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Relation of contaminants to fish intersex in riverine sport fishes

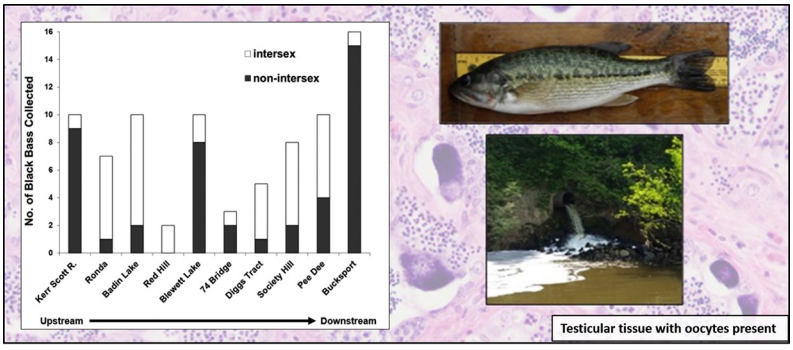
Casey A. Grieshaber [a,*], Tiffany N. Penland [a], Thomas J. Kwak [b], W. Gregory Cope [c], Ryan J. Heise [d], J. Mac Law [e], Damian Shea [f], D. Derek Aday [c], James A. Rice [c], Seth W. Kullman [f]

[a] North Carolina Cooperative Fish and Wildlife Research Unit, Department of Applied Ecology, North Carolina State University, Raleigh, NC 27607, USA
[b] U.S. Geological Survey, North Carolina Cooperative Fish and Wildlife Research Unit, Department of Applied Ecology, North Carolina State University, Raleigh, NC 27607, USA
[c] Department of Applied Ecology, North Carolina State University, Raleigh, NC 27607, USA
[d] Duke Energy, Environmental Sciences, Huntersville, NC 28078, USA
[e] Department of Population Health and Pathobiology and Comparative Medicine Institute, North Carolina State University, Raleigh, NC 27606, USA
[f] Department of Biological Sciences, North Carolina State University, Raleigh, NC 27606, USA

HIGHLIGHTS

- Fish intersex is a common condition related to endocrine disrupting contaminants.
- Contaminant concentrations and fish intersex are rarely examined simultaneously.
- Fish, water, and sediment were evaluated in a large southeastern USA regulated river.
- Intersex occurrence was greatest in black bass and varied throughout the river.
- PAHs, mercury, and pesticides correlated most with fish intersex.

GRAPHICAL ABSTRACT



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ABSTRACT

Endocrine active compounds (EACs) are pollutants that have been recognized as an emerging and widespread threat to aquatic ecosystems globally. Intersex, the presence of female germ cells within a predominantly male gonad, is considered a biomarker of endocrine disruption caused by EACs. We measured a suite of EACs and assessed their associated impacts on fish intersex occurrence and severity in a large, regulated river system in North Carolina and South Carolina, USA. Our specific objective was to determine the relationship of contaminants in water, sediment, and fish tissue with the occurrence and severity of the intersex condition in wild, adult black bass (*Micropterus*), sunfish (*Lepomis*), and catfish (Ictaluridae) species at 11 sites located on the Yadkin-Pee Dee River. Polycyclic aromatic hydrocarbons (PAHs), ethinylestradiol (EE2), and heavy metals were the most prevalent contaminants that exceeded effect levels for the protection of aquatic organisms. Fish intersex condition was most frequently observed and most severe in black basses and was less frequently detected and less severe in sunfishes and catfishes. The occurrence of the intersex condition in fish showed site-related effects, rather than increasing longitudinal trends from upstream to downstream. Mean black bass and catfish tissue contaminant concentrations were higher than that of sunfish, likely because of the latter's lower trophic position in the food web. Principal component analysis identified waterborne PAHs as the most correlated environmental contaminant with intersex occurrence and severity in black bass and sunfish. As indicated by the intersex condition, EACs have adverse but often variable effects on the health of wild sport fishes in this river, likely due to fluctuations in EAC inputs and the dynamic nature of the riverine system. These findings enhance the understanding of

* Corresponding author.
E-mail address: cagriesh@ncsu.edu (C.A. Grieshaber).

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the relationship between contaminants and fish health and provide information to guide ecologically comprehensive conservation and management decisions.

1. Introduction

Aquatic ecosystems are under threat because of their susceptibility to act as sinks or conduits, accumulating and transporting numerous chemical contaminants released from industrial, agricultural, and municipal sources (Scholz and Mayer, 2008). Such sources of contaminants may become increasingly detrimental to aquatic species, due to persistent human population growth and pollution. One class of contaminants that has been identified as a significant stressor is endocrine active compounds (EACs). The endocrine system is important to the health and reproductive success of organisms, and alterations to it can be harmful to individuals and populations (Lee Pow et al., 2017b; Solomon, 2015). EACs may interfere with the endocrine system by disrupting normal synthesis, storage, release, metabolism, transport, binding action, and elimination of endogenous hormones; those implicated in such disruption are recognized as endocrine disrupting compounds (EDCs; Kavlock et al., 1996). Further, with exposures at sufficient levels, or during critical times of development, EACs have the potential to cause toxicity to an organism (Barton and Andersen, 1998).

