Report for 2005SC11B: Toxicological effects of endocrine disrupting compounds in Lake Conestee and the Reedy River

Publications

- Other Publications:
    - Otter, RR, Schreiber, EA, van den Hurk, P, Klaine, SJ. Watershed-scale application of biomarkers to assess ecosystem health. Poster presentation at the 25th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Portland, OR Nov 14-18, 2004
    - Schreiber, E.A., R.R. Otter, S.J. Klaine, and P. van den Hurk. The Use of Biomarkers to Evaluate Contaminant Effects on Largemouth Bass from Lake Conestee, SC, USA. Poster presentation at the 25th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Portland, OR Nov 14-18, 2004
    - Schreiber, E.A, R.R. Otter, S.J. Klaine, P. van den Hurk. Effects of Legacy Contaminants on Largemouth Bass in the Saluda Watershed. Poster Presentation at the Annual Meeting of the Carolinas Chapter of SETAC. April 2005, Raleigh, NC.
    - Otter, R.R., E.A. Schreiber, P. van den Hurk, S.J. Klaine. Watershed-scale application of biomarkers to access contaminant exposure in largemouth bass. Oral platform presentation at the 26th Annual Meeting of the Society of Environmental Toxicology and Chemistry. Baltimore, MD. Nov 13-17, 2005
- Articles in Refereed Scientific Journals:
    - Schreiber, E.S., Otter, R.R., Van den Hurk, P. (2006) A biomarker approach to measure biological effects of contaminant exposure in largemouth bass from Lake Conestee, SC, USA. Environ. Toxicol. Chem. In press.
- Dissertations:
    - Schreiber, E.A. A biomarker approach to measure biological effects of contaminant exposure in largemouth bass from Lake Conestee and nearby reservoirs. Masters Thesis, August 2005.
- Unclassified:

- Otter, R.R. Spatial Characterization Of Biomarkers In A Contaminated Watershed: Usefulness In Ecological Risk Assessments. Dissertation. May 2006.

Report Follows

Progress Report

August 2006

Toxicological effects of endocrine disrupting compounds in Lake Conestee and the Reedy River.

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Executive summary

As urban development progresses wastewater treatment becomes vital as the human population in an area increases. However, wastewater treatment does not tend to include monitoring for pharmaceuticals and chemical metabolites that humans often excrete in urine or feces. Therefore, many hormone based pharmaceuticals consumed and later excreted remain in the effluent from wastewater facilities. Effluents in wastewater have previously been shown to contain elevated concentrations of hormone metabolites and pharmaceuticals that downstream wildlife is subsequently exposed to. Human hormone metabolites and pharmaceuticals have the potential to cause disruption in the normal hormone signaling mechanisms in endocrine systems of wildlife including fish, amphibians, and other animals exposed to the wastewater effluent either by direct contact and ingestion or indirect exposure such as consumption of contaminated fauna or flora.

In Greenville County the primary wastewater treatment facility is located just below the city of Greenville and the effluent is discharged into the Reedy River water basin above Lake Conestee. The Reedy River originates in the foothills of the Blue Ridge Mountains about 7 miles northwest of the city of Greenville, SC and flows south through the city of Greenville, the Piedmont, and into Laurens county. The river empties into an arm of Lake Greenwood that is formed by a dam on the Saluda River which also flows into Lake Greenwood.

The water quality in the Reedy River basin has been shown to be impacted from the diverse and highly urbanized land uses within the watershed. Recent water quality data has suggested that the wastewater treatment plant effluents comprise a significant portion of the flow in the Reedy River, especially during extended dry periods when the effluent discharge is

believed to account for greater than 60 percent of the flow of the Reedy River basin. Previous studies have demonstrated the presence of signficant amounts of endocrine disrupting chemicals in the wastewater treatment plant effluents. The objective of this study was to examine the observed toxicological effects of the endocrine disrupting compounds (EDCs) that are released into the Reedy River from the Greenville wastewater treatment facility by using a suite of biomarkers that were measured in indigenous fish to characterize the exposure and biological effects of these contaminants.

Bluegills (*Lepomis macrochirus*) were collected during three different seasons (spring, summer, and fall) from several sites along the length of the Reedy River and from an un-impacted control site at Lake Robinson. The fish were analyzed for unnatural estrogenic effects such as: 1). Vitellogenin (VTG) egg yolk protein production in male fish, 2). Activity of estrogenic compounds in bile extractions, and 3). Inhibition or induction of steroid hormone metabolizing enzyme UDP-glucuronosyltransferase (UGT).

