ORGANOCHLORINE COMPOUNDS IN MIDDLE MISSISSIPPI RIVER SHOVELNOSE STURGEON (*SCAPHIRHYNCHUS PLATORYNCHUS*): BIOACCUMULATION AND REPRODUCTIVE CONSEQUENCES

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Organochlorine compounds, including polychlorinated biphenyls and organochlorine pesticides, were used on lands adjacent to the Middle Mississippi River (MMR) from 1930 through 1988 and continue to be detected in MMR fish. These compounds are estrogenic and/or antiandrogenic and can alter hormone production and reception within the brain and gonads of male fish, resulting in intersexuality and/or suppressed gonadal development. To assess the reproductive effects of OCs on MMR fish, I quantified OC accumulation, intersexuality, and gonadal development in male shovelnose sturgeon (*Scaphirhynchus platorynchus)* collected throughout the MMR during spring 2003. Gonads were observed for intersexual characteristics, weighed to calculate a gonadosomatic index (GSI), and then stored for contaminant and histological analysis. Brains and fillets were also removed to assess tissue-specific accumulation. Four out of 59 males were macroscopically identified as intersexuals, and another was found through histology. Intersexuals accumulated significantly higher concentrations of OCs in the brain than mature males. In addition, a significant negative relationship was found between GSI and OC accumulation within brains, gonads, and fillets of mature males. Results indicate that exposure to OCs prior to sexual differentiation may induce intersexuality

in MMR shovelnose sturgeon, and exposure throughout gonadal maturation may inhibit gonadal development as well.

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TABLE OF CONTENTS

ABSTRACT i

ACKNOWLEDGEMENTS iii

LIST OF TABLES v

LIST OF FIGURES vi

CHAPTER 1: INTRODUCTION 1

MATERIALS AND METHODS 4

RESULTS 11

DISCUSSION 15

LITERATURE CITED 28

VITA 33

LIST OF TABLES

Table 1. Organochlorine compounds analyzed in shovelnose sturgeon collected during April 2003 from the Middle Mississippi River 22

LIST OF FIGURES

Figure 1. Map of sites along the Middle Mississippi River, where shovelnose sturgeon (N=59) were collected during April 2003. RKM = River kilometer 23

Figure 2. Contaminant accumulation in tissues of MII males (N=43) collected during April 2003 from the Middle Mississippi River. Letters denote significant differences in concentrations among tissues (ANOVA, Tukey's HSD, $p < 0.05$). Sum OCP, sum organochlorine pesticides; Sum PCB, sum polychlorinated biphenyls; Total OCs, sum organochlorine pesticides and polychlorinated biphenyls 24

Figure 3. Regression of GSI versus Total OCs (sum organochlorine pesticides and polychlorinated biphenyls) in **(A)** gonads (N=43), **(B)** brains (N=40), and **(C)** fillets (N=43) of MII male shovelnose sturgeon collected during April 2003 from the Middle Mississippi River 25

Figure 4. Histological sections (×100) of shovelnose sturgeon collected during April 2003 from the Middle Mississippi River. Digital images are of **(A)** a MII male gonad (FL = 621 mm; GSI = 4.37; Total OCs = 3,205 µg/kg lipid wt.), **(B)** a highly contaminated MII male gonad (FL = 610 mm; GSI 0.91; Total OCs = 15,445 µg/kg lipid wt.), **(C)** an MII intersex gonad containing primary oocytes surrounded by degenerative tissue, and **(D)** the same MII intersex gonad devoid of spermatozoa in interior regions. DT, degenerative tissue; PO, primary oocytes; SP, spermatozoa within seminiferous tubules; VT, vacant seminiferous tubules 26

Figure 5. Relationship between Total OC accumulation in tissue of MII males (N=40) and MII intersexuals (N=5) collected during April 2003 from the Middle Mississippi River. Different letters denote a significant difference of Total OC accumulation in tissues among males and intersexuals (ANOVA, Tukey's HSD, $p < 0.05$). Total OC, sum organochlorine pesticides and polychlorinated biphenyls. 27

Introduction

Organochlorine compounds (OCs) were used worldwide as agricultural pesticides and industrial agents throughout the 20th century. While most have been banned or restricted throughout much of the industrialized world, OCs still persist in the environment and may adversely affect endocrine function in exposed organisms. Many OCs have the potential to mimic natural hormones important in determining gender, as well as regulating gonadal maturation within exposed organisms, whereupon elevated exposure may induce a variety of atypical responses. Developmental and reproductive abnormalities documented in wild species exposed to OCs include decreased penis sizes and testosterone concentrations in alligators (Guillette et al. 1996), eggshell thinning in bald eagles (Faber et al. 1973), altered sex ratios in bloater populations (Monosson et al. 1995), intersexuality in gulls (Fox 1992), and premature births and abortions in seal pups (DeLong et al. 1973, Helle et al. 1976, Reijnders 1986).

Continued research on the endocrine-disrupting effects of OCs has provided insight into the mechanisms behind these occurrences. The organochlorine pesticide (OCP) 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'* -DDE), a metabolite of the OCP 1,1,1-trichloro-2-(*p*-chlorophenyl)-2-(*o*-chlorophenyl)ethane (*o,p'*- DDT), acts as an antiandrogen in male rats by binding to the androgen receptor (AR) and inhibiting androgen-induced AR transcriptional activity (Kelce et al. 1995). Further studies have found *p,p'* -DDE to bind to androgen receptors in male fish, delaying maturity, decreasing gonad size, and reducing sperm counts (Wells and Van Der Kraak 2000, Baatrup and Junge 2001). Polychlorinated biphenyls (PCBs), which

were widely used as industrial agents, are antiandrogenic as well and decrease sperm production, testes size (reviewed in Gray and Kelce 1996), and testosterone levels in rodents (Hany et al. 1999). Estrogenic OCs may adversely affect male organisms as well. Lower chlorinated PCB mixtures and *o,p'* -DDT have been found to bind to estrogen receptors in the brains and gonads of rodents (Bitman and Cecil 1970, Mussi et al. 2005), and *o,p'* -DDT has been linked to intersexuality in male gulls (Fry and Toone 1981, Fox 1992).

Hormonal interactions between the brain and gonads are instrumental in sexual differentiation and maturity in fishes (Kime 1999), and exposure to endocrine-disrupting chemicals may alter proper endocrine function of these tissues depending on the timing and severity of exposure (Gimeno et al. 1997). It has been well established that exposure to estrogenic chemicals in males prior to sexual differentiation may induce permanent intersexuality (Gimeno et al. 1996), while exposure to estrogens and antiandrogens throughout maturation may inhibit gonadal growth and development (Kinnberg et al. 2000, Wells and Van Der Kraak 2000, Baatrup and Junge 2001). Decreased gonadosomatic index (GSI; gonad size / body weight × 100) is typically indicative of decreased hypothalamic, pituitary, or gonadal activity (Kime 1999), and studies have linked decreased GSI to reductions in other reproductive parameters in fish such as sperm counts (Baatrup and Junge 2001), milt volume and sperm density (Jobling et al. 2002b). Despite the importance of the brain and gonads in controlling endocrine function, few studies have quantified OC residues within these tissues in relation to reproductive parameters such as GSI. While whole body, liver, or fillet residues are typically measured, contaminant

residues within the brains and gonads of exposed organisms may provide a better link to endocrine-disrupting effects seen in the wild.

