SETAC PRESS

Environmental Toxicology and Chemistry, Vol. 25, No. 7, pp. 1926–1932, 2006

Printed in the USA
0730-7268/06 $12.00 + .00

A BIOMARKER APPROACH TO MEASURE BIOLOGICAL EFFECTS OF CONTAMINANT EXPOSURE IN LARGEMOUTH BASS FROM LAKE CONESTEE, SOUTH CAROLINA, USA

ERIKA A. SCHREIBER,*† RYAN R. OTTER,‡ and PETER VAN DEN HURK‡
†Department of Biological Sciences, Clemson University, Clemson, South Carolina 29634, USA
‡Institute of Environmental Toxicology, Clemson University, P.O. Box 709, Pendleton, South Carolina 29670, USA

(*Received* 12 *October* 2005; *Accepted* 5 *January* 2006)

Abstract—Sediments from Lake Conestee, a former reservoir now filled with pollutant-enriched sediments, located south of Greenville, South Carolina, USA, and other nearby reservoirs were collected and analyzed for lead and polycyclic aromatic hydrocarbons (PAHs). Hepatic ethoxyresorufin-*O*-deethylase (EROD), glutathione *S*-transferase (GST), UDP-glucuronosyltransferase (UGT), sulfotransferase (SULT), and erythrocyte δ-aminolevulinic acid dehydratase (ALAD) were measured in largemouth bass (*Micropterus salmoides*) to characterize biological effects of these contaminants over three seasons. Results showed that total PAH concentrations in Lake Conestee sediments were significantly greater than the control during each season. An average 10-fold induction in EROD activity was observed at Lake Conestee compared with the control over all three seasons, indicating that PAHs present in sediment were bioavailable to fish. Significant gender effects were observed in EROD activity during the spring, in which activity in reproductively active female fish was significantly suppressed compared with the male fish. Sediments from Lake Conestee had elevated lead concentrations, but the lack of ALAD inhibition in bass indicated that lead was not biologically available. Total GST activity, UGT activity, and SULT activity were not significantly induced in fish from any of the affected sites compared with the reference site. Both EROD and UGT activities were highest during the winter, as were sediment PAH concentrations in Lake Conestee, possibly linked to seasonal resuspension events. The biomarkers measured in this study were useful as a first investigation into the biological effects of contaminant exposure, as well as in determining the bioavailability of contaminants in Lake Conestee.

Keywords—Biomarkers Largemouth bass Phase II enzymes Ethoxyresorufin-*O*-deethylase δ-Aminolevulinic acid dehydratase

INTRODUCTION

Lake Conestee was formerly a 52.5 ha (130-acre) millpond located on the Reedy River, south of Greenville (SC, USA). Because of infilling of sediments over the last 100+ years, the lake area is now roughly 8 ha (20 acres). Above Lake Conestee, the Reedy River drains a relatively small, highly urbanized watershed that includes the rapidly growing city of Greenville, including all of the older most industrialized portions of the city, and parts of the industrialized Interstate 385 corridor (B. Beasley, South Carolina Department of Natural Resources, Columbia, SC, USA, personal communication). Greenville's largest wastewater treatment plant, with a permitted capacity of 29 million gallons per day, is also located less than 3.2 km (2 miles) upstream. The development of this urbanized area has been a major contributor of contaminants, sediment, and trash to the Reedy River. Many of these pollutants ultimately settle into the sediments of Lake Conestee, which are known to contain high concentrations of heavy metals, such as lead, and polycyclic aromatic hydrocarbons (PAHs; [1–3], http://www.fcc.gov/foia/). Polycyclic aromatic hydrocarbon contamination of aquatic systems originates primarily from anthropogenic sources such as incomplete combustion of fossil fuels, former coal gas production, petroleum spills, industrial effluents, and highway runoff [4]. An example of this type of contamination occurred in June 1996 when an interstate oil pipeline ruptured, spilling more than 950,000 gallons of diesel fuel into the Reedy River several kilometers downstream of Lake Conestee (U.S. Environmental Protection Agency, Office of Enforcement and Compliance Assurance, 2003, http://www.epa.gov/compliance/resources/cases/civil/cwa/colonialfs.pdf). An estimated 35,000 fish and other species of wildlife were killed and the diesel fuel dispersed more than 55 km (34 miles) downstream. This was the worst oil spill in South Carolina's history and the fifth worst inland oil spill in U.S. history (D.L. Hargett, The Pinnacle Consulting Group—A division of North Wind, Greenville, SC, USA, personal communication).

Despite the documented contamination of Lake Conestee, nothing was known about the biological effects of these contaminants. A way of assessing biological effects in a polluted system is the measurement of biochemical markers in resident species. Biochemical markers, or biomarkers, are early warning signals that reflect adverse biological responses toward anthropogenic environmental toxins. In addition, the application of biomarkers to complement traditional chemical methods of detecting pollution can reveal the presence of contaminants that were not initially suspected; identify the presence of a biologically available contaminant, rather than a biologically inert form of contamination; or both.

Levels of Pb in the subaqueous sediments of Lake Conestee were up to 218 mg/kg [2]. Lead is a hazardous environmental toxicant largely because of its well-documented deleterious effects on biological systems, combined with a widespread global distribution [5]. Inhibition of δ-aminolevulinic acid dehydratase (ALAD) activity in fish blood is well documented as a biomarker of environmental lead exposure [6]. In fish,

* To whom correspondence may be addressed (erikas2727@yahoo.com).

erythrocyte ALAD inhibition is correlated with exposure to lead in the water and increases with Pb concentration and exposure time [7,8]. Previous studies have demonstrated negative correlations between ALAD activity and both carcass and blood Pb concentrations in fish species [7,9,10].

Polycyclic aromatic hydrocarbons are a major contaminant in Lake Conestee and the Reedy River [2]. Following uptake, these compounds are readily metabolized in fish, typically by hepatic biotransformation enzymes such as cytochrome P450 isozymes (CYP1A) and a variety of phase II conjugating enzymes. Induction of CYP1A in fish is correlated in a dose-dependent manner to environmental levels of PAHs [11,12]. According to Goksøyr and Förlin [13], the most sensitive probe for measuring induction of CYP1A in fish is the activity of ethoxyresorufin-*O*-deethylase (EROD). Induction of EROD activity by a variety of environmental contaminants such as PAHs and polychlorinated biphenyls has been well documented [14].

