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Relationship between genotoxicity, mutagenicity, and fish community structure in a contaminated stream

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Abstract

Genotoxic responses (chromosomal damage, DNA strand breakage) of redbreast sunfish (*Lepomis auritis*) populations exposed to industrial effluent and mutagenicity of the associated sediments were determined in order to compare them to changes in community structure. Data were collected from a reference stream and East Fork Poplar Creek (EFPC), a first-order stream which originates on the grounds of the Department of Energy Y-12 Plant at Oak Ridge, TN. This stream is contaminated with mercury, PCBs, and numerous other compounds. Previous studies have shown that sediment contaminant concentrations, as well as physiological biomarker responses of the local fish populations, are highest at the headwaters of EFPC and decrease with increasing distance from the DOE facility as contaminant loading decreases. Chromosomal damage was measured by flow cytometry – as reflected by variation in cellular DNA content – and strand breakage was determined by agarose gel electrophoresis using blood as the source of DNA. Mutagenicity was determined by the Salmonella/microsome assay using organic solvent extracts of sediment surface samples. Community level responses included community diversity and percent pollution-tolerant species. Biomarker responses and mutagenicity were found to be highest at the headwaters of EFPC, and tended to decrease with increasing distance from the effluent. In general, biomarker responses appeared to be correlated with mutagenicity of the sediment, and both of these related to fish community disturbance and level of stream contamination. Because responses at several levels of biological organization show similar patterns of downstream effects, this suggests that there may be a causal relationship between contamination and biological effects.

1. Introduction

The effects of contaminant exposure can be manifested at a variety of levels of biological organization, from the molecular to community levels. Molecular effects include such processes as alterations of DNA structure/function or changes in gene expression (Huggett et al., 1992). Community-level effects include perturbations of community diversity, structure, or dynamics (Newman & Jagoe, 1996). The use of indicators of exposure and effects at each of these levels of organization has particular advantages and drawbacks.

For example, molecular effects of contaminant exposure are relatively easily measured with standard laboratory assays (Huggett et al., 1992). Also, because samples of only one or a few representative species need to be collected, field sampling can be done by one or two people with a seine, dipnet or backpack electroshocker. Furthermore, manifestation of such effects usually occurs fairly early – within days, hours or even minutes – after the onset of exposure. Many of

these effects can also be specific indicators of contaminant exposure, if not for specific chemical species (Shugart et al., 1987), then for general classes of chemicals – e.g., genotoxicants (Shugart, 1992, 1998), aromatic hydrocarbons (Freely, 1995), heavy metals (Roesijadi, 1994) etc. However, it is often difficult to determine the ultimate consequences of the manifestation of these molecular effects to populations or communities in which they are expressed.

Effects on fish communities and populations, on the other hand, have high ecological relevance. However, they are often difficult to quantify, and the techniques used to quantify them are expensive and time and labor intensive (Krebs, 1989). In addition, it is especially difficult to demonstrate the effect of environmental stressors at the community or population levels because the responses which may be ultimately observed at these levels are latent and temporally removed from the initial event(s) of exposure, making it very difficult to establish causality. This is further complicated by the fact that differences between sites in terms of population or community structure and function are not necessarily indicative of contaminant exposure; there are a wide variety of environmental variables that can lead to such differences.

Thus it would be advantageous to examine the relationships between molecular, community-level, and population genetic effects of contamination. Toward this end, this study focuses on redbreast sunfish (*Lepomis auritis*) collected from East Fork Popular Creek (EFPC), a small stream which receives effluent from the Department of Energy's (DOE) Y-12 Plant, a nuclear weapons production facility. This stream was chosen because there is extensive information available on amount and types of contaminants present in water, sediments and biota, as well as on fish community structure (Adams et al., 1989; Hinzman, 1993). Redbreast sunfish were chosen as the study animal because populations of this species in EFPC have been the subject of past studies which determined biochemical, physiological and reproductive effects of Y-12 effluents (Adams et al., 1992; Ham et al. 1997). Recently, these populations have also been the focus of a population genetic study using the randomly amplified polymorphic DNA (RAPD) technique (Nadig et al., 1998). However, genotoxic effects have received less attention.

Genotoxicity is a relevant molecular effect on which to focus because, not only can it can be indicative of xenobiotic contamination, a wide variety of chemicals can elicit genotoxic responses (Shugart, 1998). Thus, genotoxic responses may be highly applicable for monitoring a large number of contaminants. Also, DNA damage has been associated with perturbations in fecundity, longevity and growth of affected organisms (Theodorakis et al., 1992; Barja, 1998; Steinert et al., 1998), and thus may have repercussions on population- and higher-level effects. Finally, genotoxic responses in feral organisms could serve as sentinels for potential chronic effects of contaminant exposure, such as carcinogenesis and teratogenesis, in human populations. The three endpoints used to assess genotoxicity here were mutagenicity of the sediment (via the Salmonella/microsome assay), DNA strand breakage (via agarose gel electrophoresis), and chromosomal damage (via flow cytometry).

Sediment mutagenicity assays provide an indication of genotoxic chemicals in the sediment (Donnelly et al., 1995). They can complement chemical analyses because genotoxic chemicals are usually present as complex mixtures. Measuring the concentrations of all genotoxic chemicals would be a daunting task, if not impossible, and the additive or synergistic effects of complex mixtures are largely unknown. In addition, there may be unknown or unsuspected genotoxic chemicals present. The use of bacterial-based mutagenic assays can provide an important link between sediment contamination and DNA damage.

