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Quality of Suspended Fine Particulate Matter in Aquatic Ecosystems and Its Role  
As a Conduit for Metals in Riverine Food Webs

(Under the Direction of DR. JUDY L. MEYER)

Suspended fine particulate matter (SFPM) is an important component of southeastern river food webs; however, variability in its quality has not previously been measured. A chironomid growth assay was used as an integrative measure of quality, SFPM was collected along the Little Tennessee River continuum. Quality of SFPM collected from the 7<sup>th</sup> order site was significantly higher than SFPM collected from the 5<sup>th</sup> order site. Traditional measures of quality (% lipids, % diatoms, calories, bacteria, % inorganic and N/C ratio) were not related to growth. Using the same methods, I measured the quality of SFPM collected from the Chattahoochee River which flows through metropolitan Atlanta, GA. Traditional measures of quality and total recoverable metal concentrations associated with SFPM were measured. Quality of SFPM was inversely related to urbanization but was not related to metal concentration. SFPM quality in the Chattahoochee River was less than in the Little Tennessee River.

I also examined the role of SFPM as a conduit for metals to macroinvertebrates. Three macroinvertebrate taxa (scraping mayflies, collecting/gathering midges and filtering caddisflies) were collected from four sites along the Chattahoochee River during four seasons. While the concentrations of metals (arsenic, copper, cadmium, lead and mercury) in macroinvertebrates were not different among sites, they were elevated compared to macroinvertebrate metal concentrations measured in other sites around the US. No other factor (diet, size, extent of exoskeleton, and sorbed and dissolved metal concentrations in the water column) was significantly related to tissue metal concentrations. Arsenic, copper, cadmium and mercury dynamics were also examined in

the food web of the Chattahoochee. Cadmium and mercury biomagnified from SFPM to macroinvertebrates and arsenic and mercury biomagnified from macroinvertebrates to fishes. Arsenic and mercury were positively related to  $\delta^{15}\text{N}$  and were significantly related to each other, suggesting that they move in similar ways through the food web.

Currently only dissolved concentrations of metals are regulated in US surface waters and Georgia adopted this standard in 1998. Metals sorbed to SFPM are no longer regulated. The criteria change and adoption of clean techniques, resulted in 943 Georgia stream miles being no longer listed for metals violations. I recommend metals criteria based on tissue residue to more adequately protect biota.

**INDEX WORDS:** Copper, Cadmium, Lead, Arsenic, Mercury, Seston, Macroinvertebrates, Fishes, Metals Criteria

QUALITY OF SUSPENDED FINE PARTICULATE MATTER AND ITS ROLE AS A  
CONDUIT FOR METALS IN RIVERINE FOOD WEBS

by

EMMA JOSEPHINE ROSI-MARSHALL

B.S. The University of Michigan, 1994

M.S. The University of Georgia, 1997

A Dissertation Submitted to the Graduate Faculty of The University of Georgia in Partial  
Fulfillment of the Requirements for the Degree

DOCTOR OF PHILOSOPHY

ATHENS, GA

2002

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May 2002

## DEDICATION

I dedicate this work to the people who use and enjoy the Chattahoochee River.

## ACKNOWLEDGEMENTS

The many people who contributed the completion of this work need to be acknowledged and thanked. First of all, I would like to thank my advisor, Dr. Judy L. Meyer. Without her guidance, support, and friendship this endeavor would not have come to fruition. Thank you, Judy for allowing me to follow my instincts and letting me conduct research that I feel is meaningful and fulfilling. Over the years, the balance of advice, structure and freedom was exactly what I needed. I would also like to thank my committee who provided additional guidance and perspective. I would like to thank The Turner Foundation for supporting this research. Berry Lyons, Klaus Neumann, and Betsy Graham conducted a majority of my metals analysis. Jeff Pollack assisted me in the field and laboratory for the bulk of the project. The Upper Chattahoochee Riverkeeper provided survey data and advice on keeping this research relevant.

Thanks to Meyerfauna, especially Mike Paul, Cathy Gibson, and Sue Eggert for all of their helpful suggestions, rousing discussions and laughter in the face of difficulty. Thanks to all of my friends in the Institute of Ecology who kept me going through this degree.

Finally, many thanks to Eric Rosi-Marshall for everything you have given to me which is too personal to mention. Specifically, thank you for reading this entire document and always acting interested in my work. Finally I would like to thank the people I met on the Chattahoochee River, who truly inspired the need for this project.

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## CHAPTER 1

### INTRODUCTION

A basal food resource for food webs in large rivers is suspended fine particulate matter (SFPM) (Wallace et al 1987, Couch et al. 1996, Benke and Wallace 1997, Hall and Meyer 1998, Rosi-Marshall and Wallace 2002). Although SFPM has been identified as an important food resource supporting food webs in large rivers (Benke and Wallace 1997, Rosi-Marshall and Wallace 2002), little is known of how its quality varies. SFPM has a high propensity to sorb metals (Rao et al. 1993, Maki and Hermansson 1994), which may have a negative effect on the quality of this food resource. In addition, metals sorbed to SFPM may be bioavailable to the aquatic food web forming an important route of metal exposure to aquatic organisms. The importance of SFPM to aquatic food webs as a food resource and a conduit for metals is the overarching focus of this dissertation.

The quality of SFPM as a food resource may be a consequence of how it is formed. As water passes over land or through the ground, it picks up dissolved and colloidal materials, including contaminants such as metals, and transports these compounds to the stream. While in the stream, biotic and abiotic processes result in aggregation of dissolved and colloidal particles and breakdown of larger particles resulting in SFPM (Lush and Hynes 1973, Alber and Valiela 1994, Wotton 1996). The first research objective, addressed in Chapter 2, was to assess the natural variability of SFPM in a river with minimal human impacts. I used sites along the Little Tennessee River continuum, Franklin Co. NC, to obtain SFPM of potentially variable quality (Vannote et al. 1980). The River Continuum Concept predicts that consumption of labile

resources upstream results in more refractory SFPM downstream (Vannote et al. 1980), and I hypothesized that SFPM quality would decline downstream. To assess the quality of SFPM I used traditional measures: N/C ratio, % lipids, caloric content, % inorganic, % diatoms, and bacterial density. In addition, I used a chironomid growth assay as an integrated measure of quality.

Practices occurring in the watershed (e.g. application of pesticides, trace metal inputs, sewage discharge) and instream conditions may also influence the quality of SFPM. The second research objective was to determine if the SFPM collected from the Chattahoochee River, which drains metropolitan Atlanta, Georgia, varies in quality (Chapter 3). Large urban rivers can have numerous point and non-point sources of metal contamination (Paul and Meyer 2001). I hypothesized that SFPM collected from an urban river would decline in quality as the cumulative amount of urbanization in the watershed increased. I also predicted that the quality of SFPM would be inversely related to the concentration of trace metals sorbed to the SFPM. The metals I examined were Cu, Cd, Pb and Zn, which are typically found in urban streams and when sorbed to particles are not regulated (US Federal Water Pollution Control Act § 307, 1997). To assess the quality of SFPM collected from this urban river I used traditional measures (N/C ratio, % lipids, caloric content, % inorganic, % diatoms, chlorophyll a concentration and bacterial density) and a chironomid growth assay. I also measured the concentrations of trace metals sorbed to the SFPM. Finally, I compared the growth rates of chironomids fed SFPM collected from the Chattahoochee River to the growth rates of chironomids fed SFPM collected from the Little Tennessee River.

Currently EPA only requires that dissolved metals be regulated in surface waters. This policy was adopted in Georgia in Nov. 1998. As a result, metals sorbed to fine particles are no longer regulated in aquatic ecosystems. The EPA found that metals sorbed to fine particles are not bioavailable to aquatic organisms; however, recent studies have demonstrated that this is not necessarily the case. Wang and Fisher (1998) developed a model which demonstrated that the tissue metal concentrations in an estuarine filterer were related to the total metal load in the water column. Roditi and Fisher (1999) found that assimilation efficiencies of metals sorbed to fine particles by zebra mussels were much greater than from uptake of the dissolved form. Schlekot et al. (1999) found that Cd associated with bacterial extracellular substance is highly bioavailable to estuarine benthos. Recently Lee et al. (2000) found that an organism's feeding behavior controls bioaccumulation of Cd, Ag, Ni, and Zn. In addition, they found that metal concentrations in tissues related to concentrations of metals associated with sediments, but not with pore-water concentrations.

These studies suggest that metals sorbed to SFPM are bioavailable, and I hypothesized that SFPM in the Chattahoochee River is a conduit of metals in the riverine food web. The third research objective was to examine the patterns of metal concentrations in macroinvertebrates inhabiting snags in an urban river and relate the concentrations measured to the dissolved and sorbed metal concentrations in the water column (Chapter 4). The metals I measured were Cu, Cd, Pb, As and Hg. Cu, Cd, and Pb are bioavailable when associated with sediments (Wang and Fisher 1998, Roditi and Fisher 1999, Schlekot et al. 1999) and these metals are typically found in urban streams (Paul and Meyer 2001, Rose et al. 2001). Arsenic was measured because coal-fired

power plants add large amounts of inorganic As to the Chattahoochee River (Froelich and Lesley 2001, Lesley and Froelich 2001). Hg was also measured because it is regulated in the total recoverable form and typically enters food webs through trophic routes. I also related tissue metal concentrations to diet, body size and life cycle. Because I was able to measure temporal patterns in macroinvertebrate tissue metal concentrations and examine diets, the macroinvertebrate patterns are presented separately from analyses of the entire food web examined in Chapter 5.

The fourth research objective was to measure the extent of trace metal contamination in snag food webs at four sites along the Chattahoochee River and determine the extent of biomagnification of Hg, As, Cu, and Cd (Chapter 5). These are the same metals measured in Chapter 4; however, Pb was excluded because all fishes had Pb concentrations lower than detection limits. I determined whether SFPM is a source of metals to higher trophic levels. In sandy-bottom rivers, such as the Chattahoochee, the only stable habitat for macroinvertebrates are snags (Wallace et al. 1987, Benke and Wallace 1997); therefore, snag food webs were examined. The components of the food web measured were SFPM, collector/gathering chironomids, scraping mayflies, filtering caddisflies, catfish, carp, shad and largemouth bass. Stable isotopic values ( $^{15}\text{N}$  and  $^{13}\text{C}$ ) were used to reconstruct trophic relationships. I also calculated the biomagnification of metals from SFPM to insects and from insects to fishes.

Chapter 6 examines legal and societal implications of these findings, including a discussion of how metals are currently regulated nationwide and in Georgia. The extent of contamination that exists in the food web of the Chattahoochee River is compared to the Chattahoochee River's status under current regulatory criteria. Finally, I discuss

factors that should be considered in the development of more protective water quality standards.

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CHAPTER 2  
QUALITY OF SUSPENDED FINE PARTICULATE MATTER ALONG A RIVER  
CONTINUUM<sup>1</sup>

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<sup>1</sup>Rosi-Marshall, E. J. and J. L. Meyer. To be submitted to the Journal of the North American Benthological Society.

## Abstract

Fine particulate organic matter is a major food resource in southeastern river food webs, but natural variability in the quality of this resource has not been assessed. We measured the quality of suspended fine particulate matter (SFPM) along the Little Tennessee River continuum at four sites ranging from 5<sup>th</sup> to 7<sup>th</sup> order. Our hypothesis, based on the River Continuum Concept, was that the quality of SFPM would decline with increasing stream order. SFPM quality was measured using traditional measures: nitrogen to carbon ratio (N/C), calories (cal g<sup>-1</sup> DM), % lipids, % inorganic matter, bacteria (# cells g<sup>-1</sup> DM) and % diatoms. As an integrated measure of food quality, we measured instantaneous growth rates of chironomids fed SFPM. Quality of SFPM collected from the 7<sup>th</sup> order site was significantly higher than SFPM collected from the 5<sup>th</sup> order site. Traditional measures of SFPM quality varied among sites, with higher N/C, % inorganic, and calories downstream, higher % lipids and bacteria upstream and no pattern in the % diatoms. Seasonal variability was different among sites and parameters. Although chironomid percent mortality did not differ among insects fed SFPM from different sites, instantaneous growth rates (IGRs) of chironomids fed SFPM from the most downstream site were significantly higher than those fed SFPM from the three upstream sites. The IGRs were not significantly different among seasons for any site. Only % diatoms were weakly related, and not significantly, to IGRs. The traditional measures in combination (using principal components analysis) were not related to IGRs. IGRs are a more realistic indicator of food quality than measures of individual attributes, because IGR integrates the consumer's response. The quality of a food resource is not merely the sum of its measurable parts (% lipids, calories, % inorganic, diatoms, etc.), and using one or a

combination of measures to predict food quality is not adequate. Our results do not support the hypothesis based on the RCC. The Little Tennessee River has very high secondary production of filtering invertebrates at the 7th order site. The quality of SFPM as a food resource and a highly favorable habitat of macrophytes on bedrock, appear to lead to the high secondary production of filtering invertebrates.

Key words: food quality, seston, Little Tennessee River, chironomids, instantaneous growth rates.

### **Introduction**

Fine particulate organic matter (FPOM) is a major food resource in stream food webs (Wallace et al 1987, Couch et al. 1996, Benke and Wallace 1997, Hall and Meyer 1998, Rosi-Marshall and Wallace 2002) and is the dominant basal resource in large southeastern river food webs (Benke and Wallace 1997, Rosi-Marshall and Wallace 2002). It is consumed by aquatic invertebrates, which support fish and other higher trophic levels; therefore FPOM supports many economically, recreationally, and ecologically important species. Despite its demonstrated importance in large river food webs few studies have examined natural variability in FPOM quality (Meyer et al. 2000, Vos et al. 2000).

FPOM quality likely reflects the nature of its formation and the types of organic matter from which it is derived. It can be formed by the breakdown of larger particles (leaves, animals, etc.) or the flocculation of dissolved organic matter (DOM). As water passes over land or through the ground, it picks up dissolved and colloidal materials and instream resources decompose or actively contribute to the pool of DOM (leaf leaching,

algal exudates, bacterial exopolymers, etc.). Both chemical (Warren and Zimmerman 1993) and physical properties (Lush and Hynes 1973, Alber and Valiela 1994, Wotton 1996) cause DOM to aggregate into particles which are a substrate for bacterial colonization (Alber and Valiela 1994, Wotton 1996). Breakdown, both chemical and physical, of large particles, such as leaves and algae, also contribute to the pool of FPOM. All of these combine to form the mixed resource called FPOM.

Because it is a mixture of various substances, FPOM quality could vary among sites with different sources of organic matter, different proportions of large particles, differing DOM quality and the amount of inorganic particles present. FPOM in streams can be found in the benthos and suspended in the water column. An integrated sample of FPOM available to aquatic insects at a site can be assessed by collecting suspended FPOM. Because natural suspended FPOM also contains inorganic particles, we use the term suspended fine particulate matter (SFPM) for this material.

The nutritional quality of food resources in streams has been examined in numerous studies. Anderson and Cummins (1979) predicted a nutritional food gradient with food quality increasing from wood, terrestrial leaf litter, fine particulate matter, decomposing vascular hydrophytes and filamentous algae, live algae, to animal tissue. While numerous studies have supported this general trend (Fuller and Mackay 1981, Pandian and Marian 1986, Fuller et al. 1988, Rosillon 1988, Fuller and Desmond 1997), fewer studies exist which examine the natural variability in quality within one food resource (Meyer et al. 2000). SFPM is a mixture of numerous food resource types, bacteria, algae, leaf particles, etc. and while there has been research on the quality of its

parts (Fuller et al. 1988, Vos et al. 2000), measurement of natural SFPM variability necessitates examination of the resource as a whole.

In order to obtain naturally varying SFPM, we collected particles along a river continuum. The River Continuum Concept (RCC) predicts that the dominant resources shift from allochthonous upstream to autochthonous downstream (Vannote et al. 1980). The quality of SFPM is most likely linked to the initial sources of its constituent parts, which will vary with stream size and with dominant resource base, i.e. allochthonous versus autochthonous, and the extent of microbial processing. In streams draining eastern deciduous forests, the dominant carbon inputs shift from allochthonous leaf material upstream to autochthonous algae downstream (Wallace et al. 1992), resulting in changes in the substances available for formation of SFPM. A river continuum provides a useful gradient along which to examine the natural variability of SFPM as a food resource.

Our objective was to determine if there is a change in the quality of SFPM along a river continuum. The RCC predicts that labile forms of dissolved compounds and fine particles are used or absorbed upstream and that downstream areas will contain more refractory compounds (Vannote et al. 1980). This would result in lower quality SFPM downstream. However, the secondary production of filtering insects that consume SFPM increases downstream which suggests that the quality of SFPM may increase downstream (Grubaugh et al. 1997). We hypothesized that the quality of SFPM would decline downstream. As a river widens along a river continuum, the potential for autochthonous production increases and the importance of allochthonous production can decline (Vannote et al. 1980, Rosi-Marshall and Wallace 2002). We also hypothesized that the seasonal variability of SFPM quality would increase in the mid-orders, where

autochthonous and allochthonous resources may vary in importance throughout the year. To test these hypotheses, we measured the quality of SFPM collected along a river continuum during four seasons using traditional measures of food quality (nitrogen to carbon ratio, % diatoms, % inorganic, caloric content, % lipids and bacteria). As an integrative measure of food quality, we measured instantaneous growth rates of chironomids fed suspended fine particles.

