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ASSESSMENT OF ENDOCRINE DISRUPTION IN FISH AND ESTROGENIC POTENCY OF WATERS IN GEORGIA

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Abstract. Recent reports of intersex fish (males with oocytes in their testicular tissue) in water bodies around the world have stimulated widespread concern about the effects that chemicals are having in the environment. Intersex fish have decreased sperm production, decreased sperm motility and decreased fertilization success compared to histologically 'normal' male fish. Estrogens and estrogen-like chemicals in the environment are known to induce intersex and other forms of endocrine disruption in fish. To date, a systematic evaluation of the severity and extent of intersex fish has not been completed in Georgia. Therefore, our objectives are (1) assess intersex condition in black bass collected from rivers and lakes across Georgia, and (2) determine spatial and temporal trends in estrogenic potency (a measure of the estrogens and estrogen-like substances) of surface waters. Study sites include the Oconee River and its major tributaries, the Ocmulgee River, the Savannah River and the Broad River as a reference (no major wastewater effluent discharges). Fish and water samples were collected upstream and downstream of municipal wastewater effluent discharges in each river (except Broad River). Fish were also sampled from lakes across Georgia with no major wastewater inputs to determine a natural 'background' rate of intersex in fish from relatively unpolluted water bodies. Gonads from all fish were examined histologically the intersex condition and incidence rates were compared among sites. We hypothesize that incidence of intersex fish will be associated with estrogens in surface waters. Potency of estrogens in surface waters will be determined by use of an in vitro yeast-based reporter gene assay. This study will provide the first investigation of intersex fish in many of Georgia's rivers and lakes and will be the first to investigate the estrogenic potency of surface waters across the state.

INTRODUCTION

Reports of intersex fish in water bodies around the world (including Georgia) have stimulated widespread concern about the effects that chemicals are having in the environment. Intersex is a term used to describe the presence of both male and female characteristics in individual fish, most commonly presence of oocytes (eggs) in testicular tissue, a pathological condition that is not routinely observed in most fish species (Hecker et al. 2006). The intersex condition has often been associated with a hormonally active component of municipal wastewater effluent discharge and has been induced in laboratory studies where fish were exposed to natural and synthetic hormones (Jobling et al. 2002), which are routinely measured in treated municipal wastewater effluent. The intersex condition has individual- as well as population-level implications; intersex male fish have been shown to have altered sperm production and reproductive success compared to non-intersex male fish (Jobling et al. 2002). These findings generate numerous questions about the ecological implications of intersex fish and fuel widespread concerns about the role of chemicals in well-documented trends in reproductive abnormalities in human health as well (Colborn et al. 1994). Understanding the extent and distribution of intersex fish in the environment and the chemicals that are known to induce this condition is a critical

first step toward developing a management strategy.

In a widely-publicized recent scientific article, Hinck et al. 2009 reported that intersex largemouth bass (*Micropterus salmoides*) were found in rivers across the US. Intersex bass were more common (up to 91%) in Southeastern US rivers than in other sampled areas of the country. The Chattahoochee, Flint and Savannah Rivers in Georgia were included in the sampling, and of the five sites sampled in these rivers, the incidence of intersex in bass ranged from 30–50%. The intersex fish all appeared macroscopically to be male but had oocytes in their testes. No clear relationship was evident between the incidence of intersex and concentrations of legacy persistent organic compounds and other chemicals within a fish. The authors did not test association between intersex and land use in areas where fish were collected. Causes for the intersex condition are currently unknown and in this study the authors did not analyze water samples for the presence of estrogens or other hormones that have previously been associated with this condition. Sample sites were not associated with wastewater effluent or particular contaminants but were stratified by land use (urban, agricultural, etc.). Other indicators of reproductive system abnormalities were not assessed. Additional sampling is required to fully understand the extent of the distribution of intersex fish in Georgia and the underlying causes for this condition. Our research will address the prevalence of estrogens in surface waters in Georgia and their association with the intersex condition. The distribution of intersex fish from the North Oconee, Broad, Ocmulgee and Savannah Rivers will be investigated as well as numerous lakes in Georgia. We will then compare incidence of intersex and estrogenic activity from river fish and water with that of lakes and ponds across Georgia.

METHODS

River sampling. Black bass sampling was conducted from April – June 2010. Fish were collected by boat electroshocking and/or hook and line from the North Oconee River, Broad River, Ocmulgee River, and Savannah Rivers in Georgia. Approximately 15 fish (age 1+) were collected. Fish from all rivers except the Broad were collected within 1 km of a municipal wastewater effluent outfall. The fish were kept alive in an aerated live well until sufficient numbers were obtained. Fish were anesthetized by buffered MS-222 overdose, weighed and measured. Each fish's gonads were examined macroscopically for confirmation of gender. Gonads were dissected from each fish, weighed and preserved in 10% buffered formalin for histological preparation by the Fish Pathology Laboratory at the University of Georgia College of Veterinary Medicine Diagnostic Lab. We determined the incidence of intersex based on presence of oocytes in the testes of apparent (macroscopic) male fish.

