The Effect of an Industrial Spill on the Macrofauna of a South Carolina Stream: Physiological to Community-Level Responses

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ABSTRACT

In 1985, a waste retainment pond ruptured at a galvanizing facility and spilled 75,700 liters of HCl and other contaminants into the groundwater above the Upper Enoree River in South Carolina, USA. In 1999, we examined the residual effects of this spill by surveying the water chemistry and biota in the main channel of the Upper Enoree River and uncontaminated tributaries. We also transplanted yellowfin shiners (*Notropis lutipinnis)* to channel and tributary sites and monitoring their survivorship and the histological condition of their gills. The two upstream sites were significantly different in chemical composition from downstream sites and control tributaries. Conductivity and the concentrations of Na^+, K^+, Ca^{2+}, Mg^{2+}, Cl^-, NO_3^-, PO_3^-, F^-, Zn^{2+}, and Mn^{2+} were highest at the headwater site and declined significantly downstream. The abundance and diversity of salamanders, fish, and macroinvertebrates correlated with these changes in water chemistry. Yellowfin shiners (*Notropis lutipinnis)* transplanted to the headwater site died within 24 hours, and fish in channel sites had significantly higher mortality than fish transplanted to tributary sites. At the tissue level, the size and density of gill pavement cells were significantly lower in fish transplanted into the two headwater sites closest to the spill compared to other channel and tributary sites. Thus, this spill continues to exert a significant effect on the chemistry and biota of the Upper Enoree River.

INTRODUCTION

Wastewater containing acids and metals can initiate a cascade of events that may affect the water chemistry of aquatic systems, the physiology of aquatic organisms, the abundance of sensitive species, and the structure of entire aquatic communities (Karuppiah and Gupta 1997). Acid influx can reduce the buffering capacity of water, reduce pH, stimulate the release of soil cations, and change solute concentrations and conductivity (Cole 1979, Karuppiah and Gupta 1997). Changes in pH, conductivity, and metal concentrations can disrupt ionic and osmotic potentials across cell membranes, affecting basic membrane transport and cell function (Newman and Jagoe 1996).

Aquatic organisms can accumulate metals from direct ingestion and diffusion across cellular membranes (Dethloff et al. 1998). Metal ions cannot passively diffuse across membranes, but they can be actively transported if they are bound to inorganic or organic ligands (Hare 1992). Since many metals, including heavy metals such as Zn^{2+}, are essential micronutrients, their uptake and excretion is normally monitored in a homeostatic manner at boundary layers such as gill epithelia (Hogstrand et al. 1996, Newman and Jagoe 1996, Hogstrand et al. 1998). Metals such as Zn^{2+} normally have concentrations in river water in the parts per trillion range (Shiller and Boyle 1985). However, exposure to elevated concentrations of metals can lead to induced nutrient deficiency (Sunda and Huntsman 1998) or even more drastic structural and physiological damage such as tissue damage to gill epithelial cells (Evans 1987).

The effect of these chemical changes depends not only on the magnitude and duration of the exposure but also on the tolerance limits of exposed organisms. Unfortunately, because toxicity varies with an organism's size, age, and life history stage, indicator species may not accurately reflect the effect of pollutants on a watershed ecosystem (Hare 1992, Sunda and Huntsman 1998). In addition, because many sensitive species are naturally rare and patchily distributed, their absence from any one locality is not necessarily an indication of poor water quality.

A more complete approach to evaluating anthropogenic impact on aquatic systems is to measure effects across several scales of biological organization, from the tissue to community level. Histological studies can reveal sublethal effects that population studies might miss. Comparisons of community structure (species richness and diversity) are affected by all species in the habitat and are less likely to be affected by the vagaries of single species analyses that can compromise studies of indicator species.

Here we report on the persisting effects of an industrial spill on the ecological integrity of a South Carolina piedmont stream. In 1985, a retainment pond at a galvanizing facility ruptured and spilled 75,700 liters of galvanizing waste (HCl, zinc, and other unknown contaminants) into the groundwater (Hagins 1988). We measured effects by 1) conducting a survey of the water chemistry and biota, and 2) conducting a transplant experiment to describe the effect of this point-source pollution on the survivorship and gill histology of yellowfin shiners (*Notropis lutipinnis*) at impacted and control sites.

METHODS AND MATERIALS

Sampling Sites

Thirteen sites were sampled within the Upper Enoree River watershed near Traveler's Rest, South Carolina, USA (Fig 1). Nine sites (C1-C9) were on the main channel of the Upper Enoree River, 0.322-7.242 km downstream from the spill site. Four sites (T1-T4) were on tributary creeks that fed into the main channel. Chemical composition of streamwater indicated that these four sites were unaffected by the industrial spill.