Despite the significant role that phase II enzymes play in homeostasis of endogenous compounds and detoxification of xenobiotics, relatively little is known about piscine phase II systems. In general, responses of phase II enzymes such as glutathione *S*-transferase (GST), UDP-glucuronosyltransferase (UGT), and sulfotransferase (SULT) are less pronounced than those of phase I enzymes [15]. However, even minor alterations in phase II activity, such as changes in phase II cosubstrate levels in response to exposure to toxicants, can be harmful to organisms [16,17]. Several studies have observed increases in hepatic GST and UGT activity after exposure of fish to PAHs [18]. Sulfotransferase is generally considered to be noninducible, although a recent study reported evidence of increased SULT activity in both catfish and mummichog after exposure to PAHs [19].

The abundance and trophic level status of largemouth bass (*Micropterus salmoides*), a freshwater species with commercial and economic importance in the southeastern United States, made them a coveted species for study. Within an aquatic system, if the principal source of chemical input is slow release from polluted sediments, as has been proposed in the case of Lake Conestee, it is likely that uptake by benthic organisms followed by predation by larger organisms such as fish could be a significant source of bioaccumulation [20]. Hence, largemouth bass are particularly susceptible to accumulation of persistent organic contaminants such as PAHs and heavy metals as a result of their top predator status and relatively long life span.

In addition to the measured chemical contamination of Lake Conestee, information is needed on the potential biological effects of these contaminants. The purpose of this study was to use a suite of biomarkers to measure biological effects of contaminants (specifically Pb and PAHs) in Lake Conestee and other nearby reservoirs with the use of largemouth bass as a resident target species.

MATERIALS AND METHODS

Field collections

Fish and sediment samples were collected from May to December 2004. The principal site of concern in this study was Lake Conestee, located approximately 11 km (7 miles) south of the city of Greenville on the Reedy River (Fig. 1). Two other reservoirs, Boyd Mill Pond and Lake Greenwood, located downstream of Lake Conestee on the Reedy River, were also sampled. As a reference, largemouth bass were collected from Lake Robinson, a relatively pristine 324-ha (800-acre) lake located on the nearby South Tyger River in upper Greenville County. This reservoir is surrounded primarily by undisturbed shorelines (Fig. 1). Saluda Lake, an impoundment on the Saluda River nearby the City of Greenville, was originally incorporated into this study as a second reference site; however, because of the high volume of boating activity and on-going dredging, this lake was dropped as a reference and simply considered another sampling site within the watershed.

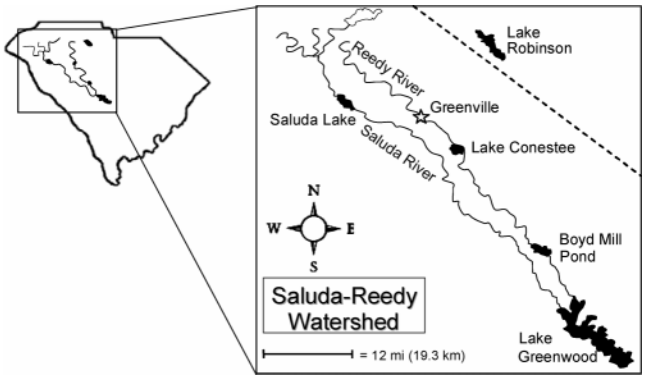

Fig. 1. Locations of the five sampling sites in upstate South Carolina, USA. The reference site Lake Robinson; Saluda Lake located on the Saluda River; and Lake Conestee, Boyd Mill Pond, and Lake Greenwood all located on the Reedy River. Dashed line indicates separation of watersheds.

Largemouth bass were collected by electrofishing from each of the five sampling sites ($n = 7$) during the spring (May), summer (July–August), and winter (October–December) of 2004. Blood samples were obtained by caudal vein puncture with a disposable needle and heparinized Vacutainer® (Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Blood was kept on ice until return to the laboratory, where it was frozen in liquid nitrogen and stored at −80°C until analysis. Fish were kept on ice until returned to the laboratory, where they were measured (cm), weighed (g), identified by gender, and dissected for removal of livers, which were wrapped in foil, frozen in liquid nitrogen, and stored at −80°C pending analysis.

Surficial (top 8 cm) sediment samples were taken from three different locations on each of the five study sites. All samples were taken from locations corresponding to areas of fish collection. A subsample of sediment was placed in a 500-ml translucent plastic Nalgene® (Nalgene, Rochester, NY, USA) bottle on ice, which on return to the laboratory was stored at 4°C until heavy metal analysis. Another sediment subsample was placed in a Teflon®-lined amber vial on ice until it could be stored at 4°C to await PAH analysis.

Cytosol and microsome preparations

All livers were homogenized and cytosolic and microsomal fractions were prepared according to standard procedures. Approximately 2 g of individual liver tissues were homogenized in 10 ml of ice-cold buffer consisting of 0.25 M sucrose, 0.05 M Tris-base, 1 mM ethylenediamine tetra-acetic acid, 1 mM dithiothreitol, and 0.2 mM phenylmethylsulfonylfluoride (pH 7.4). The homogenates were centrifuged, and the resulting fat layer was aspirated off twice before transfer and storage of the cytosolic supernatant. The microsomal pellet was resuspended in 1 ml of buffer containing 0.25 M sucrose, 0.01 *N*-(2-hydroxyethyl) piperazine-*N*′-2-ethanesulfonic acid, 1 mM

ethylenediamine tetra-acetic acid, 0.1 mM dithiothreitol, and 5% glycerol (pH 7.4). Both cytosol and microsome samples were aliquoted in 1.5-ml microcentrifuge tubes and frozen at −80°C until analyzed. Cytosolic and microsomal protein concentrations were measured on a colorimetric plate reader (Molecular Devices Spectramax 190, Sunnyvale, CA, USA) at 562 nm with a bicinchoninic acid Protein Assay Kit (Pierce, Rockford, IL, USA) with bovine serum albumin as a standard.