DNA strand breakage – measured using both alkaline unwinding and electrophoretic procedures – and chromosomal aberrations (via flow cytometry) have been detected in feral EFPC sunfish populations and in fish exposed to EFPC sediment in the laboratory (Adams et al., 1989; Theodorakis et al., 1992). Additional studies by the authors have also used these techniques to detect DNA damage in other organisms exposed to multiple contaminants (Custer et al., 1994; Lamb et al., 1995; Theodorakis et al., 1996; Bickham et al., 1998; Wickliffe & Bickham, 1998). An association between genotoxic responses in the sediment and the fish would strengthen the argument that the population and community-level responses seen in EFPC redbreast populations are due to environmental exposures to genotoxicants.

Therefore the objectives of this study are to use a combination of previously published and original data to examine the relationships between community diversity/structure, DNA strand breakage, chromosomal damage and sediment mutagenicity.

2. Methods and materials

2.1 *Study sites*

EFPC is a first-order stream that originates a few hundred meters upstream of the DOE Y-12 plant. The habitat of this stream is dominated by riparian woodlands on the banks along most of the stream, although it does pass through urbanized areas in and around the city of Oak Ridge, TN. The substrate of the stream is dominated by rock and gravel, intermixed with some muddy sediment. A number of potential genotoxic contaminants have been identified in EFPC, including polycyclic aromatic hydrocarbons (PAHs), Cd, Cu, Pb and Hg (Hinzman, 1993). The particular study sites included 4 locations along EFPC, as well as 2 reference sites (Figure 1). The locations of each of these sample sites are as follows: Site 1, EFK23.7; Site 2, EFK22; Site 3, EFK18; Site 4, EFK10.8 (EFK = "East Fork kilometer", i.e., the distance from the mouth of EFPC at Poplar Creek to the sampling site). The two reference sites were Brushy Fork and Hinds Creek, chosen because of their hydrological and physical similarity to EFPC.

2.2 *Sample collection*

For determination of community diversity and percent pollution-tolerant species, samples were collected at EFPC and Hinds Creek. Samples were collected October 7–12, 1991. Small sections of the stream were cordoned off with 0.64 cm mesh seines, and the fish within these sections were collected with a backpack electroshocking device. The length of the stream sampled for EFPC sites and Hinds ranged from 90–114 m. Samples were collected by 2 electrofishing unit operators and 3–4 assistants to retrieve the stunned fish with dipnets. Each section of stream was sampled 3 times, each time proceeding upstream. Fish were then identified to species, and their relative pollution tolerance was determined according to Ohio EPA (1988) and Plafkin (1989). The χ^2 test was used to determine statistically significant differences between sites. If the overall test was found to be significant, pairwise comparisons were made by calculating the 95% confidence interval (CI) for the estimator of $|p_1 - p_2|$, where p_1 and p_2 are the proportion of tolerant species at sites 1 and 2, respectively. If the CI included 0, then this difference was recorded as being not significantly different.

For genotoxicity analysis, adult redbreast sunfish (65–130 mm, standard length) were collected via backpack electroshocker from October 11–28, 1997. Unfortunately, at the time of sampling, high water prevented samples from being collected from Hinds Creek for genotoxicological analysis. Therefore an additional site (Brushy Fork) was chosen as the reference site for these assays. All fish were then transported in EFPC water to the laboratory for analysis. Blood was collected via caudal vein puncture in EDTA-treated Vacutainer tubes (Beckton-Dickinson, Rutherford, NJ), and immediately frozen in liquid nitrogen. For mutagenicity assays, surface sediment was collected with a trowel and placed into chemically certified, clean, amber glass jars.

2.3 *Genetic analysis*

For flow cytometric analysis, nuclei were isolated from blood cells and stained with propidium iodide according to a modification of the methods of Vindelov et al. (1983) and Vindelov and Christianson (1990). An increase in the amount of chromosomal damage is reflected by an increase in cell-to-cell variation in DNA content (Bickham, 1990). This variation was measured using an Epics Profile II Flow Cytometer (Coulter Corp., Hialeah, CA), which measures the cell to cell half-peak coefficient of variation (gated CV) in DNA content for cells in the G_1/G_0 phase of the cell cycle. Alignment, focus, and instrumental gain were set prior to analysis using 0.097 mm fluorescent microspheres (Coulter Corp.) and all samples were run in one day. Differences between sites were tested using the Kruskal-Wallis test.

For DNA extractions, 20 μl whole blood was suspended in 500 μl TEN (50 mM Tris, 10 mM EDTA, 100 mM NaCl, pH 8.0). DNA was then extracted and purified according to Theodorakis et al. (1996), and dissolved in TE (10 mM Tris, 1 mM EDTA, pH 8.0). DNA was quantified spectrophotometrically at 260 nm (1 AU = 50 μg DNA/ml).