Survey of Aquatic Macrofauna and Water Chemistry

The salamanders, fishes and macroinvertebrates at each site were sampled once between May and July 1999 using a Smith-Root© backpack electrofisher, a 4'x10'x1/8" seine, and long handled dip nets. Sampling effort was standardized by shocking for eight minutes at each site. During the shocking period, the substrate was kicked vigorously to dislodge salamanders, fish, and macroinvertebrates that were all collected downstream in the seine and dip nets. Salamanders and fishes were preserved in 10% formalin, then sorted, identified, counted, and stored in 70% ethanol. Macroinvertebrates were preserved in 75% ethanol, sorted, identified, and counted.

At each sample locality, water samples were collected weekly for seven weeks from June through July 1999. Sites C1-C6, T1 and T2 were also sampled once in December 1998 and February, April, and May 1999. Dissolved oxygen, conductivity, temperature, and pH were measured *in situ* at the time of collection using YSI dissolved oxygen and conductivity meters and an Accumet pH meter. Grab samples were collected into pre-cleaned HDPE bottles and packed on ice. In the laboratory, samples were pressure filtered using a 0.45 μm nylon membrane filter. A cation aliquot was preserved with 16 M trace metal grade nitric acid to a pH of 2.0; an anion aliquot was left unpreserved. Both were refrigerated until analysis. Cation concentrations ($Na^{=}$, K^{+}, Ca^{2+}, Mg^{2+}, $Si^{4=}$, Fe^{3+}, Al^{3+}, Mn^{2+}, Zn^{2+}) were measured with a Varian ICP-AES. Anion concentrations (Cl^{-}, SO_4^{2-}, NO_3^{-}, NO_2^{-}, F^{-}, Br^{-}, HPO_4^{2-}) were measured with a Dionex 120 ion

chromatograph. Alkalinity was measured by the potentiometric method for low alkalinity (Eaton et al. 1995). We assumed that all alkalinity was in the form of bicarbonate. Dissolved organic carbon concentrations were measured with a Dohrman 80 total organic carbon analyzer using the uv-persulfate reduction method. An unfiltered aliquot of each sample was analyzed for turbidity using a LaMotte 2020 turbidimeter.

Variation in water chemistry among the sites was described with a discriminant function analysis and correlation analyses. Discriminant function centroids were computed for each site, and the loading scores for each site centroid along the first two discriminant function axes (DFA1, DFA2) were correlated with the site means for each chemical variable to determine which variables correlated with the discriminant functions (Spearman rank correlations). In addition, the mean chemical composition of main channel sites was compared with mean chemical composition of tributary sites using Mann-Whitney U tests.

The following biotic descriptors were computed for each site: salamander abundance, fish abundance, fish species richness, fish diversity (Simpson's diversity, $D = 1/\Sigma p_i^2$), macroinvertebrate abundance, crayfish abundance, odonate abundance, odonate species richness, odonate diversity (Simpson's diversity, $D = 1/\Sigma p_i^2$), and EPT score (the number of species in the insect orders Ephemeroptera, Plecoptera, and Trichoptera). Differences in these parameters between channel sites and tributary sites were described with Mann-Whitney U tests. Means for the Upper Enoree River channel sites were also compared with means generated from a haphazard sample of 50 other sites sampled in Greenville County in summer 1999. In addition, the relationships between water chemistry and the aquatic biota were described by correlating these biotic parameters with site centroid loadings from the discriminant function analysis (DFA1 and DFA2).