## Methods

### Study sites

Suspended fine particulate matter (SFPM) was collected from four sites (Table 2.1) in the Little Tennessee River drainage (LTR), Macon county, North Carolina (35° 03' N and 83° 25' W). The LTR is in the Blue Ridge province and flows north from Georgia into North Carolina before entering Fontana Reservoir. The LTR drains an area of crystalline rock which results in low streamwater ion concentrations (Swank and Bolstad 1994). The secondary production and trophic basis of production of the macroinvertebrate assemblage were previously measured at the four sites (Grubaugh et al. 1997, Rosi-Marshall and Wallace 2002). These sites range from mid to high (5-7) stream order. SFPM is an important food resource at these sites, and its importance increases at the larger sites (Rosi-Marshall and Wallace 2002).

The first two sites, Coweeta Creek and Conley (5<sup>th</sup> order), drain predominantly forested basins (over 90%) (Swank and Bolstad 1994). Coweeta Creek has the greatest canopy closure because of a riparian evergreen (Rhododendron maxima, L.), that reduces light and potential primary production. Conley, downstream of Coweeta Creek, has a

deciduous canopy cover, which may increase the seasonality of primary production. The two lower sites, Prentiss (sixth order) and Iotla (seventh order) are on the LTR mainstem with forested riparian zones that provide little stream shading because of large stream width.

#### Collection and measurement of suspended fine particles

We collected SFPM (from 20 $\mu$ m to 1mm) from the four sites described above in January, May, July, and October 1998. Mean monthly stream flow for the months sampled were 825, 519, 177, and 147 cfs (cubic feet second) for January, May, July, and October, respectively (HUC Code 06010202, <http://waterdata.usgs.gov/nwis/monthly>, April 19, 2002). Fine particulate organic matter ranges in size from 0.45  $\mu$ m to 1mm (Wallace and Grubaugh 1996); we collected particles that encompass a large portion of this range. Samples were collected during an extended period of base flow in each season. A 20 $\mu$ m net with a 1mm sieve attached to the opening was used to collect particles. The net was placed in the flow of the channel from 10-20 minutes to collect an integrated sample of SFPM, which was placed on ice and brought back to the lab for analysis. We measured the following chemical attributes of SFPM for each site and season: lipid content, nitrogen to carbon ratios, % inorganic, and calories. In addition, we measured the proportion of leaf material, diatoms, and amorphous detritus. Four subsamples were used to measure N/C, % inorganic, % diatoms, % leaves, % amorphous detritus and bacteria. One subsample was analyzed for % lipids and calories. A subsample of the SFPM was frozen for later use in the growth experiment, and bacterial abundance was measured at the conclusion of the growth experiment.

Lipid content was measured using an ether extraction technique (APHA 1985) and is expressed as % lipids ( $100 * \text{mg lipids mgDM}^{-1}$ ). N/C ratio (by mass) was measured using a Carlo Erba NA1500 CHN Combustion Analyzer (Carlo Erba Instrumentazione). Percent inorganic material was measured by filtering SFPM onto ashed glass fiber filters, drying at  $60^{\circ}\text{C}$  for 2 days, weighing, ashing at  $500^{\circ}\text{C}$  for 24 hours and then reweighing to obtain ash free dry mass (AFDM) and % inorganic ( $100 * \text{mg ash mass mg DM}^{-1}$ ). The proportion of leaves, diatoms, and algae were measured using microscopy (Benke and Wallace 1997). SFPM was filtered onto  $0.45 \mu\text{m}$  filters (Gelman Corporation, Ann Arbor, MI); filters were cleared with immersion oil; the areal proportion of leaves, diatoms and amorphous detritus was measured using an imaging software (ImagePro©) connected to a microscope (100X). Animal material was infrequently detected and was excluded from analysis.

Regression analysis was used to determine if variables measured were related to catchment size. The extent of seasonal variability was determined by examining the spread of the samples from each season around the mean as indicated by the coefficient of variation (CV) for each site.

### Growth experiment

As an integrated measure of SFPM quality, we measured instantaneous growth rates of chironomids fed SFPM. Other studies have used chironomid growth as an indicator of food quality because of their short lifespan and rapid growth rates (Gresens 1997, Meyer et al. 2000, Vos et al. 2000). We used chironomids collected from the LTR at the 7th order site in April 1999. Chironomids were collected and placed in a cooler the

day before the experiment was begun. We removed the chironomids from the rocks and haphazardly collected individuals. Numerous chironomid taxa were present, although only non-tanypodinae (non-predatory) taxa were used for the experimental treatments (Merritt and Cummins 1996).

Individuals were measured using an ocular micrometer (total length), and a single chironomid was placed in a sterile petri dish that contained 3-5 mg of thawed SFPM and 10ml dechlorinated tapwater. Tests were done with chironomids prior to the experiment to insure that the amount of SFPM provided an adequate food supply. Sixteen treatments (four sites\*four seasons) were run concurrently using chironomids collected from a single pool of individuals. Twenty replicates (individuals) were used per treatment (resulting in 340 individuals total). Individuals of different sizes were distributed evenly across treatments to ensure that potential effects of size on growth rate would not affect the results. High replication allowed for adequate numbers for statistics given potentially high mortality from collection and handling injuries. The experiment was housed in an incubator at 16°C in a 12/12 light/dark schedule. After five days, lengths of all the surviving individuals were measured again. Mortality and emergence were also recorded. Lengths were converted to biomass using regressions from Benke et al. (1999). Instantaneous growth rates (IGRs) of the individuals were calculated as follows (Waldbauer 1968):

$$\text{IGR (d}^{-1}\text{)} = \frac{\ln \text{ final mass} - \ln \text{ initial mass}}{\text{\#days in interval}}$$

After removal of the chironomids, subsamples of the SFPM were preserved in 2% formalin for later analysis of bacteria. Samples were sonicated for one minute to remove

bacteria cells from the particles. Bacteria were stained with acridine orange and counted using a fluorescence scope (Hobbie et al. 1977). A subsample of the solution was dried to measure DM per ml, and bacteria density per g DM of SFPM was then calculated. This method does not measure bacterial density associated with SFPM in the field; rather it provides data on the bacterial density associated with the SFPM in the chambers during the experiment.

Because IGRs were not normally distributed, a non-parametric test (Kruskal-Wallis Rank Sum) was used to determine if there were significant differences among sites and seasons. Regression analysis was used to determine which variables (% lipids, % diatoms, etc.) were related to growth rates. Principal Components Analysis (PCA) can be used to reduce a large number of variables, into a smaller set of linear combinations of the variables. We used PCA (on correlations) to reduce the traditional measures of quality down to a few principal components that encompass the variability in the measurements. Then we determined if the combinations of these variables were related to IGRs. Statistical analysis was conducted using JMP© software.

## **Results**

### Composition of suspended fine particulate matter

SFPM along the Little Tennessee River continuum varied markedly. As catchment area increased, N/C of the particles increased (Figure 2.1a) and the seasonal variability of N/C increased; the CV was 3.5 for the most upstream 5<sup>th</sup> order site, 24 for the second 5<sup>th</sup> order site, 12 for the third site and 44 for the most downstream site. There was also an increase in % inorganic matter with catchment area (Figure 2.1b); however

seasonal variability in % inorganic matter was consistent among sites, with CVs ranging from 10-17. The proportions of leaves, diatoms and amorphous detritus were autocorrelated so we only report the % diatoms because diatoms are known to be a high quality food resource (Cummins 1973). Percent diatoms were not related to catchment area (Figure 2.1c), although % diatoms was highest during spring at the most downstream site (Figure 2.1c). Seasonal variability in % diatoms was high at all sites with CVs ranging from 48-88. Percent extractable lipids declined steadily downstream (Figure 2.1d). The seasonal variability in % lipids was similar across all sites. % Lipids may be associated with fungi on leaves that typically decrease downstream (Vannote et al. 1980). Calories (cal/g DM) did not show a clear pattern along the gradient with no seasonal patterns (Figure 2.1e). Bacteria density (#bacteria cells/g DM of SFPM) decreased slightly with catchment area (Figure 2.1f). Seasonal variability decreased with catchment area; the CV of the most upstream 5<sup>th</sup> order site was 61, the second 5<sup>th</sup> order site was 37, the third site was 31 and the most downstream site was 23.

#### Chironomid growth as a measure of SFPM quality

Percent mortality in the chironomid growth experiment ranged from 40-60% but were not significantly different among sites or seasons ( $p = 0.31$ ), and there was minimal emergence (less than 10 for the entire experiment). Dead or emerged individuals were not included in the analysis. Despite mortality, the high replication (20 replicates per treatment) resulted in an adequate number of survivors for statistical analysis. Instantaneous growth rates were not significantly different among seasons for any site, therefore we used data from all seasons for each site to detect differences among sites.

Instantaneous growth rates differed among sites (Kruskal –Wallis Rank Sum,  $p = 0.025$ ) (Figure 2.2). The instantaneous growth rates of the two 5<sup>th</sup> and the 6<sup>th</sup> order sites were not significantly different (Nemenyi test,  $p > 0.05$ ). However, the 7<sup>th</sup> order site had significantly higher instantaneous growth rates than the first 5<sup>th</sup> order site (Nemenyi test,  $p < 0.05$ ).

#### Relationships of SFPM attributes and growth

No single variable explained the variability in IGR (Table 2.2); however, % diatoms were weakly related to IGR. Principal component analysis (PCA) on correlations was used to describe the variability in the independent variables (% lipids, % inorganics, % diatoms, N/C and bacteria). Calories were excluded because of missing values. Using PCA, two principal components were obtained that explained 55% and 23% of the variability (Table 2.3). Hence PCA was effective at reducing the five variables down to two variables which together explain 78% of the variability among the independent variables. The first PCA was most weighted by % lipids (negatively), N/C (positively) and % inorganic (positively). The second PCA was most weighted by % diatoms (positively) and bacteria (negatively). The first PCA was not related to IGR ( $r^2 = 0.06$ ,  $p = 0.80$ ), while the second PCA was weakly related to IGR ( $r^2 = 0.18$ ,  $p = 0.10$ ). This indicates that the combination of bacteria (negative) and % diatoms (positive) may explain some of the variability in IGR.

## Discussion

Suspended fine particulate matter quality along the Little Tennessee River continuum varied based on several traditional measures of organic matter quality. The chemical composition of SFPM changed along the continuum with increases in N/C and inorganic matter and decreases in % lipids and bacteria. % Lipids may be associated with fungi on leaves that typically decrease downstream (Vannote et al. 1980). In addition, temporal variability of these variables differed among sites: % lipids, % diatoms and % inorganic had similar variability across all sites, N/C was more temporally variable downstream and bacteria was more temporally variable upstream. Given this variability in composition of SFPM, one would anticipate a change in the quality of SFPM as a food resource for aquatic organisms.

The food quality of SFPM as measured by chironomid instantaneous growth rates also varied along the Little Tennessee River continuum. The instantaneous growth rates were highest at the downstream site. These results do not support the hypothesis based on the RCC prediction that downstream areas would have more refractory compounds, but rather suggest that SFPM is more labile downstream. While there were differences in composition of SFPM among the three upstream sites, these differences were not enough to result in significant differences in growth rates among these sites. However, the differences between the first site and the large river site were great enough to result in significant differences in food quality: the large river site had higher food quality. Percent mortality was not a useful indicator of quality. The high percent mortality in this experiment may be due to the abundance of Rheotanytarsus at the site chironomids were collected. Rheotanytarsus are sensitive to changes in flow and the conditions in the

experimental chambers may have caused them to die. However, because chironomids were haphazardly selected for each treatment and there were no differences in percent mortality across treatments, mortality of Rheotanytarsus should not affect the overall conclusions.

While % diatoms were weakly related to IGR, no single variable or combination of variables was effective at explaining the patterns in measured IGR. These results may be a consequence of failure to measure the appropriate variables. It is more likely that IGR, as an integrative measure, does not simply reflect the sum of the SFPM parts alone. The variables that were used to measure SFPM are analogous to multiple stressors, which other studies have shown are not necessarily additive (Schindler et al. 1996, Breitberg et al. 1999).

Numerous studies demonstrate that different types of foods (leaves, wood, diatoms, etc) vary in quality measured by growth rates or assimilation efficiencies (Anderson and Cummins 1979, Fuller and Mackay 1981, Pandian and Marian 1986, Webb and Merritt 1987, Fuller et al. 1988, Rosillon 1988, Fuller and Desmond 1997). However, in combination, the constituents of SFPM do not affect growth rates in relation to their proportions (Vos et al. 2000, this study). Insects encounter FPOM as a conglomeration of food types and cannot separate these food types before ingestion. Some components of the food resource may be beneficial, such as calories, % lipids, % diatoms, while others may be negative, such as % inorganics. The combination of these different components represent what the insects ingest. In order to accurately assess the quality of SFPM as a food resource, it is necessary to measure growth rates of insects fed naturally occurring particle mixtures (Vos et al. 2000). In this study, we used particles

that organisms would encounter in the field to obtain an estimate of the quality of SFPM. Using chironomids as test organisms, we were able to demonstrate that the quality of suspended fine particles increased downstream in the Little Tennessee River.

#### Food quality and secondary production

Increasing quality of SFPM may contribute to the increase in secondary production of filtering insects along the Little Tennessee River (Table 2.1) (Grubaugh et al. 1997). More than 70% of the dominant filterer's secondary production (hydropsychid caddisflies) was supported by the consumption of amorphous detritus (Rosi-Marshall and Wallace 2002), which is an important component of SFPM. The observed increase in filterer secondary production (Grubaugh et al. 1997) could be the result of four factors: 1) increased temperature (Grubaugh et al. 1997), 2) increased quantity of SFPM downstream (Rosi-Marshall and Wallace 2002), 3) increased habitat availability and 4) increased quality of SFPM (this study). The secondary production of filtering insects increased by 2 orders of magnitude at the most downstream site; however, temperature and FPOM quantity increased linearly (Table 2.1). Therefore, temperature and FPOM quantity did not appear to control filterer secondary production.

The macrophyte Podostemum ceratophyllum (L.) provides an ideal habitat for filtering caddisflies, as it is a stable structure for net construction (Grubaugh et al. 1997). The three most downstream sites all have P.ceratophyllum on cobbles, but only the most downstream site has P.ceratophyllum covered bedrock as a dominant habitat (38%, compared to 0% at all other sites) (Table 2.1). The stability of P.ceratophyllum covered

bedrock appears to be more important to filterer secondary production than P.ceratophyllum on cobbles only.

In addition, the quality of suspended fine particulate matter was significantly higher at the downstream site. This suggests that filtering macroinvertebrates are able to exploit high quality habitat and thrive because a high quality food resource is constantly delivered by the flow of the river. Secondary production is an important ecosystem function (Benke 1993), so it is noteworthy that it can be affected by food quality. Through SFPM's influence on the secondary production of its consumers, the food quality at a site could have repercussions throughout the food web, thereby affecting many more trophic levels than the initial consumer of the resource.

Our hypothesis, based on the RCC, was that the quality of SFPM would decline downstream because more labile fractions would be used upstream. These data suggest the opposite: SFPM quality tends to increase downstream. Suspended fine particles are a dominant food resource in southeastern river systems (Wallace et al. 1987, Couch et al. 1996, Hall and Meyer 1998, Benke and Wallace 1997, Rosi and Wallace 2002), and the quality of this resource could have a significant impact on food webs in these river ecosystems. Anthropogenic factors that degrade or enhance this resource could have repercussions throughout the food web.

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Table 2.1. Study site characteristics along the Coweeta Creek - Little Tennessee River, Macon Co. North Carolina (from Rosi-Marshall and Wallace 2002, modified from Grubaugh et al. 1996,\* from Rosi 1997 and † from Grubaugh et al. 1997).

Site	Coweeta Creek	Conley Road	Prentiss	Iotla
Stream System	Coweeta Creek	Coweeta Creek	Little Tennessee River	Little Tennessee River
Stream order	5	5	6	7
Catchment area (ha)	1548	4163	36260	83660
Elevation (m above sea level)	671	633	620	597
Mean annual discharge ( $\text{m}^3 \cdot \text{s}^{-1}$ )	0.58	1.35	10.85	22.18
Mean width (m)	7.2	15	25	60
Mean depth (cm)	25	25	50	50
Annual degree days	4078	4389	4763	4922
<u>Podostemum ceratophyllum</u> covered cobbles	0	62%	14%	27%
<u>P.ceratophyllum</u> covered bedrock	0	0	0	38%
FPOM concentration ( $\text{g AFDM L}^{-1}$ ) *	0.002	0.001	0.003	0.004
Habitat weighted collector/filterer secondary production ( $\text{gm}^{-2}\text{y}^{-1}$ )†	1.1	9.9	13.8	122.8

Table 2.2. Relationships of traditional measurements of quality and the instantaneous growth rates measured.

Variable	$r^2$	p value
% Lipids	0.02	0.82
N/C	0.03	0.50
Calories	0.00	0.86
% Diatoms	0.14	0.16
Bacteria	0.00	0.77
% inorganic	0.06	0.35

Table 2.3. The relationship of independent variables to each other (from the principal components analysis). The percents show the amount of variability in the data set that can be attributed to each principal component (PC1 and PC 2). The eigenvector loading for each variable is given. A variable with a high loading (absolute value) is one that is driving a large amount of the variability in the principal component.