The DNA was subjected to electrophoresis with alkaline or neutral running buffer (Theodorakis et al., 1996). Alkaline electrophoresis was performed with 30 mM NaOH, 2 mM EDTA (pH 12.5) as the running buffer. A total of 0.5 μg of DNA was loaded into each well of a 0.8% agarose gel and subjected to electrophoresis at 5 V/cm for 5 h. The buffer was constantly recirculated and cooled in an icebath. For neutral gel electrophoresis, TBE (45 mM Tris, 45 mM borate, 0.5 mM EDTA, pH 8.0) was used as the running buffer. A total of 0.05 μg DNA was loaded into a 0.3% agarose gel and subjected to electrophoresis at

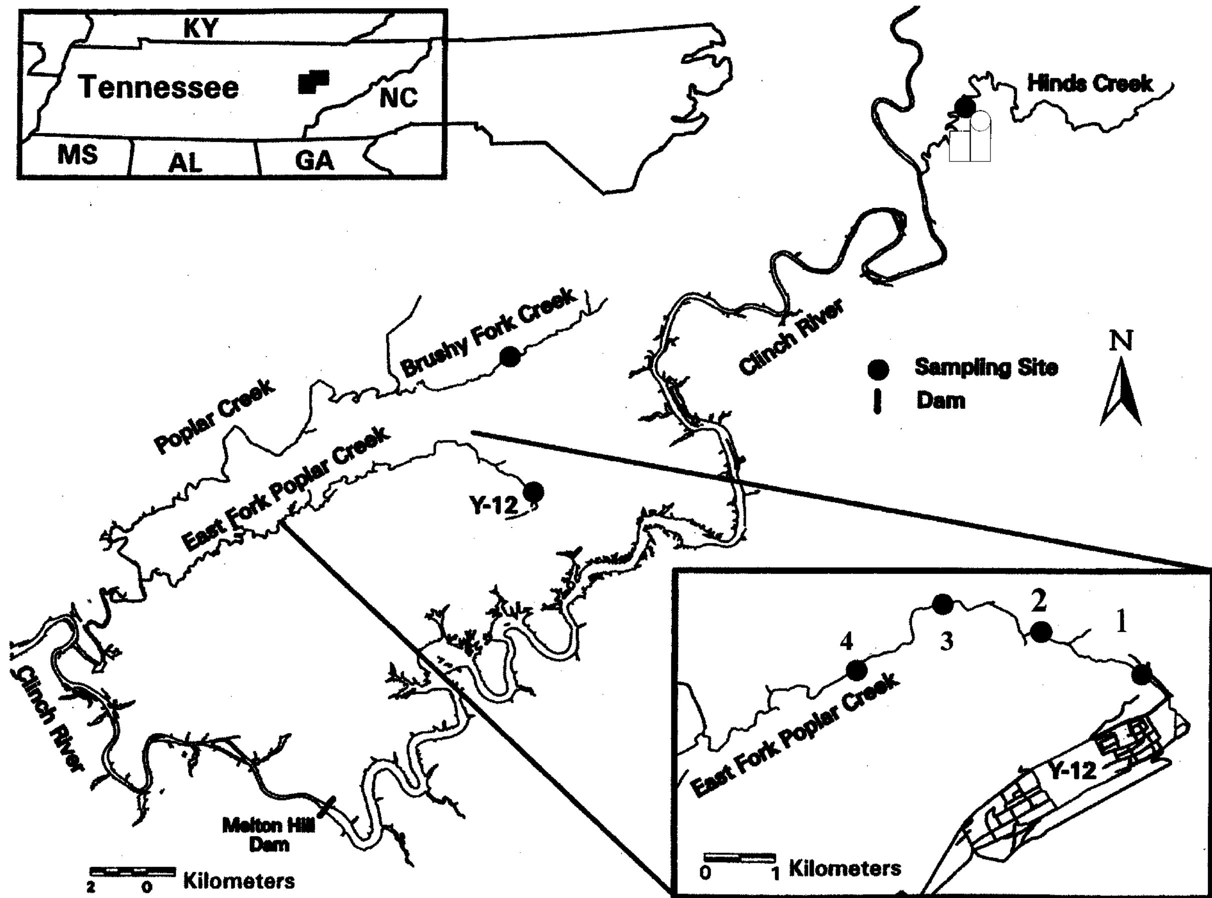

Figure 1. Map of sampling locations along East Fork Poplar Creek, Brushy Fork and Hinds Creek.

0.75 V/cm for 18 h. Because such a low percentage gel is very fragile, the gels were cast on a basement of 3% agarose. After electrophoresis, gels were stained in ethidium bromide and photographed under UV light. The average molecular length (Ln) of the DNA in each sample was calculated as in Theodorakis et al. (1993).

The Ln calculated under alkaline conditions is affected by both single- and double-strand breaks, while the Ln of neutral gels is affected by double strand breaks alone. Because DNA is a long and fragile molecule, double-strand breaks can be caused not only by genotoxicant exposure, but also by physical shearing which may take place during extraction, purification and analysis. In order to take this into account, the number of single-strand breaks (per 10^5 bases) was determined by subtracting the double-strand Ln from the single-strand Ln according to the following formula, as modified from Freeman et al. (1990):

$$\#SSB = \left(\frac{1}{\text{Ln(single)}} - \frac{1}{\text{Ln(double)}}\right) \times 100.$$

Comparisons between sites were tested with the Kruskal-Wallis test. Because there seemed to be differences between sites in the amount of variability, differences between sites in the degree of data dispersion were tested with the Anseri-Bradley test (Hollander & Wolfe 1973). Correlations between molecular (DNA content CV and single-strand breaks) and community-level responses (community diversity and % pollution tolerant species) were tested with non-parametric Kendall correlation tests.