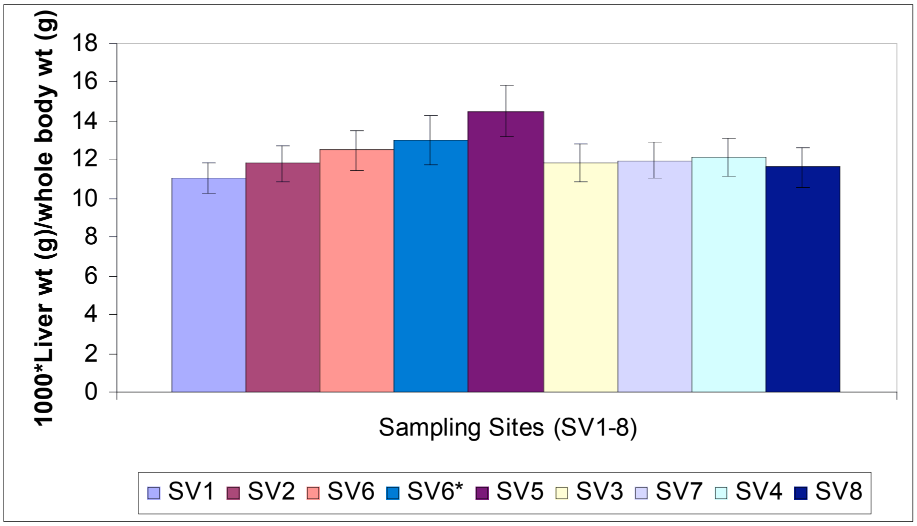
The hepatosomatic indices (HSI) measured for the bluegill showed seasonal variations between spring and summer sampling (Fig.1). No significant differences in H.S.I from along the sampling sites were apparent during the spring season. However, during the summer season the H.S.I for most of the sites was found to be higher than those measured during the spring season, with the fish at the SV5 site directly below the Greenville wastewater treatment facility having significantly elevated H.S.I compared to the fish collected at control sites.

Bluegill that were collected in the sites downstream of the Greenville wastewater treatment facility were found to have significantly elevated levels of estrogenic activity in their bile conjugates relative to the specimens collected in reference sites during each season (Fig.2), providing evidence that the fish were exposed to elevated concentrations of xenoestrogenic compounds downstream of the water treatment plant and that the xenoestrogens were also bioavailable to the bluegill.

Two biomarkers of effect were also examined: Vitellogenin concentrations in plasma of male and juvenile specimens and UDP-glucuronosyltransferase (UGT) activity in liver S9 fractions. The plasma VTG concentrations for fish collected in the spring season showed that several sites downstream of the Greenville wastewater treatment facility as far downstream as Boyd Mill Pond showed significantly elevated plasma VTG concentrations relative to the fish from control sites (Fig.3). The same general trend was seen during the summer season as well, however the SV5 site immediately downstream of the wastewater facility showed a two fold increase in plasma VTG concentration compared to the same site during the spring season. The measured UGT activity showed little variation between sites during either the spring or summer seasons, except immediately downstream of the wastewater treatment facility, where the measured UGT activity was significantly elevated during both seasons (Fig.4). The general trend in UGT activity that was seen during both seasons showed elevated UGT activity after the wastewater treatment plant, where UGT activity was highest, followed by a subsequent decline in activity the farther the sites were downstream from the facility.

The cumulative information provided by these biomarkers provided a more comprehensive understanding of the extent of EDCs contamination, its effects on biota, and may serve as a foundation for an ecological and human health risk assessments for the watershed.