Biomarkers

δ-Aminolevulinic acid dehydratase activity was measured in whole blood samples by a modification of the method of Murase et al. [21]. Because of insufficient blood sampling, ALAD activity was not measured in the spring samples. In brief, tubes containing heparinized blood diluted (1:5) with lysing reagent (0.1% Triton-X-100 detergent in deionized water), 0.5 M phosphate-buffered saline (pH 6.8), deionized water, and 60 mM δ-aminolevulinic acid in phosphate-buffered saline were incubated in a shaking water bath at 27°C for 2 h. After centrifugation, a subsample of supernatant was combined with Ehrlich's reagent (0.2 g of *p*-dimethylamino-benzaldehyde, 6 ml of glacial acetic acid, and 3.2 ml of 70% perchloric acid diluted up to 10 ml with acetic acid). Subsamples were added in duplicate to a clear, flat-bottom, 96-well microliterplate, and absorbance was measured at 555 nm with a colorimetric microplate reader. δ-Aminolevulinic acid dehydratase activities were normalized (mg hemoglobin/g blood) as determined by cyanmethemoglobin assays [22].

Cytochrome P450-1A isozyme activity was measured as EROD in a reaction volume of 250 μl containing 0.1 M Tris-HCl buffer (pH 7.8), 0.2% bovine serum albumin, 5 mM $MgCl_2$, 2 μM ethoxyresorufin, 25 μg of microsomal protein, and 0.5 mM nicotinamide adenine dinucleotide phosphate in its reduced form. Enzyme kinetics were measured by fluorescence spectrophotometry at 5-min intervals for a total of 30 min [23].

Total GST activity with the use of 1-chloro-2,4-dinitrobenzene as a substrate was measured in a reaction volume of 3 ml containing 1 M *N*-(2-hydroxyethyl) piperazine-*N*′-2-ethanesulfonic acid buffer (pH 7.6), 3 mM glutathione, 30 mM 1-chloro-2,4-dinitrobenzene, 300 μg of cytosol of varied volumes, and deionized water to bring the volume up to 3 ml. Reaction kinetics were analyzed spectrophotometrically for 2 min at 344 nm [24].

Glucuronidation of 9-OH-benzo[*a*]pyrene (BaP) was measured in a reaction volume of 500 μl containing 1 μM 9-OH-BaP, 0.1 M Tris-HCl buffer (pH 7.8), 5 mM $MgCl_2$, 50 μg of microsomal protein (mixed 5:1 w/w) with Brij 58 (Sigma, St. Louis, MO, USA), and 200 μM UDP-glucuronic acid. Glucuronidated product was measured by fluorescence spectrophotometry at pH 11 [25,26]. Sulfation was measured in a reaction volume of 500 μl containing 1 μM 9-OH-BaP, 0.05 M Tris-HCl buffer (pH 7.0), 0.4% bovine serum albumin, 25 μg of cytosolic protein, and 20 μM 3′-phosphoadenosine 5′-phosphosulfate. Sulfonated product was quantified as described for glucuronidation.

Sediment analysis

Sediment PAH concentrations were determined for all surficial sediment samples collected and stored in amber vials by enzyme-linked immunosorbent assay. Briefly, sediment was extracted (Sample Extraction Kit User Guide, Strategic Diagnostics, Newark, DE, USA) and analyzed for PAHs with a RaPID Assay® PAH Test Kit (Strategic Diagnostics) on an RPA-1™ RaPID Photometric Analyzer (Strategic Diagnostics). Total PAH concentrations are reported as the sum of the phenanthrene, fluoranthene, benzo[*a*]pyrene, pyrene, chrysene, anthracene, indeno(1,2,3-*cd*)pyrene, heating fuel, JP-5, JP-4, gasoline, kerosene, jet A fuel, benzo[*a*]anthrecene, fluorene, benzo[*b*]fluoranthene, acenaphthylene, benzo[*k*]fluoranthene, acenaphthalene, and benzo[*ghi*]perylene concentrations in the sample (mg phenanthrene/kg sediment, wet wt). Surficial sediment samples collected during the summer season were digested and analyzed for Pb by inductively coupled plasma–mass spectrometry (Lab for Environmental Analysis, Department of Crop and Soil Sciences, University of Georgia, Athens, GA, USA).

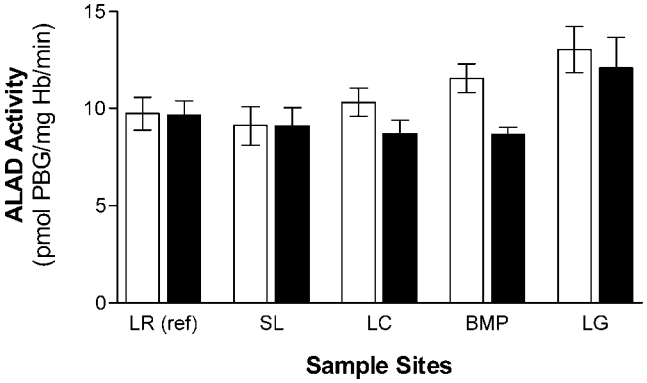

Fig. 2. δ-Aminolevulinic acid dehydratase (ALAD) activity in whole blood of *Micropterus salmoides* collected during the summer and winter from LR (ref) = Lake Robinson reference site; SL = Saluda Lake; LC = Lake Conestee; BMP = Boyd Mill Pond; LG = Lake Greenwood, USA. Data are mean ± standard error of the mean (n = 7 for each site, except n = 6 for LC summer and LG winter). □ Summer; ■ Winter.