	PC 1	PC 2
Percent of variance explained	55	23
Eigenvectors		
% Lipids	-0.53	0.05
Nitrogen to carbon ratio	0.52	-0.21
% Diatoms	-0.11	0.87
% Inorganic	0.55	0.15
Bacteria	-0.36	-0.42

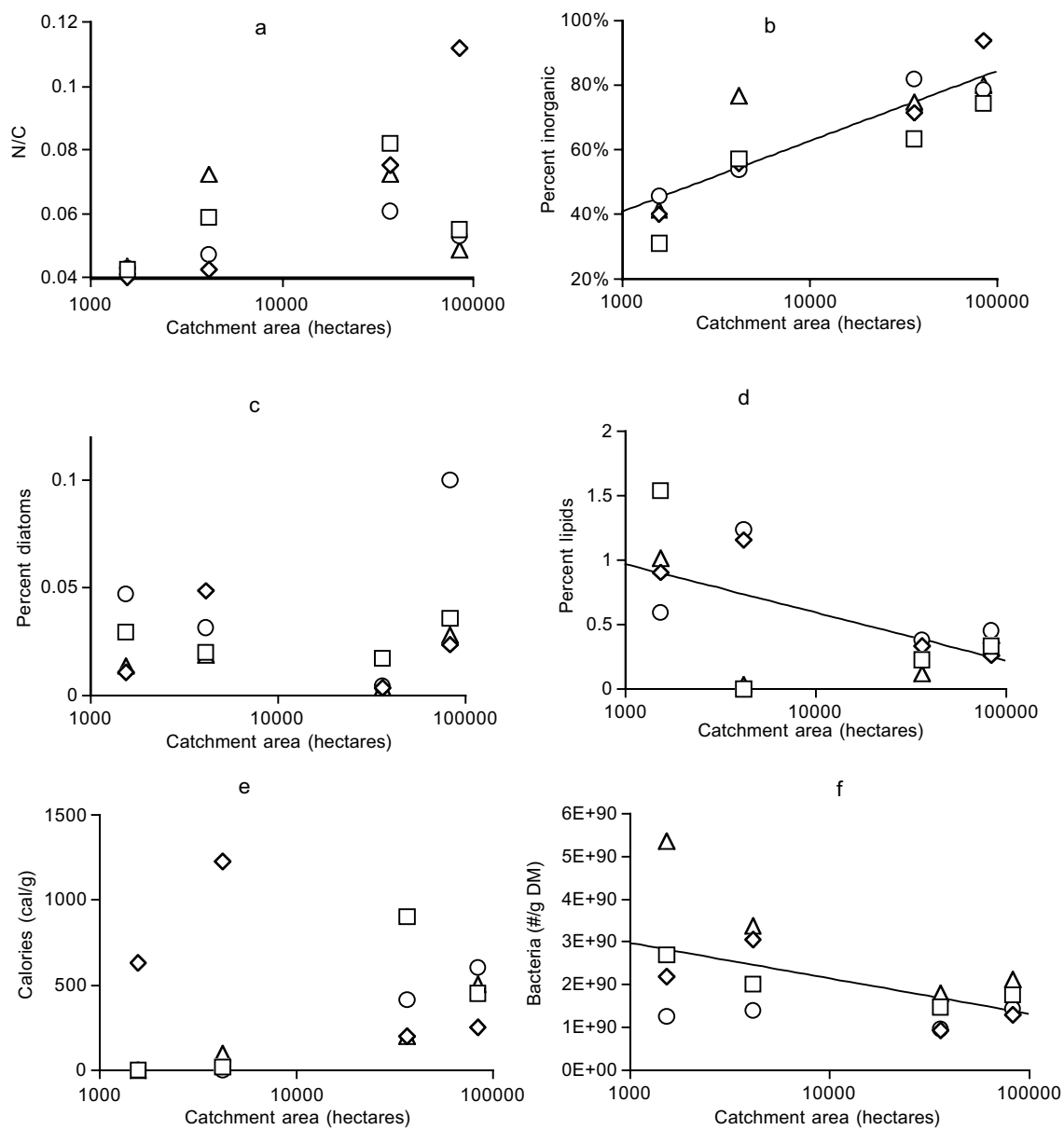
### Figure Legends

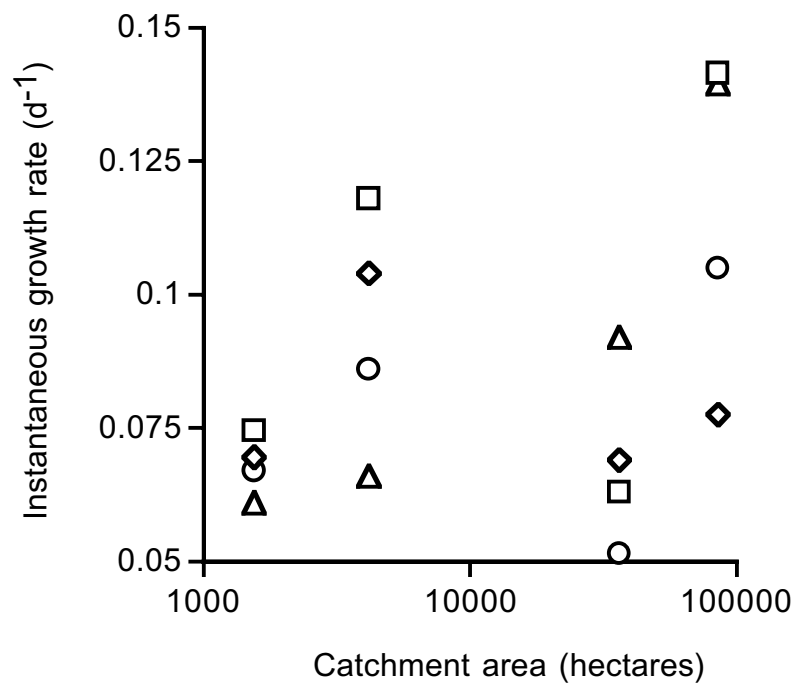
Figure 2.1. Relationship between catchment area (CA in ha) and quality of SFPM collected during each season [January ( $\diamond$ ), May ( $\circ$ ), July ( $\Delta$ ) and October ( $\square$ ), 1998].

SFPM quality is measured in terms of:

- a. N/C ( $r^2 = 0.20$ ,  $p = 0.09$ ),
- b. % inorganic ( $r^2 = 0.76$ ,  $p = 0.001$ ,  $y = -0.25 + 0.09 \log CA$ )
- c. % diatoms ( $r^2 = 0.09$ ,  $p = 0.25$ ),
- d. % lipids ( $r^2 = 0.45$ ,  $p = 0.005$ ,  $y = 2.3 - 0.18 \log CA$ ),
- e. calories (cal/gram DM) ( $r^2 = 0.002$ ,  $p = 0.88$ ),
- f. bacteria (cells/g DM) ( $r^2 = 0.28$ ,  $p = 0.03$ ,  $y = 5.45 - 3.6 \times 10^8 \log CA$ ).

Figure 2.2. Mean instantaneous growth rates of chironomids fed suspended fine particulate matter collected from Coweeta Creek, Conley Road, Prentiss and Iotla in January ( $\diamond$ ), May ( $\circ$ ), July ( $\Delta$ ) and October ( $\square$ ) 1998.





CHAPTER 3  
QUALITY OF SUSPENDED FINE PARTICULATE MATTER  
ALONG AN URBAN RIVER<sup>1</sup>

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<sup>1</sup>Rosi-Marshall, E. J. To be submitted to Limnology and Oceanography.

### Abstract

The quality of suspended fine particulate matter (SFPM) as a food resource for aquatic insects could decline if it is contaminated. Metals, in particular, readily bind to fine particles in the aquatic environment. I hypothesized that the quality of SFPM (20 $\mu$ m to 1mm) from the Chattahoochee River, which drains metropolitan Atlanta, declines due to contamination associated with urbanization. SFPM was collected during four seasons at base flow and high flow at four sites along the mainstem of the Chattahoochee River. The quality of SFPM was measured using traditional indicators: bacteria, N/C ratio, caloric content, % inorganic, % lipids, and chlorophyll a concentration. The concentration of metals (Cd, Cu, Pb, and Zn) associated with the SFPM was also measured. The cumulative permitted wastewater treatment discharge increased along the Chattahoochee River and was used as an indicator of urbanization. In SFPM samples collected during base flow conditions, the % inorganic matter, calories, Cu, Pb, and Zn concentrations increased with urbanization. In SFPM samples collected during high flow, the % diatoms, chlorophyll a, Cu, Pb and Zn increased with urbanization. A growth assay was used as an integrated measure of SFPM quality as a food resource for aquatic insects. The instantaneous growth rate (IGR) of chironomids fed SFPM collected from the river during base flow declined downstream of the city, but percent mortality did not differ among sites. The IGRs of chironomids fed SFPM collected during high flow were as low as the most downstream site. The insects fed SFPM collected from the Chattahoochee River had IGRs 5X lower than the IGRs of chironomids fed SFPM collected from the Little Tennessee River, a relatively undisturbed river in western North Carolina. However, mortality of insects fed SFPM collected from the two rivers did not differ.

Nitrogen to carbon ratio, calories, % lipids, % diatoms, chlorophyll a concentration, bacteria, % inorganic matter, and the concentration of total recoverable metals associated with the SFPM were not individually related to IGR. The variables in combination (principal components analysis) were not related to IGR. The decline in SFPM quality in the Chattahoochee River could not be attributed to traditional measures or metal contamination. However, the low quality of SFPM in the Chattahoochee River downstream of Atlanta is likely attributed to some aspect of extensive urbanization in the Chattahoochee River basin.

Key words: Chattahoochee River, chironomids, seston, growth rates, copper, cadmium, lead, and zinc.

### **Introduction**

In 1995, the US Environmental Protection Agency promulgated a rule that metals criteria in surface waters could be expressed as dissolved concentrations, revising previous regulations based on total recoverable concentrations (US Federal Water Pollution Control Act § 307, 1997). The reasoning behind this change was that metals sorbed to suspended particles were thought to be unavailable to organisms in the aquatic environment (SAB 2000a). Recent studies have called this earlier finding into question (Roditi and Fisher 1999, Schlekat et al. 1999, Wang and Fisher 1999, Lee et al. 2000, SAB 2000b). The metals that compose the total recoverable concentrations are sorbed to suspended fine particulate matter (SFPM) (both organic and inorganic) in aquatic

ecosystems. SFPM is the dominant food resource for aquatic insects in large river food webs (Wallace et al 1987, Couch et al. 1996, Benke and Wallace 1997, Hall and Meyer 1998, Rosi-Marshall and Wallace 2002). In addition to being bioavailable, metals sorbed to particles could alter the quality of SFPM as a food resource in aquatic ecosystems.

Suspended fine particulate matter is a food resource that has great potential to be adversely affected by human activities in the watershed. The quality of this food resource reflects the larger particles and dissolved organic matter (DOM) from which it is formed. Human activities in a watershed affect the quality of the particles and DOM entering the stream (Markosova 1991, Servais and Garnier 1993) and hence the quality of SFPM. In addition, contaminants entering the stream, such as metals and pesticides, are incorporated into suspended particles in remarkably high concentrations (Rao et al. 1993 and references therein, Maki and Hermansson 1994).

The first objective of this research was to measure the quality of SFPM collected in an urban river using both traditional parameters and growth rates of aquatic insects fed SFPM. The second objective was to determine which factors influence the quality of SFPM as a food resource for aquatic insects. Factors examined were traditional measures of food quality (N/C ratio, % diatoms, % inorganics, % lipids, caloric content, chlorophyll a concentration, and bacterial content) and metal (Cu, Cd, Pb and Zn) concentrations sorbed to particles. Urbanization, with its associated point and non-point sources of pollution, may lead to metal-contaminated SFPM. I hypothesized that SFPM collected from sites within an urban area would have higher concentrations of metals than SFPM collected upstream. I hypothesized that SFPM samples with higher concentrations of metals would have lower food quality for aquatic insects. I hypothesized that this trend

would be stronger in samples collected during high flow than those collected during base flow, because high flow samples would contain contaminants from non-point source runoff. I used a chironomid growth assay and hypothesized that growth would be a more sensitive indicator of food quality than percent mortality .

## Methods

### Study sites

The upper Chattahoochee River flows through metropolitan Atlanta. Point and non-point source pollution contribute to the problem of inorganic and organic pollution throughout the basin (Frick et al. 1998). Trace metal concentrations in sediment and *Corbicula* tissues pose an ecological risk to native fauna (<http://wwwga.usgs.gov/nawqa> Jan. 2002). I collected SFPM samples within and below the city of Atlanta on an unimpounded section of the river between two reservoirs (Table 3.1). The first site (MF) is in a northern section of the city of Atlanta, just downstream of the Morgan Falls impoundment, which is used for hydroelectric power generation. While this site is upstream of most of the city, it is downstream of suburban wastewater treatment plants and suburban runoff; hence, it is not an upstream reference site. The second site (ATL) is located at the Atlanta Road crossing of the Chattahoochee River. This site is just downstream of Peachtree Creek, the tributary that drains downtown Atlanta and is downstream of a major wastewater treatment facility. The third site (166) is located at the Georgia Route 166 crossing of the Chattahoochee River. This site is downstream of most of the urban runoff, a number of tributaries draining industrialized areas, and a majority of the point sources in the major metropolitan area, including municipal and industrial facilities. The final site (FRA) is at

the Georgia Route 27 crossing of the Chattahoochee River. This site is approximately 160 km downstream of Atlanta, in Franklin, GA, just upstream of West Point Reservoir. This site was chosen to determine the extent of contamination from upstream sources.

#### Collection and measurement of SFPM

I collected suspended fine particulate matter (SFPM) from the four sites in October 1998, January, April, and August 1999. Particles were collected once during base flow and once following large rainfall during each season (Table 3.2). High flow samples were collected after a storm that caused an increase in the hydrograph (Whitesburg gage, HUC 03130002, <http://waterdata.usgs.gov/ga/nwis/>). The high flow samples were collected while discharge was high; however, because of the distance between the sites, I was not able to sample the rising limb at all sites.

Particles between 40-60  $\mu\text{m}$  have the highest concentration of sorbed metals (Bremer and Geesey 1993), so a non-metal 20 $\mu\text{m}$  mesh net was used to collect SFPM. The net was placed in the flow of the channel from 10-20 minutes to collect an integrated sample of SFPM, which was placed on ice and brought back to lab for analysis. Any large particles, such as leaves, macrophytes, etc. were removed in the field. Subsamples of SFPM were preserved in the field in 2% formalin for later analysis of bacteria. Water samples from each site were collected, placed on ice and returned to the lab for measurement of chlorophyll a concentration in the water. Upon arrival at the lab, the water samples for chlorophyll a concentration were filtered onto ashed glass fiber filters and then frozen for later spectrophotometric analysis (Wetzel and Likens 1991). I measured the following chemical attributes of SFPM: % lipids, N/C ratios, % inorganic,

total calories, chlorophyll a concentration and bacteria. In addition, I measured the proportion of leaf material, diatoms, and amorphous detritus. Subsamples of the SFPM were placed in acid-washed HDPE containers in the field and frozen for metals analysis and for later use in the growth experiment.

Lipid content was measured using an ether extraction technique (APHA 1985) and is expressed as % lipids ( $100 * \text{mg lipids mgDM}^{-1}$ ). N/C (by mass) was measured using a Carlo Erba NA1500 CHN Combustion Analyzer (Carlo Erba Instrumentazione). Percent inorganic material was measured by filtering SFPM onto tared and ashed glass fiber filters, drying at 60°C for 2 days, weighing, ashing at 500°C for 24 hours and then reweighing to obtain ash free dry mass (AFDM) and % inorganic ( $100 * \text{mg ash mass mg DM}^{-1}$ ). Samples for bacteria were sonicated for one minute to remove bacteria cells from the particles. Bacteria were stained with Acridine orange and counted using a fluorescence scope (Hobbie et al. 1977). A subsample of the solution was dried to measure  $\text{g ml}^{-1}$ , and bacteria density per g dry mass (gDM) of SFPM was then calculated. The proportion of leaves, diatoms, and algae were measured using microscopy (Benke and Wallace 1997). SFPM was filtered onto 0.45  $\mu\text{m}$  filters (Gelman Corporation, Ann Arbor, MI); filters were cleared with immersion oil; the areal proportion of leaves, diatoms and amorphous detritus was measured using an imaging software (ImagePro©) connected to a microscope (100X). Animal material was infrequently detected and was excluded from analysis.

Metals analysis was conducted at the University of Georgia Chemical Analysis Lab. Frozen samples of SFPM were freeze-dried and digested with nitric acid to obtain

the total recoverable metals associated with the particles. Metals (Cu, Cd, Pb and Zn) were analyzed using Thermo Jarrell-Ash 965 Inductively Coupled Argon Plasma (ICP).

### Growth experiment

As an integrated measure of SFPM quality, I measured the growth rates of chironomids fed SFPM collected from the Chatthahoochee River. Other studies have used chironomid growth as an indicator of food quality, because of their short lifespan and rapid growth rates (Hax 1995, Gresens 1997, Meyer et al. 2000, Vos et al. 2000). I collected chironomids from Little Tennessee River, in April 2000, at a relatively uncontaminated site in Macon Co. North Carolina (7th order). This site was selected to insure that the organisms have not developed a tolerance for heavy metals (Clements 1999). I removed the chironomids from the rocks and haphazardly collected individuals for the experimental treatments. Numerous chironomid taxa were present, although only non-tanypodinae (non-predatory) taxa were used (Merritt and Cummins 1996).