A. Spring



B. Summer

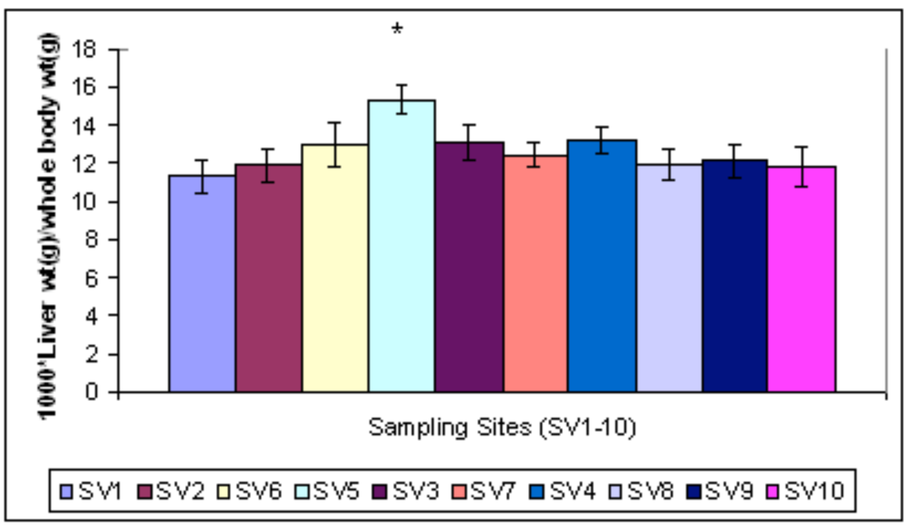
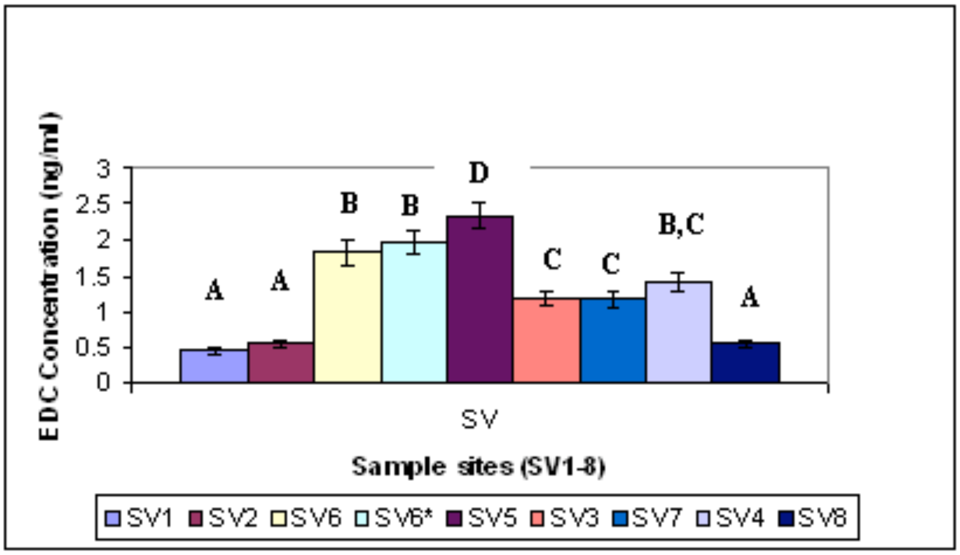


Figure 1. Hepatosomatic indices for *Lepomis macrochirus* collected in (a) spring, (b) summer, and (c) fall, from sites along the Reedy River watershed (SV1-10). SV1,SV2 = reference sites; SV6, SV6* = above WWTF1; SV5 = directly below WWT1; SV3 = above Conestee dam; SV7 = directly below Conestee dam; SV4 = below WWTF2; SV8 = 2 miles down of WWTF2; SV9 = Boyd Mill Pond; SV10 = Lake Greenwood. Significant difference from the reference site was determined (*; $p<0.01$, ANOVA). Data presented are mean ± SEM of n=10 from each site.