Statistical analysis

All treatments were analyzed by analysis of variance (ANOVA) followed by Tukey's test for significant differences. Two-way ANOVA tables were calculated to determine whether significant differences existed among gender, site, or both. One-way ANOVA relationships were analyzed by season, treatment, and overall differences. Activities of EROD, UGT, and SULT enzymes were determined from their standard curves by performing linear regressions with 95% confidence intervals. All data are reported as mean ± standard error of the mean. The level of statistical significance was set at $\alpha = 0.05$.

RESULTS

ALAD and sediment Pb

No significant inhibition of erythrocyte ALAD activity was observed in bass from Lake Conestee compared with the control, Lake Robinson (Fig. 2). Gender variation was not significant; therefore, male and female samples were pooled. It was found that Pb concentrations in surficial sediment samples from Lake Conestee (81.2 ± 7.5 mg/kg dry wt) were approximately 27 times greater than the reference site (3.04 ± 0.74 mg/kg dry wt; Fig. 3).

EROD and sediment PAHs

Spring data (Fig. 4A) showed a general trend of greater EROD activity in males compared with females in all loca-

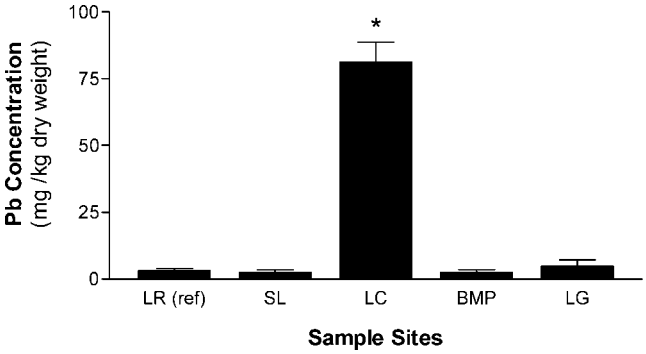

Fig. 3. Lead concentrations in surficial sediment from LR (ref) = Lake Robinson reference site; SL = Saluda Lake; LC = Lake Conestee; BMP = Boyd Mill Pond; LG = Lake Greenwood, USA, during the summer season. Significant difference from the reference site was determined (* $p < 0.001$). Data are mean ± standard error of the mean ($n = 3$ from each site).

tions, and a two-way ANOVA revealed that a significant gender effect did exist ($p < 0.05$). The EROD activity in male bass from Lake Conestee was significantly induced ninefold (132.7 ± 43.9 pmol/mg/min) over males from the reference site (14.41 ± 2.76 pmol/mg/min), whereas EROD activity in the females was significantly suppressed at this location in the spring. EROD activity was not significantly different by gender across sites during the summer and winter sampling seasons ($p > 0.05$); therefore, statistical analysis was performed after male and female data were pooled at each site for the summer and winter seasons. The EROD activity in summer samples was significantly induced eightfold and 14-fold at Boyd Mill Pond (42.82 ± 10.50 pmol/mg/min) and Lake Conestee (76.25 ± 9.68 pmol/mg/min), respectively, over the reference site Lake Robinson (5.26 ± 0.84 pmol/mg/min; Fig. 4B). During the winter, only bass from Lake Conestee exhibited a significant increase in EROD activity (135.10 ± 26.30 pmol/mg/min) over the reference site (23.97 ± 4.54 pmol/mg/min; Fig. 4C).

Total PAH concentrations in surficial sediments from Lake Conestee were significantly greater than those in the reference site for each season (Fig. 5). Sediment PAH concentrations below the detection limit were reported at the detection limit of 0.05 mg phenanthrene/kg sediment wet weight.

Phase II enzymes

No significant differences were found in the activity of the phase II enzymes GST, UGT, or SULT between males and females within the same site for any of the three seasons ($p > 0.05$). Therefore, the reported results are pooled data of males and females for each site. Average GST activity was approximately 546.6 ± 10.01 nmol/mg protein/min and sites within the three seasons sampled did not differ significantly. Although no significant differences were observed in UGT activity between affected sites and Lake Robinson in largemouth bass within the three seasons sampled, overall, UGT activity in all sites increased from spring to winter, and seasonal variation was significant for all sites except Saluda Lake. Average UGT activities in bass collected during the spring and winter were 102.2 ± 3.94 and 207.3 ± 9.71 pmol/mg protein/min, respectively. Largemouth bass SULT activity did not differ significantly between the affected sites and the reference site during any sampling season. However, SULT activity in bass from Saluda Lake, Lake Conestee, and Boyd Mill