Individuals were measured using an ocular micrometer (total length) and a single chironomid was placed in a sterile petri dish which contained 3-5 mg of thawed SFPM and 10ml dechlorinated tapwater. Tests were done with chironomids prior to the experiment to insure that the amount of SFPM provided an adequate food supply. Thirty-two treatments (four sites\*four seasons\*two flows) were run concurrently using chironomids collected from a single pool of individuals. Twenty replicates (individuals) were used per treatment (resulting in 640 individuals total). Individuals of different sizes were distributed evenly across treatments to ensure that potential effects of size on growth rate would not affect the results. High replication allowed for adequate replicates

given potentially high percent mortality from collection and handling injuries. The experiment was housed in an incubator at 16°C in a 12/12 light/dark schedule. After five days, lengths of all the surviving individuals were measured again. Mortality and emergence were also recorded. Lengths were converted to biomass using regressions from Benke et al. (1999). Instantaneous growth rate (IGR) of the individuals was calculated as follows (Waldbauer 1968):

$$\text{IGR (d}^{-1}\text{)} = \frac{\ln \text{ final mass} - \ln \text{ initial mass}}{\text{\#days in interval}}$$

#### Relationship of urbanization to SFPM quality

Urbanization in a watershed leads to a number of changes, e.g. increased wastewater effluent discharge, increased impervious surface area, increased non-point source discharge (e.g., Paul and Meyer 2001). At the four sites sampled along the Chattahoochee River, the cumulative amount of permitted wastewater discharge (PWD) steadily increases downstream (Table 3.1). I used PWD as an indicator of urbanization (Frick et al. 1996). To determine if variables measured were related to urbanization, the relationship between PWD and each variable (N/C ratio, % lipids, calories, etc) was explored using regression analysis. A significant regression indicates a relationship, either positive or negative, with increasing urbanization. Attributes of SFPM from base flow and high flow samples were regressed separately.

To determine if IGR was related to urbanization, IGR was regressed against PWD for SFPM collected during base flow and highflow. Regression analysis was also used to determine which properties of the SFPM such as calories, % lipids, etc., influenced

growth rates. Principal Components Analysis (PCA) can be used to reduce a large number of variables into a smaller set of linear combinations of the variables. I used PCA (on correlations) to reduce the traditional measures of quality and metals (Cu, Cd, Pb and Zn) down to a few principal components that encompass the variability in the measurements. Then I determined if the combinations of these variables were related to IGR. Statistical analysis was conducted using JMP© software.

## Results

The composition of suspended fine particulate matter varied spatially and temporally in the Chattahoochee River (Appendix 1). In SFPM collected during high flow had an increase in chlorophyll a concentration with an increasing in urbanization (Table 3.3). No other traditional measure of quality was significantly related to urbanization (PWD).

The concentrations of metals associated with SFPM varied along the Chattahoochee River (Appendix 2). Some trace metal concentrations associated with the particles were related to urbanization. In SFPM samples collected during base flow and high flow, Cu, Pb and Zn concentrations increased with increased urbanization (Table 3.3).

Chironomid growth rates as a measure of quality

Percent mortality in the growth experiments was transformed ( $\arcsin X^{1/2}$ ) to achieve a normal distribution. The percent mortality was not statistically different among sites for both base flow (ANOVA, mean = 55%;  $p = 0.81$ ) and high flow (ANOVA,

mean = 55%;  $p = 0.72$ ). Only living individuals were used for growth measurements. The high initial replication allowed for sufficient numbers of surviving individuals for statistical analysis. The average annual instantaneous growth rates of chironomids fed particles collected during base flow declined steadily with urbanization ( $r^2 = 0.37$ ;  $p = 0.02$ ) (Figure 3.1). Instantaneous growth rates of chironomids fed particles collected during high flow were not related to urbanization ( $r^2 = 0.14$ ;  $p = 0.15$ ) (Figure 3.1); however, the high flow samples were all as low as the most downstream site.

#### Relationships of IGR with other variables

Instantaneous growth rates were positively related to % diatoms ( $r^2 = 0.13$ ;  $p = 0.05$ ) and negatively related to bacterial density ( $r^2 = 0.14$ ;  $p = 0.04$ ), but were not significantly related to any other individual measure of food quality. PCA was effective at reducing the six traditional variables (chlorophyll a concentration, N/C, % diatoms, % inorganics, % lipids, and bacteria) to three variables that in combination explained 72% of the variability in the data set (Table 3.4). The first principal component was most weighted by % diatoms (negatively), bacteria (positively) and N/C (positively). The second principal component was most weighted by chlorophyll a concentration (positively), % inorganic (positively) and % lipids (negatively). The third principal component was most weighted by N/C (positively) and % lipids (positively). IGRs were positively related to the third principal component ( $r^2 = 0.13$ ;  $p = 0.05$ ), but not with the first two principal components. These results indicate that in combination, N/C and % lipids were positively related to IGR.

No single metal had a significant relationship with IGR. PCA was effective at reducing metals down to two variables accounting for 93% of the variability in the data set (Table 3.5). The first principal component was weighted by Cu, Pb and Zn and the second principal component was weighted by Cd. Instantaneous growth rates were not significantly related to either of the principal components. These results suggest that metals individually and in combination are not related to instantaneous growth rates measured in this experiment.

### **Discussion**

SFPM quality in the Chattahoochee River was related to permitted wastewater discharge, a correlate of increased urbanization. During base flow and high flow conditions there were changes in the biological attributes and metal concentrations of the SFPM. During base flow, the % inorganic matter, calories, Cu, Pb and Zn concentrations increased with increased urbanization. During high flow, the % diatoms, chlorophyll a concentration, Cu, Pb and Zn increased with increased urbanization. Given the large number of varying factors, predicting whether the quality of SFPM increased or decreased downstream of Atlanta is very difficult based on measures of individual factors either alone or in combination (i.e. by PCA). Some of the factors may enhance quality such as calories, % diatoms and chlorophyll a concentration; at the same time other factors may degrade quality, such as % inorganics and metals. Under these circumstances, an integrated measure is needed. IGRs of chironomids fed SFPM provided an integrated measure to assess the quality of SFPM as a food source.

## Growth rates

Measurement of IGRs revealed differences in SFPM quality in the Chattahoochee River. IGRs of chironomids fed SFPM collected during base flow from the Chattahoochee River declined with increasing PWD, a correlate of urbanization. This supports the initial hypothesis that the quality of SFPM would decline downstream of Atlanta. IGRs of chironomids fed SFPM collected during high flow were not related to urbanization. The original hypothesis that samples collected during high flow would show a stronger trend with urbanization was not supported by these results. The concentration of contaminants associated with SFPM should be highest as the river rises during or following a storm (e.g., during the rising limb of the hydrograph); although I collected particles within 24 hours of a storm, this may not have represented the rising phase at all sites. This may help explain why no apparent pattern was observed in the high flow samples. However, while there was no trend with increasing urbanization, the mean IGRs for SFPM collected during high flow at all sites were as low as IGRs for SFPM collected during base flow at the most downstream site. During high flow conditions, the quality of SFPM from all sites measured was consistently low.

IGRs of chironomids fed SFPM collected from the Chattahoochee River were negatively related to bacterial density and positively related to the % diatoms in the suspended fine particulate matter. However neither one of these relationships was strong. In combination, the variables were more strongly related to IGR, indicating that a combination of variables has a higher predictive power than one surrogate measure. The N/C ratio and % lipids in SFPM, in combination, were highly related to IGR. No single metal or a combination of all metals related to IGR. Chironomids are particularly

tolerant of metals (Richardson and Kiffney 2000); however, they are very useful in laboratory growth experiments due to rapid growth rates. Their tolerance to metals may be why I found little relationship between metals and growth rates or the metal concentrations associated with the SFPM may have been too low to affect growth.

Although the decline in quality of SFPM collected from the Chattahoochee was not related to the concentration of metals associated with SFPM that I measured, the relationship of IGR and PWD and the low IGR after storms suggests that contaminants associated with urbanization may be the cause of the decline in quality. I did not measure the concentrations of As and Hg or other organic contaminants associated with urbanization, although elevated concentrations of As and Hg have been observed in macroinvertebrates and fishes in this river (Chapter 5). Detectable concentrations of PAHs, phenol, phthalates, chlordane, DDT, other pesticides and pharmaceutical products were found in urban tributaries and the Chattahoochee River mainstem (Frick et al. 1998, Frick et al. 2001). These contaminants may contribute to the low SFPM quality in the Chattahoochee River.

To further examine the effect of urbanization on the quality of SFPM, I compared the IGR of chironomids fed SFPM collected at base flow from the Chattahoochee River with those of chironomids fed SFPM collected at base flow from four sites along the Little Tennessee River, which drains an area of low population density in western North Carolina (Chapter 2). Similar traditional parameters were used to measure the quality of SFPM in both rivers (Table 3.6). The N/C, % diatoms, % lipids, and % inorganics were significantly higher in Chattahoochee River SFPM samples than in Little Tennessee River SFPM samples. These traditional measures of quality suggest that SFPM quality is

higher in the Chattahoochee River. However, instantaneous growth rates of chironomids fed SFPM collected from the Chattahoochee River were 5X lower than those measured on samples collected from the Little Tennessee River (Table 3.6). The significant difference in the IGRs of chironomids fed SFPM collected from the two rivers further emphasizes the utility of IGR as an integrated measure of quality and further suggests the potential role of urban contaminants.

The relationship of % diatoms to IGR from the Little Tennessee River can be used to predict IGR's for the Chattahoochee River. In the Little Tennessee River, IGR increased as % diatoms in SFPM increased ( $r^2 = 0.14$ ,  $p = 0.16$ , Chapter 2). This relationship predicts that IGRs for chironomids fed SFPM collected from the Chattahoochee River should be higher than observed (Figure 3.2). Higher content of diatoms, % lipids and N/C should result in a higher quality food resource (Cummins 1973, Vos et al. 2000, Chapter 2), but this was not the case. The presence of contaminants may mask the contribution of other attributes important to quality (Breitburg et al. 2001).

### Mortality

An important result from the growth experiment is that mortality of chironomids is a poor indicator of differences in the quality of SFPM in the Chattahoochee River. This finding suggests that mortality, while a useful endpoint in some cases, is not an adequate measure of the quality of this particular food resource. The percent mortality was not significantly different in experiments conducted with SFPM collected from the Little Tennessee than the Chattahoochee River (Table 3.6). The similarity of percent mortality

in experiments conducted with SFPM collected from the two rivers despite striking differences in IGR (Figure 3.2) further supports the conclusion that mortality is not an effective indicator of quality of SFPM. The high percent mortality in this experiment may be due to the abundance of Rheotanytarsus at the site chironomids were collected. Rheotanytarsus are sensitive to changes in flow and the conditions in the experimental chambers may have caused them to die. However, because chironomids were haphazardly selected for each treatment and there were no differences in percent mortality across treatments, mortality of Rheotanytarsus should not affect the overall conclusions.

#### Metal Bioavailability

Contaminants associated with particles are bioavailable to aquatic consumers through trophic transfer ( Roditi and Fisher 1999, Schlekot et al. 1999, Wang and Fisher 1999, Lee et al. 2000). Although these experiments did not demonstrate that metals caused a decline in growth rates, the metals may have been bioavailable to the insects. I was unable to determine the bioavailability of the metals associated with SFPM because the small biomass of the test organisms precluded measuring their metal content at the conclusion of the experiment. There have not been any studies that measure the assimilation efficiencies for metals associated with fine particles for aquatic insects; however, zebra mussels can assimilate metals associated with their food (Roditi and Fisher 1999). Although metal concentrations were not associated with chironomid growth rates, there are numerous other endpoints that could have been affected by exposure (see review by Luoma and Carter 1991). Specific effects of trace metals on aquatic biota

include delayed sexual maturation (Kemble et al. 1994), increased drift rate (Richardson and Kiffney 2000), increased risk of predation (Clements 1999), and increased metallothionein production and its associated metabolic costs (Di Giulio et al. 1995).

## Conclusion

The quality of SFPM collected during base flow from the Chattahoochee River, as measured by chironomid growth rate, steadily declined downstream of Atlanta. Quality of SFPM collected during high flow was also low at all sites. The comparison with the Little Tennessee River experiment (Chapter 2) provides further evidence for the degradation in the quality of SFPM in the Chattahoochee River. Although many SFPM components (e.g., calories, % diatoms and chlorophyll a concentration) increased with urbanization, the quality of SFPM declined with increased urbanization. This suggests that SFPM quality may be low due to other factors associated with urbanization. The decline cannot be attributed to metal contamination; however, other contaminants, such as PAHs, phenols, and phthalates, chlordane, DDT, other pesticides and pharmaceutical products, have been detected in the Chattahoochee River, (Frick et al. 1998, Frick et al 2001) and may be the cause of the decline in SFPM quality.

Decreases in the quality of riverine basal food resources that may accompany urbanization can have serious repercussions for organisms other than its consumers. System secondary productivity is an important ecosystem function that is tightly linked with quality of the basal food resources (Polis and Hurd 1995). The secondary production of SFPM consumers could decline if the SFPM quality decreases which would lead to fewer food resources available to higher trophic levels (Rosi-Marshall and Wallace

2002). The decline in SFPM quality may also contribute to decreases in economically important fisheries in southeastern rivers.

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Table 3.1. Characteristics of the four study sites along the Chattahoochee River. The four sites include: Morgan Falls (MF), Atlanta Road crossing (ATL), Route 166 crossing (166) and Franklin, GA (FRA).

Variable	MF	ATL	166	FRA
Drainage area (km <sup>2</sup> )*	4110	4121	5325	6941
Mean annual flow (m <sup>3</sup> s <sup>-1</sup> )*	71.47	72.04	100.9	115.6
Runoff (cm <sup>yr</sup> <sup>-1</sup> )*	60.7	48.78	59.72	57.99
Temperature (C°)★				
Oct. 2 1998	17.3	19.6	22.2	23.4
Jan. 22, 1999	11.2	12.9	15.9	14.9
May 4, 1999	16.9	18.1	21.6	23.0
Aug. 17, 1999	20.8	24.4	29.6	32.7
FPOM Concentration (g AFDM L <sup>-1</sup> )	0.002	0.004	0.01	0.003
Permitted Wastewater Discharge (PWD) (10 <sup>2</sup> Ls <sup>-1</sup> )	7.58	38.76	72.05	91.9

\*From Frick et al.1996

★ Measured by Neumann, K. pers.comm.

Table 3.2. Discharge during sampling dates at USGS station, Whitesburg, GA (HUC 03130002). Whitesburg is downstream of site 166 on the mainstem of the Chattahoochee, with a drainage area of 6061 km<sup>2</sup> (<http://waterdata.usgs.gov/ga/nwis/>). Conditions are base flow (BF) and high flow (HF).

Season	Condition	Date	Mean Daily Discharge (m <sup>3</sup> s <sup>-1</sup> )
Fall	BF	October 2, 1998	74.47
	HF	November 11, 1998	91.75
Winter	BF	January 22, 1999	54.09
	HF	January 24, 1999	235.9
Spring	BF	May 4, 1999	45.31
	HF	May 8, 1999	122.9
Summer	BF	August 17, 1999	33.7
	HF	August 25, 1999	110.7

Table 3.3. Relationships of traditional measures of SFPM quality and permitted wastewater discharge (PWD) ( $\text{Lsec}^{-1}$ ) along the Chattahoochee River, within and below Atlanta. Only relationships with  $p < 0.20$  are shown. Slope is given when slope is significantly different from zero ( $\alpha = 0.05$ ). Samples were collected during base flow and during high flow in October 1998, January, May and August 1999.

PWD X SFPM measure	$r^2$	$p$	Slope
During base flow			
Calories	0.24	0.15	NS
% Inorganic	0.17	0.12	NS
Cu	0.50	0.001	+
Pb	0.29	0.002	+
Zn	0.34	0.001	+
During high flow			
Chlorophyll a	0.22	0.03	+
% Diatoms	0.13	0.08	NS
Cu	0.49	<0.0001	+
Pb	0.29	0.0008	+
Zn	0.34	0.0003	+

Table 3.4. Results of principal components analysis of traditional measures of food quality of SFPM collected at four sites along the Chattahoochee River, within and below Atlanta. The samples were collected during base and high flow in October 1998, January, May, and August 1999.

	Principal Component 1	Principal Component 2	Principal Component 3
Percent variance explained	32.5	23.44	15.94
Eigenvectors			
Chlorophyll a	0.26	0.59	0.28
N/C	0.39	0.29	0.61
% Diatoms	-0.56	0.09	0.29
% Inorganic	-0.36	0.52	-0.26
% Lipids	-0.18	-0.49	0.58
Bacteria	0.55	-0.20	-0.23

Table 3.5. Results of principal components analysis of Cu, Cd, Pb and Zn concentrations in SFPM collected at four sites along the Chattahoochee River, within and below Atlanta. The samples were collected during base and high flow in October 1998, January, May, and August 1999.

	Principal component 1	Principal component 2
Percent variance explained	68.5	24.3
Eigenvectors		
Cu	0.13	0.98
Cd	0.58	-0.04
Pb	0.56	-0.11
Zn	0.58	-0.09

Table 3.6. Comparison of measures of SFPM quality for samples collected during base flow in the Little Tennessee River and during base flow in the Chattahoochee River.