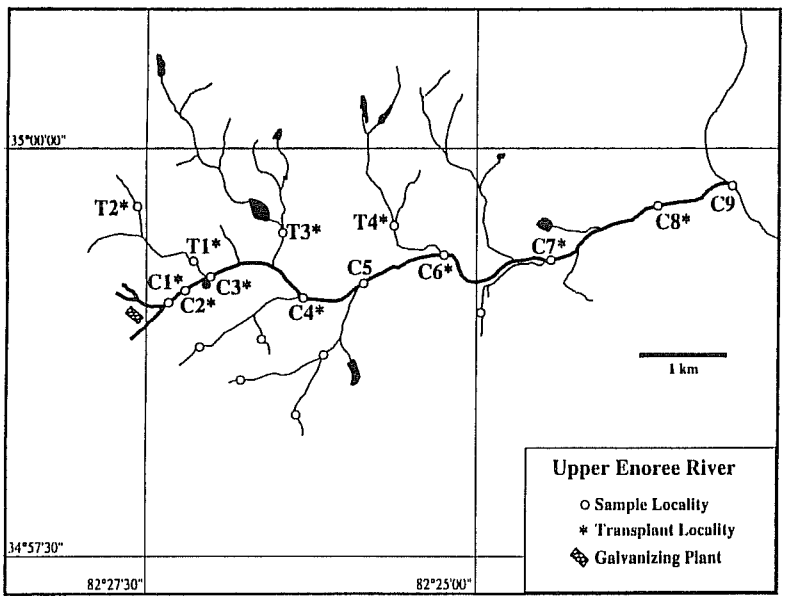

Figure 1. The location of 13 sampling sites used for biological and chemical surveys in the Upper Enoree River watershed, Greenville Co., South Carolina, USA. Nine sites were on the main channel of the Upper Enoree (C1- C9), and four sites were on tributaries that fed into the main channel (T1-T4). Sites with an asterisk (*) were also used in the fish transplant experiment.

Transplant Study

We conducted a transplant experiment to determine whether putative changes in water chemistry resulting from the industrial spill might affect fish survivorship. *Notropis lutipinnis* were collected from Gilder Creek in Greenville, SC, using the electrofishing sampling technique described above. The fish were transported to the laboratory in aerated water from the collection site; they were held overnight in aerated and filtered aquaria. The next morning, ten fish were placed in each of two closed minnow traps at each of ten sites in the Upper Enoree River watershed. Six sites were located in the main channel 0.322 - 6.276 km from the spill site (Fig. 1). The four tributary sites (T1-T4) were again used as controls. Survivorship in each trap was recorded after three hours, and daily for eight days. Upon death and/or completion of the experiment the fish were removed, preserved in Carnoy's fixative and transferred to 70% ethanol. Differences in daily fish survivorship at these sites over time were described with a repeat-measures analysis of variance.

Histology Study

Histological preparations were made of first and second right-side gill arches of preserved transplanted fish (5-10 fish per site). Gills were dehydrated through an ethanol series from 70% to 100%, embedded in paraffin, and serially sectioned at 10μm. Tissue was stained with 1% aqueous toluidine blue and examined at 400X magnification. Images were captured using Xclaim® software and were analyzed using NIH Image 1.61 software viewed at 400X magnification. For each gill, we measured the areas of fifty randomly selected pavement cells and the number of pavement cells in a randomly selected 50μm^2 area (cell density). Analyses of variance were used to describe differences in mean pavement cell size and mean pavement cell density in fish from different transplant sites, and to compare means for channel and tributary sites.

RESULTS

Water Chemistry

A discriminant function analysis separated the sites into three groups (Fig. 2). The first discriminant function (DFA1) explained 88.6% of the variance ($p < 0.0001$) and separated the two upstream channel sites (C1, C2; centroid loadings > 20.0) from three of the four tributary sites (T1, T2, T4; centroid loadings < -7.50) and the remaining channel sites (centroid loadings -0.374 to -5.40; Fig. 2). Several chemical variables were significantly positively correlated with the site centroid loading scores along DFA1, including conductivity, cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), anions (Cl^-, NO_3^-, PO_4^{3-}) and metals (Zn^{2+} and Mn^{2+}; Table 1). Zinc concentrations in the channel ranged from an average of 13.5 mg/l at site C1 to 0.3 mg/l at site C9. These concentrations are several orders of magnitude greater than levels normally found in river water (Shiller and Boyd 1985). The tributary sites had zinc concentrations below our detection limit of 0.03 mg/l. Only pH and silicate concentrations were negatively correlated with DFA1 (Table 1). DFA2 separated three of the four tributary sites (T1, T2, T4) from the other sites (Fig. 2) and explained 5.7% of the variance ($p < 0.0001$). Site centroid loading scores along DFA2 were positively correlated with site means for aluminum and negatively correlated with site means for temperature, total organic carbon, and iron (Table 1).

The mean chemical composition of control tributary sites was significantly different from channel sites. On average, channel sites had significantly higher conductivity and higher concentrations of K^+, Ca^{2+}, Mg^{2+}, Cl^-, Zn^{2+}, Mn^{2+}, and Fe^{2+} than tributary sites (Mann-Whitney U tests, Table 1). These differences occur even though site T3 was more similar in water chemistry to the downstream channel sites than to the other tributary sites

(discriminant function analysis, Fig. 2). Site T3 was immediately downstream from a man-made pond and had higher temperature, lower dissolved oxygen, higher total organic carbon, lower silicate, and lower Al^{3+} levels than the other tributaries.