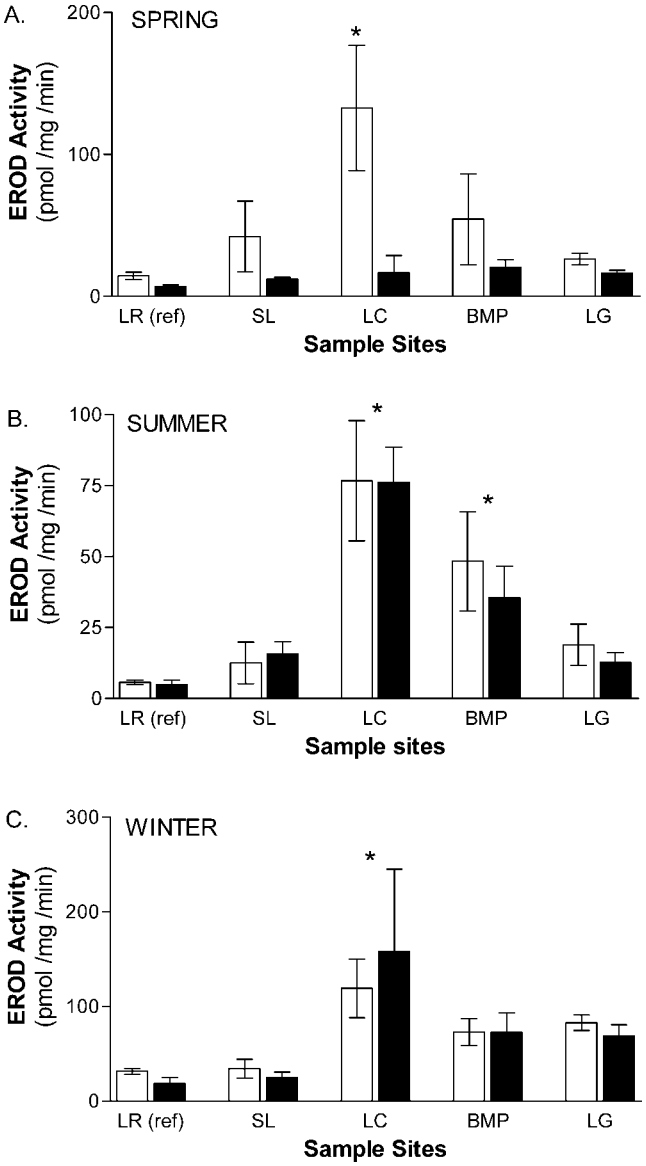

Fig. 4. Ethoxyresorufin-*O*-deethylase (EROD) activity in *Micropterus salmoides* collected during (**A**) spring, (**B**) summer, and (**C**) winter from LR (ref) = Lake Robinson reference site; SL = Saluda Lake; LC = Lake Conestee; BMP = Boyd Mill Pond; LG = Lake Greenwood, USA. Significant difference from the reference site was determined (* $p < 0.05$). Data are mean ± standard error of the mean ($n = 6$–7 from each site). □ Male; ■ Female.

Pond was significantly lower, with an average of 59.5 ± 9.45 pmol/mg protein/min, than Lake Greenwood during the spring ($p < 0.05$). The highest SULT activities were observed in bass collected during the summer from Lake Robinson, Saluda Lake, and Lake Greenwood, and the average activity measured in bass from these sites was 156.7 ± 22.5 pmol/mg protein/min.

DISCUSSION

In fish, erythrocyte ALAD activity can be depressed by exposure to Pb in the water column [7,8]. In this study, significant inhibition of ALAD activity in largemouth bass from affected sites compared with the control was not observed during any season sampled (Fig. 2). Our findings were similar to results of a study by Martin and Black [27], in which inhibition of ALAD in channel catfish exposed at a Pb-contam-

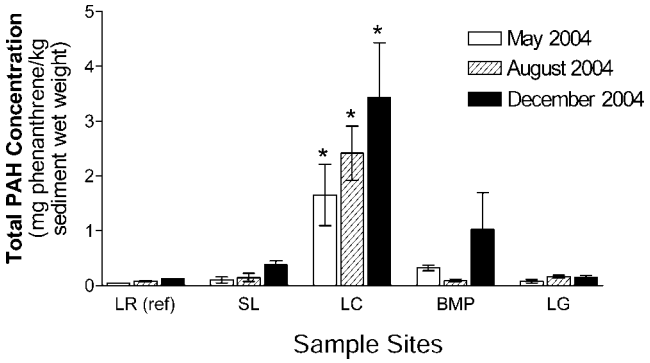

Fig. 5. Total polycyclic aromatic hydrocarbon (PAH) concentrations in surficial sediment from LR (ref) = Lake Robinson reference site; SL = Saluda Lake; LC = Lake Conestee; BMP = Boyd Mill Pond; LG = Lake Greenwood, USA, during three seasons. Significant difference from the reference site within a season was determined (* $p < 0.05$). Samples below detection limit are reported at the concentration of the detection limit 0.05. Data are mean ± standard error of the mean ($n = 3$ per season per site).

inated site was not observed. The mean Pb concentration in surficial sediment from Lake Conestee was 81.2 ± 7.5 mg/kg dry weight, which was significantly higher than the other sites ($p < 0.001$; Fig. 3) and much higher than the Ecological Comparison Criteria of 30.2 mg/kg for Pb ([28], http://www.epa.gov/region4/waste/ots/ecolbul). In aquatic environments, sediments are typically a sink for metals, and in anoxic sediments, acid-volatile sulfides play a major role in affecting the bioavailability of many divalent metals having high affinity for sulfide (i.e., Pb) [29,30]. Although not measured, the observed high amounts of organic material and the silt/clay composition of sediment suggest that anaerobic conditions are likely to exist in the sediment of Lake Conestee. It is known that if the molar quantity of acid-volatile sulfides in sediment exceeds the molar quantity of sulfide-associating metals, the Pb concentration in the overlying water column would not be detectable [31]. The suggested possibility of anoxic, sulfate-reducing conditions and subsequent binding of Pb by acid-volatile sulfides in Lake Conestee would explain the lack of inhibition of ALAD activity in fish that was observed in this study. This result emphasizes the importance of bioavailability of heavy metals and the usefulness of a specific biomarker to measure whether certain potentially toxic compounds are indeed bioavailable.

Because it is known that PAH compounds accumulate in sediments because of their hydrophobicity and partitioning to organic carbon-coated particles [11,32], it is useful to correlate EROD activity to PAH concentration in sediments. Total PAH concentrations in surficial sediments from Lake Conestee were significantly greater than those in the reference site within each season ($p < 0.05$; Fig. 5). Contrary to the Pb–ALAD data presented in this study, for PAHs sediment concentrations and biomarker response are positively correlated. Ethoxyresorufin-*O*-deethylase activity for the pooled male and female data was significantly induced at Lake Conestee compared with the reference site for all three seasons. The EROD activity in summer samples (males and females pooled) displayed a downstream trend of decreased EROD activities from Lake Conestee to Lake Greenwood, with bass from the latter site having EROD activities that did not differ significantly from the reference site (Fig. 4B). This clear reduction in EROD activities would suggest that EROD-inducing chemicals are present in high enough concentrations in water, sediments, or both from only Lake Conestee and Boyd Mill Pond and that by the time they reach Lake Greenwood, they are diluted enough for EROD activity to fall near control levels. In the past decade, two major events on the Reedy River triggered interest into the investigation of possible downstream effects. In June 1996, the fifth worst inland oil spill in U.S. history occurred on the stretch of Reedy River between Lake Conestee and Boyd Mill Pond, and in June 2000, the gate orifice of the Lake Conestee dam abruptly dislodged and released approximately 90,000 cubic yards of contaminated sediment downstream. Both of these events contributed high concentrations of PAHs into the Reedy River below Lake Conestee but upstream of Boyd Mill Pond and Lake Greenwood, which would theoretically be expected to become more dilute as distance downstream increased. This trend is observed in both the sediment concentrations and the biomarker response.