Organochlorine compounds are highly lipophilic and tend to accumulate in the organic substrate of contaminated waters, placing a benthic species such as the shovelnose sturgeon (*Scaphirhynchus platorynchus*) at risk to elevated exposure and consequent reproductive effects. Although the shovelnose sturgeon is the only North American river sturgeon species not listed as endangered under the Federal Endangered Species Act, the distribution and abundance of shovelnose sturgeon has declined throughout many areas due to habitat alterations, overharvest, and pollution (Keenlyne 1997). Organochlorine compounds have been continually detected in Middle Mississippi River (MMR) shovelnose sturgeon in the past (Bush and Grace 1989), and elevated concentrations of PCBs and OCPs within shovelnose sturgeon have prompted several health advisories either restricting or banning the consumption of shovelnose sturgeon eggs and fillets (Hornshaw, 2005). Organochlorine compounds continue to be detected in MMR shovelnose sturgeon today, and recent research suggests that shovelnose sturgeon reproduction may be adversely affected by these endocrine-disrupting chemicals (Harshbarger et al. 2000). Intersexual shovelnose sturgeon occur in the MMR, but not in adjacent reaches upstream, where OCs are less prevalent (Harshbarger et al. 2000). Intersexuality in a declining, commercially fished sturgeon population may be cause for concern. Intersexual roach (*Rutilus rutilus*) inhabiting U.K. rivers polluted with endocrine-disrupting chemicals have decreased gonadosomatic index (GSI; gonad size / body weight × 100) (Jobling et al. 2002b). Further, these fish have reduced milt volume, sperm

motility, fertility, and offspring survival compared to roach from reference sites (Jobling et al. 2002ab). Males within the same rivers are similarly affected, and have decreased sperm density and milt volume compared to specimens from reference sites (Jobling et al. 2002a). Exposure to OCs in the MMR may similarly diminish the reproductive abilities of intersexual and male shovelnose sturgeon and exacerbate the effects of commercial harvest.

The reproductive effects that OCs may have on MMR shovelnose sturgeon are presently unknown. Despite the relative importance of the brain and gonads in controlling sexual development of fishes, the relationship between GSI and organochlorine accumulation within these tissues has not been studied to date, nor has tissue-specific accumulation within males and intersexuals been addressed. My aim was to determine how OCs affect the reproductive potential of MMR shovelnose sturgeon, specifically by quantifying GSI and OC accumulation in the brains, gonads, and fillets of males and intersexuals.

Materials and Methods

Field Collection and Laboratory Procedures

Organochlorine compounds were previously used as industrial agents and agricultural pesticides on lands adjacent to the MMR, therefore urban and agricultural sites were selected for sample collection. The upper region of the MMR includes the highly populated and industrialized Alton and St. Louis region, which receives large amounts of urban runoff as well as navigation-related industrial activity. The middle and lower regions of the MMR are primarily surrounded by agricultural areas and

small non-industrious communities. I chose four urban and four agricultural sites (Figure 1) within the MMR before sampling by field observation and digital orthophoto quadrangle (DOQ) maps. Urban sites were designated as having greater than 50% of surrounding land being commercial, residential, or industrial areas, and agricultural sites as greater than 50% of the surrounding land used for farming. Up to eight male shovelnose sturgeon were collected from each site with overnight gill net (5.08 cm mesh) sets during through April 2003. Fish were transported to the Fisheries and Illinois Aquaculture Center, Southern Illinois University, where fork length (mm), total length (mm), and weight (g) were quantified after a lethal dose of carbon dioxide was administered. Gonadal tissue was grossly inspected to determine if fish were male, female, or intersexual using the classification of Colombo (2004). Paired gonads were removed, weighed (g wet mass) to calculate GSI, then stored for histological and contaminant analysis. Gonads intended for histology were fixed in 10% neutral buffered formalin, while those used for contaminant analysis were wrapped in aluminum foil and frozen at -80° C. Brains and fillets were also removed for contaminant analysis and stored following the same protocol.

Histology

Gross examination alone is often insufficient in identifying intersexuality (Van Aerle et al. 2001, Gercken and Sordyl 2002, Jobling et al. 2002ab, Barnhoorn et al. 2004), therefore I used histology to determine sex as well as stage of maturity. Gonad samples intended for histology were sent to the Southern Illinois University histology lab for processing. Five transverse sections (3-5 mm) were taken

equidistantly throughout each gonad (N=59) and processed using standard histological techniques. Sections (5 µm) were mounted on slides stained with hematoxylin and counterstained with eosin, examined by light microscopy (40× - 630×, Nikon Optiphot-2), and colored digital images were captured using a top mounted digital camera (Olympus 3000Z). Gonadal sections were examined to identify stage of maturity following Colombo (2004), males were classified as either MI (reproductively immature with seminiferous tubules containing spermatogonia dispersed in a homogenous fashion) or MII (reproductively mature with mature spermatozoa in seminiferous tubules but with spermatogonia absent). Intersexuals were similarly classified using male characteristics and were identified by ovarian folds and/or primary oocytes in the presence of male gametes.

Contaminant Analysis

Shovelnose sturgeon brain, gonad, and skinless fillet samples were analyzed by gas chromatography for 14 OCPs and 40 PCB congeners, eight of which are coplanar and similar to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Table 1). Organochlorine pesticide standards were purchased from Supelco, Bellefonte, PA, USA, and PCB standards from Ultra Scientific, North Kingstown, RI, USA. Anhydrous Na_2SO_4, anhydrous $MgSO_4$, and various pesticide grade solvents were purchased from Fisher Scientific (Pittsburgh, PA, USA). 4,4'-Dibromooctafluoro-biphenyl (DBOFB) (Supelco, Bellefonte, PA, USA) was used as a surrogate to verify the extraction and cleanup efficiency of the newly developed method.

Extraction Procedures

I thawed individual brain, gonad, and fillet samples and homogenized them by mortar and pestle. Approximately 2 g of ground tissue was placed in a 50 ml centrifuge tube and 50 ppb of the surrogate DBOFB was added. The samples were cooled by ice and mixed with anhydrous $MgSO_4$ until dry. A 25 ml aliquot of extraction solvent (mixture of acetone and hexane 1:1, v/v) was added as extraction solvent and the mixture sonicated for 3 minutes in pulse mode (3 seconds on, 1 second off) using a high intensity ultrasonic processor (Model VCX 400, Sonics and Materials Inc., Newtown, CT, USA). The extract was centrifuged for 5 min with an IEC Clinical Centrifuge (International Equipment Company, Needham Heights, MA, USA). The supernatant was then decanted into an evaporation vial and evaporated under a stream of nitrogen to approximately 5 ml using a TurboVap II evaporator (Zymark, Hopkinton, MA, USA). After being solvent exchanged to hexane, the extract was then split into two equal aliquots for lipid determination and contaminant analysis.