2.4 *Sediment mutagenicity*

Mutagenic potential of the sediment samples was determined by the Salmonella plate incorporation assay as described by Ames et al. (1975) and revised by Maron and Ames (1983). This assay determines

Table 1. Relative abundance (%) of species captured in EFPC and Hinds Creek and their relative pollution tolerance

Species	EFPC Sites				Hinds Creek	Tolerance[a]
	Site 1	Site 2	Site 3	Site 4		
Bluntnose minnow (*Pimephales notatus*)	0	0	0	0	0.8	T
Fathead Minnow (*P. promelas*)	1	0	0	0	0	T
Blacknose dace (*Rhinichthes atratulus*)	3.8	6.3	1.2	1.2	3	T
Creek Chub (*Semolitus atromaculatus*)	0.1	3.8	2.1	1.2	2.2	T
Rosefin shiner (*Notropis ardens*)	0	0	0	0	0.2	MI
Striped shiner (*Luxilus chrysocephalus*)	34.2	50	62.4	29.1	22.1	T
Common stoneroller (*Campostoma anomalum*)	52.5	34.5	17.4	37.6	43.7	MT
Goldern redhorse (*Moxostoma erythrurum*)	0	0	0	1.2	1.9	MI
Northern hogsucker (*Hypentilium nigricans*)	0	0	0.4	8.5	6.8	MI
White sucker (*Catostomus commersoni*)	1.7	1.7	21	0	0	T
Yellow bullhead (*Amerius natalis*)	0.4	0	0	1.8	0	T
Mosquitofish (*Gambusia affinis*)	0.7	0.8	0.8	0	0	T
Bluegill sunfish (*Lepomis macrochirus*)	1.7	0.4	0.4	4.8	0.5	MT
Green sunfish (*L. cyanellus*)	0.1	0	0	0	0	T
Redbreast sunfish (*L. auritus*)	3.5	2.5	13.2	10.9	0.8	MT
Rockbass (*Ambloplites rupetrus*)	0	0	0	0.6	4.4	SI
Stripetail darter (*Etheostoma kennicotti*)	0	0	0	0	0.8	SI
Snubnose darter (*E. simoterum*)	0	0	0	0	3.3	SI
Banded sculpin (*Cotus carolinae*)	0	0	0	3	9.3	MI

[a]T = tolerant; MT = moderately tolerant; MI = moderately intolerant; SI = slightly intolerant (Ohio EPA 1989).

the mutagenic potential of a chemical or complex mixture as its ability to revert a histidine-requiring strain of *Salmonella typhimurium* to a wild type phenotype. These strains of *S. typhimurium* are unable to synthesize the amino acid histidine due to various mutations in the histidine operon and, thus, require histidine supplementation in order to grow. Different strains vary in the position and nature of the mutation in the operon and respond to different types of mutagens (e.g. point vs. frameshift) (DeMarini, 1993) and, hence, to different sets of chemicals. In this way, the assay is able to detect mutagens capable of reversing these muta-

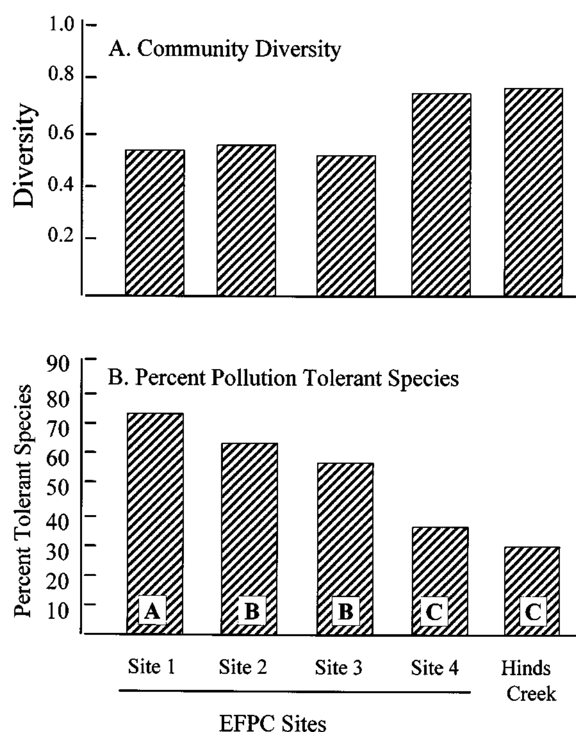

Figure 2. (A) Relative diversity (Shannon Weiner Index) of fish communities and (B) percent pollution tolerant species at four East Fork Poplar Creek and one Hinds Creek locations. In (B), bars labeled with the same letter are not significantly different ($P > 0.05$, $\chi^2 = 222.3$).

tions, thus removing the histidine requirement. The strain used in the present study is most sensitive to PAHs and their metabolites as well as some nitroaromatics. Samples are tested both with and without the addition of a liver microsome preparation (S9) that simulates the metabolic activation required for some chemical classes (e.g. PAHs) to exert their genotoxic effect.