Within the channel, conductivity, cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), anions (Cl^-, NO_3^-, PO_4^{3-}, F^-), and heavy metals (Zn^{2+}, Mn^{2+}) were highest at the impacted headwater site and declined significantly downstream (Fig. 3; Spearman rank correlations with distance, Table 1). In contrast, pH, total organic carbon, bicarbonate, and silicate were lowest at the headwater site and increased downstream, with significant positive correlations with distance from the spill site (Spearman rank correlations, Table 1). In fact, when only channel sites were considered, the variable that was most strongly correlated with site loadings along DFA1 was "distance from the spill site" (Spearman rank correlation, $r = -0.904$, $p = 0.001$, $n = 9$). Thus, DFA1 is an excellent proxy for distinguishing between the chemical environments of the heavily impacted upstream sites (C1 and C2), the other channel sites and T3, and the other three tributary sites (T1, T2, T4).

Table 1. Mean comparisons of chemical characteristics of channel and tributary sites in the Upper Enoree River watershed, Traveler's Rest, SC (Mann-Whitney U tests). Spearman rank correlations between site means for each variable (n = 13) and discriminant function 1 and 2 (DFA1 and DFA2; see Fig. 2), and distance from an industrial spill site (only channel sites included, n = 9). Significance symbols for all comparisons: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$; **** = $p < 0.0001$). bd = below detection limit

	Channel	Tributaries	Mann-Whitney		Spearman Rank Correlations		
VARIABLE	(X + 1 sd, n = 9)	(X + 1 sd, n = 4)	U	Z	DFA1	DFA2	Distance
Temperature (°C)	21.61 ± 0.61	21.34 ± 2.88	9.0	-1.39 ns	0.326 ns	-0.929****	0.183 ns
pH	6.61 ± 0.25	6.73 ± 0.29	10.0	-1.23 ns	-0.586*	-0.313 ns	0.717*
Conductivity (uS/L)	89.26 ±72.55	36.25 ± 3.20	0.0	-2.77**	0.890****	-0.132 ns	-0.950****
Alkalinity (mg l^{-1})	10.95 ± 4.35	12.26 ± 1.35	17.0	-0.15 ns	-0.464 ns	-0.511 ns	0.683*
Dissolved O_2 (mg l^{-1})	7.78 ± 0.20	7.93 + 0.43	14.5	-0.54 ns	-0.346 ns	0.300 ns	0.583 ns
Turbidity	17.72 +17.55	7.96 ± 2.98	10.0	-1.23 ns	0.033 ns	-0.027 ns	0.317 ns
Total organic carbon	2.03 ± 0.54	1.77 ± 0.43	13.0	-0.77 ns	-0.199 ns	-0.802***	0.667*
Na^+ (mg l^{-1})	3.74 ± 0.90	3.04 ± 0.34	6.0	-1.85 ns	0.558*	0.181 ns	-0.800**
K^+ (mg l^{-1})	1.43 ± 0.40	1.04 ± 0.19	5.0	-2.01*	0.691**	0.000 ns	-0.783*
Ca^{2+} (mg l^{-1})	5.78 ± 3.33	2.89 ± 0.27	0.0	-2.77**	0.818***	-0.077 ns	-0.917****
Mg^{2+} (mg l^{-1})	1.99 ± 1.14	0.91 ± 0.16	0.0	-2.77**	0.856****	-0.143 ns	-0.933****
Cl^- (mg l^{-1})	21.23 ±28.84	2.30 ± 0.27	0.0	-2.77**	0.901****	-0.060 ns	-1.000****
SO_4^{2-} (mg l^{-1})	1.38 ± 0.21	1.26 ± 0.89	10.0	-1.23 ns	0.204 ns	0.022 ns	-0.100 ns
HCO_3^- (mg l^{-1})	13.36 ± 5.31	14.96 ± 1.64	17.0	-0.15 ns	-0.464 ns	-0.511 ns	0.683*
NO_3^- (mg l^{-1})	2.13 ± 0.70	1.30 ± 0.67	7.0	-1.70 ns	0.591*	0.324 ns	-0.783*
PO_4^{3-} (mg l^{-1})	0.03 ± 0.04	0.00 ± 0.00	10.0	-1.51 ns	0.648*	0.275 ns	-0.676*
NO_2^- (mg l^{-1})	0.01 ± 0.01	0.01 ± 0.01	18.0	0.00 ns	-0.128 ns	-0.168 ns	0.040 ns
Br^- (mg l^{-1})	0.01 ± 0.01	0.00 ± 0.00	10.0	-1.51 ns	0.412 ns	0.074 ns	-0.438 ns
F^- (mg l^{-1})	0.07 ± 0.01	0.06 ± 0.03	13.0	-0.77 ns	0.348 ns	0.170 ns	-0.667*
SiO_2 (mg l^{-1})	15.36 ± 0.98	15.81 ± 3.32	12.0	-0.93 ns	-0.712**	0.379 ns	0.883**
Zn^{2+} (mg l^{-1})	3.34 ± 5.14	0.02 ± 0.01bd	0.0	-2.77**	0.890****	-0.049 ns	-0.983****
Mn^{2+} (mg l^{-1})	0.63 ± 0.83	0.02 ± 0.01bd	0.0	-2.77**	0.884****	-0.214 ns	-0.950****
Al^{2+} (mg l^{-1})	0.24 ± 0.16	0.21 ± 0.07bd	16.0	-0.31 ns	0.188 ns	0.707**	-0.504 ns
Fe^{2+} (mg l^{-1})	0.36 + 0.24	0.16 + 0.06bd	5.0	-2.01*	0.475 ns	-0.659*	-0.083 ns