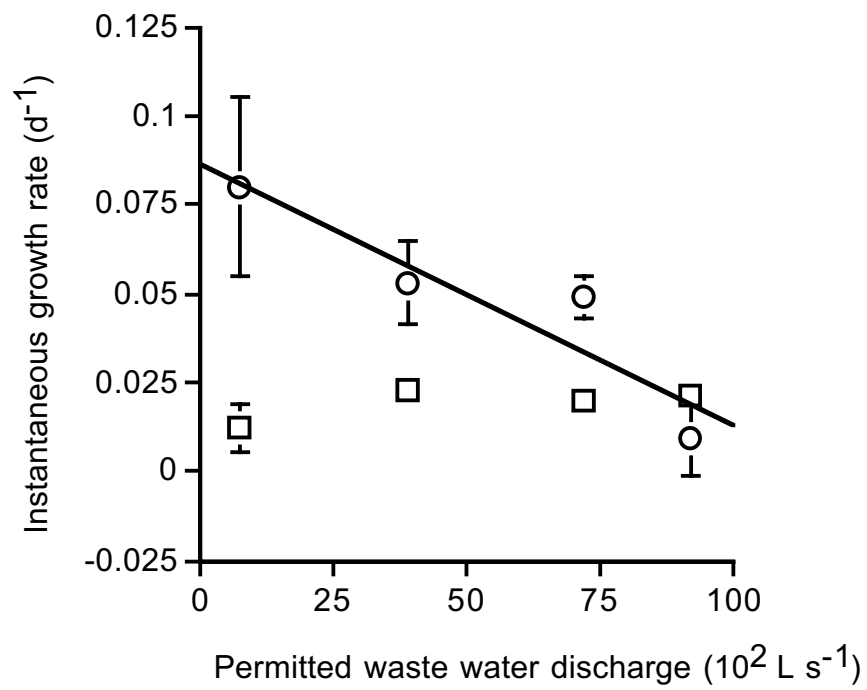
Annual means from all sites are provided. The variables were all compared with Students t-tests. For statistical analysis, non-normally distributed data, % mortality, % diatoms, % lipids, and % inorganics, were transformed using the formula ( $\arcsin X^{1/2}$ ).

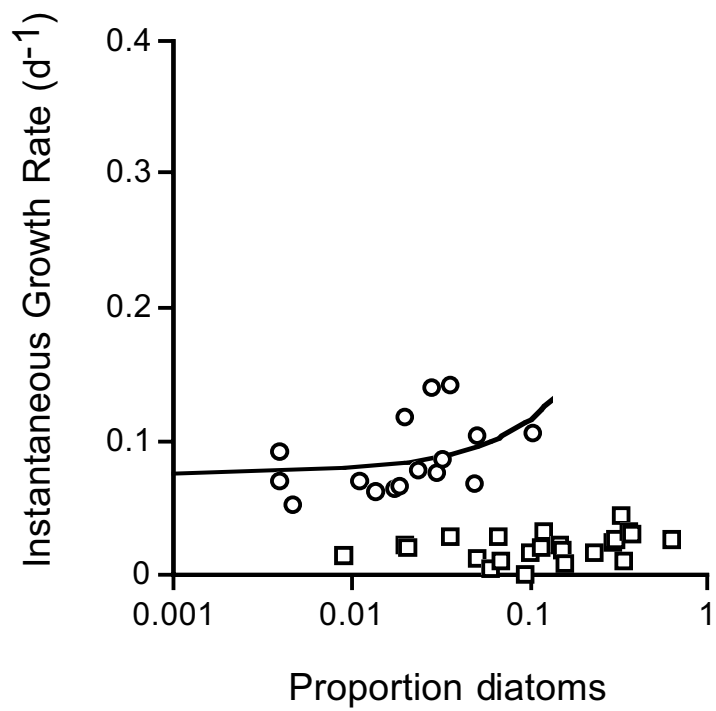
Variable measured	Little Tennessee		Chattahoochee		<i>p</i>
	Mean	SE	Mean	SE	
Instantaneous Growth Rate (d <sup>-1</sup> )	0.086	0.007	0.018	0.003	0.0001
Mortality %	53	2	52	4	0.10
N/C (mass)	0.059	0.005	0.077	0.005	0.02
Calories (cal/g DM)	460	101	392	37	0.33
Lipid content (% DM)	0.56	0.11	0.98	0.17	0.06
% Inorganic	63.5	5	83	3	0.001
% Diatoms	2.8	0.6	13	4	0.001

### Figure Legends

Figure 3.1. The average annual instantaneous growth rates of chironomids fed suspended particles collected during high flow ( $\square$ ) ( $r^2 = 0.14$ ,  $p = 0.15$ ) and during base flow ( $\circ$ ) ( $y = -0.001x + 0.081$ ,  $r^2 = 0.37$ ,  $p = 0.02$ ) as a function of PWD. Error bars represent the standard error of the mean; high error indicates high temporal variability.

Figure 3.2. The relationship of % diatoms in the SFPM and instantaneous growth rates of chironomids fed SFPM collected from the Little Tennessee ( $\circ$ ) and the Chattahoochee River ( $\square$ ). The relationship of % diatoms and IGR in the Little Tennessee River is given ( $IGR = 0.406 \% \text{ diatoms} + 0.075$ ).





Appendix 1a. Morgan Falls data for SFPM collected during base flow and following a high precipitation event. NA indicates where samples were not available for analysis.

Variable	Fall	Winter	Spring	Summer
Base flow				
% Diatoms	0.01	0.33	0.12	0
% Inorganic	0.46	0.86	0.73	NA
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	1.00	2.69	1.63
N/C (mass)	NA	0.09	0.08	0.09
% Lipids	NA	0.91	0.14	1.44
Calories (cal/g DM)	NA	323	250	NA
Bacteria (cells/g DM)	$2.46 \times 10^9$	$1.55 \times 10^6$	$2.12 \times 10^8$	$2.23 \times 10^9$
High flow				
% Diatoms	0.095	0.12	0.06	0.07
% Inorganic	0.9	0.90	0.88	NA
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	4.46	6.73	4.31
N/C (mass)	NA	0.08	0.002	0.08
% Lipids	NA	NA	0.03	0.99
Calories (cal/g DM)	NA	NA	456	150
Bacteria (cells/g DM)	NA	$1.47 \times 10^6$	$6.82 \times 10^8$	$1.53 \times 10^9$

Appendix 1b. ATL data for SFPM collected during base flow and following a high precipitation event. NA indicates where samples were not available for analysis.

Variable	Fall	Winter	Spring	Summer
<b>Base flow</b>				
% Diatoms	0	0.15	0.07	0
% Inorganic	0.86	0.89	0.78	0.88
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	3.18	3.77	3.98
N/C (mass)	NA	0.05	0.08	0.06
% Lipids	2.13	0.69	0.56	0.35
Calories (cal/g DM)	NA	368	359	NA
Bacteria (cells/g DM)	NA	$2.23 \times 10^6$	$1.43 \times 10^9$	$1.22 \times 10^9$
<b>High flow</b>				
% Diatoms	0.38	0.31	0.04	0.05
% Inorganic	0.91	0.80	0.84	0.78
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	4.47	6.98	4.71
N/C (mass)	NA	0.07	0.06	0.07
% Lipids	0.64	0.38	NA	1.01
Calories (cal/g DM)	NA	NA	224	249
Bacteria (cells/g DM)	NA	$1.30 \times 10^8$	$5.77 \times 10^8$	$8.36 \times 10^8$

Appendix 1c. 166 data for SFPM collected during base flow and following a high precipitation event. NA indicates where samples were not available for analysis.

Variable	Fall	Winter	Spring	Summer
<b>Base flow</b>				
% Diatoms	0	0.10	0	0.02
% Inorganic	0.82	0.77	0.80	0.84
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	0.84	3.14	2.17
N/C (mass)	NA	0.11	0.09	0.07
% Lipids	2.61	0.51	1.20	0.91
Calories (cal/g DM)	353	NA	569	430
Bacteria (cells/g DM)	$2.37 \times 10^9$	$2.16 \times 10^9$	$1.24 \times 10^9$	$1.49 \times 10^9$
<b>High flow</b>				
% Diatoms	0.30	0.23	0.00	0.00
% Inorganic	0.80	0.83	0.79	0.86
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	5.47	8.59	5.13
N/C (mass)	NA	0.06	0.06	0.07
% Lipids	1.99	1.10	0.52	0.93
Calories (cal/g DM)	NA	1437	572	507
Bacteria (cells/g DM)	NA	$1.76 \times 10^6$	$7.46 \times 10^8$	$2.53 \times 10^9$

Appendix 1d. FRA data for SFPM collected during base flow and following a high precipitation event. NA indicates where samples were not available for analysis.

Variable	Fall	Winter	Spring	Summer
<b>Base flow</b>				
% Diatoms	0.02	0.34	0	0.16
% Inorganic	0.78	0.90	0.81	0.88
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	2.15	2.37	12.41
N/C (mass)	NA	0.07	0.06	0.08
% Lipids	1.12	1.12	0.37	0.76
Calories (cal/g DM)	586	459	NA	230
Bacteria (cells/g DM)	NA	$1.47 \times 10^6$	$4.64 \times 10^9$	$2.15 \times 10^9$
<b>High flow</b>				
% Diatoms	0.62	0.36	0.16	0
% Inorganic	0.88	0.90	0.83	0.87
Chlorophyll a ( $\mu\text{gL}^{-1}$ )	NA	11.99	9.22	17.95
N/C (mass)	NA	0.06	0.07	0.07
% Lipids	1.27	0.42	1.27	0.75
Calories (cal/g DM)	456	NA	717	211
Bacteria (cells/g DM)	NA	$1.28 \times 10^6$	$1.28 \times 10^9$	$1.16 \times 10^9$

Appendix 2a. Concentration of total recoverable metals associated with the SFPM ( $\mu\text{g/g}$ ) collected at MF during base flow and following a high precipitation event. NA indicates where samples were not available for analysis.

Variable	Fall	Winter	Spring	Summer
Base flow				
Al	25803	34035	18945	NA
B	8	5268	7	NA
Ba	234	168	279	NA
Ca	1753	2659	2038	NA
Cd	2.1	8.6	1.2	NA
Co	13.4	24.6	12.4	NA
Cr	24.6	27.0	21.1	NA
Cu	32.5	32.2	26.8	NA
Fe	30821	44985	24461	NA
K	4445	2992	3078	NA
Mg	3723	5143	2549	NA
Mn	2783	6868	6984	NA
Mo	9.5	3.1	8.3	NA
Na	163	4957	167	NA
Ni	15.4	22.2	11.7	NA
P	810	1223	119	NA
Pb	86	52	69	NA
Si	295	3253	241	NA
Sr	18.8	20.2	22.0	NA
Zn	103	87	107	NA
Highflow				
Al	19189	19181	21750	22301
B	529	122	17	9
Ba	108	185	211	131
Ca	1577	1417	1306	1657
Cd	2.3	2.0	1.9	1.7
Co	8.2	11.4	11.9	10.8
Cr	22.6	20.3	22.9	18.9
Cu	26.8	26.0	27.8	30.2
Fe	31796	27060	26061	35538
K	3442	3790	4390	2652
Mg	4780	3506	3281	4227
Mn	3046	2910	2587	3371
Mo	0.0	7.9	9.1	0.0
Na	774	268	164	238
Ni	7.8	10.4	13.1	10.8
P	583	983	763	1101
Pb	22	61	76	64
Si	1897	349	233	2042
Sr	11.5	13.4	13.7	11.2
Zn	58	91	96	89

Appendix 2b. Concentration of total recoverable metals associated with the SFPM ( $\mu\text{g/g}$ ) collected at ATL during base flow and following a high precipitation event. NA indicates where samples were not available for analysis.

Variable	Fall	Winter	Spring	Summer
Base flow				
Al	20375	21488	22609	29745
B	6	188	7	26
Ba	134	145	153	170
Ca	1762	2509	2964	2263
Cd	1.5	0.7	2.2	1.2
Co	7.9	7.6	9.9	11.0
Cr	15.6	16.5	18.0	21.9
Cu	24.0	29.0	32.0	34.8
Fe	21710	25755	28561	28850
K	2990	2929	2689	3270
Mg	3033	3695	3863	3265
Mn	1482	2831	3898	2450
Mo	7.4	6.3	5.1	11.2
Na	131	305	371	198
Ni	8.8	6.9	8.4	14.8
P	560	866	1203	974
Pb	68	75	74	101
Si	323	584	748	322
Sr	11.6	12.6	14.7	12.9
Zn	95	119	120	131
Highflow				
Al	NA	29821	24872	34375
B	NA	444	7	8
Ba	NA	197	167	177
Ca	NA	2505	2065	2516
Cd	NA	2.2	1.6	2.9
Co	NA	11.3	10.1	12.9
Cr	NA	22.5	20.0	24.4
Cu	NA	36.4	40.1	40.7
Fe	NA	33855	28239	36193
K	NA	4540	4060	3512
Mg	NA	4625	3970	3988
Mn	NA	3659	2053	3180
Mo	NA	9.9	9.5	9.1
Na	NA	518	181	241
Ni	NA	11.4	13.2	18.6
P	NA	1169	769	1087
Pb	NA	109	89	125
Si	NA	838	467	1035
Sr	NA	14.1	12.3	12.4
Zn	NA	164	180	178

Appendix 2c. Concentration of total recoverable metals associated with the SFPM ( $\mu\text{g/g}$ ) collected at 166 during base flow and following a high precipitation event.

Variable	Fall	Winter	Spring	Summer
Base flow				
Al	24034	23066	24455	33252
B	6	19	572	14
Ba	149	89	184	136
Ca	2157	4546	2352	2471
Cd	2.3	0.0	1.6	1.7
Co	9.6	4.4	11.2	11.6
Cr	21.1	17.0	25.9	23.6
Cu	61.3	73.1	76.1	73.9
Fe	26981	36451	26239	31252
K	3710	2579	4069	3512
Mg	3684	4523	3092	3962
Mn	1378	1572	1446	1620
Mo	8.0	0.0	11.0	11.6
Na	214	510	826	150
Ni	12.9	17.5	14.3	16.0
P	1187	3127	2434	1308
Pb	102	78	124	138
Si	295	3461	197	357
Sr	15.8	20.1	19.1	16.0
Zn	182	182	260	230
Highflow				
Al	20667	23718	25083	22571
B	168	28	7	74
Ba	178	202	189	149
Ca	3386	2311	3048	2021
Cd	1.8	1.8	2.2	1.7
Co	12.0	12.0	12.6	10.1
Cr	21.4	21.7	21.2	19.1
Cu	88.3	59.1	75.2	55.5
Fe	30557	31363	30109	28136
K	3371	4447	4082	3108
Mg	4149	4771	4342	3745
Mn	3408	2191	2730	2214
Mo	5.7	5.5	9.3	3.1
Na	442	175	212	264
Ni	12.3	14.8	15.1	12.5
P	1388	935	1331	911
Pb	118	97	122	111
Si	417	489	391	284
Sr	19.1	16.0	19.3	13.4
Zn	306	210	279	208

Appendix 2d. Concentration of total recoverable metals associated with the SFPM ( $\mu\text{g/g}$ ) collected at FRA during base flow and following a high precipitation event.

Variable	Fall	Winter	Spring	Summer
Base flow				
Al	38868	21807	30435	10473
B	20	6	9	3
Ba	167	95	151	46
Ca	3473	1444	2882	645
Cd	1.5	2.5	2.0	0.9
Co	10.4	4.9	9.4	4.3
Cr	24.4	16.3	20.7	9.0
Cu	99.8	37.2	70.1	16.2
Fe	40596	25099	34716	11858
K	3535	3009	2791	1655
Mg	4835	3978	4346	1944
Mn	2305	893	3539	308
Mo	12.7	3.5	8.6	2.7
Na	362	87	455	81
Ni	18.0	8.1	19.9	3.9
P	1458	678	1310	274
Pb	157	70	101	35
Si	1494	824	1310	318
Sr	28.3	9.8	20.3	3.9
Zn	220	109	184	49
Highflow				
Al	25625	29107	29197	47657
B	23	355	17	18
Ba	97	187	153	171
Ca	1842	2127	2896	2387
Cd	3.0	1.7	3.2	1.9
Co	5.6	11.9	10.7	14.2
Cr	13.8	23.2	20.1	26.3
Cu	51.7	74.6	70.5	82.3
Fe	27663	29496	35700	37061
K	2015	4619	3152	3435
Mg	35	4468	5153	3979
Mn	1530	1843	3660	2923
Mo	7.0	11.8	5.5	17.2
Na	95	526	198	366
Ni	7.2	16.2	10.3	23.4
P	1053	1112	1430	1342
Pb	86	129	105	168
Si	986	430	1497	670
Sr	12.3	17.8	16.8	21.5
Zn	115	209	221	226

CHAPTER 4

METAL CONTAMINATION PATTERNS IN

MACROINVERTEBRATES INHABITING AN URBAN RIVER<sup>1</sup>

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<sup>1</sup>Rosi-Marshall, E. J., J. L. Meyer, K. Neumann, E. Y. Graham, and W. B. Lyons. To be submitted to Environmental Toxicology and Chemistry.

### Abstract

The Chattahoochee River flows through metropolitan Atlanta, GA. Point and non-point sources add metals (Cu, Cd, Pb, As, and Hg) to the river as it passes through Atlanta. We measured the concentrations of these metals in the tissues of three snag-dwelling taxa, Hydropsychidae, Chironomidae and Stenonema, during four seasons at four sites from Morgan Falls dam to Franklin, GA. Tissue concentrations of Cu, Cd, Pb, As and Hg were not significantly different among taxa or sites. The temporal variability of Cu, Cd, As and Pb in Stenonema and chironomid tissues increased at the site just downstream of the major point and non-point sources in Atlanta. The temporal variability in the concentrations of metals in hydropsychids did not show a consistent pattern among sites. Several factors were examined to try to determine what influenced the metal concentrations in macroinvertebrate tissues. We examined the gut contents of the taxa to determine if diet played a role in tissue metal concentrations. Macroinvertebrates consumed mainly amorphous detritus and some algae. No significant relationship was found between diet and macroinvertebrate tissue concentrations of metals. In addition, we measured the concentration of metals in the water column, dissolved and sorbed to particles, at the time of macroinvertebrate sample collection. There was no relationship between the concentrations of dissolved or sorbed metals measured in the water column and the concentrations in the macroinvertebrate tissues, with the exception of a weak relationship with dissolved Cd. These findings call into question the wisdom of using water column metals criteria to protect aquatic macroinvertebrates and the food webs they support from metal exposure. Despite the fact that metal concentrations in the macroinvertebrates inhabiting the Chattahoochee River were equal and at times higher

than concentrations measured in macroinvertebrates inhabiting other metal contaminated sites, water column metal concentrations never exceeded established Georgia water quality standards.