Lipid Determination

Because OCs are hydrophobic and accumulate in the lipids of exposed organisms, I calculated the lipid content of each sample in order to limit the variability among samples. One aliquot of extract (2.5 ml) was transferred into a pre-weighed disposable vial and evaporated under a gentle stream of nitrogen using a Pierce Model 1878 Reactivap™ (Rockford, IL, USA) until total dryness of solvent. The vial was weighed again, and the lipid content was then calculated from the

sample weight, the weight of the empty vial, and the weight of the vial after solvent evaporation.

NPLC Cleanup and Fractionation of PCBs and Pesticides

The remaining aliquot of extract (2.5 ml) was evaporated to 1 ml and used to detect PCBs and OCPs after normal phase liquid chromatography (NPLC) cleanup and fractionation. Depending on the lipid content of the extract, a suitable dilution factor was used to lower the lipid content to less than 20% for NPLC cleanup. Cleanup was performed on an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA, USA). PCB and OCP separation was completed on a 250×4.6mm i.d. × 5μm ZORBAX RX-Sil column with a ZORBAX guard column (Agilent Technologies, Palo Alto, CA, USA) and fractions collected with a Foxy® Jr. Fraction collector (ISCO Inc., Lincoln, NE, USA). The mobile phase (1% ethyl acetate in hexane) was set at a flow rate of 1.0 ml/min and 0.5 ml of extract was injected into NPLC with a Rheodyne 7225 injector with 0.5mL loop (Cotati, CA, USA). A UV detector (280 nm) was used to detect the analytes. Two fractions were collected, the first for PCB analysis (2-6 min, fraction 1), and the other for pesticide (6-20 min, fraction 2) analysis. Finally, 3 ml of ethyl acetate was injected into the column to elute the remaining lipids. Fraction 1 was concentrated to 1mL under a gentle flow of nitrogen prior to analysis of PCBs by GC-ECD. After PCB analysis, fraction 1 was then combined with fraction 2, and evaporated to 1 ml for OCP analysis by GC-ECD.

Instrumentation and Calibration for GC-ECD Analysis

I analyzed the final extracts on an Agilent 6890 series GC equipped with an

Agilent 7683 autosampler and an electron capture detector (Agilent Technologies, Palo Alto, CA, USA). Two columns, an HP-5MS (30m*0.25mm*0.25μm film thickness, Agilent Technologies) and a DB-608 (30m*0.25mm*0.25μm film thickness, Agilent Technologies) were used to confirm the analytical results. Helium and nitrogen were employed as the carrier and makeup gas, respectively. A 2 μL sample was injected into the GC using a pulsed split-less mode. For OCP analysis on the DB-608 column, the oven was set at 100°C, heated to 250°C at 10°C /min, then to 280°C at 3°C /min and held at 280°C for 10 min and the flow rate of carrier gas was 1.8 ml/min. For OCP analysis on the HP-5 column, the oven was set at 100°C, heated to 180°C at 10°C /min, to 205°C at 3°C /min and held at 205°C for 4min, then to 280°C at 20°C /min and held at 280°C for 11 min and the flow rate of carrier gas was 3.5 ml/min. For PCB analysis on the DB-608 column, the oven was set at 100°C, heated to 240°C at 10°C /min and held for 2min, then to 280°C at 3°C /min and held at 280°C for 10 min and the flow rate of carrier gas was 1.8 ml/min. For PCB analysis on the HP-5 column, the oven was set at 100°C, heated to 180°C at 10°C /min, to 200°C at 6°C /min and held for 4min, to 220°C at 5°C /min and held for 4min, then to 270°C and the flow rate of carrier gas was 4.0 ml/min.

Calibration was based on area using four external standards. The standard solutions contained 1, 10, 50 and 100 ng/ml each of PCB, OCP, and surrogate standard. These solutions were analyzed using the GC-ECD methods detailed above, with the calibration curves linear within this concentration range. Qualitative identity was established using a retention window of 1% with confirmation on a second column, the lowest value between the two columns was reported for each analyte.

Method detection limits ranged from 0.36 – 1.37 µg/kg, but a detection threshold of 2 µg/kg was set for all analytes.

Data Analysis

Due to the variability of lipid content within tissue types and among fish, all data were lipid normalized for statistical analyses. Total concentrations of PCBs (Sum PCBs) were calculated by summing the masses of individual congeners and total OCPs (Sum OCPs) were calculated by summing the masses of individual pesticides. Total OCs refers to the sum of total PCBs and total OCPs. Individual PCB congeners were used to estimate Aroclor mixtures 1242, 1254, and 1260. The composition of each Aroclor mixture was obtained from Frame et al. (1996), and concentrations were determined by extrapolating from the average mass of 7 to 8 reference congeners that make up a known percentage of each Aroclor (USEPA, 1996). Aroclor 1254 and 1260 were distinguished from one another by the ratio of PCB congeners 180 and 118 (Shelton et al. 2004). TCDD equivalents (TEQs) were estimated based on toxic equivalency factors (TEFs) of PCBs for fish (Van den Berg et al. 1998). Concentrations of TCDD-like congeners were multiplied by their corresponding TEF, summed, and reported as Sum TEQs. Non-detectable concentrations of all contaminants were assigned a numerical value one-half the detection limit for statistical analysis (JMP IN Version 4.0.2, 2000), and a significance level for all tests was set at the effect level $p<0.05$. A one-way analysis of variance was used to determine whether there were significant differences of bioaccumulation among tissue type, land use, and phenotypic sex (males and

intersexuals); Tukey's test (HSD) was used as the multiple comparison statistic. Bivariate regressions were used to assess the relationship between contaminant bioaccumulation and GSI of males.

Results

Fish Collection and Sexual Determination

A total of 59 shovelnose sturgeon were collected throughout the MMR, 54 fish were identified as males and five as intersexuals by gross observation and histology. Forty-five of 54 males were identified as MII, and the remaining 11 males were MI. All five intersexuals possessed spermatozoa and were of MII maturity, but were also found to have varying amounts of primary oocytes, often located in periphery regions. One intersexual was grossly identified as an MII male, but histological analysis found primary oocytes in one of five cross-sections taken.

OC Analysis

Mean residue concentrations (Table 1) were reported on a lipid normalized basis because of the variability of mean lipid content among tissues (gonads 51.4% ± 20.0; brains 9.9% ± 4.1; fillets 3.3% ± 3.4). The surrogate recovery data for all tissues were within acceptable limits (% mean DBOFB recovery ± SD: PCB analysis; gonad 90.0% ± 18.7, brain 84.5% ± 8.5, fillet 80.5% ± 10.4; OCP analysis; gonad 95.9% ± 12.8, brain 88.4% ± 10.0, fillet 81.1% ± 10.5). The concentrations of PCBs and OCPs were positively correlated with one another in each tissue (gonad, $r^2 = 0.48$, $p < 0.0001$; brain, $r^2 = 0.57$, $p < 0.0001$; fillet, $r^2 = 0.17$, $p < 0.005$), thereby

masking the effects that individual compounds may have on GSI. Therefore, further results will be presented as total OCs. Total OC concentrations were highest in fillets and lowest in brains (Figure 2), and PCBs, *p,p'* -DDT and its metabolites *p,p'* -DDE and *p,p'* -DDD, comprised 94% of total OCs in all tissues.