Sediment samples were collected from the same EFPC and Brushy Fork sites as were used for the DNA damage analyses. Each sample was thoroughly mixed and an aliquot dried overnight in a 70 °C oven. Ten grams of dried sediment were aliquoted into extraction thimbles and 2–4 thimbles were extracted per site. Extractions were performed on a Tecator Soxtec HT-6 automatic extraction unit according to EPA Method #3541 (EPA, 1989). Samples were extracted with boiling 1:1 hexane:acetone (v/v) for 60 minutes, then rinsed with the same solvent for an additional 60 minutes. Residues from the same site were combined, dried under a nitrogen stream, and weighed. Serial dilutions of 20, 10, 5, 2, and 1 mg/ml residue were made in DMSO. Top agar containing 0.05 ml of the test chemical, 0.1 ml of a fresh overnight culture of the test strain and 0.5 ml of a liver microsome preparation (S9 mix) or phosphate buffer was plated on a minimal glucose agar base. Each concentration of the dilution series was run on duplicate plates. The plates were incubated for 72 hrs at 37 °C. Revertant colonies were counted using an automated colony counter. Duplicate assays were performed for each dilution series. The number of revertants for each dose was calculated as the average of total revertants per dose (4 plates) minus the average spontaneous revertants with or without the S9 mix. Responses were scored as positive or negative. A positive response was defined as a doubling of the absolute number of revertants over solvent controls in two consecutive doses. Net revertants (number of revertants at the highest responding dose (optimal dose) – average number of spontaneous revertants) with and without S9 are also reported for each site.

3. Results

Community diversity was lower at EFPC sites 1–3 than it was at EFPC site 4 or Hinds Creek (Figure 2). Percent pollution-tolerant species was greatest at site 1 and decreased with distance from the Y-12 plant (Figure 2). The total number of individuals of all species captured at EFPC sites 1–4 and Hinds Creek was, respectively, 690, 238, 242, 165,and 366. The relative abundance of each species, as well as pollution tolerance classification, are listed in Table 1. As noted before, the sites are numbered according to their distance from the Y-12 plant, with site 1 being the closest.

The CV in DNA content also decreased with distance from the Y-12 plant. The median CV was greater at sites 1–3 than at site 4. The CV at Brushy Fork Creek was not significantly different from site 3 or site 4 (Figure 3). There were no significant differences between the median number of DNA strand breaks between sites. However, the amount of dispersion at sites 1–3 was significantly higher than at site 4 and Brushy Fork ($P < 0.05$, Anseri-Bradley test). There was a significant correlation between CV and percent pollution-tolerant species, but none of the other correlations were statistically significant (Figure 4).

Mutagenicity responses of sediments from the five sampled stations are presented in Table 2. Sediment from site 1 was not directly mutagenic, but showed

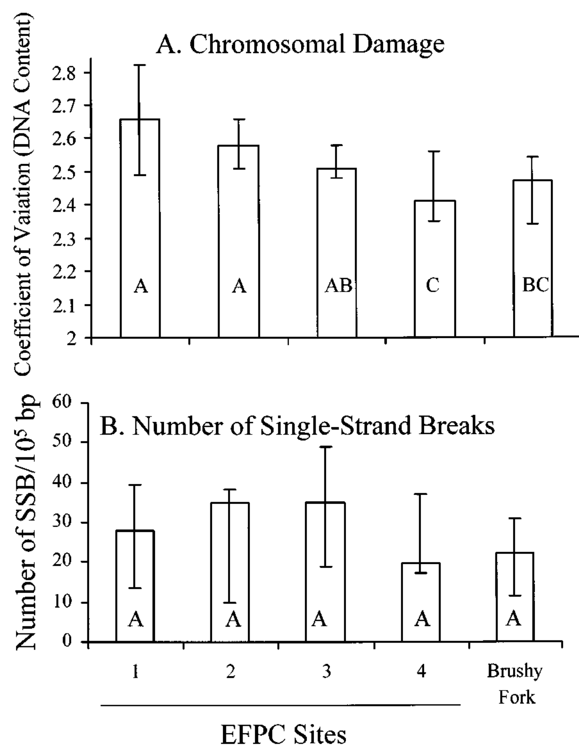

Figure 3. Half-peak coefficient of variation (CV) in DNA content (A) and number of DNA single-strand breaks (B) in redbreast sunfish from four East Fork Poplar Creek and one Brushy Fork locations. Bars and error bars represent medians and first and third quartiles, respectively. Bars which are labeled with the same letter are not significantly different ($P > 0.05$, Kruskal-Wallis test; Kruskal-Wallis test statistic = 9.8 (A) and 1.55 (B)). Sample sizes for EFPC Sites 1–4 and Brushy Fork are, respectively (A): 11, 12, 8, 9 and 7; (B): 6, 9, 7, 6 and 5.

a moderate mutagenic response with metabolic activation and gave the strongest mutagenic response of the five samples tested. Sediment residues from sites 2, 3, and 4 produced weak mutagenic responses without metabolic activation and moderate responses with metabolic activation, although the magnitude of response differed among the sites. The number of revertants induced by samples from sites 2 and 3 was similar and was less than the number induced by the site 4 sample. The magnitude of response from all three of these sites was less than that from site 1. The reference site gave the lowest response of the five sites, although the response was still weakly positive.

4. Discussion

The results of this study show that, in general, the trends of changes in community structure are similar to the trends of genotoxic response. These are consistent with previous studies that have shown trends in biochemical responses that are concordant with distance from the DOE Y-12 plant (Adams et al., 1996). These data are also consistent with the findings that concentrations of contaminants in the water, sediment, and biota generally decrease with distance from the Y-12 plant (Hinzman, 1993). All of these studies indicate that there are a wide variety of contaminants in EFPC, so that exposure to genotoxic agents is undoubtedly not the only cause for changes in community structure, nor DNA damage the only biological effect. However, genotoxicity was the only biomarker endpoint measured here, so the discussions below will focus on the potential contributions of using genotoxic effects to the perturbations of biological communities seen in EFPC.

