FISH HEALTH IN THE REEDY-SALUDA WATERSHED, AS MEASURED THROUGH BIOCHEMICAL MARKERS

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Abstract. The rivers in the Upstate of South Carolina are known for their richness in species, including some endemic species. However, these rivers have suffered anthropogenic influences for a long time, including chemical pollution from point sources and non-point sources. Water quality assessment through chemical measurements has identified a variety of sources; but little is known about the effects of chemical pollutants on fish health in these rivers. This paper reviews several recent studies on the effect of water quality on biochemical responses in sunfish and bass in the Reedy and Saluda Rivers. Most prevalent are effects of polynuclear aromatic hydrocarbons in urban run-off and estrogenic effects from wastewater treatment plant outfalls. In addition, fish tissue arsenic concentrations were elevated in a variety of locations in the Saluda River.

INTRODUCTION

The Reedy-Saluda watershed is one of the watersheds in the Upstate of South Carolina. The Reedy and Saluda Rivers originate in the Blue Ridge Mountains, and combine in the upper reaches of Lake Greenwood, which is an important reservoir for drinking water supply and recreational activities. The water quality of the rivers in the Upstate has been of concern for years. Legacy pollutants that were produced and discharged over the last century have accumulated in the sediments of a variety of reservoirs. Relatively recent environmental disasters, like the 1996 rupture of the Colonial Pipeline over the Reedy River, and continuous discharge of environmental pollutants through wastewater treatment plants and other wastewater producers keep putting strains on the quality of essential surface water resources. In 2009 the Saluda River was ranked as # 6 of America’s Most Endangered Rivers by American Rivers, a leading conservation organization.

Water quality in the upstate rivers has been measured extensively over the last two decades. Both the SC Department of Health and Environmental Control, and the Saluda-Reedy Watershed Consortium (http://www.saludareedy.org/index.htm) have produced a number of papers evaluating the water quality condition of the watershed. However, very little has been contributed to effects that reduced water quality might have on fish health in the rivers that are part of the Saluda-Reedy watershed. Fish health can be quantified at a number of levels: from biochemical changes within cells and tissues, up to population and ecosystem changes. Changes at higher levels of organization often take years to develop, and once dramatic changes have occurred they may be difficult to reverse. Therefore more and more attention is being directed towards investigating changes at the cellular and sub-cellular level, which may serve as early warning signals for impending changes at higher levels of organization.

Biomarkers are biochemical measurements that indicate an abnormal change within the sentinel fish as a result of exposure to environmental pollutants (Van der Oost, 2003). These biomarkers can be measured in a variety of tissues, like liver, blood, gall bladder, gonads and spleen. The changes that are measured can be classified as enzyme inductions or inhibitions, metabolite formation, membrane damage and specific effects of hormonal modifications. These changes can be induced by a variety of pollutants, like heavy metals, oil and gasoline components, pesticides, and hormone-like chemicals.

We started investigating fish health through biomarker studies in the Reedy River reservoirs in 2004. In subsequent years the main stem of the Reedy River was sampled, followed by samples from urban creeks in the City of Greenville and an extensive sampling program in the Saluda River in 2010. The objective of this paper is to summarize these studies and to draw some general conclusions about the most important chemical pollutants in the rivers and to identify the sources of these pollutants. This information can direct further research and support management decisions that will help to
prevent further contamination of the rivers and increase fish health and human health for those who use the river ecosystem for recreation, food and drinking water supply.

