. salmoides</i>	Raisin River	Spring 2012	20
GL14A	<i>A. nebulosus</i>	Rochester - Long Pond	Spring 2012	20
GL14B	<i>A. nebulosus</i>	Rochester - Irondequoit Bay	Spring 2012	20
GL13A1F	<i>A. nebulosus</i>	Maumee – Maumee River	Fall 2012	20
GL04	<i>A. nebulosus</i>	Detroit River – Trenton Channel	Spring 2012	20
GL2	<i>C. commersoni</i>	St. Louis River	Fall 2012	14
GL16A (same as GL2A & B)	<i>C. commersoni</i>	St. Louis River - WLSSD	Spring 2012	20
GL16B	<i>C. commersoni</i>	St. Louis River - Superior Bay	Spring 2012	13
			Total	451

Plasma Analyses, Micronuclei, Other Nuclear Abnormalities and Microscopic Pathology

The sampling plan initiated in 2012 allows for comparisons of sites along a gradient of particular rivers (Maumee and St. Louis) or of upstream and downstream sites (Fox and Raisin) and to compare fish health among these sites in association with landuse, point and nonpoint chemical sources and available chemical data. A journal article comparing the health of largemouth bass collected at four sites within the Maumee River AOC in Fall 2012 is in review. A journal article comparing the health of smallmouth bass collected at three sites along the St. Louis River in Fall 2012 is in preparation. A third manuscript comparing the health of bass collected at upstream and downstream sites on the Fox and Raisin rivers in Spring 2012 is also in preparation.

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Appendix 1 Site Key

	Site Designation	City - River
GL14A	Rochester	- Long Pong
GL14B	Rochester	--Irondequoit Bay
GL16A	St, Louis	Bay
GL16B	Superior	Bay
GL13A	Maumee	
GL13B	Maumee	
GL 4	Detroit	- Detroit River
GL17A	River Raisin	
GL17B	River Raisin	
GL15A	Fox River	- Green Bay
GL15B	Fox River	- Green Bay

Appendix 2 White Sucker (WHS) Data

WHITE SUCKER (WHS) DATA

Fish #	Date	Species	Sex	Age	T.L. (mm)	Wt (gms)	Kil	Skin/Fin Lesions			GI/Cross Lesions			Eyes		Liver Cross		Spleen Cross		Conad Wt (gms)	GSI		
								mel.sp	parasites	red/erod	w.spots	grubs	eroded	Other	opaque	Other	w.spots	Other	gen discolor			Other	w.spots
GL16A-21	5/22/2012	WHS	F	10	466	999	0.97																
GL16A-22	5/22/2012	WHS	F	9	444	1045	0.98																
GL16A-23	5/22/2012	WHS	F	8	438	869	0.89																
GL16A-24	5/22/2012	WHS	F	8	460	1071	1.08																
GL16A-25	5/22/2012	WHS	F	9	467	1135	1.10																
GL16A-26	5/22/2012	WHS	F	8	476	1197	0.97																
GL16A-27	5/22/2012	WHS	F	8	488	1400	1.05																
GL16A-28	5/22/2012	WHS	F	6	445	1082	1.09																
GL16A-29	5/22/2012	WHS	M	7	376	552	0.99																
GL16A-30	5/22/2012	WHS	M	10	437	855	1.00																
GL16A-31	5/22/2012	WHS	M	7	452	978	1.03																
GL16A-32	5/22/2012	WHS	M	17	442	916	1.03																
GL16A-33	5/22/2012	WHS	M	9	397	668	1.03																
GL16A-34	5/22/2012	WHS	F	7	418	888	1.02																
GL16A-35	5/22/2012	WHS	F	8	459	1157	1.11																
GL16A-36	5/22/2012	WHS	F	10	430	1506	1.53																
GL16A-37	5/22/2012	WHS	M	4	285	228	0.95																
GL16A-38	5/22/2012	WHS	F	10	453	978	0.93																
GL16A-39	5/22/2012	WHS	F	7	413	782	0.96																
GL16A-40	5/22/2012	WHS	F	4	425	936	1.11																
GL16B-1	5/22/2012	WHS	F	12	483	1274	0.86																
GL16B-2	5/22/2012	WHS	F	8	468	1324	1.08																
GL16B-3	5/22/2012	WHS	F	4	343	500	1.23																
GL16B-4	5/22/2012	WHS	M	8	404	653	0.95																
GL16B-5	5/22/2012	WHS	M	8	415	830	1.14																
GL16B-6	5/22/2012	WHS	F	7	407	762	0.98																
GL16B-7	5/22/2012	WHS	F	6	391	677	1.02																
GL16B-8	5/22/2012	WHS	F	5	415	863	1.05																
GL16B-9	5/22/2012	WHS	F	6	408	846	1.11																
GL16B-10	5/22/2012	WHS	F	8	411	795	0.99																
GL16B-11	5/22/2012	WHS	F	5	336	426	1.12																
GL16B-12	5/22/2012	WHS	F	8	315	355	1.13																
GL16B-13	5/22/2012	WHS	F	10	412	783	0.88																