The spring data (for all sites) showed a general trend of approximately fourfold greater EROD activity in males compared with females, and a two-way ANOVA confirmed that a significant gender effect did exist ($p < 0.05$; Fig. 4A). These results are comparable to the twofold higher induction Sepúlveda et al. [33] reported in male largemouth bass over females exposed to CYP1A-inducing compounds. Reynolds et al. [34] also reported EROD levels in male flounder that were significantly higher than those in females following a six-month exposure to PAH-contaminated food. In fish that exhibit strong sex differences, EROD activity was typically highest and most similar among both males and reproductively inactive females, whereas reproductively active females typically displayed lower activity levels [13]. The suppression of EROD activity is likely related to increases in 17β-estradiol in spawning female fish, but the exact mechanism of CYP1A suppression by estrogens is still unknown [35]. The data from this study emphasize once more that care has to be taken in selecting species, gender, and season when planning a biomarker study that includes CYP1A measurements.

Hepatic GST activity has been reported to increase in several field studies after exposure of fish to PAHs, polychlorinated biphenyls, and polychlorinated dibenzo-*p*-dioxins [18] and, therefore, was included in the suite of biomarkers investigated in this study. However, in this study, no significant seasonal or gender variation was observed, and cytosolic GST activities measured in largemouth bass did not differ significantly from one another between sites. A controlled laboratory study on the effects of β-naphthoflavone on biotransformation and glutathione biosynthesis in liver of largemouth bass was conducted by Hughes and Gallagher [36]. They observed significant increases in EROD activity and the glutathione biosynthesis pathways, but a lower responsiveness was seen in GST catalytic activity. The authors proposed that these results indicated that the GST pathway in largemouth bass might not be markedly affected by PAH-type inducers, which could help explain the unexpected GST results at Lake Conestee. Despite the important role GST enzymes play in major detoxification processes, hepatic total GST activity (measured by 1-chloro-2,4-dinitrobenzene conjugation) might not be a reliable biomarker of PAH exposure in fish. Instead, it is suggested that specific GST isoenzymes that exhibit more sensitive and selective responses to pollutants be investigated in the future.

Sulfotransferases are not considered to be readily induced by pollutants [37], and little is known about the regulation of hepatic GST and SULT in fish exposed to pollutants. In this

study, no induction of SULT was observed in the contaminated sites. It is possible that the capacity of largemouth bass for sulfation versus glucuronidation compared with other vertebrates is relatively low, although no known information appears to be available on the expression of SULT and UGT in largemouth bass. Despite the observation that SULT activity from the affected sites did not differ significantly from the reference site during any of the sampling periods, SULT activity in bass from Saluda Lake, Lake Conestee, and Boyd Mill Pond were significantly lower than Lake Greenwood during the spring. This inhibition could possibly be attributed to the presence of SULT-inhibiting compounds at those sites, which should be investigated further.

Unlike GST, it is well documented that both CYP1A and UGT are under regulation of the Ah receptor [38]. Because of the abilities of both CYP1A and UGT to biotransform some of the same classes of compounds, the expectation follows that exposure to chemicals that induce CYP1A will also trigger UGT induction. However, it is noteworthy to mention that this proposed relationship between CYP1A and UGT is complicated by the considerably greater constitutive levels of UGT than EROD. Therefore, EROD typically exhibits much stronger induction over a control compared with the average two- to threefold induction of UGT reported by numerous studies [39,40]. In this study, gender variation was not significant; however, seasonal variation did differ significantly, with UGT activity being the highest during the winter. This did correspond with higher EROD activities in the winter, which were linked to higher sediment PAH concentrations in the winter.

The pattern of increased enzyme activity and PAH concentration during the winter could be closely related to the hydrology of the Reedy River, which feeds into Lake Conestee. Although it is known that historical contamination, from both industrial and municipal sources, contributed high concentrations of PAHs to Lake Conestee, the existence of current sources of PAH pollutants to Conestee or upstream regions of the Reedy River is plausible. Thus, it would be logical to conclude that highly contaminated historic sediments are buried under layers of less contaminated sediments brought in from the Reedy River more recently. However, this study found that surficial (top 8 cm) sediment samples from Lake Conestee still had high concentrations of total PAHs compared with the reference site. This is primarily attributable to runoff events that cause resuspension and redistribution of contaminated sediments in depositional areas of the lake and downstream areas, although migration of recently contaminated sediment into Lake Conestee might also play a role.

The relationship between the observed increases in UGT and EROD activity, sediment PAH concentrations, and hydrology of the Reedy River is further demonstrated by analysis of the discharge pattern of the Reedy River during the sampling period. The normal runoff events that occurred from March 2004 to the end of June 2004 were small in nature and less than 42.5 m^3/s (1,500 $feet^3/s$; Fig. 6). Two substantially higher runoff events occurred in July and August 2004 ($\sim$71 m^3/s [2,500 $feet^3/s$]). These events corresponded with both biomarker activity and sediment PAH concentrations, which were higher in samples collected in August (summer) compared with May (spring). September 2004 was an especially wet month, with runoff events generated from the remnants of three hurricanes that swept through South Carolina. During this month, three runoff events were greater than 100 m^3/s (3,500 $feet^3/s$; Fig. 6). It is postulated that these three large events were able to resuspend large quantities of buried PAH-contaminated sediments in Lake Conestee, thereby increasing the bioavailability of PAHs in the system. This resuspension and subsequent increased bioavailability of PAH compounds would explain the increases that were observed in UGT activity, EROD activity, and sediment PAH concentration in the winter.