GENERAL METHODS

Fish were collected using electrofishing equipment. In wadeable parts of the rivers a backpack shocking unit was used, in deeper water an aluminum johnboat outfitted with a generator and a 1.5kV shocking unit was used. For most studies only largemouth bass and sunfish species were selected; but in the urban creeks of Greenville study also bluehead and creek chubs were collected. The fish were anaesthetized with MS-222, after which a blood sample was collected from the caudal vein. Then the fish were dissected and tissue samples of gallbladder, liver, spleen, gonads and muscle were harvested. Blood samples were centrifuged to separate blood cells from plasma; tissue samples were frozen in liquid nitrogen and stored at -80°C until further analysis. Biomarker measurements were performed according to published methods. Bile fluorescence and bile protein were measured following the methods described in Van den Hurk (2006). The modified EROD activity assay and ALAD inhibition assay were presented in Schreiber et al. (2006). Total concentration of estrogenic compounds in bile samples were measured according to the methods described in Truman & Van den Hurk (2010).

RESULTS AND DISCUSSION

Reedy River reservoirs

To study the effects of legacy pollutants that have accumulated in the sediments of Lake Conestee, sediment samples and tissue samples from largemouth bass were collected in 2004 and 2005. Two examples of correlations between sediment contamination and biomarker effects are presented here. The first example is the distribution and biological effect of lead (Schreiber et al., 2006). Chemical analysis demonstrated that sediment concentrations of lead were significantly elevated in Lake Conestee, compared to the reference location in Lake Robinson and compared to the downstream reservoirs in the Reedy River. Despite these high lead levels in the sediment, no significant inhibition of the ALAD enzyme was observed. This enzyme forms part of the cascade of enzymes that synthesize hemoglobin, and it is extremely sensitive to lead. Thus, these results demonstrate that lead in Lake Conestee sediments is very prevalent, but it is not bioavailable as measured through this biomarker for lead exposure. These results were later confirmed through heavy metal analysis of muscle tissue, which did not show any elevated lead concentrations in this tissue. The lead contents in Lake Conestee sediments are clearly sequestered, most likely to organic matter and fine particulates, and therefore not available for uptake by pelagic fish like largemouth bass.

The second example of how biomarkers can be used to establish the bioavailability of sediment pollutants is provided by the measurements of concentrations and effects of polycyclic aromatic hydrocarbons (PAHs). Sediment analysis revealed again that PAH concentrations in Lake Conestee were significantly higher than in the reference site in Lake Robinson and in the downstream reservoirs (Schreiber et al., 2006). Contrary to the lead example, the PAHs did have a significant effect on a biomarker that was measured: the EROD assay showed a significant increase in activity in the Lake Conestee fish. This assay measures the induction of a specific enzyme that is considered a part of a detoxification pathway that renders these PAHs into more water soluble products that are easier to excrete in aqueous media like urine and bile. An upregulation of this enzyme activity is induced by PAH exposure, and therefore the conclusion is that PAHs in Lake Conestee are bioavailable and could be the cause of other health effect in these fish. A number of PAHs are known carcinogens, and could cause tumors in liver and other organs. In general, PAHs do not accumulate in vertebrate species, and thus chemical analysis of muscle tissue would not reveal any elevated concentrations. The EROD assay is therefore an excellent tool to measure bioavailability of PAHs, and predict potential health effects of PAH exposure.

Reedy River estrogens

In 2006 a study was performed in the Reedy River to specifically investigate the occurrence and effects of estrogenic compounds in the river. For this study juvenile bluegills were collected at a number of locations along the Reedy River. Juvenile fish were used because they naturally have low levels of estrogenic compounds in their system, and thus any exposure to environmental estrogens will prematurely increase the internal levels of these hormones and induce specific effects. The level of estrogenic compounds in bile samples was measured using a competitive estrogen receptor binding assay (Truman & Van den Hurk, 2010). When estrogenic compounds are found in these bile samples it follows that the fish were exposed to these compounds and that they processed them in the liver and excreted the biotransformation products in the bile. The activity of the enzyme responsible for this biotransformation process was measured (estrogen-type glucuronosyltransferase), and also a biological effect of estrogen exposure: the formation of vitellogenin was analyzed. Vitellogenin is a yolk protein precursor; it is normally formed in the liver of pre-spawning females when estrogen levels in plasma
increase, and is then transported to the egg cells in the female gonads where it is incorporated in the ripening egg cells as yolk protein. Vitellogenin should not occur in plasma of unexposed juvenile fish; so when it is measured at elevated concentrations it is a good biomarker for exposure to and absorbance of estrogenic compounds. The results of this study demonstrated that especially just downstream of the Mauldin Road wastewater treatment plant significant responses were measured for all three biomarkers described above. This means that most likely estrogenic compounds are released by the treatment plant, which was expected because numerous other studies have shown that especially the synthetic estradiol that is incorporated in anti-conceptive medication is not well broken down in wastewater treatment plants, and is therefore discharged in surface waters in measurable concentrations.