4.1 *Genotoxic responses*

Although the DNA repair mechanisms in fish are not as efficient as those in mammals (Espina & Wies, 1995; Wirgin & Waldman, 1998), they do exist. DNA damage is a steady-state process, i.e., it is constantly being formed and repaired (Freidberg, 1985). Thus, any DNA damage that is apparent is that which remains unrepaired at the time the tissue is collected and frozen. The fact that any damage is observable indicates that this steady-state has been altered such that the equilibrium has been shifted more towards accumulation of DNA damage – either through an increase in the number of DNA-damaging events or a decrease in DNA repair. This would be an argument in favor of viewing DNA damage as a biomarker of contaminant effect, and not simply of exposure.

In general, DNA content CV showed a stronger trend with increasing distance from the Y-12 plant and less variability than did DNA strand breaks. Differences between the flow cytometry and DNA-damage assays could be due to differences in sensitivities of the assays or the type of damage that they measure. The electrophoretic assay measures single-strand DNA breaks, which could be due to direct nicking of the sugar-phosphate backbone or to conversion of certain DNA base modifications (also indicative of contaminant exposure) to single strand breaks *in vitro* at alkaline pH (so-called "alkaline labile sites").

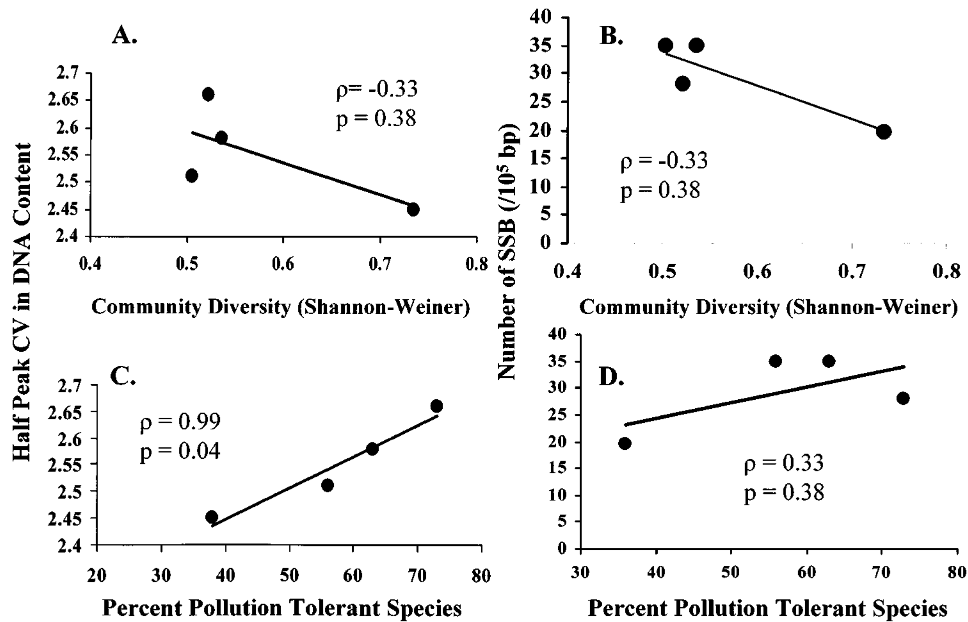

Figure 4. Correlations between community diversity and CV in DNA content (A) or number of DNA single-strand breaks (B), and between % pollution tolerant species and CV (C) or single strand breaks (D). ρ = Kendal's correlation coefficient. p = probability of Type I error (H_0: no dependance between variables).

Table 2. Results from the Ames assay of organic extracts from East Fork Poplar Creek and Brushy Fork Creek sediment. Data represent number of net revertants (back mutations) with microsomal activation of the sediment extracts (with S9) and without

	Net Revertants		
Site	With S9	Without S9	Overall Response
EFPC1	167	29	+/–
EFPC2	121	47	+
EFPC3	122	61	+
EFPC4	138	77	+
Brushy Fork	62	32	+

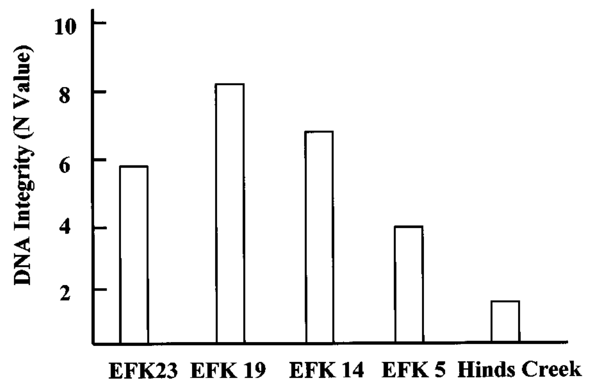

Figure 5. Relative number of strand breaks (N) in redbreast sunfish at four East Fork Poplar Creek and one Hinds Creek locations.

The flow cytometric assay, on the other hand, measures chromosomal damage, a result of double strand breaks. Double-strand breaks are more difficult to repair (Ward, 1988), so they may be a less transient effect than single-stand breaks or alkaline-labile sites. These differences could also be due to the nature of the assays themselves, since the flow cytometry assay used intact nuclei and the electrophoretic assay used extracted DNA. Future studies will incorporate electrophoresis using intact nuclei or whole blood cells (Theodorakis et al., 1993) to determine if this results in lower variation between samples.