Survey of Macrofauna

Macrofaunal indices of channel sites were compared with those of the control tributaries, as well as those of 50 other randomly selected sites in Greenville County, SC (Mann-Whitney U tests, Table 2). Vertebrate communities in sites in the main channel of the Upper Enoree River were significantly different from vertebrate communities in other streams in the region. On average, sites in the channel had significantly lower salamander abundance, fish abundance, fish species richness, and fish diversity than other sites in the county. Even the smaller tributaries of the Upper Enoree River had higher means for these parameters than the channel sites; however, only the difference in fish abundance was statistically significant. Only 10 species of fishes were collected in the Upper Enoree River, all within the families Centrarchidae and Cyprinidae. A total of 203 fish was

collected in the watershed; the most common fishes were *Nocomis leptocephalus* (n = 25), *Semotilus atromaculatus* (n = 48), *Micropterus salmoides* (n = 16), and *Lepomis macrochirus* (n = 73), which together accounted for 80% of the total sample.

On average, invertebrate communities in the channel were similar to invertebrate communities in the tributaries and in other streams in the county (Table 2). The channel sites had significantly fewer crayfish than tributaries and other county sites, but most other means were comparable. A total of 1256 macroinvertebrates was collected in the Upper Enoree, representing eight orders. Odonates accounted for 49.8% of the total sample, with a disproportionate number of *Progomphus obscurus* (n = 228) and *Cordulegaster maculata* (n = 222).

The effects of pollution on the biota of the Upper Enoree River are somewhat obscured in these mean comparison tests because all channel sites are pooled together, even though there are significant differences in the chemistry of upstream and downstream sites. To describe the relationship between general chemical composition of the sites and their biotas, the biotic parameters were correlated with the loadings for the site centroids along the first two discriminant functions (DFA1 and DFA2, Table 2). To correct for differences in sampling volume, we conducted partial correlations between the biotic parameters and DFA1 and DFA2 controlling for stream width. In addition, abundance values were $\log_{10}$ transformed prior to these parametric partial correlation analyses (Sokal and Rohlf 1995). All biotic parameters except crayfish abundance were significantly negatively correlated with DFA1 (Table 2). Crayfish abundance was significantly correlated with DFA2 ($r = 0.7406$, $p = 0.006$).

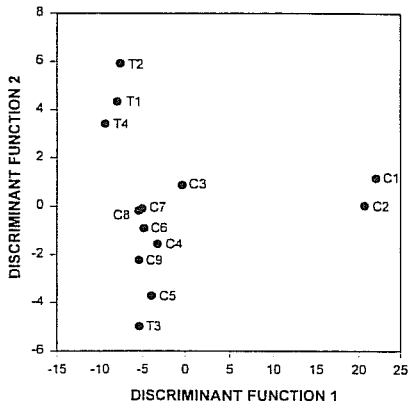

Figure 2. A plot of site centroids for the 13 sampling sites in the Upper Enoree River watershed, Greenville Co., SC, along the first two discriminant functions from the discriminant function analysis of site variation in water chemistry.