All PCB congeners examined were frequently detected in gonad samples, but several congeners were below detection limits in fillet and brain samples (Table 1). Fillets accumulated the highest mean concentrations of sum PCBs followed by gonads and brains, and congener patterns within tissues suggested that Aroclor mixtures 1254 and 1260 are predominant. Aroclor 1254 and 1260 mixtures share similar congeners, but can be distinguished from one another by the abundance of congeners 180 and 118 (Shelton et al. 2004). In pure Aroclor 1254 the 180/118 ratio is 0.09, whereas in pure Aroclor 1260 the 180/118 ratio is 24. The mean 180/118 ratio in our fish was 2.30, indicating a prevalence of Aroclor 1260. All TCDD-like PCB congeners were detected at low levels and tissue accumulation was similar to that of other PCBs, with fillets accumulating highest concentrations followed by gonads and brains (Table 1).

Eleven of 14 OCPs were detected in fish; *alpha*-BHC, *beta*-BHC, and *gamma*-BHC were not detected in any samples; and mean concentrations of several other OCPs were below detection limits in brain and fillet samples (Table 1). Sum OCP concentrations were mainly comprised of DDT metabolites, *p,p'* -DDE was detected at the highest mean levels followed by *p,p'*-DDT and *p,p'*-DDD (Table 1). Dieldrin, *alpha*-chlordane, and *gamma*-chlordane were the only other analytes routinely detected above detection limits.

No difference of OC accumulation was found among fish from agricultural and urban sites (gonads, $p = 0.31$; brains, $p = 0.29$; fillets, $p = 0.30$), although fish from urban areas had higher mean concentrations of OCs in each tissue (gonads, agricultural = 6,755 µg/kg, urban = 8,357 µg/kg; brains, agricultural = 4,359 µg/kg, urban = 5,347 µg/kg; fillets, agricultural = 7,034 µg/kg, urban = 9,297 µg/kg). Sum PCB and Sum OCP accumulation were not different between agricultural and urban sites as well (Sum OCP: gonads, $p = 0.52$; brains, $p = 0.59$; fillets, $p = 0.18$; Sum PCB: gonads, $p = 0.31$; brains, $p = 0.29$; fillets, $p = 0.22$).

Analysis of Males

MI males (n=11) were analyzed separate of MII males because of significantly lower GSIs (MI = 2.28 ± 0.46, MII = 4.2 ± 0.23, $p < 0.0005$). Total OCs and GSI were negatively related in gonads ($r^2 = 0.33$, $p < 0.0001$), brains ($r^2 = 0.27$, $p < 0.001$), and fillets ($r^2 = 0.21$, $p < 0.005$) of MII males (Figure 3). Sum TEQs were negatively related with GSI, but only within gonads ($r^2 = 0.28$, $p < 0.0005$) and fillets ($r^2 = 0.12$, $p < 0.05$). Sum PCBs and *p,p'* -DDE in gonads appeared to have the greatest negative effect on GSI of MII males ($r^2 = 0.34$, $p < 0.0001$). MI males also displayed a significant negative relationship between GSI and accumulation of total OCs in gonads ($r^2 = 0.70$, $p < 0.005$), brains ($r^2 = 0.61$, $p < 0.005$) and fillets ($r^2 = 0.58$, $p < 0.001$). Sum TEQs and GSI were negatively related in gonads ($r^2 = 0.75$, $p < 0.001$), brains ($r^2 = 0.42$, $p < 0.05$), and fillets ($r^2 = 0.58$, $p < 0.01$) as well. Residues of *p,p'* -DDE within the gonads had the greatest effect on MI GSI ($r^2 = 0.78$, $p < 0.0005$). Histological images were useful as indicators of reproductive

limitations in highly contaminated males. MII males with relatively low concentrations of OCs were characterized by large seminiferous tubules filled with spermatozoa (Figure 4a), while highly contaminated MII males possessed smaller tubules with visibly fewer numbers of spermatozoa (Figure 4b).

Analysis of Intersexuals

Four intersexuals were identified by the gross observation, and another was discovered through histology by the presence of primary oocytes in one of five cross-sections. Mean GSI of intersexuals (4.05 ± 0.02) and males (4.22 ± 1.72) did not differ ($p = 0.79$). Intersexual gonads displayed a predominately male appearance but contained masses of primary oocytes surrounded by black degenerative tissue (Figure 4c). One intersexual was found to have a long, single gonad stretching along both walls of the body but positioned as paired gonads. In another instance an intersexual possessed one gonad being primarily testicular, and the other primarily ovarian. Histologically, intersexual gonads possessed MII characteristics with clutches of primary oocytes located in periphery regions. In some cases the tunica albuginea (covering of the gonad) was extended and formed an island of primary oocytes, a structure which is typically seen in the ovarian folds of females (Figure 4c) (Colombo 2004). Because interior regions of the gonads were primarily devoid of spermatozoa in four of five fishes (Figure 4d), intersexuals were likely limited reproductively.

No difference of total OC accumulation was found among intersexual tissues ($p = 0.59$), but tissues of MII males and MII intersexuals accumulated OCs in a different manner (Figure 5). Total OCs in the gonads and fillets of males and

intersexuals did not differ (gonads, $p = 0.92$; fillets, $p = 0.43$), but intersexuals accumulated significantly higher concentrations of total OCs in the brain ($p < 0.05$).

Discussion

Discoveries of intersexual fishes inhabiting estrogenic and antiandrogenic waters have become widespread in recent years (reviewed in Kavanagh et al. 2004), and reproductive limitations in these organisms have been well documented (Jobling et al. 2002a,b). Body residues of endocrine-disrupting chemicals within fishes have not yet been linked to intersexuality, and accumulation within the brains and gonads are likely important in inducing intersexuality. Organochlorine compounds are estrogenic and/or antiandrogenic and are capable of inducing intersexuality (Fry and Toone 1981, Kelce et al. 1995) as well as suppressing gonadal development in exposed organisms (Baatrup and Junge 2001, Foster et al. 2001, Bayley et al. 2002). Intersexual MMR shovelnose sturgeon have been reported in the past (Carlson et al. 1985, Harshbarger et al. 2000, Colombo 2004), but occurrences have not been reported prior to the mass production and dispersal of OCs into the environment. The aim of this study was to determine how OCs affect the reproductive potential of MMR shovelnose sturgeon, specifically by quantifying GSI and OC accumulation in the brains, gonads, and fillets of males and intersexuals. Results clearly indicate that OCs inhibit gonadal maturation in male shovelnose sturgeon. Furthermore, there is also evidence to suggest that shovelnose sturgeon intersexuality is induced through elevated OC exposure within the brain.