Fish #	Date	Species	Sex	Age	T.L. (mm)	WT (gms)	Kil	Skin/Fin Lesions			Gill Gross Lesions			Eyes			Liver wt (gms)	HSI	Liver Gross		Spleen Gross		Ant/Kid w. spots	Post. Kid w. spots	GSI			
								reference	parasites	b. spot	raised	med. spot	Other	w. spots	grubs	eroded			Other	opaque	Other	w. spots				Other	w. spots	Other
GL15B-13	4/18/2012	SMB	M	6	410	1089	1.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.72	0.82			
GL15B-14	4/18/2012	SMB	M	7	470	1759	1.68	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	13.60	0.77		
GL15A-1	4/23/2012	SMB	F	7	455	1635	1.57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	159.74	9.77		
GL15A-2	4/23/2012	SMB	M	7	440	1515	1.46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	12.78	0.84		
GL15A-3	4/23/2012	SMB	F	7	451	1528	1.48	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	173.05	11.33		
GL15A-4	4/23/2012	SMB	M	8	460	1550	1.57	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	19.05	1.23		
GL15A-5	4/23/2012	SMB	F	10	507	2261	1.54	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	260.00	11.50		
GL15A-6	4/23/2012	SMB	F	6	438	1602	1.71	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	167.75	11.72		
GL15A-7	4/23/2012	SMB	F	5	403	1308	1.76	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	155.19	11.86		
GL15A-8	4/23/2012	SMB	M	8	440	1358	1.58	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.41	0.84		
GL15A-9	4/23/2012	SMB	F	5	380	1147	1.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	74.83	9.16		
GL15A-10	4/23/2012	SMB	M	5	388	856	1.46	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.12	0.47		
GL15A-11	4/23/2012	SMB	M	5	309	448	1.51	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.63	0.54		
GL15A-12	4/23/2012	SMB	M	5	279	355	1.63	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.67	0.47		
GL15A-13	4/24/2012	SMB	M	6	470	1775	1.69	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	16.79	0.95		
GL15A-14	4/24/2012	SMB	F	6	425	1351	1.75	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.8	0.80		
GL15A-15	4/24/2012	SMB	F	6	431	1204	1.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	127.78	10.20		
GL15A-16	4/24/2012	SMB	F	5	391	1002	1.52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	95.57	9.54		
GL15A-17	4/24/2012	SMB	F	5	349	640	1.38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54.97	8.59		
GL15A-18	4/24/2012	SMB	M	5	295	343	1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.30	0.09		
GL15A-19	4/24/2012	SMB	F	4	298	435	1.64	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.94	0.46		
GL15A-20	4/24/2012	SMB	M	6	308	472	1.60	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.17	0.67		
GL15B-1	4/25/2012	SMB	M	6	266	240	1.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.88	0.37	
GL15B-2	4/25/2012	SMB	M	4	307	370	1.27	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.11	0.57	
GL15B-3	4/25/2012	SMB	M	7	318	416	1.29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.29	0.55		
GL15B-4	4/25/2012	SMB	M	7	327	444	1.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.04	0.46		
GL15B-5	4/25/2012	SMB	F	6	350	575	1.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	54.43	9.47		
GL15B-6	4/25/2012	SMB	F	7	266	235	1.21	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	7.57	3.22		
GL15B-7	4/25/2012	SMB	F	4	280	266	1.16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	11.92	4.48		
GL15B-8	4/25/2012	SMB	F	4	290	269	1.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4.05	1.84		
GL15B-9	4/25/2012	SMB	F	5	277	272	1.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3.77	1.39		
GL15B-10	4/25/2012	SMB	M	6	286	305	1.29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2.39	0.78		
GL15B-11	4/25/2012	SMB	F	7	284	261	1.11	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6.47	2.48		
GL15B-12	4/25/2012	SMB	F	8	380	737	1.22	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	65.64	8.91		
GL15B-13	4/25/2012	SMB	F	7	350	607	1.26	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	67.77	11.16		
GL15B-14	4/25/2012	SMB	M	6	288	280	1.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.55	0.55		
GL15B-15	4/25/2012	SMB	F	11	458	1439	1.33	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	162.85	11.32		
GL15B-16	4/25/2012	SMB	M	5	293	312	1.23	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1.56	0.50		
GL15B-17	4/25/2012	SMB	F	4	272	250	1.19	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	10.48	4.19		
GL15B-18	4/25/2012	SMB	F	4	273	224	1.10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.99	0.44	
GL17-01	5/8/2012	SMB	F	6	400	979	1.47	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	39.49	4.03	
GL17-02	5/8/2012	SMB	F	8	388	804	ND	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	ND	ND	
GL17-03	5/9/2012	SMB	F	6	391	935	1.32	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	147.36	15.76	
GL17-04	5/9/2012	SMB	F	7	384	835	1.34	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	116.84	12.50	
GL17-05	5/9/2012	SMB	F	5	351	825	1.35	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	47.21	7.47	
GL17-06	5/9/2012	SMB	F	7	428	1150	1.38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	66.42	12.62	
GL17-07	5/9/2012	SMB	F	7	325	642	1.36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	150.66	12.37	
GL17-08	5/9/2012	SMB	F	6	405	1218	1.61	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.93	0.28
GL17-09	5/9/2012	SMB	M	6	390	693	1.17	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1.93	0.28
GL17-10	5/9/2012	SMB	F	7	406	1147	1.52	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	129.04	11.25	
GL17-11	5/9/2012	SMB	F	5	372	756	1.45	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	46.06	5.79	
GL17-12	5/9/2012	SMB	F	7	375	747	1.36	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	27.73	3.71	
GL17-13	5/9/2012	SMB	F	7	428	1150	1.38	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	69.85	6.06	
GL17-14	5/9/2012	SMB	F	6	395	852	1.39																					