Urban creeks in Greenville

After completing both projects described above, the question arose if the smaller tributary creeks in the city of Greenville do contribute to the toxicological effects that were seen in the fish residing in the main stem of the Reedy River. A sampling campaign was set up to collect fish from a number of urban creeks in Greenville in 2009. Five different creeks were sampled, and 3 sites in the Reedy River. The most upstream location, north of the city of Greenville was used as reference site. In addition to the sunfish species that were used in previous studies, it was observed that two chub species were also relatively abundant in these creeks. To obtain a large enough sample size for each location, both taxa were collected and analyzed for biomarker responses.

While analyzing the liver samples for enzyme activities, it became clear that there were significant differences in EROD and GST activities for both taxa: the sunfish species had overall much higher EROD activities that the chub species, while the other way around the chub species had much higher GST activity than the sunfish species. Both enzymes are part of detoxification pathways, and it was unexpected that both species groups had such different expression of these enzymes. These results were later confirmed by exposing both taxa in a controlled laboratory experiment to a known inducer for these enzymes. The results of this experiment confirmed that sunfish have much higher expression of the EROD enzyme and a lower expression of GST. In both species EROD activity was significantly induced by exposure to the model PAH benzo[a]pyrene, but it was also observed that at the highest dosage of 50 mg/kg the chub species started to show mortality, while the sunfish were not suffering lethal effects. This demonstrates that while the chubs have higher expression of the GST enzyme, this does not protect them enough against effects of higher levels of PAH exposure. The sunfish species have higher levels, and good inducibility, of the EROD enzyme, which protects them against PAH exposure. This makes the sunfish species less sensitive species than the chubs. The conclusion drawn from these results is that considerable species differences can exist in biomarker inducibility, which should be taken in consideration when results of comparable studies are evaluated.

The biomarker results from the fish collected in the urban creeks showed that some of these urban creeks contribute significant levels of contaminants to the Reedy River, while others are significantly cleaner than even the reference site in the upstream part of the river. Of concern are especially the measured PAH effects, both in the biomarkers presented here and in the bile fluorescence assay (results not presented). All these biomarkers show that some urban creeks contribute significant amounts of PAHs to the Reedy River. These PAHs originate most likely from run-off from roads, parking lots and tar roofs. Especially the application of coal tar sealants on asphalt surfaces has received scrutiny in recent years. These sealants are loaded with PAHs, and so far there are no regulations for their use. As a result several studies have linked the occurrence of PAHs in aquatic systems to nearby coal tar sealant use. The unregulated use and potential environmental toxicity of this product deserves further study and possibly limitations on where it can be applied.