Intersexuality

Hormonal interplay between the brain and gonads is essential in the sexual differentiation of fishes. Pituitary secretions stimulate steroid production in the gonads, and depending on genotypic sex, androgenic (11-ketotestosterone (KT)) or estrogenic (17β-estradiol (E_2)) hormones are received at receptor sites within the brain which stimulate further steroid production (Kime 1999). This developmental process is much more plastic in fishes than in mammals and is more readily influenced by exogenous factors (Shapiro 1992). Chemicals that mimic E2 and/or 11-KT action, such as OCs (Fry and Toone 1981, Kelce et al. 1995), may bind to receptors in the brain and/or gonads and inhibit proper sex steroid synthesis. It is now well known that intersexuality in gonochoristic fishes may be induced with treatments of estrogenic chemicals prior to or throughout sexual differentiation (Gimeno et al. 1997, reviewed in Piferrer 2001). While sexual differentiation occurs within 110 days post hatch in species such as common carp (*Cyprinus carpio*) (Gimeno et al. 1997), shovelnose sturgeon are a relatively slow-maturing species and differentiate at a later age. By utilizing age and growth estimates by Jackson (2004) and length at maturity estimates by Colombo (2004), shovelnose sturgeon likely undergo sexual differentiation between age two and three, which allows considerable time for this species to accumulate elevated concentrations of endocrine-disrupting chemicals prior to sexual differentiation.

Intersexual shovelnose sturgeon were found to accumulate higher concentrations of OCs in brains compared to males, but similar results were not found in the fillets and gonads, although fillet concentrations were elevated. These results

are very interesting, given the importance of the brain in directing sexual differentiation in fishes. Elevated exposure to OCs in the brain prior to and throughout sexual differentiation may stimulate intersexuality by creating an estrogenic environment within exposed organisms. It is the relative ratio of androgens to estrogens within an organism that creates an androgenic or estrogenic hormonal milieu (Guillette et al. 1996), and a combination of suppressed 11-KT production and spiked E2 levels in response to OC exposure may be important in inducing intersexuality in shovelnose sturgeon. MMR shovelnose sturgeon are exposed to a mixture of estrogenic and antiandrogenic OCs (reviewed in Schantz and Widholm 2001), and further studies on white sturgeon (*Acipenser transmontanus*) suggest that endocrine function in sturgeons may be altered by OC exposure (Kruse and Scarnecchia 2002, Feist et al. 2005). Organochlorine compounds suppress 11-KT levels and increase E2/11-KT ratios in white sturgeon from the Kootenai River (Kruse and Scarnecchia 2002), and threshold levels of gonadal OC exposure whereupon 11-KT levels are suppressed were observed at 2.5 µg/kg total PCBs (wet wt.) in Colombia River white sturgeon (Feist et al. 2005). Mean wet weight concentrations of total PCBs in MMR shovelnose sturgeon were measured at 2.25 µg/kg (wet wt.), and the added insult of OCPs such as *p,p'* -DDE may further increase E2/11-KT ratios as well, thus creating an estrogenic environment during sexual differentiation and inducing intersexuality.

The lack of significantly elevated OC accumulation within the gonads and fillets of intersexuals may be explained by life history attributes of male shovelnose sturgeon. Sexual differentiation occurs between ages 2-3 in male shovelnose

sturgeon (Jackson 2004, Colombo 2005) and sexual maturity is reached at age five (Keenlyne 1997). Spawning takes place every 2-3 years (Keenlyne and Jenkins 1993), whereupon contaminated gametes are released and maturity, as well as contaminant accumulation, begins once again. Thus, OCs within the gonads represent only 2-3 years of exposure and are not representative of exposure prior to or during sexual differentiation. Because males and intersexuals accumulated nearly identical concentrations of OCs in the gonads, elevated brain residues in intersexuals were likely accumulated in previous years, possibly prior to sexual differentiation. Shovelnose sturgeon are capable of long-range movements (Bramblett and White 2001), and the turnover of endogenous reserves during spring migrations may decrease OC concentrations within fillet tissue. Furthermore, growth following sexual differentiation may lead to a dilution of OCs residues within the muscle of intersexuals (Anderson et al. 1987). The brain undergoes little turnover (Levenson and Janghorbani 1994) and may serve as a better indicator of past OC exposure compared to fillet and gonadal tissue. Elevated OCs in the brains of intersexuals may therefore be indicative of high exposure early in life, which may have intervened with proper sexual differentiation.

Reproductive Limitations

The reproductive ability of male and intersexual shovelnose sturgeon appears to be limited in response to elevated OC exposure. Histological observation of four of five intersexuals found reduced numbers of spermatozoa within interior regions, and highly contaminated males showed similar results. Such occurrences are typical

of exposures to estrogenic chemicals, and laboratory studies have found that reproductive limitations may range from reduced GSI to complete sterility depending on the severity of exposure (reviewed in Piferrer 2001). Wild intersexual roach exposed to estrogenic sewage effluent exhibited a variety of gonadal malformations, and their reproductive potential was diminished through decreased gamete production and fertility (Jobling et al. 2002ab).

Decreased GSI is often a sign of decreased hypothalamic, pituitary, or gonadal activity (Kime 1999), thus males with high OC residues and low GSI are likely reproductively limited. Because gonadal residues of sum PCBs and *p,p'* -DDE were most strongly negatively related to GSI, they may be most influential in the suppression of gonadal development of males. Recent research suggests that antiandrogenic compounds may depress steroidogenesis through the competitive inhibition of androgen receptors (AR) within testes. The antiandrogen *p,p'* -DDE competes with AR in goldfish (*Carassius auratus*) testes, but not in brains or ovaries (Wells and Van Der Kraak 2000). Further, *p,p'* -DDE in male guppies (*Poecilia reticulate*) decreases sperm counts, GSI, and delays maturity (Baatrup and Junge 2001, Bayley et al. 2002), and 11-KT and GSI Columbia River white sturgeon are suppressed by DDT derivatives and PCBs in gonadal tissue as well (Kruse and Scarnecchia 2002, Feist et al. 2005). The antiandrogen TCDD delays maturity, and decreases testis size and spermatogenesis in male rats, and TCDD-like PCBs (TEQs) are capable of producing similar patterns of developmental effects (reviewed in Peterson et al. 1993, reviewed in Gray and Kelce 1996, Faqi et al. 1998). Gonadal residues of sum TEQs were most strongly negatively related to GSI, which is

interesting considering that the antiandrogen *p,p'* -DDE preferentially binds to AR in the testes. The strong correlation between GSI and gonadal OC residues suggests gonadal inhibition in MMR shovelnose sturgeon is exerted through antagonistic AR binding within the testes.