In the present study the average number of single-strand breaks was not significantly different between EFPC and the reference site. This could have been an artifact of low statistical power due to a small sample size and high variability. The high variability seen in this assay could have a number of sources. First, it could stem from the transient nature of single-strand breaks. As discussed before, single-strand breaks are easier to repair and hence more transient than double-strand breaks. Differences in stage or capacity of DNA repair could lead to increased inter-individual varia-

tion in the amount of DNA strand breakage, resulting in increased within-population variation relative to between-population variation. Another causal factor could be migration of fish between contaminated and reference areas. Again, this would result in increased within-population variation and decreased between-population variation, thus obscuring any differences between contaminated and reference populations. A third reason why single-strand breaks reveal no differences between the contaminated and reference sites may be due to the fact that EFPC is undergoing remediation efforts, and the amount of DNA strand breakage and expression of other biomarkers has been gradually approaching that seen in reference sites as a result.

Although the average number of single-strand breaks was not different, there was more variation in the more highly contaminated sites (EFPC sites 1–3) than in site 4 or Brushy Fork. This is true not only for the single-strand break data, but also to a lesser extent, the flow cytometry data. A larger amount of variation in data obtained from contaminated relative to reference fish has been observed previously (Theodorakis et al., 1992), and it has been suggested that increased variation in biological processes is in itself an effect of contaminant exposure (Suter, 1993). One possible explanation for this could be genetic variation in susceptibility to DNA damage, in that such variation would not be expressed until the individuals are exposed to a DNA-damaging agent. Migration between contaminated and non-contaminated areas could also result in an increase in variation of responses.

There was also another result from the present study that reflected previous findings. Note that, although the medians are not statistically different, the trend is that the number of single-strand breaks is greater at site 2 than site 1, then gradually decreases downstream (Figure 3). This same trend was also found in previous studies using the alkaline unwinding technique with liver tissue (Figure 4; Nadig et al., 1998). The fact that this trend was seen in two separate studies employing different methods of strand-breakage determination (alkaline unwinding vs. electrophoresis) in different tissues (blood vs. liver) implies that this is a real effect and not a sampling artifact. The finding that alkaline unwinding and electrophoretic methods for determination of DNA strand breakage both give comparable results has been substantiated in previous studies (Theodorakis et al., 1993).

4.2 *Community-level responses*

The data presented here indicate that community structure is affected by exposure to genotoxicants in EFPC, and that % pollution tolerant species shows a clearer downstream trend than does community diversity. This may suggest that % pollution tolerant species is a more appropriate indicator of biological effects than is community diversity, as has been argued in previous studies (Karr, 1991). Such changes in community structure are believed to be caused by loss of "pollution sensitive" species and an increase in "pollution tolerant" species. Although the ultimate causes of this process are not entirely clear, possible mechanisms could include differential effects on survival and fitness between tolerant and resistant species, resulting in loss of species due to lower recruitment or alterations of patterns of competition. Because an increase in the amount of DNA-damage may be associated with detrimental effects on fitness parameters such as fecundity (Theodorakis et al., 1992) and growth rates (Steinert et al., 1998), genotoxicity may be a contributing factor to such differential effects. The relationship between genotoxicity and percent pollution tolerant species was most evident for chromosomal damage (Figure 4c). This may be influenced by the fact that the single-strand break data had a higher variance than the CV data. It may also be attributable to the relative consequences of single-strand vs. double-strand breaks. In general, double-strand DNA breaks have more dire consequences – in terms of cell survival, carcinogenesis, and heritable DNA lesions (Kampf & Eichhorn, 1983; Kraft et al., 1989; Olive, 1998) – than do single-strand breaks or alkaline-labile damage, and are more strongly correlated with curtailment of reproduction (Theodorakis et al., 1996). However, it should be noted that the correlation between percent pollution tolerant species and chromosomal damage does not necessarily imply a direct cause and effect relationship. Rather it implies that the same set of chemicals that cause changes in community structure also lead to genetic perturbations. Nevertheless, the correlation of an organism-based biomarker and a community-level metric has important implications both for ecological risk assessment (see later discussion) and for mechanistic studies of population and community-level responses (e.g. pollution tolerance) to contaminant exposure.

The work presented in this study provides a basis for more detailed studies to explore the underlying mechanisms of pollution tolerance, and the relation-

ship between biomarker response and community composition. For example, it could be that the "pollution sensistive" species are more prone to the effects of pollution due to relatively less efficient detoxification, repair, or other protective mechanisms. Or it may be that they receive a greater dose of toxicants than the more "tolerant" individuals due to habitat requirements or feeding behaviors. Thus comparative studies that examine relative biomarker exoression between species could be used to help elucidate the mechanisms that lead to changes in community structure. Studies that involve multiple species and integrate biomarker responses, body burdens, fitness components (fecundity, growth etc.) and relative toxicity, both in the laboratory and in the field, would be particularly useful in this regard.

4.3 *Sediment mutagenicity*

Mutagenicity has long been of interest in toxicological research because of the strong potential for a relationship between mutagenicity and carcinogenicity. An enormous number of short term mutagenicity assays have been proffered in an attempt to develop a simple screen for possible carcinogens. One of the most widely used of these short term assays is the bacterial Salmonella/microsome assay. It provides a rapid, cost effective method to screen many chemicals, including complex mixtures obtained from environmental samples, in a single assay. Despite these advantages however, questions still remain as to the relevance of these *in vitro* short term tests in relation to prediction of future carcinogenicity and manifestation of genotoxicity *in vivo* (Ashby, 1991).