**Key words:** metals, copper, cadmium, lead, arsenic, mercury, aquatic insects, diets, Chironomidae, Hydropsychidae, Stenonema.

### Introduction

Macroinvertebrates can be useful for determining the extent of contamination of metals in a river system (Cain et al. 1992). Metal contamination is typically associated with western streams with mine drainage (Beltman et al. 1999, Cain et al. 1992, Cain et al. 2000); however, urban rivers also have metals entering them through numerous point and non-point sources (Frick et al. 1998, Paul and Meyer 2001). Runoff from parking lots and roads can often have elevated concentrations of metals (Rose et al. 2001). Waste water treatment effluent contains metals associated with particles and in the dissolved form (Frick et al. 1998). Metals (such as copper (Cu), cadmium (Cd), lead (Pb), and mercury (Hg) and the metalloid arsenic (As)) associated with urbanization may be bioavailable to macroinvertebrates inhabiting the streams flowing through these areas; however, this has not been previously examined.

Our first objective was to determine the patterns of metal concentration in macroinvertebrates inhabiting an urban river. We hypothesized that macroinvertebrates inhabiting sites in the Chattahoochee River downstream of Atlanta, GA would have higher concentrations of metals than those upstream of the major metropolitan area. As

discharge changes throughout the year, the concentration of metals in the water column changes (Yang and SanudoWilhelmy 1998, Sherrell and Ross 1999) which may lead to temporal variability in metal concentrations in the biota. We hypothesized that temporal variability in tissue concentrations of macroinvertebrates would be greater downstream of the city because temporal variability in discharge could lead to variation in metal loading from urban sources.

Our second objective was to determine if diet composition was a factor affecting the metal concentrations in macroinvertebrates inhabiting an urban river. The diet composition of macroinvertebrates inhabiting urban rivers has not been previously examined and may influence tissue metal concentrations (Besser et al. 2001). We hypothesized that the relative proportion of different food types in macroinvertebrate diets is related to tissue metal concentrations.

Our third objective was to determine if metal concentrations measured in macroinvertebrates inhabiting an urban river were related to dissolved or sorbed metals measured in the water column at the time of insect collection. In aquatic environments, macroinvertebrates are exposed to dissolved metals, through gills and body surface contact, and to particulate metals, through consumption of particles. In 1995, the US Environmental Protection Agency (US EPA) promulgated a change in water quality criteria which allow states to regulate only the dissolved fraction of metals (US FWPCA §307 1997). The US EPA argues that only metals in the dissolved phase are bioavailable (SAB 2000); however metals associated with particles have been shown to be bioavailable to zebra mussels (Roditi and Fisher 1999) amphipods (Schlekat et al. 1999), clams, and marine polychaetes (Wang and Fisher 1999, Lee et al. 2000). We

hypothesized that the metal concentrations in macroinvertebrates would be related to dissolved metals if these were the only metals that were bioavailable.

## **Methods and Study Sites**

### Study sites

The upper Chattahoochee River flows through metropolitan Atlanta. Point and non-point source pollution contribute to the problem of inorganic and organic pollution throughout the basin (Frick et al. 1998). Trace metal concentrations in sediment and *Corbicula* tissues pose an ecological risk to native fauna (<http://wwwga.usgs.gov/nawqa> Jan. 2002). We collected macroinvertebrate samples within and below the city of Atlanta on an unimpounded section of the river between two reservoirs (Table 4.1). The first site (MF) is in a northern section of the city of Atlanta, just downstream of the Morgan Falls impoundment, which is used for hydroelectric power generation. While this site is upstream of most of the city, it is downstream of suburban wastewater treatment plants and suburban runoff; hence, it is not an upstream reference site. The second site (ATL) is located at the Atlanta Road crossing of the Chattahoochee River. This site is just downstream of Peachtree Creek, the tributary that drains downtown Atlanta and is downstream of a major wastewater treatment facility. The third site (166) is located at the Georgia Route 166 crossing of the Chattahoochee River. This site is downstream of most of the urban runoff, a number of tributaries draining industrialized areas, and a majority of the point sources in the major metropolitan area, including municipal and industrial facilities. The final site (FRA) is at the Georgia Route 27 crossing of the Chattahoochee River. This site is approximately 160 km downstream of Atlanta, in Franklin, GA, just

upstream of West Point Reservoir. This site was chosen to determine the extent of contamination from upstream sources.

#### Sample collection and analysis

Macroinvertebrates were collected from woody debris (snags) during October 1998, January, April, and July 1999 and preserved in Kahles solution for gut content analysis. Additional macroinvertebrates were collected, allowed to depurate for 24 hours, and frozen for metal analysis. Three macroinvertebrate trophic groups were collected: hydropsychid caddisflies (filterers) (Hydropsyche and Cheumatopsyche), snag-dwelling chironomids (collector/ gatherers), and heptageniid mayflies (scrapers) (Stenonema). These taxa were chosen because they had the highest densities on snags and were observed at all sites.

From 4-6 individuals of each non-predatory taxa (Stenonema and chironomids) were used for gut content analysis from each site and each season. The gut contents of Stenonema were removed, filtered onto 0.45  $\mu\text{m}$  gridded metricel filters (Gelman Sciences, Ann Arbor, MI) and affixed to slides for preservation and measurement (Cummins 1973). The gut contents of chironomids were placed directly on slides and preserved, due to their small size. For hydropsychids, ten individuals were analyzed when available. Hydropsychid gut contents were dissected and preserved on a slide to retain the integrity of any animal parts for identification purposes. In addition, from 4-6 other individuals' guts were removed, filtered onto 0.45  $\mu\text{m}$  gridded metricel filters (Gelman Sciences) and affixed to slides for preservation and measurement (Cummins 1973). The gut contents were identified and quantified using an image analysis software (Image Pro)

(Benke and Wallace 1980, Benke and Wallace 1997). The proportion of each food resource consumed was estimated using the relative area of the particles found in the gut. Particles were identified and classified into six major food resources: amorphous detritus, animal, diatoms, filamentous algae, fungi, and leaf tissue.

### Metal Analysis

The macroinvertebrates were placed in pre-weighed teflon-containers, freeze-dried, and weighed again. Then 5ml Optima HNO<sub>3</sub> was added, the sample was covered and heated to sub-boiling temperatures for one to two days. A sub-sample was diluted to 2% acid using DI water and then analyzed by Inductively Coupled Plasma (ICP) to obtain concentrations of metals (e.g., Cu, Cd, Zn, Pb, etc.). A portion of the sample material was used for Hg measurement, using a Brooks-Rand cold-vapor atomic fluorescence spectrometer (CV-AFS).

We collected water samples from the four sites in October 1998, January, April, and August 1999. The concentration of dissolved and sorbed particulate metals in the water column were measured. Teflon bottles (1000ml for Hg, 250ml for other metals) were soaked in 10% HNO<sub>3</sub> for a week, then filled with fresh acid solution and kept at a sub-boiling temperature overnight. Finally they were rinsed with DI, filled with DI water and acidified to a pH < 2 with Optima HNO<sub>3</sub>. The bottles were double-bagged in the lab and stored until sampling.

In the field, the metal samples were collected using the clean hand/dirty hand technique, i.e., one person (dirty hand) opening only the outer bag, and the second person (clean hand) opening the inner bag, taking the sample, putting the bottle back in the inner

bag, and the first person closing the outer bag. In addition, 1L samples were collected for total suspended solid determination.

One aliquot of each water sample was filtered through acid-cleaned 0.4 $\mu$ m nucleopore filters and then acidified to a pH < 2 with Optima<sup>®</sup>HNO<sub>3</sub>, while the other aliquot was acidified without filtration. The filtered aliquots were analyzed for dissolved metal concentrations ( $M_{\text{diss}}$ ). The unfiltered/acidified samples were filtered just before analysis (acid-cleaned 0.4 $\mu$ m nucleopore filters), and termed environmentally active metal concentration ( $M_{\text{tot}}$ ). The concentrations of metals on the suspended particles ( $M_{\text{part}}$ ) can be calculated ( $(M_{\text{tot}} - M_{\text{diss}}) / \text{TSS}$ ), where TSS = total suspended solids. Mercury samples were treated the same way as the other trace metal samples. They were analyzed using cold vapor atomic fluorescence spectrometry (CV-AFS). TSS was determined by filtering 1L of water through a pre-weighed nucleopore filter, drying and re-weighing the filter.

Individuals of each taxon were pooled from each season from each site. Therefore one concentration was measured for each taxon at the four sites during four seasons. We used ANOVA to detect differences in tissue metal concentrations among taxa (sites and seasons were treated as replicates with a maximum N = 16 for each taxon), among sites (seasons and taxa were treated as replicates with a maximum N = 12 for each site) and among sites within each taxon (seasons were used as replicates with a maximum N = 4 for each site). We used coefficient of variation (CV) to examine which sites had the highest temporal variability in macroinvertebrate metal tissue concentrations.

## Results

### Metal concentrations in macroinvertebrate tissues along the Chattahoochee River

The tissues of Stenonema, chironomids and hydropsychids collected from the Chattahoochee River had measurable concentrations (dry mass) of Cu, Cd, As, Pb and Hg (Appendix 1). The tissue metal concentrations in the insects were not normally distributed, and the square root of the concentrations was used in ANOVA. Tissue metal concentrations were not significantly different among taxa when all sites and seasons were pooled (ANOVA, Cu  $p > 0.05$ , Cd  $p > 0.10$ , As  $p > 0.10$ , Pb  $p > 0.10$ ). When all taxa and seasons were pooled, macroinvertebrate tissue metal concentrations were not significantly different among sites (Figures 4.1a-d), except for Pb (Figure 4.1e). Pb concentrations were significantly lower at FRA than at ATL. Although not significantly different, Cu concentrations were slightly higher at the two middle sites in chironomids and Stenonema, and the opposite was true for hydropsychids (Figure 4.1a). Cd concentrations were slightly higher downstream in all taxa (Figure 4.1b). Using seasons pooled, macroinvertebrate tissue concentrations were not significantly different among sites for any taxon (Figures 4.2, 4.3 and 4.4).

### Temporal variability in macroinvertebrate tissue concentrations

The Hg data were not sufficient to analyze temporal trends. The greatest temporal variability in Stenonema tissue concentrations of Cu, Cd, and As occurred at the third site (Figure 4.2a, b and c), with CVs of 90, 80 and 119 respectively. Pb concentrations in Stenonema tissues were most variable at the second site with a CV of 116 (Figure 4.2d).

Chironomid tissue concentrations generally followed the same patterns. The highest temporal variability of Cu, Cd and Pb concentrations in chironomid tissues occurred at the third site (Figure 4.3a, b, and c ) with CVs of 130, 159 and 110 respectively. The number of As (Figure 4.3d) samples was not large enough to detect temporal variability. Hydropsychid tissue concentrations did not follow similar patterns. The last site had the highest temporal variability of Cd concentrations in the tissues of hydropsychids (Figure 4.4a) with a CV of 115. Pb concentrations in hydropsychid tissues were most variable at the upstream site (Figure 4.4b), with a CV of 93. The concentration of Cu in hydropsychid tissues was similar at the first three sites (Figure 4.4c) with CVs ranging from 38-52, and the temporal variability was low at the downstream site, with a CV of 3.5. The As concentrations in hydropsychid tissues were most variable at the second site (Figure 4.4d), with a CV of 59.

#### Factors influencing tissue concentrations

Hydropsychids consumed amorphous detritus, diatoms, and other (which includes leaves, animal and fungi) (Figure 4.5a). The proportion of diatoms in their guts was similar among sites and seasons with the exception of fall at the most downstream site, when consumption of diatoms increased by nearly 4X. Stenonema, a scraping mayfly (Merritt and Cummins 1996) consumed diatoms and amorphous detritus (Figure 4.5b). Stenonema diets varied by site and season with generally higher proportion of diatoms in fall and winter. Chironomid diets were homogenous, with 100% amorphous detritus found in their guts in all sites and seasons.

Diet was not related to macroinvertebrate tissue concentrations of metals (Table 4.2). Water column concentrations of dissolved and sorbed metals also were not related to macroinvertebrate tissue concentrations of any metal measured (Table 4.2). The one exception was dissolved Cd which was significantly related to tissue concentrations, although with a low  $r^2$ . This relationship was driven by one very high tissue Cd concentration in Stenonema at site 166 (Figure 4.2b and Appendix 1c).

### **Discussion**

The concentrations of metals in macroinvertebrates along the Chattahoochee River downstream of Atlanta are variable. The three taxa measured had similar concentrations of each metal; therefore no taxon measured bioaccumulates more than the others. Tissue metal concentrations did not differ among sites, although Cd concentration increased slightly downstream. The sites sampled were all within or downstream of the metropolitan area; hence an appropriate upstream reference site was not available. This may account for the lack of variation among sites in the concentrations of metals in the macroinvertebrate tissues; all appear elevated.

We used values from the literature to determine if macroinvertebrate tissue concentrations were elevated in the Chattahoochee River (Table 4.3). The references used for comparison were western acid mine drainage sites (Cain et al 1995, Beltman et al. 1999, Cain et al. 2000, Besser et al. 2001) and the Upper Mississippi River (Dukerschein et al. 1992). The macroinvertebrates from the Chattahoochee River are equally, and at times more, contaminated than macroinvertebrates collected from smaller streams which contain acid mine drainage. Macroinvertebrates inhabiting all four sites on the

Chattahoochee River had elevated tissue metal concentrations compared to the reference conditions (minimum values in Table 4.3) measured in other studies.

Macroinvertebrate tissue metal concentrations varied temporally as well. The extent of temporal variability differed among sites and metals, with the highest temporal variability most often measured at the more downstream sites. This suggests that the variability in metal exposure increased downstream of the city of Atlanta. The Chattahoochee River is regulated by Buford dam and Morgan Falls dam. As the Chattahoochee River flows through Atlanta, the cumulative amount of point and non-point source discharge increases. Large areas of non-point source runoff may provide a large but variable source of metals downstream of the city. In addition, the upstream sites have lower water temperatures (Table 4.1) due to hypolimnetic releases from a large upstream reservoir (Lake Sydney Lanier, GA). There is a gradient of increasing water temperature downstream, and in August the water temperature increased 12 degrees from the first site to last site. Because of this temperature gradient, macroinvertebrates likely have longer life cycles upstream than downstream (Sweeney 1984, Huryn 1990). Longer life cycles may lead to lower variability in metal concentrations throughout the year. Higher temperatures also lead to higher rates of bioaccumulation in organisms (Newman 1998). Increased water temperatures and more variable metal inputs may be the cause of the higher variability in macroinvertebrate tissue metal concentrations at downstream sites in this river.

The temporal variability in tissue concentrations suggests that although these macroinvertebrates are exposed to metals, the extent and duration of exposure varies. Exposure may vary with discharge or with the nature of source dynamics; i.e., point and

non-point sources may have different concentrations. Given the highly variable nature of an urban river with numerous sources of input, the variability measured in the macroinvertebrates is not unexpected. We were not able to ascertain the inputs into the system, because of its complexity and size, and the lack of information on effluent concentrations. Hence we could not use variation in exposure to predict macroinvertebrate tissue concentrations. Although macroinvertebrate tissue concentrations may relate to exposure, without continuous monitoring, estimating actual exposure is difficult.

#### Insect tissue concentrations and food web consequences

The concentration of metals measured in macroinvertebrates may have numerous consequences for the insects and the river ecosystem at large. Elevated metal concentrations in aquatic biota can delay sexual maturation (Kemble et al. 1994), increase drift rate (Richardson and Kiffney 2000), increase predation risk (Clements 1999), and increase metabolic costs due to metallothionein production or other factors (Di Giulio et al. 1995). In addition, insects with elevated metal concentrations may be a sub-optimal prey item. Macroinvertebrates collected from the Clark Fork River, which have similar concentrations as macroinvertebrates from the Chattahoochee River (Table 4.3), were a low quality food resource for fishes (Woodward et al. 1995). Therefore the macroinvertebrates inhabiting the Chattahoochee River may be suffering from sub-lethal effects and represent low food quality for fishes, because of their tissue metal concentrations.

### Factors influencing tissue concentrations

The diets of the macroinvertebrates measured varied with taxa, site and season, although in all cases macroinvertebrates consumed primarily amorphous detritus. The importance of amorphous detritus in the diets of these macroinvertebrates from the Chattahoochee River was greater than has been measured in other systems (Benke and Wallace 1980, Benke and Wallace 1997, Rosi-Marshall and Wallace 2002). Amorphous detritus in this system may come from both autochthonous sources (e.g., algal exudates) and allochthonous sources (e.g., leaf leachate, DOC and waste water treatment effluent) (Lush and Hynes 1973, Warren and Zimmerman 1993, Alber and Valiela 1994, Wotton 1996). Although the importance of amorphous detritus to macroinvertebrates in this system is greater than elsewhere, there is no evidence to suggest that it is a factor influencing macroinvertebrate tissue metal concentrations. Diet composition was not significantly related to tissue metal concentrations for any taxa measured.

