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Contaminant-associated health effects in fishes from the Ottawa and Ashtabula Rivers, Ohio

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ABSTRACT

The health of resident fishes serves as a biologically relevant barometer of aquatic ecosystem integrity. Here, the health of the Ottawa River and Ashtabula River (both within the Lake Erie Basin) were assessed using morphological and immunological biomarkers in brown bullheads (*Ameiurus nebulosus*) and largemouth bass (*Micropterus salmoides*). Biomarker metrics were compared to fish collected from a reference site (Conneaut Creek). Data utilized for analyses were collected between 2003 and 2011. Fish collected from all three river systems had markedly different contaminant profiles. Total PCBs were the dominant contaminant class by mass. In bullhead, PCBs were highest in fish from the Ashtabula River and there were no differences in fish collected pre- or post-remediation of Ashtabula Harbor (median = 4.6 and 5.5 mg/kg respectively). Excluding PCBs, the Ottawa River was dominated by organochlorine pesticides. Liver tumor prevalence exceeded the 5% trigger level at both the Ashtabula (7.7%) and Ottawa Rivers (10.2%), but was not statistically different than that at the reference site. There was no statistically significant association between microscopic lesions, gross pathology and contaminant body burdens. Collectively, contaminant body burdens were generally negatively correlated with functional immune responses including bactericidal, cytotoxic-cell and respiratory burst activity in both species. Exceptions were positive correlations of HCB and heptachlor epoxide with respiratory burst activity in largemouth bass, and HCB with respiratory burst activity in bullhead and ΣBHC for all three functional assays in bullhead. Data here provide additional support that organochlorine contamination is associated with immunomodulation, and that species differences exist within sites.

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Introduction

Areas of Concern (AOC) are specific geographic areas in the Great Lakes Basin in which environmental degradation has been demonstrated based on established impairments. These AOCs were originally identified by the U.S.–Canada Joint Commission based on the impairment of one or more of 14 beneficial uses for the area (IJC, 1989). In 2001, a stepwise approach was adopted for delisting AOCs that was based on restoration of beneficial use impairments (BUIs). Two Ohio AOCs are the Maumee and Ashtabula Rivers. The Maumee River Area of Concern (AOC) encompasses 2007 km^2 and includes the lower reach of the Maumee River and tributaries that include the Ottawa River. The Maumee AOC was first designated following the Stage 1 Remedial Action Report in 1984 that identified heavy metals and organic chemical sediment contamination as the initial reason for the listing. Subsequent investigations found other problems such as contaminated industrial sites, abandoned dumps, combined sewer overflows and disposed dredging materials. The BUIs identified included "degradation of fish and wildlife populations", "restrictions of fish and wildlife consumption" and "fish tumors and other deformities". The Ottawa River enters Lake Erie at Toledo, Ohio. Decades of industrial activity and improper waste disposal have resulted in contamination of the sediment, water and fishes with various hazardous substances including polychlorinated biphenyls (PCBs), polycyclic aromatic hydrocarbons (PAHs) and lead (USFWS, 2004). In 2004, the U.S. Fish and Wildlife Service and the Ohio Environmental Protection Agency launched a Natural Resource Damage Assessment (NRDA) governed by provisions in the Comprehensive Environmental Response, Compensation and Liability Act (CERCLA) or Superfund. The goal of the assessment was to determine the level of injury to natural resources and require responsible parties to restore, replace or acquire the equivalent of the injured resources (USFWS, EPA O, 2007).

The Ashtabula River is located in northeastern Ohio and drains into the central basin of Lake Erie. This river is contaminated with a multitude of industrial chemicals, and the lower 2 mi. are listed as an AOC. Similar to the Maumee River, this AOC includes the three BUI listed

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above. Chemical production and waste disposal along Fields Brook, a U.S. Environmental Protection Agency (USEPA) Superfund site, has caused much of the contamination within the Ashtabula River. Fields Brook is a tributary of the Ashtabula River and is the major source of hazardous substances present in the lower Ashtabula. The confluence of these lotic systems is at approximately river mile 1.6 of the Ashtabula. Biological impairment of largemouth bass (*Micropterus salmoides*) and brown bullhead (*Ameiurus nebulosus*) inhabiting the Ashtabula River was documented in this river system via biological monitoring between 2003 and 2004 (Iwanowicz et al., 2012). Results of this and other monitoring led to remediation efforts that included dredging a 1.77 km stretch of lower river in 2006 (Meier et al., 2015). Additional contaminated sediment was removed above this location the following year. An estimated 497,000 yd^3 of contaminated sediment and 11,000 kg of PCBs was removed during this remediation effort.

Historically, brown bullhead (BB) have been utilized as resident indicator species throughout the Great Lakes primarily to monitor the prevalence of fish tumors or other deformities (Baumann and Harshbarger, 1998; Blazer et al., 2009a, 2009b; Rafferty et al., 2009). Largemouth bass (LMB) have been used as indicators of ecosystem health in national monitoring programs such as the U.S. Geological Survey (USGS) Biomonitoring and Assessment (BEST) program (Hinck et al., 2009) as well as other studies (Blazer et al., 1987; Sepúlveda et al., 2003; Iwanowicz et al., 2012; Iwanowicz et al., 2016). Given that these species have different life histories and inhabit benthic or pelagic environments, respectfully, they potentially experience different contaminant exposure regimes. A suite of biomarker end-points was used to compare the health of fish collected from three sections of the lower Ottawa River, the lower Ashtabula River and Conneaut Creek as a non-AOC reference. These included morphometric (age, length, weight, condition factor and hepatosomatic indices), necropsy-based (visible lesions), immunological (bactericidal activity, cytotoxic cell activity and respiratory burst), and histopathological biomarkers. The objectives of this study were 1) to determine if there was evidence of biological impairment in fishes from the Ottawa River compared to individuals from a reference (Conneaut Creek) stream and an impaired (Ashtabula River) river and 2) determine if the remediation effort in the Ashtabula River improved the biological heath of resident fishes.