Transplant Study

The percentage of *N. lutipinnis* surviving in two minnow traps (n = 10 fish/trap) were recorded after three hours and daily for eight days. Two replicates of the experiment were performed (n = 40 traps) and the variation in daily survivorship among sites was analyzed with a repeated-measures ANOVA (percentage data were arcsin square-root transformed before analysis, Sokal and Rohlf 1995). There was a significant difference among sites in fish survivorship over the eight-day period ($F = 11.777$, $df = 9, 30$, $p = 0.0001$). Survivorship decreased more dramatically at channel sites than at tributary sites (Fig. 4). The effect was most pronounced at sites C1 and C2 where there was 100% mortality within 24 hours of transplantation (Fig. 4). However, survivorship also declined to 0% at

almost all the channel sites, even at site C8 that was 6.27 km from the spill site. In contrast, survivorship in tributary sites stabilized near 60% after four days and remained near 60% for the rest of the eight-day exposure period (Fig. 4). Even after one day of exposure, mean survivorship in channel sites (0.49 ± 0.39, n = 24) was significantly less than mean survivorship in tributary sites (0.88 ± 0.22, n = 16; $t = 4.11$, $p < 0.0001$; analysis performed on arcsin square-root transformed values). This difference became greater as the eight-day exposure period progressed.

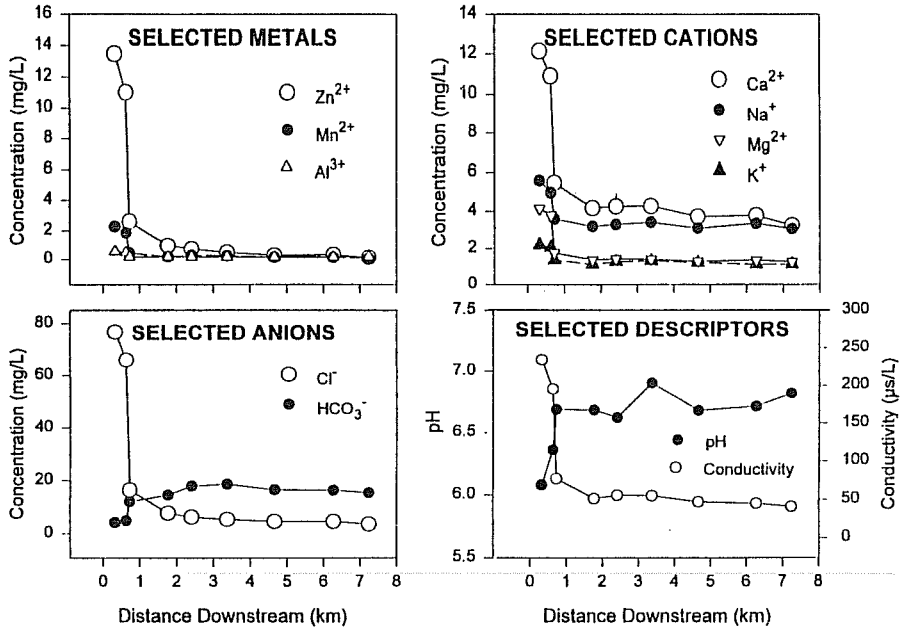

Figure 3. Summary of changes in water chemistry (mean values) in the main channel of the Upper Enoree River, Greenville Co., SC, downstream from a containment pond that ruptured in 1985.

Table 2. Comparisons of biotic descriptors for the 13 sampling sites in the Upper Enoree River watershed in Greenville County, SC (Mann-Whitney U tests, *= $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.0001$). Species = species richness. Diversity = Simpson's diversity index ($1/\Sigma p_i^2$), and EPT = the number of species in the orders Ephemeroptera, Plecoptera, and Trichoptera.

	Salamanders	Fish			Invert.	Crayfish	Odonate			EPT
	(n)	n	n	Species	Diversity	n	n	n	Species	Diversity	Species
Mean Comparisons - Channel vs. Tributaries											
Channel Mean (9)	1.0	9.1	2.2	1.48	102.0	1.3	47.1	3.1	1.61	3.1	
Tributary Mean (4)	10.5	27.0	3.5	2.15	84.8	9.0	50.0	3.5	1.95	3.5	
Mann-Whitney U	7.5	3.0	11.0	12.0	17.0	3.5	16.0	17.0	15.0	16.5	
Z, significance	0.10, ns	-2.3*	-1.1, ns	-0.9, ns	-0.2, ns	-2.4*	-0.3, ns	-0.2, ns	-0.4, ns	-0.2, ns	
Mean Comparisons - Channel vs. Other Greenville County Sites											
Channel Mean (9)	1.0	9.1	2.2	1.48	102.0	1.3	47.1	3.1	1.61	3.1	
County Mean (50)	6.9	103.1	5.5	2.45	79.2	6.6	48.7	4.7	2.51	1.6	
Mann-Whitney U	125.0	24.0	70.5	115.0	195.0	123.0	198.0	154.5	168.0	126.0	
Z, significance	-2.1*	-4.2***	-3.3***	-2.3*	-0.6, ns	-2.2*	-0.6, ns	-1.5, ns	-1.2, ns	-2.1*	
Partial Correlations with DFA1 (Proxy for Chemical Composition)											
Correlation Coeff. (r)	-0.831	-0.858	-0.634	-0.626	-0.808	-0.521	-0.710	-0.655	-0.861	-0.671	
p	0.001	0.0001	0.027	0.029	0.001	0.08, ns	0.01	0.02	0.0001	0.017	