EACs that are often introduced into aquatic systems include polychlorinated biphenyls (PCBs), current use pesticides (CUPs), organochlorine pesticides (OCPs), polycyclic aromatic hydrocarbons (PAHs), pharmaceuticals, bisphenol A (BPA), and heavy metals, forming chemical mixtures with potential additive or synergistic effects (Hinck et al., 2009; Muthumbi et al., 2003). EACs vary widely in their persistence in aquatic environments, and those resistant to environmental degradation are classified as persistent organic pollutants (POPs); common POPs in aquatic systems are PCBs and OCPs, which are known for persistent legacy effects on biota (El-Shahawi et al., 2010; Harding et al., 1998). Originating from numerous routes of entry and anthropogenic influences, EACs have been detected in aquatic environments globally and are likely to remain ever-present with associated ecological effects (Abdel-Moneim et al., 2015; Kolpin et al., 2002; Ternes et al., 1999).

The estrogenic potency of some EACs has been cause for concern because of the potential for negative effects on reproduction and sustainability of wildlife populations. EACs have been linked to skewed sex ratios, reduced fecundity, production of vitellogenin (an egg-yolk precursor) in male fish, intersex condition, and population collapse in fish (Bhandari et al., 2015; Brian et al., 2007; Jobling et al., 1998; Kidd et al., 2007; Lee Pow et al., 2017b; Puy-Azurmendi et al., 2013; Williams et al., 2009; Woodling et al., 2006). Intersex, the presence of female germ cells within a predominantly male gonad (Nolan et al., 2001), has been suggested as a biomarker of endocrine disruption from environmental, and especially estrogenic EACs (Bahamonde et al., 2013; Barnhoorn et al., 2004; Leino et al., 2005). To assess the impacts of EACs on wildlife, intersex has been evaluated in various wild fish populations worldwide (Adeogun et al., 2016; Allen et al., 1999; Bizarro et al., 2014; Blazer et al., 2007; Tetreault et al., 2011). By examining intersex and EACs, researchers are able to better understand the dynamics and health of an ecosystem. Fish are particularly applicable for this area of study because they are susceptible to EACs through the aquatic environment, can act as sentinel species, and are indicators of ecosystem health (Kolpin et al., 2002; Kwak and Freeman, 2010; Lee Pow et al., 2017b; Penland et al., 2018).

In the southeastern United States (USA), fish intersex has been documented in numerous fish populations (Hinck et al., 2009; Kellock et al., 2014; Lee Pow et al., 2017a; Penland et al., 2018). This region is especially relevant for the study of EAC effects on fish because of impacts caused by dense human populations, agriculture, industry, wastewater treatment, and concentrated animal feeding operations, which have all been described as significant sources of EACs (Kolpin et al., 2002; Mills and Chichester, 2005; Vajda et al., 2008). A USA nation-wide study of intersex was conducted by Hinck et al. (2009) in which researchers assessed the intersex condition in black basses at 111 sites in nine river basins. Their study found that the highest incidence of intersex within the USA occurred in the Yadkin-Pee Dee River basin of North Carolina and South Carolina. Another study, by Sackett et al. (2015), identified sites that were associated with point source contaminants (EACs), and these were sites where Hinck et al. (2009) found high occurrence of intersex. These previous studies provided the motivation to undertake an investigation into high incidences of fish intersex in the Yadkin-Pee Dee River and to examine their potential relationships to EACs. The specific objectives of our research were to (1) determine intersex occurrence and severity in common sport fishes in a large, regulated, southeastern USA river, (2) measure and evaluate contaminants in surface water and sediments of the same river, (3) investigate relationships between fish intersex and EACs, and (4) determine trends in EAC concentrations, intersex occurrence, and intersex severity, longitudinally within the Yadkin-Pee Dee River system. The ultimate goal of this research was to enhance the understanding of the relationship between contaminants and fish health to facilitate ecologically comprehensive conservation and management decisions.

2. Methods

2.1. Study system

The Yadkin-Pee Dee River of North Carolina and South Carolina was chosen as our study system. A total of 11 sites were selected longitudinally along the river in North Carolina (8 sites) and South Carolina (3 sites) and site coordinates and descriptions are included in Fig. 1. Sites exhibited varying levels of anthropogenic influence, land use, and habitat types and were also selected for ease of boat access and sampling logistics. Eight sites were located in riverine habitats and three were located in reservoirs impounded by high dams. Three of the sites we studied (74 Bridge, Pee Dee, Bucksport) were sampled previously by Hinck et al. (2009) as sites PRB 336, 337, and 338.

2.2. Fish collection and histopathology

In April and May 2014, boat-mounted electrofishing (pulsed direct current) was conducted to capture wild, adult fish at all sites (within 2 km upstream or downstream of a river site or within the sampled reservoir). Additional sampling was conducted during June 2015 at the Bucksport, South Carolina, site because of the high incidence of intersex observed by Hinck et al. (2009) at this site. Collections from 2014 and 2015 catches from the Bucksport site were combined for analyses. Up to 10 male black bass (*Micropterus* spp.), 10 male sunfish (*Lepomis* spp.), and 10 male catfish (Ictaluridae) were collected at each site whenever possible. These taxa represent fish with varying life history strategies and are recreationally important sport fish within the river. Black bass have special relevance because of previous research conducted on both Largemouth Bass (*Micropterus salmoides*) and Smallmouth Bass (*Micropterus dolomieu*) examining intersex occurrence (Blazer et al., 2014; Hinck et al., 2009; Yonkos et al., 2014). Fish were collected at sizes indicative of sexual maturity, and any obvious females (eggs apparent when pressure was applied to the abdomen) were released back into the river. Euthanasia of male fish was completed following NC State University approved IACUC guidelines, with a lethal overdose of pH-buffered tricaine methanosulfate (MS-222,