Land Usage

Organochlorine pesticides were widely used in the farmed floodplains adjacent to the MMR in southern regions, while PCBs were utilized by industrial facilities in the St. Louis and East St. Louis metropolitan area (Young 1996). However, OC accumulation within shovelnose sturgeon was unrelated to land use and individual site, and PCBs and OCPs were significantly correlated with one another. Shovelnose sturgeon were collected in the spring prior to spawning, and telemetry observations of have found individuals to move upwards of 250 km during spring migrations (Bramblett and White 2001). Shovelnose sturgeon also exhibit a large home-range throughout other seasons (Bramblett and White 2001), which may further diminish the importance of site location on OC accumulation. Furthermore, PCB and OCP accumulation may be ubiquitous throughout the MMR, as suggested by previous health advisories that have restricted the consumption of shovelnose sturgeon eggs and flesh throughout the entire MMR (Bush and Grace 1989, Hornshaw 2005).

Conclusions

This study provides novel information regarding the endocrine-disrupting effects that OCs may induce within exposed organisms. I have demonstrated that OC

accumulation within the brain may be critical in inducing intersexuality in shovelnose sturgeon, and that OC exposure throughout sexual maturation inhibits gonadal development, possibly through the antagonistic binding of antiandrogenic OCs within the testes. The effects of endocrine disrupting chemicals on reproduction in males and intersexuals have been quantified in other species, and similar limitations may be occurring in MMR shovelnose sturgeon as well, which may be further exacerbated by continued commercial harvests. Furthermore, results suggest that OCs are prevalent throughout much of the MMR and may impact other resident species. Although more research is needed to quantify the effects that OCs have on MMR shovelnose sturgeon reproduction, this study is another indication that the presence of endocrine-disrupting chemicals in the environment may significantly impact exposed organisms.

Table 1. Organochlorine compounds analyzed in shovelnose sturgeon (N=59) collected during April 2003 from the Middle Mississippi River.

	Mean OC Concentration in Tissue (µg/kg lipid wet wt.)				Mean OC Concentration in Tissue (µg/kg lipid wet wt.)		
Analyte[a]	Gonad	Brain	Fillet	Analyte	Gonad	Brain	Fillet
PCB 8	5.5	BDL[b]	BDL	PCB 200	12.4	BDL	BDL
PCB 18	12.0	BDL	BDL	PCB 180	915.5	550.1	808.7
PCB 31	30.8	36.5	BDL	PCB 157[d]	5.4	BDL	BDL
PCB 28	36.9	BDL	BDL	PCB 170	244.5	108.1	139.1
PCB 52	218.3	191.1	492.6	PCB 201	109.2	31.4	BDL
PCB 49	126.1	136.2	211.4	PCB 203	103.3	32.5	BDL
PCB 43	126.3	101.2	189.0	PCB 195	39.4	BDL	BDL
PCB 48	55.3	65.6	84.9	PCB 194	122.3	37.5	BDL
PCB 44	69.6	36.0	BDL	PCB 206	93.9	BDL	BDL
PCB 70	113.8	87.6	235.2	Sum PCBs	6248.5	3795.8	6950.2
PCB 66	97.3	53.2	119.8	Aroclor 1242	BDL	BDL	BDL
PCB 95	109.2	60.8	159.4	Aroclor 1254	4948.2	3751.6	6822.5
PCB 101	521.3	327.5	732.9	Aroclor 1260	5478.6	2590.1	463.9
PCB 99	204.5	129.3	293.6	Sum TEQs	0.018	0.010	0.025
PCB 97	29.4	BDL	BDL	α-BHC	ND[c]	ND	ND
PCB 86	5.3	BDL	BDL	β-BHC	ND	ND	ND
PCB 87	125.6	66.9	172.7	γ-BHC	ND	ND	ND
PCB 77[d]	162.8	105.1	250.7	heptachlor	3.6	BDL	BDL
PCB 110	198.4	144.5	367.7	aldrin	9.7	BDL	BDL
PCB 118[d]	279.7	219.7	488.0	heptachlor epoxide	41.6	BDL	BDL
PCB 123[d]	85.6	32.3	BDL	γ-chlordane	93.0	27.8	61.6
PCB 153	1051	676.9	1106.6	endosulfan I	8.6	BDL	BDL
PCB 114[d]	100.4	32.7	BDL	α-chlordane	107.9	45.0	76.3
PCB 105[d]	99.2	60.3	159.1	dieldrin	253.3	121.9	220.3
PCB 138	779.6	508.3	857.1	*p,p'*-DDE	680.8	439.7	665.3
PCB 187	406.0	203.2	282.2	endrin	30.4	23.1	BDL
PCB 183	129.1	48.9	BDL	*p,p'*-DDD	106.1	58.0	71.3
PCB 128	108.2	58.2	82.5	*p,p'*-DDT	56.1	104.4	171.2
PCB 167[d]	22.4	BDL	BDL	Sum OCPs	1334.8	1092.4	1235.0
PCB 174	148.4	49.4	75.5	Total OCs	7582.3	4852.2	8185.2
PCB 156[d]	60.4	20.2	BDL				

[a] Method detection limits range from 0.10 – 1.39 µg/kg, reporting limits were set at 2 µg/kg.
[b] BDL = Mean value is below detection limit.
[c] ND = Not detected.
[d] TCDD-like congeners