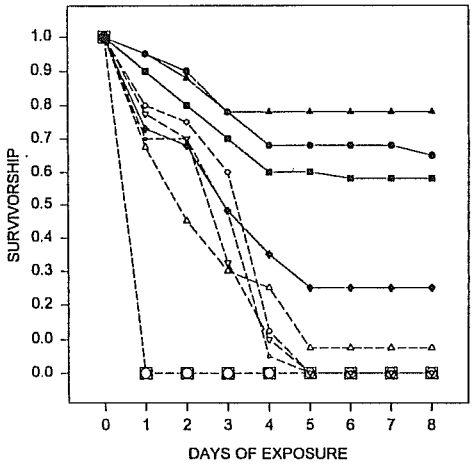

Figure 4. Mean survivorship of yellowfin shiners (*Notropis lutipinnis)* transplanted to ten sites in the Upper Enoree River watershed, Greenville Co., SC, over an eight-day exposure period. Open symbols = sites on the main channel; filled symbols = sites on tributaries.

Histology Study

The size and density of pavement cells in gill tissue from *N. lutipinnis* transplanted into channel and tributary sites in the Upper Enoree River watershed were analyzed with one-way ANOVA tests. Pavement cell area ($F=75.73$, $p < 0.001$) and pavement cell density ($\log_{10}$ transformed prior to analysis to achieve satisfactory homogeneity of variance; $F=9.037$, $p < 0.001$) differed significantly among sites in a manner consistent with survivorship data (Table 3). Mean pavement cell area in fish from site C1 was significantly lower than the mean from site C2 (Tukey HSD, $p < 0.001$, Table 3). Both of these sites had means significantly lower than means from other sites, which did not differ from one another (Tukey HSD, $p < 0.001$, Table 3). Mean pavement cell density was also lowest in fish from site C1, although they were only significantly different than means from sites C3, C5, C7, C8 and T2, T3, T4 (Tukey HSD, $p < 0.01$, Table 3). There were no other significant differences in mean pavement cell density among fish from these sites (Table 3). On average, fish in channel sites had significantly smaller, less dense gill pavement cells than fish transplanted to tributary sites.

DISCUSSION

We described downstream changes in water chemistry in the main channel of the Upper Enoree River, and compared channel sites with the water chemistry of control tributaries. All analyses suggest that the discharge of this metal-laden wastewater continues to have a profound, significant effect on the water chemistry of the Upper Enoree River. In particular, the two upstream sites (C1 and C2) that are within 0.65 km of the spill site had lower pH, reduced alkalinity, higher conductivity, and higher concentrations of anions (Cl^-), cations (Na^+, K^+, Ca^{2+}, Mg^{2+}), and metals (Al^{2+}, Zn^{2+}, Mn^{2+}) than downstream sites or control tributaries.

These differences are consistent with acid and heavy metal deposition of the spill from the galvanizing plant. Adding HCl to the groundwater should lower pH, reduce the buffering capacity of the water, reduce alkalinity, raise Cl^- levels, and increase the concentrations of cations (Na^+, K^+, Ca^{2+}, Mg^{2+}, Al^{3+},Mn^{2+}) released from soils by proton (H^+) displacement. Spent HCl from a galvanizing facility would also include zinc

used in the galvanizing process. These were precisely the combination of changes observed in the impacted headwater sites when compared to downstream sites, control tributaries, and other sites in the county.