Lake sampling. Black bass were collected (also in April – June 2010) by boat electroshocking from six lakes across Georgia. Ten to 15 adult bass adult (age 1+) were obtained from each lake. The fish were kept alive in an aerated live well until sufficient numbers were obtained. Fish were anesthetized by buffered MS-222 overdose, weighed and measured. Gonads were dissected from the fish, weighed and preserved in 10% buffered formalin for histological analysis. Rates of intersex from the lakes were compared to the intersex rate in males from rivers receiving high volume municipal wastewater effluent.

Estrogenic potency. River water samples (2 L) were collected from at least 1 km upstream and less than 1 km downstream of point source effluent discharges. Lake water samples were collected as close to the center of the lake as possible. The water samples were analyzed for total estrogenic activity by the yeast estrogen screen (YES) assay, an in vitro assay with yeast (*Saccharomyces cerevisiae*) cells that have been transfected with the human estrogen receptor and an enzyme

reporter gene. Estrogenic compounds in water samples bind the receptors and stimulate production of an enzyme, the activity of which can be measured with a colorimeter. The YES assay has been previously validated for rapid, sensitive detection of estrogenic compounds in water samples (Routledge and Sumpter 1996).

Table 1. Intersex black bass collected in 2010 from Georgia rivers and l

Water Body Type	Site	GA County	Collection Date	Male Bass	Intersex	% Intersex Males
Lake	Lake Paradise	Barrien	4/1/2010	6	4	66.7
Lake	Antitoch East	Floyd	5/26/2010	5	0	0
Lake	Walter George	Clay	5/11/2010	14	3	21.4
Lake	Lake Seminole	Seminole	5/11/2010	9	0	0
Lake	Lake Blackshear	Lee	5/12/2010	9	0	0
Lake	Private pond	Hancock	4/12/2010	15	13	86.7
River	North Oconee	Clarke	4/30/2010	3	2	66.7
River	Broad River	Elbert	6/24/2010	12	0	0
River	Ocmulgee River	Bibb	6/28/2010	8	0	0
River	Savannah River	Richmond	6/30/2010	8	1	12.5

RESULTS AND DISCUSSION

Preliminary results suggest that intersex is prevalent in some water bodies across Georgia and that intersex is not confined to rivers that receive wastewater effluent (Table 1). Of the total 89 male bass collected in 2010, 25.8% were intersex. Bass from the North Oconee River downstream of wastewater effluent had the highest incidence of intersex at 66.7%, but the sample size from the river was only 3 fish. The average percentage of intersex in male fish collected from lakes was 34.5% were intersex, whereas 9.7% of males from rivers were intersex. We expected to see the highest rates of intersex in fish collected from rivers; however, our results to date suggest that black bass from small lakes and ponds are possibly even more susceptible to the intersex condition. The largest lakes in our study are indeed impoundments of major rivers and may serve as sinks for estrogenic compounds; however, preliminary data suggests that the largest lakes (i.e. reservoirs) have among the lowest incidence of intersex of all samples to date. Additional sampling from major rivers and lakes is critical to fully elucidate the extent of intersex fish in the state. Additional sampling will allow analyses of spatial trends and correlations with other variables such as lake surface area.

We are presently analyzing water samples collected from each of the rivers and lakes with the YES assay to determine estrogen activity in these waters. The YES data from 2010 will be informative; however, temporal sampling is required because estrogen levels may not be stable throughout the year and annual patterns (i.e., year to year) are unknown. We hypothesized that the highest estrogen concentrations would be found in water samples from areas with greater incidence of intersex fish; however, high concentrations of estrogens seem unlikely in lake samples because few of the lakes we sampled receive effluent or had homes or other facilities located nearby.

CONCLUSIONS

This study will provide the first investigation of estrogens in Georgia's surface waters and intersex fish in many of Georgia's rivers and lakes. The results are crucial for understanding the spatial and temporal distribution of estrogens in surface waters. Intersex is currently thought to be an abnormal condition for bass, but little research on the background incidence of intersex has been

reported. Comparison of intersex in fish from rivers and lakes will allow insight into the 'normal' background incidence of intersex in basses and provide additional evidence to determine if the condition is indeed linked to estrogens in the water. Our preliminary results suggest that intersex rates are high in some bass populations, including those in lakes, but the factors influencing intersex are currently poorly understood. Results of our sampling suggest that intersex is not confined to fish in Georgia rivers but occurs in lake populations as well. Additional sampling is required to elucidate the incidence and severity of intersex in Georgia fish and to determine potential causes of the condition.

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