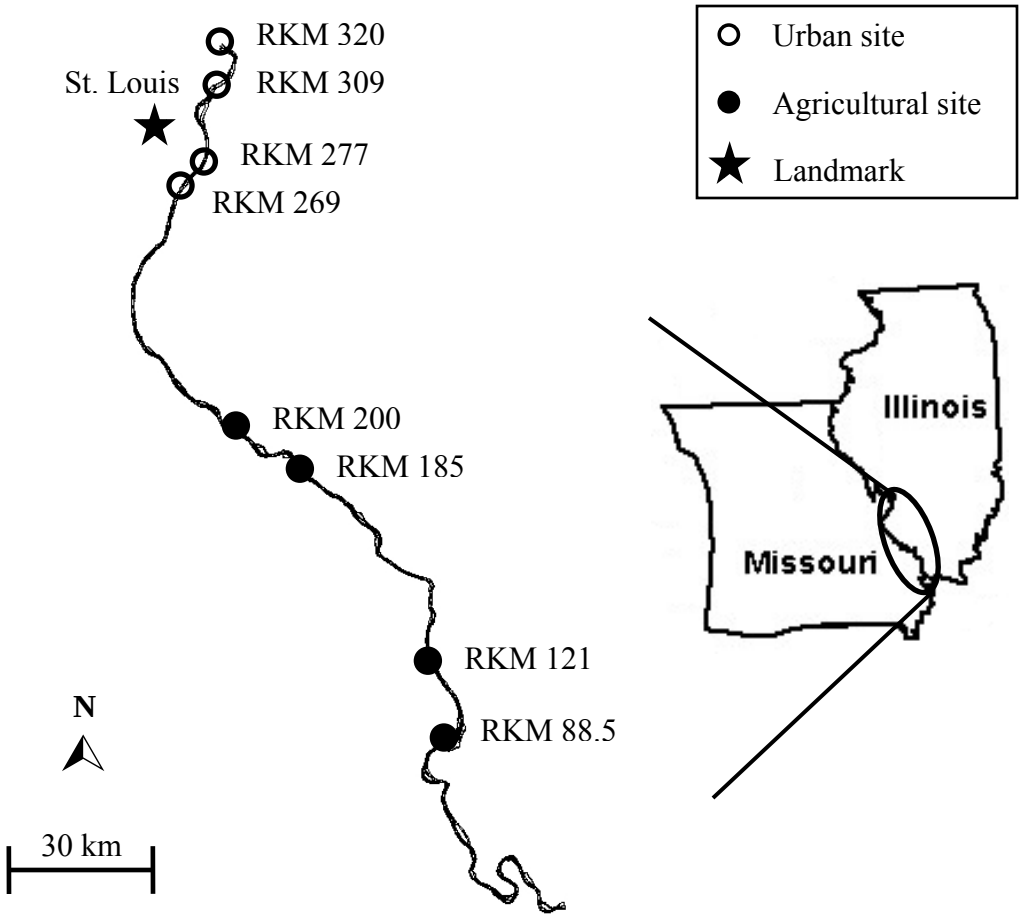

Figure 1. Map of sites along the Middle Mississippi River, where shovelnose sturgeon (N=59) were collected during April 2003. RKM = River kilometer

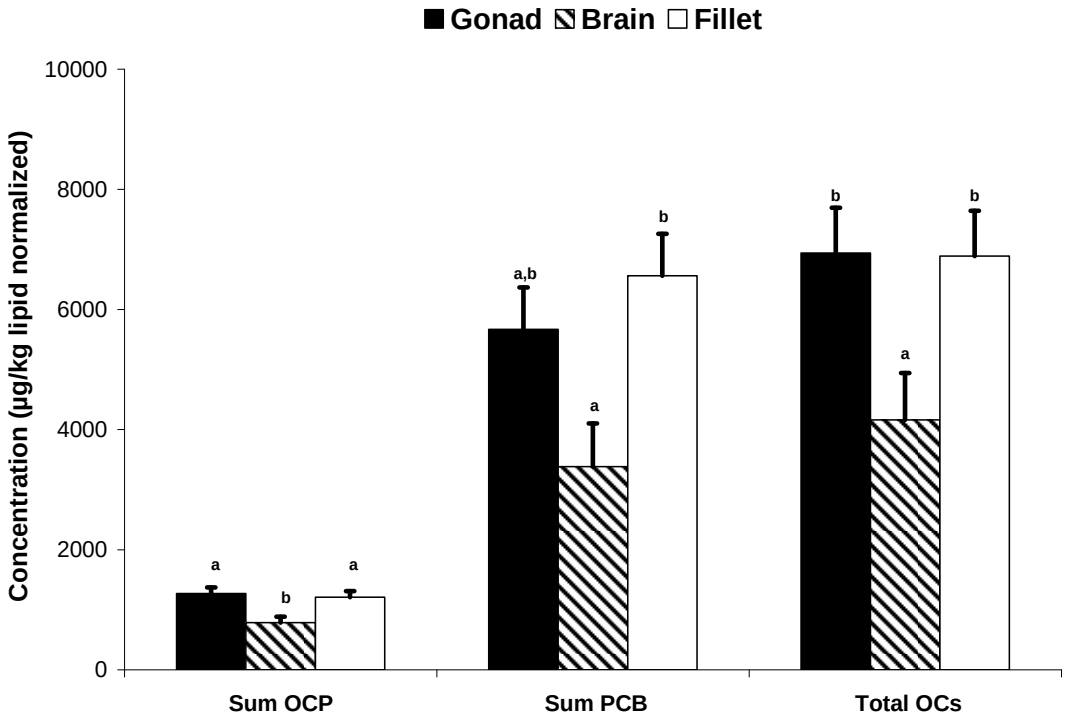

Figure 2. Total contaminant accumulation in tissues of MII males (N=43) collected during April 2003 from the Middle Mississippi River. Letters denote significant differences in concentrations among tissues (ANOVA, Tukey's HSD, $p < 0.05$).

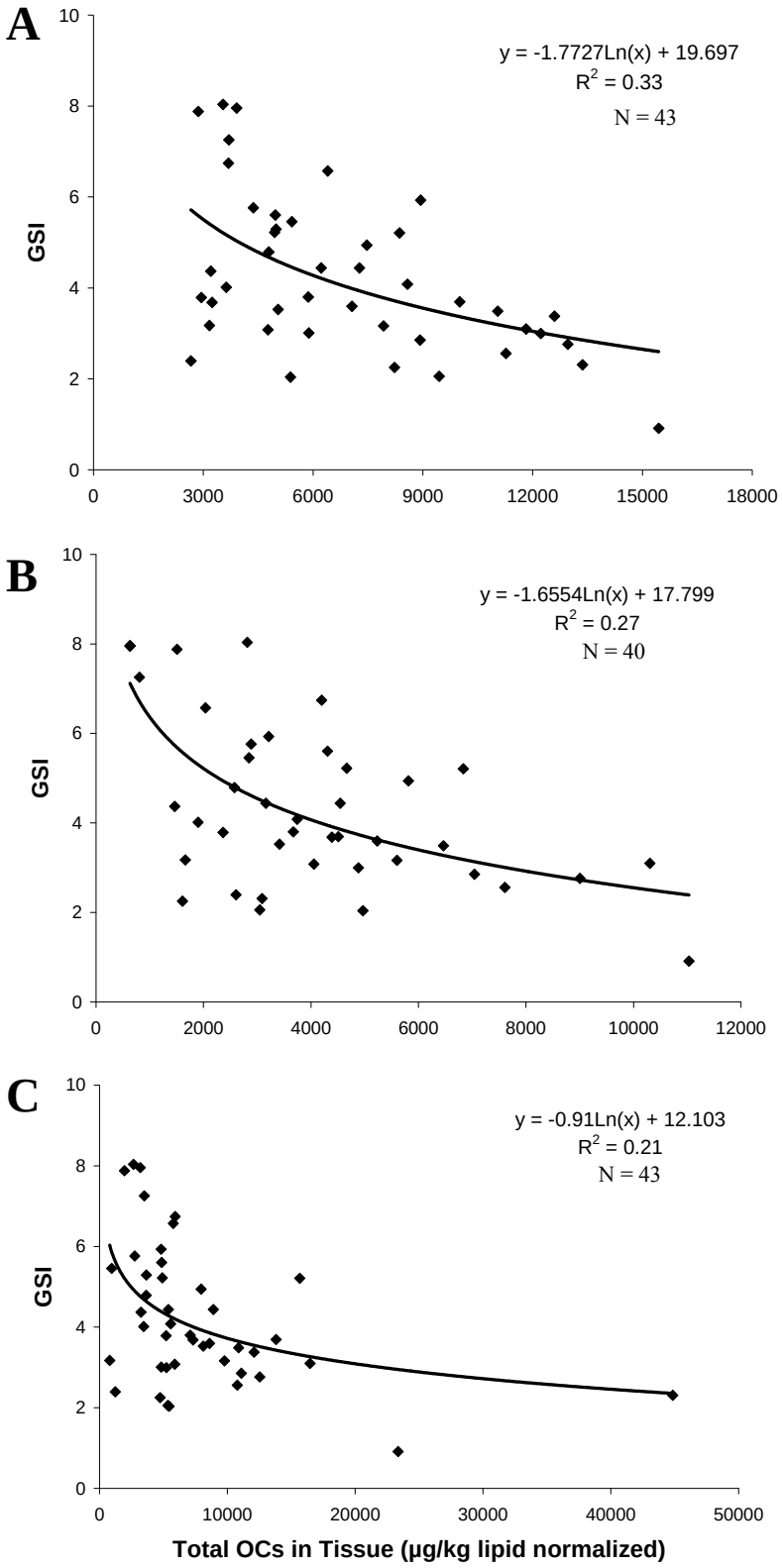

Figure 3. Regression of GSI versus total contaminants in **(a)** gonads, **(b)** brains, and **(c)** fillets of MII male shovelnose sturgeon collected during April 2003 from the Middle Mississippi River.