A. Spring



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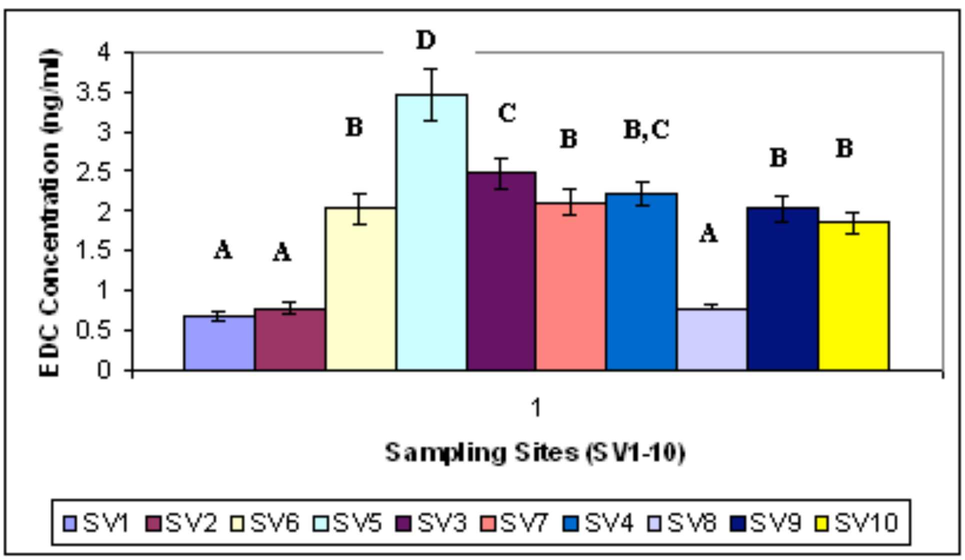
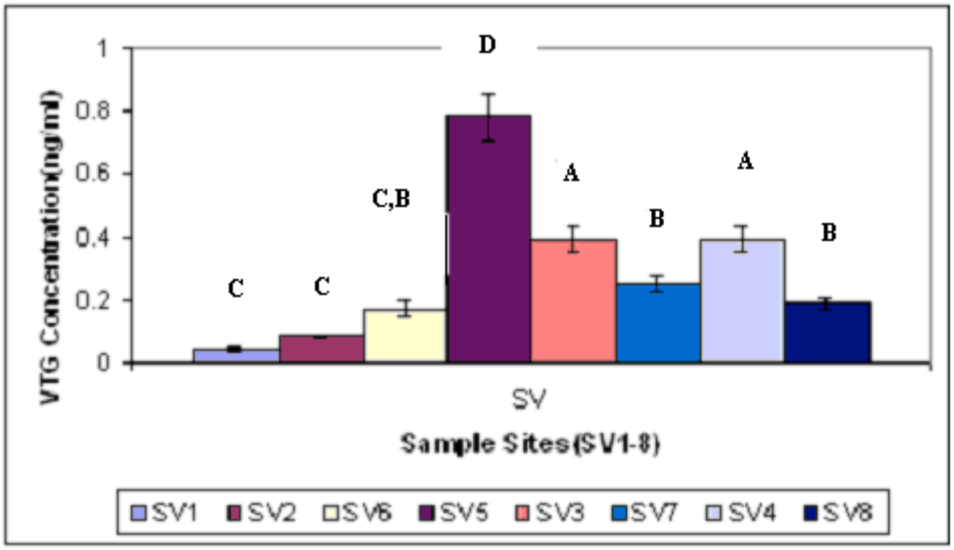


Figure 2: Estrogenic activity of bile conjugates of *Lepomis macrochirus* collected during (a) spring, (b) summer, and (c) fall along the Reedy River watershed (SV1-10). SV1,SV2 = reference sites; SV6, SV6* = above WWTF1; SV5 = directly below WWT1; SV3 = above Conestee dam; SV7 = directly below Conestee dam; SV4 = below WWTF2; SV8 = 2 miles down of WWTF2; SV9 = Boyd Mill Pond; SV10 = Lake Greenwood. Significant differences between sites during the same season were determined and are indicated by different letters (A-D) ($p<0.05$, ANOVA). Data presented as mean ± SEM of n=10 from each site.

A. Spring



B. Summer

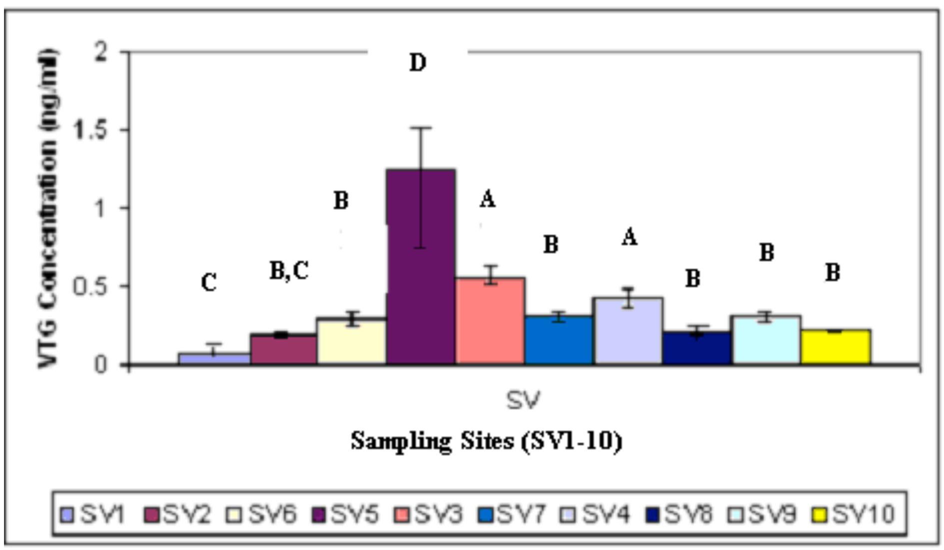
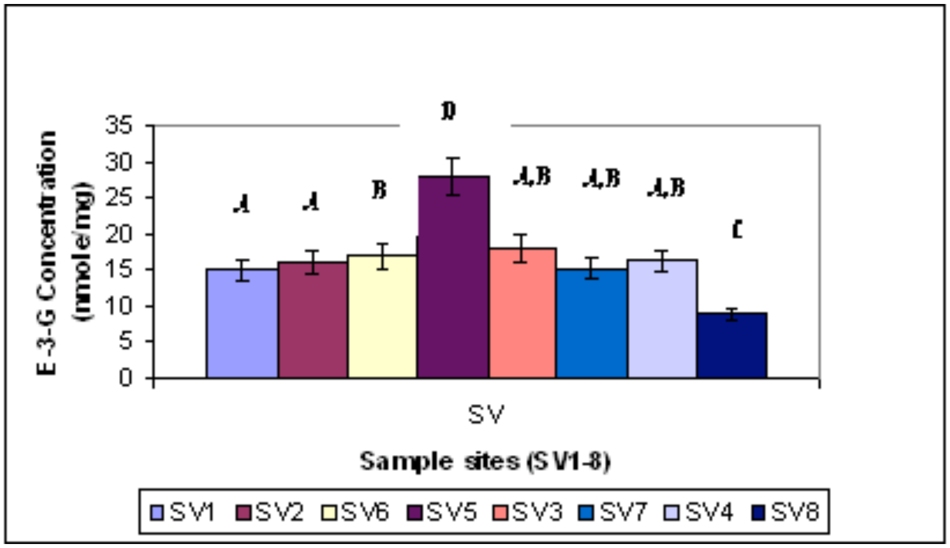