The only anomaly in the chemical data was tributary site T3, which was more similar to downstream channel sites than to other tributary sites. However, this site was directly downstream from a eutrophic man-made pond. Warm surface water pours over a spillway to site T3, contributing to the high temperature at this site. Dissolved oxygen is low, probably as a function of higher temperature and higher productivity in the pond. High productivity may also contribute to the high total organic carbon levels and low nitrate levels recorded at T3. Finally, if diatoms are settling in the pond, the pond may act as a sink for silicates and reducing levels at site T3 below those at other tributary sites.

Changes in water chemistry can affect the integrity of ecological communities (Cole 1979, Newman and Jagoe 1996, Basnyat et al. 1999). Specifically, increased concentrations of trace metals, such as those found in the Upper Enoree River (Al^{3+}, Mn^{2+}, Zn^{2+}), are believed to be the key contaminants in toxic aquatic environments (Sunda and Huntsman 1998). We measured the effect of these chemical changes on the histology, survivorship, and community composition of the biota. There were consistent, significant effects for all parameters measured. Salamander abundance, fish abundance, fish species richness, fish diversity, invertebrate abundance, odonate abundance, odonate species richness, odonate diversity, and EPT scores were all significantly correlated with the variation in chemical composition among sites (as summarized by DFA1) after correcting for differences in stream size.

All biotic parameters were lowest at the upstream sites, increased downstream, and were greater in tributaries than in channel sites. However the effects were more pronounced on vertebrate abundance and diversity than invertebrate abundance and diversity. Unlike fish, invertebrates have granules that store, sequester, and excrete trace metals (Hare 1992). Once formed, granules may be excreted, stored throughout life, or shed with the molting exoskeleton (Hare 1992). This may benefit the invertebrate in highly polluted waters, allowing the animal to accumulate and shed increased levels of metals without toxic effects. Also, because the number of granules increases with age, tolerance to metals increases with age (Hare 1992).

Table 3. Mean (± 1 se) comparison of gill histology for *Notropis lutipinnis* transplanted to main channel (C) and tributary (T) sites in the Upper Enoree River watershed. Site means with the same letter were non-significantly different ($p > 0.05$).

Site	Pavement Cell Area (μm^2)			Pavement Cell Number ($n/50\mu m^2$)		
	n	Mean + s.e.		n	Mean + s.e.	
C1	10	127.55 ± 3.92	a	10	20.9 ± 1.75	a
C2	5	211.98 ± 6.58	b	5	30.6 ± 3.91	ab
C3	10	254.06 ± 4.16	c	11	32.0 ± 2.11	b
C5	5	253.02 ± 5.60	c	6	37.5 ± 2.26	b
C7	5	256.25 ± 5.08	c	5	34.4 ± 0.68	b
C8	5	252.52 ± 5.25	c	6	38.7 ± 1.75	b
T1	5	276.71 ± 11.0	c	5	29.2 ± 2.38	ab
T2	10	268.74 ± 3.92	c	11	38.0 ± 1.10	b
T3	5	265.02 ± 10.8	c	6	36.0 ± 1.77	b
T4	5	251.69 ± 5.35	c	10	39.3 ± 3.38	b

In addition, there were complementary patterns between *N. lutipinnis* mortality and histology. All fish (n = 80) transplanted to sites C1 and C2 died within 24 hours of transplantation. These fish had damaged gills and other characteristics of gross morbidity. At the tissue level, fish from these sites had smaller, less dense gill pavement cells than fish from other sites. These results are consistent with previous studies that have shown severe tissue damage to gills following exposure to aqueous zinc (Evans 1987, Galvez et al. 1998). The most common consequence of exposure of fish to acutely toxic waterborne zinc (1.5–40 mg l^{-1}), such as seen in this study, is an irreversible interruption of oxygen transfer across the gills because of severe tissue damage (Spry and Wood 1985). Our results support this, as we demonstrated a significant impairment to gill respiratory (pavement) cells. Furthermore, our results indicate that these tissue effects develop extremely rapidly, as fish were removed and preserved as soon as they died (within 3-24 hours of exposure at sites C1 and C2).

Mortality of *N. lutipinnis* in the transplant study reached 100% at almost all channel sites, even those more than 6 km downstream from the spill site where zinc concentrations were 0.3 mg l^{-1}. In contrast, survivorship in tributary sites dropped to only 60% after four days, and then remained at this level for the rest of the exposure period. Fish transplanted to tributaries also had a higher number and density of gill pavement cells than fish transplanted to channel sites. The absence of macroscopic life at sites C1 and C2, the rapid morbidity and mortality of fish transplanted to these sites, and the histological evidence suggesting heavy metal toxicity is strong evidence that the 1985 industrial spill continues to exert a significant negative impact on this aquatic system.

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