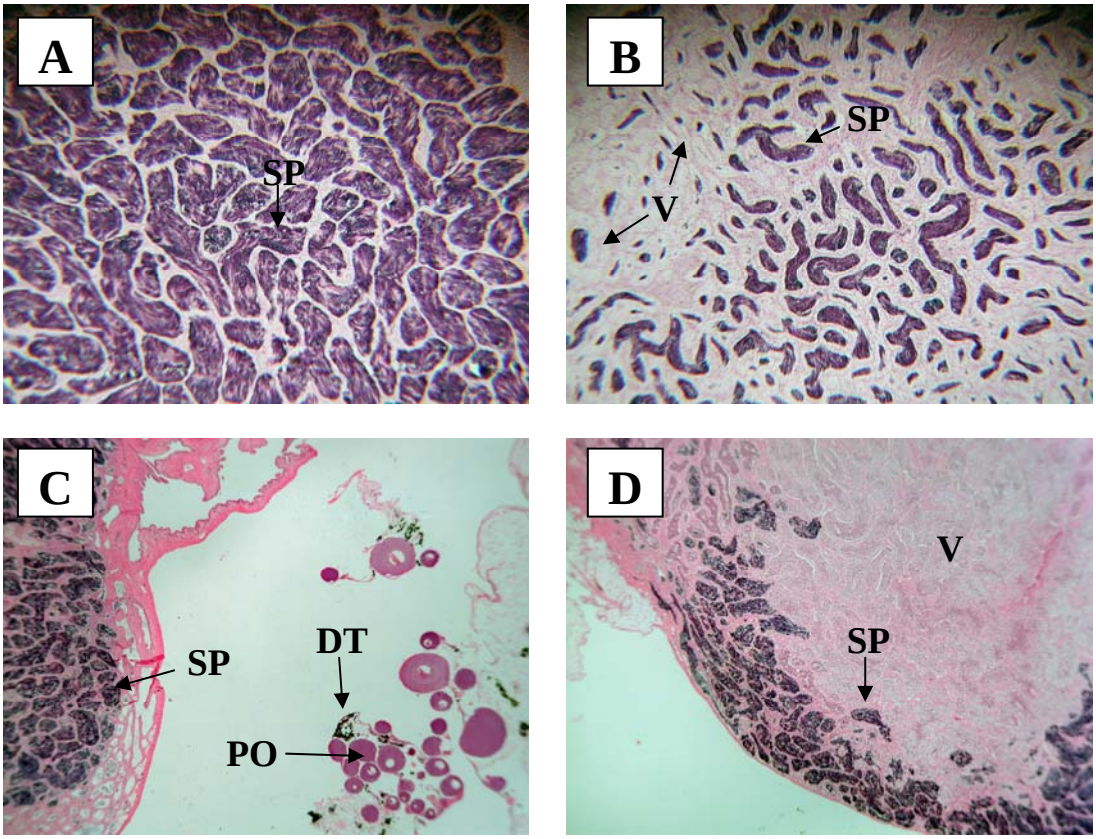

Figure 4. Histological sections (×100) of shovelnose sturgeon collected during April 2003 from the Middle Mississippi River. Digital images are of **(A)** a MII male gonad (FL = 621 mm; GSI = 4.37; total OCs = 3,205 µg/kg lipid wt.), **(B)** a highly contaminated male MII gonad (FL = 610 mm; GSI = 0.91; total OCs = 15,445 µg/kg lipid wt.), **(C)** an intersex MII gonad containing primary oocytes surrounded by degenerative tissue, and **(D)** the same intersex MII gonad devoid of spermatozoa in the interior region. DT, degenerative tissue; PO, primary oocytes; SP, spermatozoa within seminiferous tubules; VT, vacant seminiferous tubules.

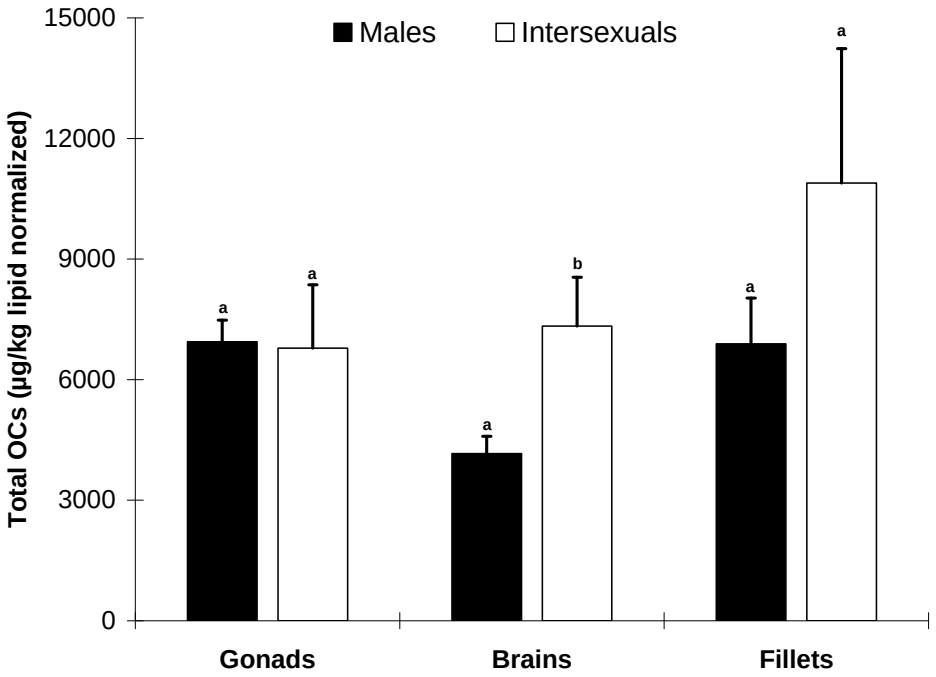

Figure 5. Relationship between total OC accumulation in tissues of MII males (N=40) and MII intersexuals (N=5) collected during April 2003 from the Middle Mississippi River. Different letters denote a significant difference of Total OC accumulation in tissues among males and intersexuals (ANOVA, Tukey's HSD, $p < 0.05$).

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