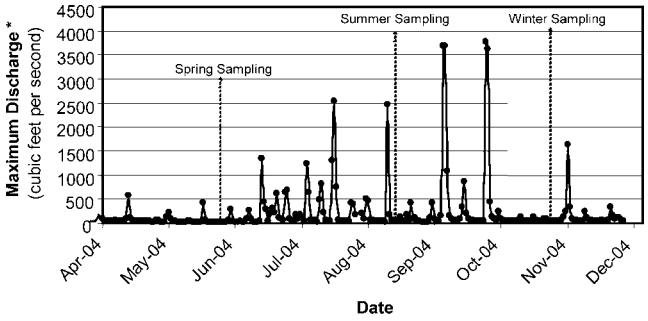

Fig. 6. Maximum daily discharge of Reedy River; Mauldin Road Greenville, South Carolina, USA (March–December 2004). * Data from U.S. Geological Survey 02164000 Reedy River near Greenville (http://waterdata.usgs.gov/sc/nwis/uv?02164000).

CONCLUSION

Although Pb concentrations in Lake Conestee sediment were found to be 27-fold higher than the reference site, ALAD activity in bass from Lake Conestee was not significantly inhibited, indicating that sediment-bound Pb was nonbioavailable. In contrast, sediment PAH concentrations were strongly correlated with EROD activity, indicating that PAH-type pollutants were biologically available to fish. Also, EROD activity was significantly suppressed in female bass during the spring spawning season. The results from this study indicate indeed a decreasing trend of sediment PAHs and correlated EROD activity downstream of Lake Conestee. In addition, UGT and EROD activity, as well as sediment PAH concentrations, were highest during the winter season.

The suite of biomarkers measured in this study provided useful information on some of the biological responses, in the form of enzyme activities, of largemouth bass exposed to various contaminants such as Pb and PAHs. One of the possible risk assessment implications of this study is that biochemical markers can be applied not only to characterize biological effects of contaminant exposures, but also to determine the bioavailability of contaminants in aquatic systems.

Acknowledgement—We thank Steve Klaine and Dave Hargett for their assistance with this project.

REFERENCES

1. Pinnacle Consulting Group. 2001. Initial targeted Brownsfield assessment report—Lake Conestee site—Greenville County, South Carolina, USA. Final Technical Report. North Wind, Greenville, SC, USA. (In print and available from author)
2. Pinnacle Consulting Group. 2004. Final combined data report, targeted Brownsfield assessment—Phases I & II, Lake Conestee, Greenville County, South Carolina, USA. Final Technical Report. North Wind, Greenville, SC, USA. (In print and available from author)
3. Zapata Engineering and Pinnacle Consulting Group. 2003. Targeted Brownsfield assessment—Phase two, Lake Conestee, Greenville County, South Carolina, USA. Final Technical Report. North Wind, Greenville, SC, USA. (In print and available from author)
4. Meador JP, Stein JE, Reichert WL, Varanasi U. 1995. Bioaccu-