Factors such as extent of exoskeleton, individual size (Smock 1983, Cain et al. 1992, Newman 1998) and life cycle may influence insect tissue metal concentrations. An individual's size can influence tissue metal concentrations; Co, Cr, Fe, Sb, and Se decrease with increasing body size due to the importance of surface adsorption of these metals (Smock et al. 1983). Due to the importance of external adsorption to chitin, the extent of exoskeleton may also be a factor influencing tissue metal concentrations. Individuals of many sizes were pooled in our study and the taxa chosen have varying extents of exoskeleton. The macroinvertebrates that we chose range from small size and minimal exoskeleton (chironomids) to medium with extensive exoskeleton (Stenonema) to relatively large with moderate exoskeleton (hydropsychids). Life cycles may also

influence bioaccumulation of elements. Shorter life cycles may result in lower tissue metal concentrations (Newman 1998). The insects that we chose had variable life cycles, from very short (chironomids typically live for 7-14 days) to long (Stenonema and hydropsychids can have year-long life cycles) (Merritt and Cummins 1996). However, tissue metal concentrations were not significantly different among taxa, suggesting that size, exoskeleton and life cycle duration were not major factors influencing macroinvertebrate tissue metal concentrations in this river.

The concentration of sorbed metals in the river also was not related to macroinvertebrate tissue concentrations. In addition to these analyses of water column samples SFPM samples were collected concurrently with a 20  $\mu\text{m}$  net (Chapter 3). Because these SFPM samples represent a different size fraction than the sorbed metals, I compared the metal concentrations in the SFPM to the macroinvertebrate tissue samples (Table 4.2). The total recoverable concentrations of metals associated with suspended fine particulate matter (SFPM) > 20 $\mu\text{m}$  (Chapter 3) were not related to macroinvertebrate tissue concentrations. Metals associated with fine particles are available to aquatic organisms through trophic exposure (Roditi and Fisher 1999, Schlekat et al. 1999, Wang and Fisher 1999, Lee et al. 2000); however, the experiments that demonstrated this were conducted under laboratory conditions. These experiments used radio-isotopes of trace elements [ $^{110\text{m}}\text{Ag}$ ,  $^{109}\text{Cd}$ ,  $^{75}\text{Se}$ ,  $^{51}\text{Cr}$ , and  $^{203}\text{Hg}$  (Roditi and Fisher 1999)], [ $^{109}\text{Cd}$  (Schlekat et al. 1999)], [ $^{51}\text{Cr}$ , (Wang and Fisher 1999)] and [ $^{110\text{m}}\text{Ag}$  and  $^{109}\text{Cd}$  (Lee et al. 2000)] to detect assimilation efficiencies of metals associated with particles. It is harder to establish the bioavailability of metals in samples collected in the field because exposure regime is difficult to measure.

Dissolved concentrations measured during four seasons were not related to macroinvertebrate tissue concentrations in the Chattahoochee River, with the exception of dissolved Cd. Therefore, the use of dissolved water quality criteria to protect aquatic biota (US FWPCA §307 1997) from metal contamination is questionable. The macroinvertebrates that inhabit the Chattahoochee River have tissue concentrations of metals that are comparable to macroinvertebrates that inhabit other contaminated streams (Table 4.3). According to Georgia metals criteria (GA DNR 1999), the dissolved metal concentrations in the Chattahoochee River are not at levels which pose a threat to biota. However, the macroinvertebrate tissue concentrations measured were elevated compared to reference systems (Cain et al 1995, Beltman et al. 1999, Cain et al. 2000, Besser et al. 2001) and indicate that the biota are exposed to metals which may pose a threat to them. Simply applying criteria based on sorbed data is also not likely to be effective, because we observed no relationships between sorbed concentrations and insect tissue concentrations. Some metals associated with particles are bioavailable (Roditi and Fisher 1999, Schlekot et al. 1999, Wang and Fisher 1999, Lee et al. 2000) and may be the source of metals to the macroinvertebrates measured in the Chattahoochee River. However, accurate assessment of the exposure regime of sorbed and dissolved metals in a river system is plagued with difficulties. Continuous monitoring of metal concentrations in surface waters are currently not feasible; however, biota, such as macroinvertebrates, integrate the exposure regime throughout their life cycle. Criteria based simply on dissolved or sorbed metal concentrations are inadequate. Prediction of bioavailability would be more accurate and protection of biota would be more effective if criteria were designed to take advantage of macroinvertebrates as integrative measures.

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Table 4.1. Characteristics of the four study sites along the Chattahoochee River. The four sites include: Morgan Falls (MF), Atlanta Road crossing (ATL), Route 166 crossing (166) and Franklin, GA (FRA).

Variable	MF	ATL	166	FRA
Drainage area (km <sup>2</sup> )*	4110	4121	5325	6941
Mean annual flow (m <sup>3</sup> s <sup>-1</sup> )*	71.47	72.04	100.9	115.6
Runoff (cm <sup>yr</sup> <sup>-1</sup> )*	60.7	48.78	59.72	57.99
Temperature (C°)★				
Oct. 2 1998	17.3	19.6	22.2	23.4
Jan. 22, 1999	11.2	12.9	15.9	14.9
May 4, 1999	16.9	18.1	21.6	23.0
Aug. 17, 1999	20.8	24.4	29.6	32.7
Permitted Wastewater Discharge (PWD) (10 <sup>2</sup> Ls <sup>-1</sup> )	7.58	38.76	72.05	91.9

\*From Frick et al.1996

★ Measured by Neumann, K. pers.comm.

Table 4.2. Relationship of macroinvertebrate tissue metal concentrations and other variables measured.

Variable	$r^2$	$p$ value
% Diatoms vs.		
Cu	0	0.99
Cd	0.11	0.09
As	0.02	0.47
Pb	0.08	0.12
Hg	0.03	0.46
Dissolved Concentration vs.		
Cu	0.01	0.66
Cd	0.27	0.01
As	0.00	0.94
Pb	NA	NA
Hg	0.05	0.27
Sorbed Concentration vs.		
Cu	0	0.9
Cd	0.06	0.43
As	0	0.8
Pb	0.02	0.51
Hg	0.06	0.28
Total recoverable concentration in SFPM >20 $\mu\text{m}^*$ vs.		
Cu	0.03	0.35
Cd	0.009	0.68
Pb	0.009	0.69

\*data from Chapter 3.

Table 4.3. Comparison of the concentrations of metals in the macroinvertebrates inhabiting the Chattahoochee River with macroinvertebrates in other rivers.

Concentrations are all

$\mu\text{g g}^{-1}$  dry mass.

River Basin	Cu		Cd		Pb		As		Hg	
	Min	Max	Min	Max	Min	Max	Min	max	Min	max
Sacramento	14.5*	37.7	0.06*	2.16	0.59*	1.29			0.03*	0.08
<u>Hydropsyche californica</u> , Cain et al 2000										
Clark Fork	30*	36	0.6*	0.9	2.2*	9.3				
<u>Hydropsyche occidentalis</u> , Cain et al 1995										
Chattahoochee	13	63	0.1	3.8	3	16.5	0.31	3.8	0.02	0.26
<u>Hydropsyche</u> spp. This study										
Blackbird Creek		3020						76.3		
Macroinvertebrate comm., Beltman et al. 1999										
Upper Animas	10*	119	0.4*	2.0	0.8*	49				
<u>Arctopsyche Rhithrogena</u> Besser et al. 2001	19*	214	6.1*	34.4	2*	129				
Upper Mississippi			0.01	0.22					0.04	1.77
<u>Hexagenia bilineata</u>										

Dukerschein et al.1992

\* concentrations are from reference sites

### Figure Legends

Figure 4.1. Annual average metal tissue concentrations measured in Stenonema (□), hydropsychids (◇) and chironomids (○) along the Chattahoochee River: (a) Cu, (b) Cd, (c) As, (d) Hg and (e) Pb. Four sites were sampled: Morgan Falls (MF), Atlanta Road (ATL), 166 Road Crossing (166) and Franklin (FRA). ANOVA was used to detect differences among sites (seasons and taxa were treated as replicates with a maximum N= 12 for each site). When there is a significant difference among sites (Tukey-Kramer HSD  $\alpha = 0.05$ ), the uppercase letters across the top of the graph are different.

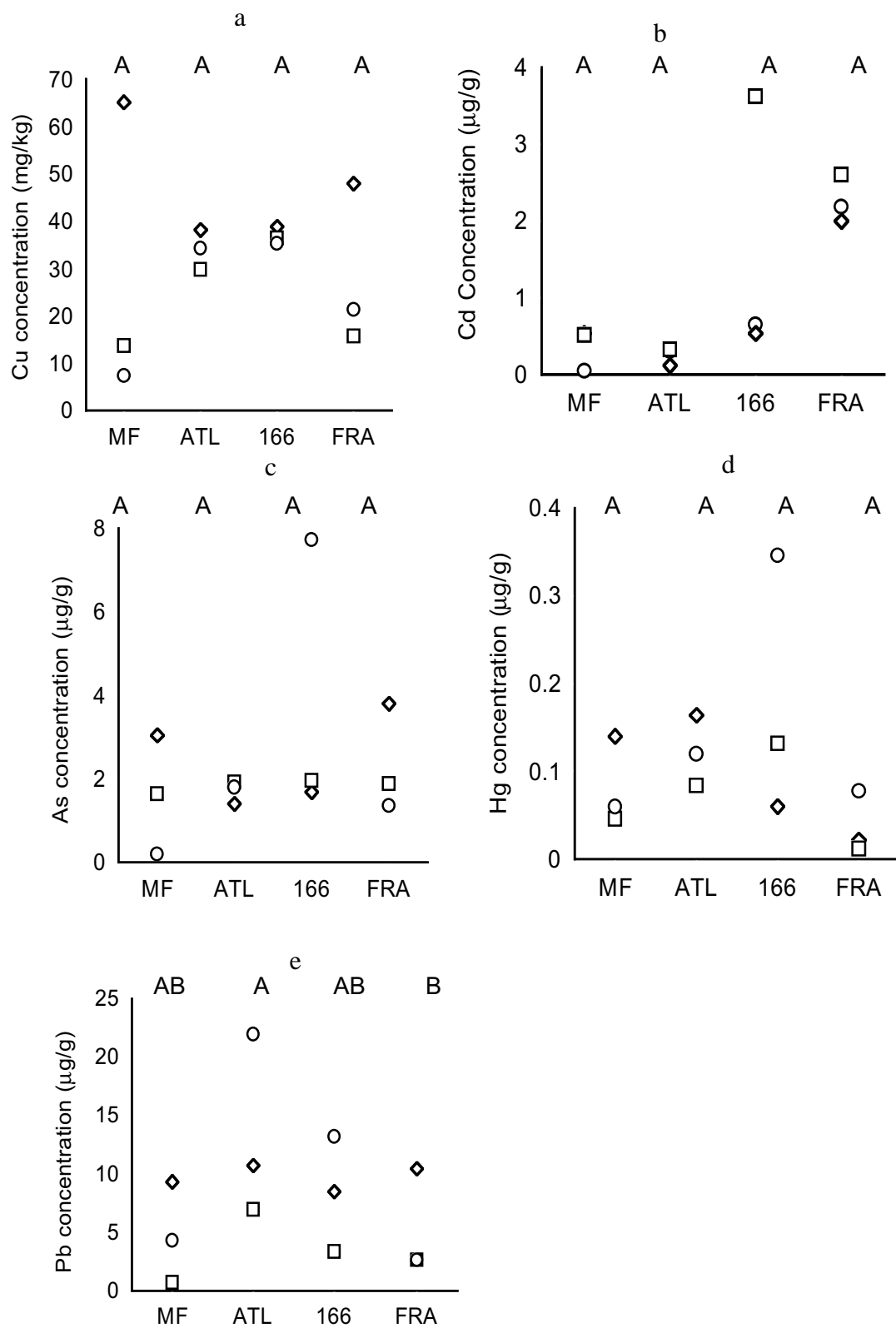
Figure 4.2. Temporal metal tissue concentrations measured in Stenonema along the Chattahoochee River: (a) Cu, (b) Cd, (c) As, and (d) Pb. Samples were collected during fall (□), winter (◇), spring (○) and summer (Δ). Site abbreviations as in Figure 4.1. ANOVA was used to detect differences among sites (seasons were treated as replicates with a maximum N= 4 for each site). When there is a significant difference among sites (Tukey-Kramer HSD  $\alpha = 0.05$ ), the uppercase letters across the top of the graph are different. Coefficients of variation (CV) are given for each site in parentheses.

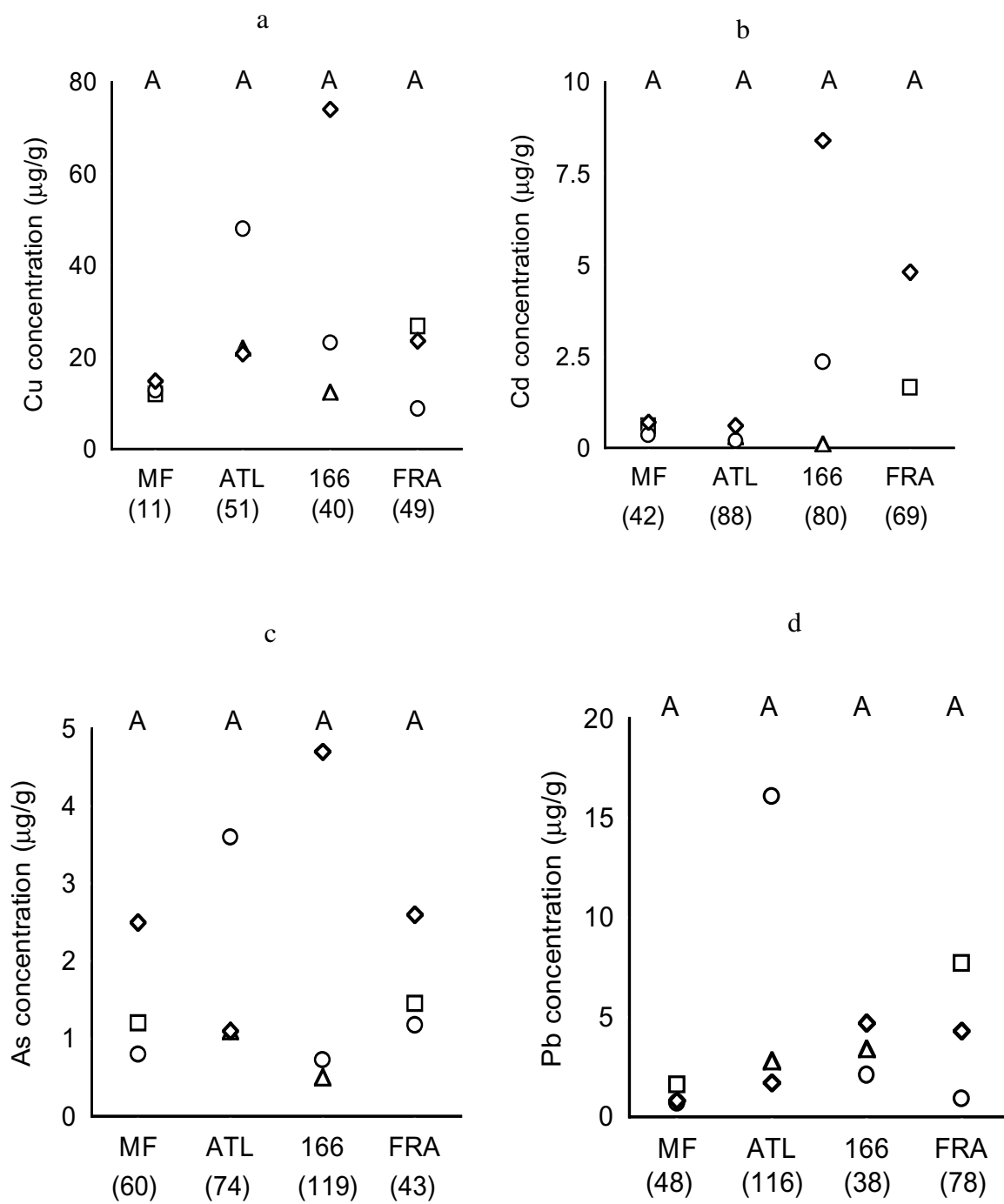
Figure 4.3. Temporal metal tissue concentrations measured in chironomids along the Chattahoochee River: (a) Cu, (b) Cd, (c) As, and (d) Pb. Samples were collected during fall (□), winter (◇), spring (○) and summer (Δ). Site abbreviations as in Figure 4.1. ANOVA was used to detect differences among sites (seasons were treated as replicates with a maximum N= 4 for each site). When there is a significant difference among sites