Methods

Fish collection and necropsy-based assessment

Fish were collected by electroshocking and/or trap nets in three reaches of the Ottawa River May 4–6, 2009. The upstream site was between river mile (RM) 7.0 and 9.0, the middle site was between RM 4.5 and 6.5 and the downstream site between RM 2.5 and 1.5. Largemouth bass (LMB) and brown bullhead (BB) were targeted for sampling at each site. Fish were collected from the Ashtabula River between RM 2.0 and 1.0, April 25 and October 4, 2011 and between RM 2.1 and Conneaut Harbor of the reference site, April 26 and October 5, 2011 (Fig. 1). A minimum length of 250 mm was targeted for both species to conservatively target sexually mature fishes of 3 years old or greater. Fish were euthanized with a lethal dose of tricaine methanesulfonate (MS-222, Argent Chemical Company, Redmond, WA) prior to necropsy and typically within 60–120 min of capture.

A comprehensive necropsy-based fish health assessment was completed. All fish capture, handling and euthanasia protocols were approved by the USGS, Leetown Science Center, Institutional Animal Care and Use Committee. Mass and total length of each fish was determined. Phenotypic sex was noted and later confirmed histologically. External abnormalities including raised lesions in the oral cavity and body surface, melanistic spots, and missing, shortened or deformed barbels (for BB only) were recorded. Fish were aseptically dissected and a portion of the anterior kidney (AK) was removed and placed into Processing Medium (PM; isotonic Leibovitz-15 medium supplemented with 2% fetal bovine serum, 100 U mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 10 U mL^{-1} sodium heparin) at 4 °C. Within an hour the tissue was homogenized with a sterile, hand-held tissue grinder, returned to the tubes which were placed on wet ice and shipped overnight to the USGS Leetown Science Center for leukocyte isolation and functional analysis. Liver was extracted and weighed to calculate organosomatic index. Pieces of liver and any observed lesions were removed and preserved in Z-Fix® (Anatech LTD, Battle Creek, MI) for histological analyses. For the Ottawa collections, scales (right side above the pectoral fin and lateral line) were removed from LMB for aging; BB were not aged. For the Ashtabula and Conneaut collections otoliths were removed from both species for aging. Carcasses were wrapped in foil and frozen for later chemical analyses.

The condition factor (KtL), hepatosomatic index (HSI) and gonadosomatic (GSI) were calculated by $KtL = 10^5 \times ((\text{body weight} - \text{gonad weight}) / \text{length}^3)$, $HSI = 100 \times (\text{liver weight} / (\text{body weight} - \text{gonad weight}))$, $GSI = 100 \times \text{gonad weight} / \text{body weight}$, respectively. The prevalence of raised lesions, melanistic spots and barbel abnormalities were determined at each site. Prevalence was defined as the number of individuals with each type of abnormality ÷ total number of fish per site × 100.

Functional immune responses

Leukocyte isolation

Anterior leukocytes were isolated and processed as described previously (Iwanowicz et al., 2012, 2009). Osmolality of the media was adjusted to 296 and 270 mOsm for bass and bullheads, respectively. Briefly, aseptically processed anterior kidney tissue from both LMB and BB were received within 24 h. Tissue preparations were resuspended and allowed to settle for 30 min on wet-ice. Supernatants were transferred to a new sterile polypropylene and centrifuged at 500*g* for 10 min at 4 °C. Cells were washed by suspension in PM followed by centrifugation as above and suspended in PM. Bass cell suspensions were layered on 32% Percoll in Hanks Balanced Salt Solution without phenol red, pH 7.2 (HBSS). Bullhead cell suspensions were added to Histopaque® 1077. The cells were centrifuged at 500*g* for 40 min or 300*g* for 20 min at 4 °C for LMB and BB respectively. The leukocyte fraction was removed and washed. The number of viable leukocytes was determined by the trypan blue (0.1% trypan blue in PM) exclusion method. Leukocytes were suspended at 2×10^7 viable cells mL^{-1} in culture medium (CM; L-15 media supplemented with 5% FBS, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin) or adherence medium (AM; L-15 supplemented with 0.1% FBS, 100 U mL^{-1} penicillin and 100 μg mL^{-1} streptomycin). All tissues and cell suspensions were maintained at 4 °C in an ice bath during processing.

Bactericidal activity

The capability of adherent anterior kidney leukocytes to inactivate the salmonid pathogen *Yersinia ruckeri* was evaluated using methods described elsewhere (Harms et al., 2000) and modified for these species (Iwanowicz et al., 2012). Briefly, 2×10^6 leukocytes suspended in AM were added in quadruplicate to the wells of a 96-well plate for bacterial challenge and in triplicate on the same plate for subsequent adherent cell enumeration. Leukocytes from LMB or BB for all treatments were included on all plates in this assay and those to follow to account for inter-plate variability. Plates were incubated at 20 °C for 2 h following cell plating. Media was then removed from all wells, replaced with CM and cells were cultured at 20 °C in a humidified chamber for 36 h. Culture media was then aspirated, wells were washed with antibiotic-free unsupplemented L-15, and replaced with antibiotic-free L-15 supplemented with 5% FBS. A 48 h culture of *Yersinia ruckeri* (Hagerman strain; NFHRL # 11.40) washed and suspended in HBSS ($OD_{600} = 1.5$) was added to the treatment and control wells. Plates were then incubated in a humidified chamber at 20 °C for 4 h. Media was then removed from the wells, cells were lysed with lysis buffer (0.2% Tween 20 in

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