Saluda River project

In 2010 a biomarker study was set up to evaluate fish health in the Saluda River (Mierzejewski, 2012). Sunfish species and largemouth bass were collected at 13 locations along the river, ranging from the upstream North Saluda Reservoir down to Lake Greenwood in the south. A number of biomarkers were measured on collected fish tissues, of which only the ones that showed significant results will be discussed here. The biomarkers for bile fluorescence and the EROD activity are both indicative of PAH exposure. The results showed that especially in the middle section of the river several locations had significantly increased responses for these biomarkers. It appears that PAH exposure is more predominant in this section of the river, which is closely correlated to the urbanization of this area. As argued above, increased urbanization leads to increased amounts of impervious surfaces from roads, parking lots, roof tops etc., which are sources for PAH discharge into the rivers and streams. This is clearly reflected in the biomarker results. Another concern is the effluents of wastewater treatment plants. There are about a dozen wastewater treatment plants in the Saluda watershed that discharge on tributaries or directly on the Saluda River. These treatment plants were identified as the predominant sources for excessive nutrient levels in the river. In
addition, as demonstrated above for the Reedy River, there is a big concern for other environmental pollutants that are discharged by these treatment plants. When bile samples were analyzed for estrogenic compounds, we found several locations where these estrogenic compounds were significantly increased. This indicates that also in the Saluda River the fish are under stress from this class of environmental pollutants, and it warrants further research into potential population and fish community effects.

Not only were significant results obtained in bile components and enzyme based biomarkers, for this study also metal concentrations in muscle tissue were measured. Several metals showed increased concentrations, most notably arsenic, which was significantly elevated in 4 locations. Arsenic is more often found to be elevated in fish tissue in the Upstate of South Carolina. This can be a legacy effect of arsenic that was used in pesticides when this area was heavily used for cotton and tobacco cultivation. Or the source can be from wood preservation chemicals that are used to treat pilings and planks for docks on the reservoirs in the river. Of special concern were the metal concentrations in two fish that were caught downstream of the Easley tributary that showed elevated levels of cadmium and silver. These are very toxic metals which should not exceed baseline levels in potential consumption species.

The overall pattern of biomarker results in the Saluda River showed that the middle part of the river is the most severely impacted section. On two locations (3 and 4) there were a total of four biomarkers that showed significant effects. These effects were initially compared to the responses in the fish from the North Saluda Reservoir. This location was selected as reference site, assuming that the remote location in an undeveloped area would reflect a more-or-less pristine location. However, when the first biomarker results became available, there were indications that this reservoir was not as pristine as we had assumed. Further evaluation of the collection site revealed that the fish were collected close to a boat ramp that had recently been repaved. This may have been the source of the PAH effects we observed, which made this site less suitable as a reference site. Further evaluation of the results revealed that the fish collected from Lake Greenwood did not show any significant biomarker results. This location was therefore used as internal reference site to compare all other locations to. The fact that the Lake Greenwood fish appeared to be healthiest is remarkable in itself: it appears that the Saluda River has a self-cleaning ability which causes the observed pollution effects in the middle section of the river to taper off towards the downstream locations and Lake Greenwood. There are numerous shallow sections with rapids in the lower part of the river, which may enhance oxidation and UV breakdown of organic pollutants, while persistent pollutants like chlorinated compounds and metals may get sequestered in the sediments of the various reservoirs.

Conclusions

The results of the biomarker studies presented here show that there should be considerable concern for the water quality of the Reedy and Saluda Rivers. In addition to known pollutants like nitrogen and phosphorous compounds several other chemical contaminants were shown to induce toxicological effects in fish species. Most abundant are the effects of polynuclear aromatic hydrocarbons. These compounds enter the rivers mostly through non-point sources like urban run-off. The other group of compounds that are of concern are the pharmaceutical compounds that are not retained or broken down in wastewater treatment plants. It was demonstrated that on several locations below wastewater treatment plants that fish had been exposed to elevated levels of estrogenic compounds. Improvement of the cleaning operations could take care of this problem, and further protect the water quality and fish health of the Reedy and Saluda Rivers. Future analyses will focus on the connection between biomarker responses in less sensitive fish species and the disappearance of more sensitive species from the species spectrum. The rivers in the Upstate of SC have an exceptional reputation of species richness and uniqueness; biomarker studies will help to monitor and protect this precious natural resource which is also essential for a variety of societal uses like recreation and drinking water supply.

LITERATURE CITED