Figure 3: Plasma Vitellogenin concentration in *Lepomis macrochirus* collected during (a) spring, (b) summer, and (c) fall along the Reedy River watershed (SV1-10). SV1,SV2 = reference sites; SV6, SV6* = above WWTF1; SV5 = directly below WWT1; SV3 = above Conestee dam; SV7 = directly below Conestee dam; SV4 = below WWTF2; SV8 = 2 miles down of WWTF2; SV9 = Boyd Mill Pond; SV10 = Lake Greenwood. Significant differences between sites during the same season were determined and are indicated by different letters (A-D) ($p<0.05$, ANOVA). Data presented as mean ± SEM of n=10 from each site.

A. Spring



B. Summer

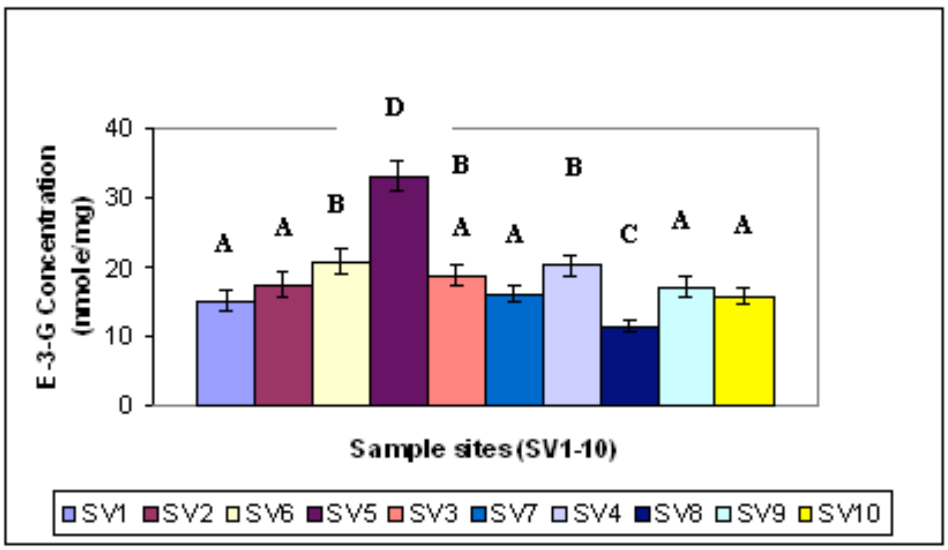


Figure 4: UGT activity in *Lepomis macrochirus* collected during (a) spring, (b) summer, and (c) fall along the Reedy River watershed (SV1-10). SV1,SV2 = reference sites; SV6, SV6* = above WWTF1; SV5 = directly below WWT1; SV3 = above Conestee dam; SV7 = directly below Conestee dam; SV4 = below WWTF2; SV8 = 2 miles down of WWTF2; SV9 = Boyd Mill Pond; SV10 = Lake Greenwood. Significant differences between sites during the same season were determined and are indicated by different letters (A-D) ($p<0.05$, ANOVA). Data presented as mean ± SEM of n=10 from each site.