Sediments from EFPC are not only contaminated with PAHs, but also with compounds (e.g. mercury, PCBs, and chlorinated pesticides) that are not mutagenic in this Salmonella assay, so these compounds probably to not contribute to the mutagenicity seen in this study. In general, the results from the assay show that the sediment at EFPC contains higher amounts of microsomal(S9)-activated genotoxicants (e.g. PAHs) than the reference site, and the highest mutagenic activity is found at site 1. The fact that there is not much difference in the responses between sites could indicate that there is not much difference between them in terms of contamination by PAHs or other as yet unidentified metabolically – activated genotoxicants. This may explain why there are also no differences between sites for single-strand breakage data. However, there does seem to be an increase in microsomally-associated mutagenic activity in sediment farther downstream (see site 4, Table 2). In addition, the non-S9 activated mutagenicity increased with distance from EFPC (Table 2). This finding may be the result of the presence of as yet unidentified contaminants that can act as direct mutagens in this assay. Alternatively, the acquisition of mutagenicity at downstream sites could be due to the presence of increasing PAH metabolites as a result of microbial activity upstream.

The relationship between *in vitro* sediment mutagenicity results and *in vivo* genotoxicity effects due to exposure to these sediments is still unknown. Similarities in the trends of the strand break data and the Salmonella/microsome data suggests that the strand breaks may at least in part be related to PAH exposure. Additional mutagenicity assays sensitive to different types of mutagens as well as examination of mechanistic correlations of *in vitro* and *in vivo* responses produced by various contaminants and contaminant mixtures may provide further insight into these relationships.

4.4 *Implications for risk assessment*

The fact that changes in community composition are correlated with genotoxic responses and, to some extent, sediment mutagenicity, has several implications for ecological risk assessments. First, changes in DNA integrity can occur relatively quickly (Shugart et al., 1989; Black et al., 1996), while changes in community structure may take much longer times to be manifested. Thus, changes in the state of molecular biomarkers could provide an early warning of the potential for future ecological damage. Conversely, during remediation processes, the relative magnitude of biomarker expression would return to pre-exposure levels much more quickly than would community structure. Monitoring molecular biomarker responses during remediation could be used as a rapid assessment as to whether the remediation activities would result in recovery of the affected populations. Secondly, one or two people can accomplish collecting samples for molecular biomarker analysis, while sampling fish communities usually requires larger sampling crews. Plus it is often advisable to collect additional information on other environmental variables (habitat structure, productivity) which could affect community responses, and this entails additional time and effort. If there are many such sites to sample, this could be a daunting task. In this case,

sampling for biomarkers may be used as a preliminary assessment in order to determine particular sites on which to focus more intensive sampling efforts. Third, many molecular biomarkers, such as DNA damage, are indicators of specific groups of contaminants, while changes in community structure could be influenced by numerous factors that may not have anything to do with environmental contamination. In this case, association of molecular with community-level responses could be used as a diagnostic tool to provide evidence that the community-level effects are due to contamination and not extraneous factors, especially if multiple taxa were used. It has been suggested that ecological effects assessments could use lower-level responses in order to ascertain possible effects at higher levels (Suter, 1993) – in this case, using molecular responses as an indicator of possible community-level effects – and this would be most appropriate if there were a linear relationship between responses at the two levels. Thus the fact that there was a linear relationship between percent pollution tolerant species and chromosomal damage is particularly useful for the three applications described above.

It should be noted, however, that this is just a preliminary study, and further research is needed in order for these applications to be widely used. Foremost, more contaminated and reference sites need to be compared, because at this point all that can be said is that EFPC is different from Brushy Fork or Hinds Creeks. Also, more taxa need to be sampled in order to determine if the patterns seen here occur widely. As mentioned above, the correlations presented here are only associations and do not provide cause and effect. However, additional evidence may be gathered for this purpose by examining comparative responses from multiple taxa at different trophic levels or feeding/reproductive guilds. For example, suppose that the abundance of a particular species is inversely proportional to the level of environmental contamination, while the abundance of a second species is not different between the two sites or is even greater at the contaminated site. If it is found that the amount of DNA damage in species 1 exceeds that of species 2, this would be evidence that the patterns of abundance are influenced by exposure to genotoxic compounds. Also, the use of the "pollution tolerant species" concept has come under criticism (Suter, 1993). Further studies could incorporate alternative endpoints such as changes in trophic or guild structure (VanWinkle et al., 1993), or the use of principal component or ordination techniques for examining changes in community structure (e.g., Kedwards et al., 1999), in conjunction with comparative studies of biomarker responses. Rather than relying on *a priori* assumptions of pollution tolerance among species, these types of studies could be used to identify how contaminated and reference communities differ. Comparison of biomarker expression among species could then be used as an aide in determining if contaminant exposure contributes to these differences.

It could also be that the responses being measured are not a reflection of environmental contamination. Even if there are concordant patterns of molecular and community-level responses, they may both covary as a consequence of stream flow, food availability, etc., and not necessarily due to anthropogenic effects. Hence it is up to the ecological effects assessors to decide how much evidence is required to be reasonably certain that such effects are actually occurring. The authors feel that an approach such as presented in this paper – incorporating chemical analyses, bioassays, biomarker and community-level responses – would be adequate to achieve these goals.

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