mulation of polycyclic aromatic hydrocarbons in marine organisms. *Rev Environ Contam Toxicol* 143:79–165.
5. Ogunsetian OA, Yang S, Ericson J. 2000. Microbial δ-aminolevulinic acid dehydratase as a biosensor of lead bioavailability in contaminated environments. *Soil Biol Biochem* 32:1899–1906.
6. Hodson PV, Blunt BR, Whittle DR. 1984. Monitoring lead exposure in fish. In Cairns VW, Hodson PV, Nriagu JO, eds, *Contaminant Effects on Fisheries.* John Wiley, New York, NY, USA, pp 87–98.
7. Schmitt CJ, Wildhaber ML, Hunn JB, Nash T, Tieger MN, Steadman BL. 1993. Biomonitoring of lead-contaminated Missouri streams with an assay for erythrocyte δ-aminolevulinic acid dehydratase activity in fish blood. *Arch Environ Contam Toxicol* 25:464–475.
8. Hodson PV. 1976. δ-Amino levulinic acid dehydratase activity of fish blood as an indicator of a harmful exposure to lead. *J Fish Res Board Can* 33:268–271.
9. Haux C, Larsson A, Lithner G, Sjobeck ML. 1986. A field study of physiological effects on fish in lead-contaminated lakes. *Environ Toxicol Chem* 5:283–288.
10. Schmitt CJ, Dwyer FJ, Finger SE. 1984. Bioavailability of Pb and Zn from mine tailings indicated by erythrocyte δ-aminolevulinic acid dehydratase (ALA-D) activity in suckers (Pisces: Catastomidae). *Can J Fish Aquat Sci* 41:1030–1040.
11. Whyte JJ, Karrow NA, Boermans HJ, Dixon DG, Bols NC. 2000. Combined methodologies for measuring exposure of rainbow trout (*Oncorhynchus mykiss*) to polycyclic aromatic hydrocarbons (PAHs) in creosote contaminated microcosms. *Polycyclic Aromat Compd* 18:71–98.
12. Stegeman JJ, Hahn ME. 1994. Biochemistry and molecular biology of monooxygenases: Current perspectives on forms, functions, and regulation of cytochrome P450 in aquatic species. In Malins DC, Ostrander GK, eds, *Aquatic Toxicology; Molecular, Biochemical, and Cellular Perspectives.* Lewis, Boca Raton, FL, USA, pp 87–206.
13. Goksøyr A, Förlin L. 1992. The cytochrome P450 system in fish, aquatic toxicology, and environmental monitoring. *Aquat Toxicol* 22:287–312.
14. Schnitz AR, O'Conner JM. 1992. In vivo DNA/RNA adduction of 7,12-dimethylbenz[*a*]anthracene (DMBA) and benzo[*a*]pyrene (benzo[*a*]pyrene) in the liver of rainbow trout (*Oncorhynchus mykiss*). *J Environ Pathol Toxicol Oncol* 11:229–233.
15. George SG. 1994. Enzymology and molecular biology of phase II xenobiotic-conjugating enzymes in fish. In Malins DC, Ostrander GK, eds, *Aquatic Toxicology; Molecular, Biochemical, and Cellular Perspectives.* Lewis, Boca Raton, FL, USA, pp 37–85.
16. Stegeman JJ, Brouwer M, Richard TDG, Förlin L, Fowler BA, Sanders BM, van Veld PA. 1992. Molecular responses to environmental contamination: Enzyme and protein systems as indicators of chemical exposure and effect. In Huggett RJ, Kimerle RA, Mehrle PM Jr, Bergman HL, eds, *Biomarkers: Biochemical, Physiological, and Histological Markers of Anthropogenic Stress.* Lewis, Boca Raton, FL, USA, pp 235–335.
17. Van der Oost R, Goksoyr A, Celander M, Heida H, Vermeulen NPE. 1996. Biomonitoring of aquatic pollution with feral eel (*Anguilla anguilla*) II. Biomarkers: Pollution-induced biochemical responses. *Aquat Toxicol* 36:189–222.
18. Van der Oost R, Beyer J, Bermeulen NPE. 2003. Fish bioaccumulation and biomarkers in environmental risk assessment: A review. *Environ Toxicol Pharmacol* 13:57–149.
19. Gaworecki KM, Rice CD, van den Hurk P. 2004. Induction of phenol-type sulfotransferase and glucuronosyltransferase in channel catfish and mummichog. *Mar Environ Res* 58:525–528.
20. Farrington JW. 1991. Biogeochemical processes governing exposure and uptake of organic pollutant compounds in aquatic organisms. *Environ Health Perspect* 90:75–84.
21. Murase T, Horiba N, Goto I, Yamato O, Ikeda T, Sato K. 1993. Erythrocyte ALA-d activity in experimentally lead-poisoned ducks and its change during treatment with disodium calcium EDTA. *Res Vet Sci* 55:252–257.
22. Stoskopf MK. 1993. Clinical pathology. In Stoskopf MK, ed, *Fish Medicine,* 1st ed. W.B. Saunders, Philadelphia, PA, USA, pp 113–131.
23. Pohl RJ, Fouts JR. 1980. A rapid method for assaying the metabolism of 7-ethoxyresorufin by microsomal subcellular fractions. *Anal Biochem* 107:150–155.
24. Habig WH, Pabst MJ, Jakoby WB. 1974. Glutathione-*S*-transferases. The first enzymatic step in mercapturic acid formation. *J Biol Chem* 249:7130–7139.
25. Singh J, Wiebel FJ. 1979. A highly sensitive and rapid fluorometric assay for UDP-glucuronyltransferase using 3-hydroxybenzo[*a*]pyrene as substrate. *Anal Biochem* 98:394–401.
26. Yagi J, Holder GM, Dansette PM, Hernandez O, Yeh HJC, LeMahieu RA, Jerina DM. 1976. Synthesis and spectral properties of the isomeric hydroxybenzo[*a*]pyrenes. *J Org Chem* 41:977–985.
27. Martin LK, Black MC. 1996. Biomarker assessment of the effects of petroleum refinery contamination on channel catfish. *Ecotoxicol Environ Saf* 33:81–87.
28. U.S. Environmental Protection Agency. 2001. Supplemental guidance to RAGS: Region 4 bulletins, ecological risk assessment. Ecological Risk Assessment Bulletin 2. Region 4, Waste Management Division, Atlanta, GA.
29. Di Toro DM, Mahony JD, Hansen DJ, Scott KJ, Hicks MB, Mayr SM, Redmond MS. 1990. Toxicity of cadmium in sediments: The role of acid volatile sulfide. *Environ Toxicol Chem* 9:1487–1502.
30. Ankley GT, Di Toro DM, Hansen DJ, Berry WJ. 1996. Technical basis and proposal for deriving sediment quality criteria for metals. *Environ Toxicol Chem* 15:2056–2066.
31. Ankley GT, Phipps GL, Leonard EN. 1991. Acid volatile sulfide as a factor mediating cadmium and nickel bioavailability in contaminated sediments. *Environ Toxicol Chem* 10:1299–1307.
32. Means JC, Wood SG, Hassett JH, Banwart WL. 1980. Sorption of polynuclear aromatic hydrocarbons by sediment and soils. *Environ Sci Technol* 14:1524–1528.
33. Sepúlveda MS, Gallagher EP, Wieser CM, Gross TS. 2004. Reproductive and biochemical biomarkers in largemouth bass sampled downstream of a pulp and paper mill in Florida. *Ecotoxicol Environ Saf* 57:431–440.
34. Reynolds WJ, Feist SW, Jones GJ, Lyons BP, Sheahan DA, Stentiford GD. 2003. Comparison of biomarker and pathological responses in flounder (*Platichthys flesus* L.) induced by ingested polycyclic aromatic hydrocarbon (PAH) contamination. *Chemosphere* 52:1135–1145.
35. Whyte JJ, Jung RE, Schmitt CJ, Tillitt DE. 2000. Ethoxyresorufin-*O*-deethylase (EROD) activity in fish as a biomarker of chemical exposure. *Crit Rev Toxicol* 30:347–570.
36. Hughes EM, Gallagher EP. 2004. Effects of β-naphthoflavone on hepatic biotransformation and glutathione biosynthesis in largemouth bass (*Micropterus salmoides*). *Mar Environ Res* 58:675–679.
37. Runge-Morris M, Wilusz J. 1994. Suppression of hydroxysteroid sulfotransferase-a gene expression by 3-methylcholanthrene. *Toxicol Appl Pharmacol* 125:133–141.
38. Besselink HT, van Santen E, Vorstman W, Vethaak AD, Koeman JH, Brouwer A. 1997. High induction of cytochrome P4501A activity without changes in retinoid and thyroid hormone levels in flounder (*Platichthus fleusus*) exposed to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. *Environ Toxicol Chem* 16:816–823.
39. George SG, Young P. 1986. The time course of effects of cadmium and 3-methylcholanthrene on activities of enzymes of xenobiotic metabolism and metallothionein levels in the plaice, *Pleuronectes platessa*. *Comp Biochem Physiol C* 83:37–44.
40. Förlin L, Andersson T, Bengtsson B-E, Hardig J, Larsson A. 1985. Effect of pulp bleach plant effluents on hepatic biotransformation enzymes in fish: Laboratory and field studies. *Mar Environ Rev* 17:109–112.