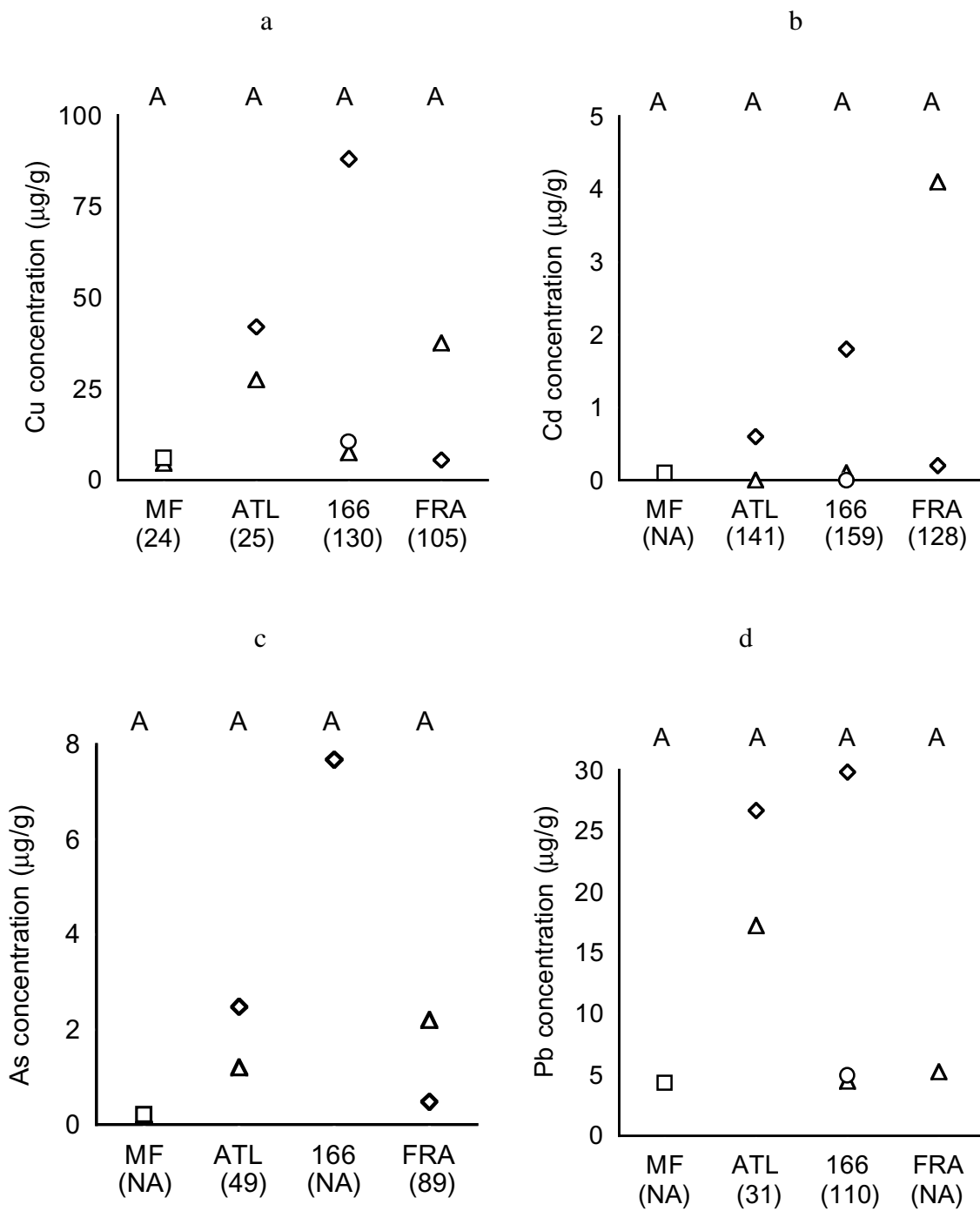
(Tukey-Kramer HSD  $\alpha = 0.05$ ), the uppercase letters across the top of the graph are different. Coefficients of variation (CV) are given for each site in parentheses.

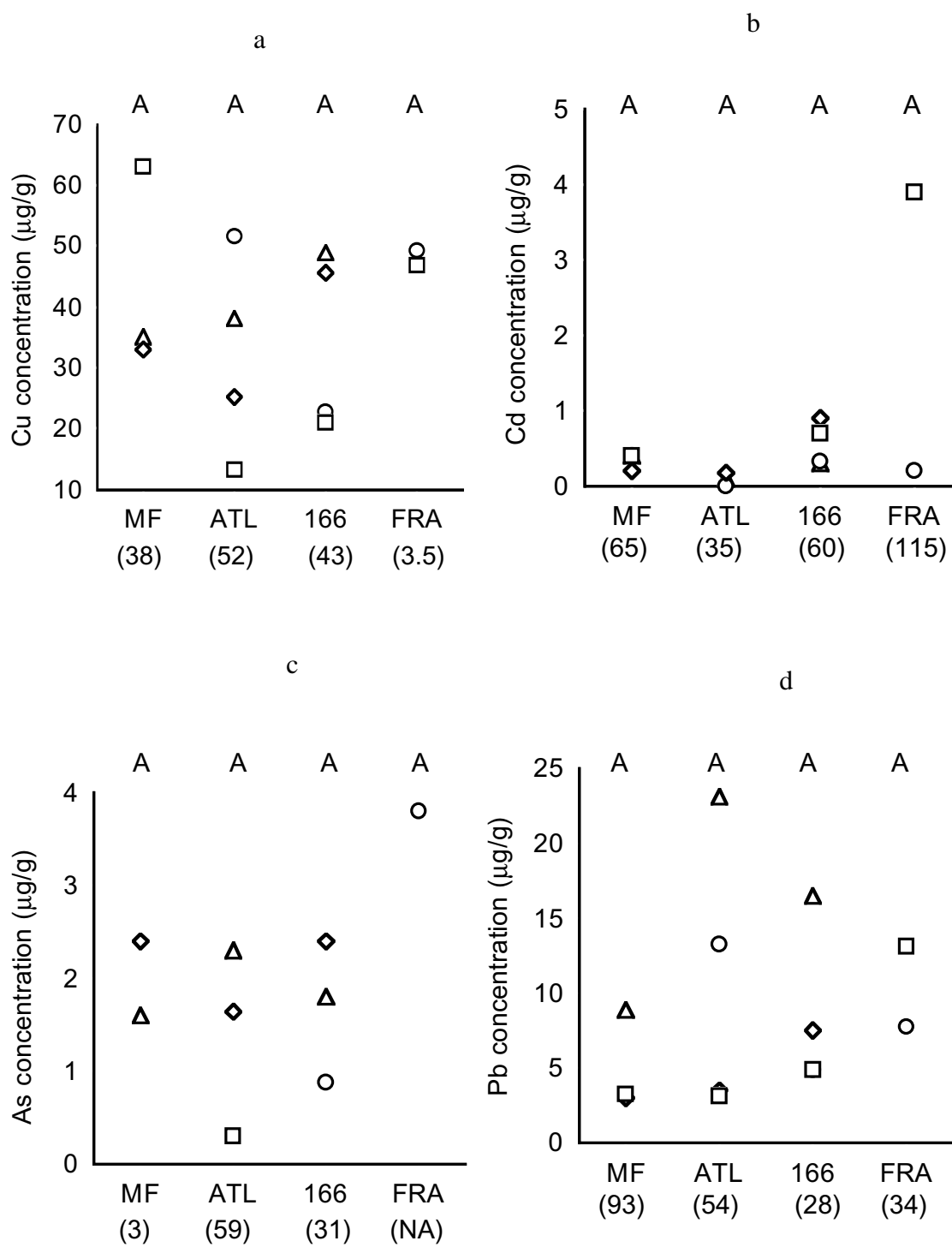
Figure 4.4. Temporal metal tissue concentrations measured in hydropsychids along the Chattahoochee River (a) Cu, (b) Cd, (c) As, and (d) Pb. Samples were collected during fall ( $\square$ ), winter ( $\diamond$ ), spring ( $\circ$ ) and summer ( $\Delta$ ). Site abbreviations as in Figure 4.1. ANOVA was used to detect differences among sites (seasons were treated as replicates with a maximum  $N=4$  for each site). When there is a significant difference among sites (Tukey-Kramer HSD  $\alpha = 0.05$ ), the uppercase letters across the top of the graph are different. Coefficients of variation (CV) are given for each site in parentheses.

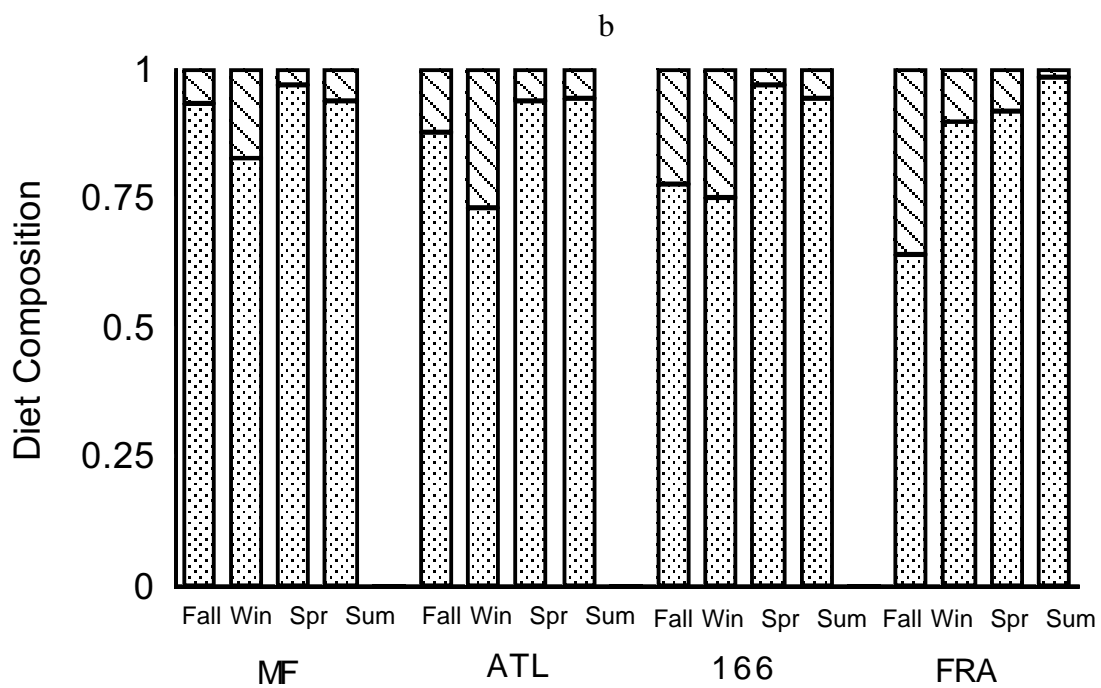
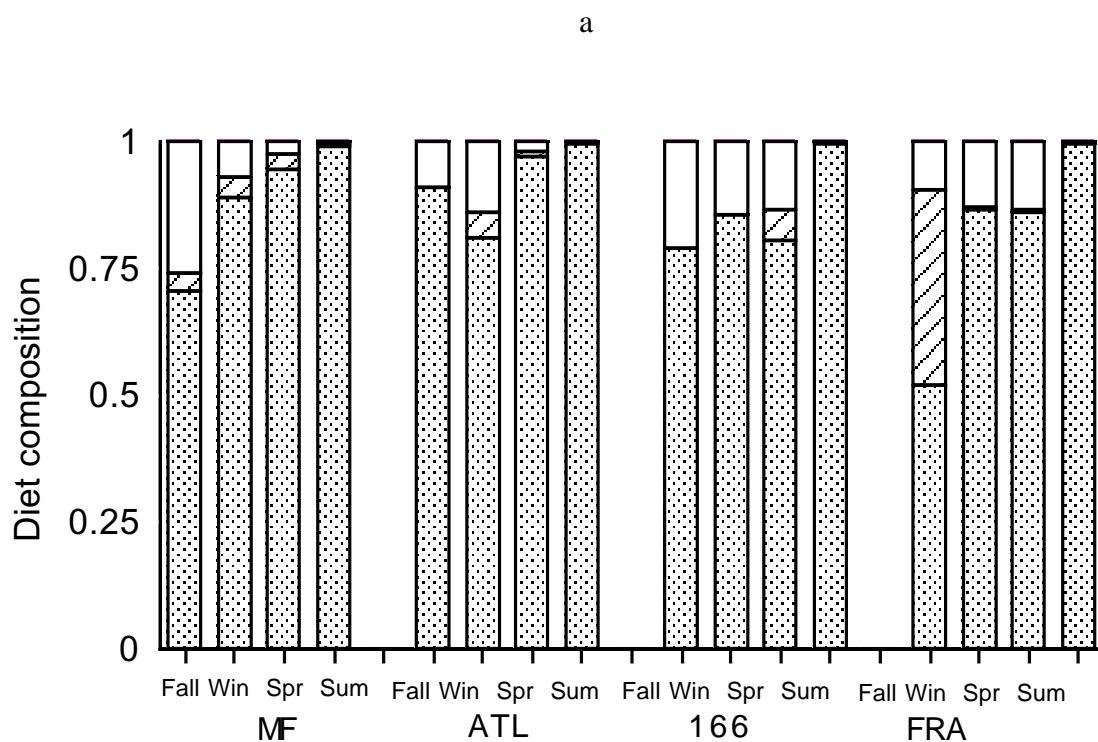
Figure 4.5. Proportions of food items measured in the gut contents of (a) hydropsychids and (b) Stenonema during four seasons at four sites. Site abbreviations as in Figure 4.1. Food types include amorphous detritus ( $\square$ ), diatoms ( $\boxtimes$ ) and other ( $\square$ ).











Appendix 1a. Tissue metal concentrations ( $\mu\text{g/g}$  dry mass) in hydropsychids, chironomids and Stenonema at Morgan Falls, Chattahoochee River, GA. The concentrations are from a single analysis of a pooled sample of individuals of each taxon. NA indicates that insufficient biomass was collected for metal analysis.

Taxa	Season	Cu	Cd	As	Pb	Hg
<u>Hydropsychids</u>						
	Fall	63	0.4	NA	3.2	NA
	Win	33.1	0.2	2.4	3	0.26
	Spr	NA	NA	NA	NA	NA
	Sum	35	0.4	1.6	8.9	0.03
<u>Chironomids</u>						
	Fall	5.97	0.09	0.22	4.32	0.05
	Win	NA	NA	NA	NA	NA
	Spr	NA	NA	NA	NA	0.10
	Sum	4.2	NA	0.22	NA	0.01
<u>Stenonema</u>						
	Fall	NA	NA	NA	NA	NA
	Win	14.5	0.7	2.5	0.8	NA
	Spr	12.65	0.32	0.79	0.7	NA
	Sum	NA	NA	NA	NA	NA

Appendix 1b. Tissue metal concentrations ( $\mu\text{g/g}$  dry mass) in hydropsychids, chironomids and Stenonema at Atlanta Road, Chattahoochee River, GA The concentrations are from a single analysis of a pooled sample of individuals of each taxon. NA indicates that insufficient biomass was collected for metal analysis.

Taxa	Season	Cu	Cd	As	Pb	Hg
<u>Hydropsychids</u>						
	Fall	13.07	NA	0.31	3.1	NA
	Win	25.27	0.16	1.65	3.49	0.19
	Spr	51.6	NA	NA	13.2	0.26
	Sum	38	0.1	2.3	23.1	0.05
<u>Chironomids</u>						
	Fall	NA	NA	NA	NA	NA
	Win	41.6	0.6	2.5	26.7	0.2
	Spr	NA	NA	NA	NA	NA
	Sum	27.2	NA	1.2	17.2	0.04
<u>Stenonema</u>						
	Fall	NA	NA	NA	NA	NA
	Win	20.5	0.6	1.1	1.7	0.10
	Spr	47.7	0.2	3.6	16.1	0.13
	Sum	21.7	0.3	1.1	2.8	0.02

Appendix 1c. Tissue metal concentrations ( $\mu\text{g/g}$  dry mass) in hydroptychids, chironomids and Stenonema at Rt. 166, Chattahoochee River, GA. The concentrations are from a single analysis of a pooled sample of individuals of each taxon. NA indicates that insufficient biomass was collected for metal analysis.

Taxa	Season	Cu	Cd	As	Pb	Hg
<u>Hydroptychids</u>						
	Fall	20.84	0.7	NA	4.91	0.08
	Win	45.6	0.9	2.4	7.5	NA
	Spr	22.64	0.32	0.88	4.85	NA
	Sum	49	0.3	1.8	16.5	0.04
<u>Chironomids</u>						
	Fall	NA	NA	NA	NA	NA
	Win	88	1.8	7.7	29.9	NA
	Spr	10.1	NA	NA	5	0.35
	Sum	7.2	0.1	NA	4.5	NA
<u>Stenonema</u>						
	Fall	NA	NA	NA	NA	NA
	Win	73.7	8.4	4.7	4.7	NA
	Spr	23.08	2.31	0.72	2.09	0.13
	Sum	12.2	0.1	0.5	3.4	NA

Appendix 1d. Tissue metal concentrations ( $\mu\text{g/g}$  dry mass) in hydropsychids, chironomids and Stenonema at Franklin, Chattahoochee River, GA. The concentrations are from a single analysis of a pooled sample of individuals of each taxon. NA indicates that insufficient biomass was collected for metal analysis.

Taxa	Season	Cu	Cd	As	Pb	Hg
<u>Hydropsychids</u>						
	Fall	46.9	NA	NA	13.1	NA
	Win	NA	NA	NA	NA	NA
	Spr	49.3	0.2	3.8	7.8	0.02
	Sum	NA	NA	NA	NA	NA
<u>Chironomids</u>						
	Fall	NA	NA	NA	NA	NA
	Win	5.4	0.2	0.5	NA	0.08
	Spr	NA	NA	NA	NA	NA
	Sum	37.1	4.1	2.2	5.3	.
<u>Stenonema</u>						
	Fall	NA	NA	NA	NA	0.01
	Win	23.5	4.8	2.6	4.3	NA
	Spr	8.45	NA	1.17	0.95	0.02
	Sum	NA	NA	NA	NA	NA

## CHAPTER 5

### MERCURY, ARSENIC, CADMIUM AND COPPER DYNAMICS IN AN URBAN RIVER FOOD WEB<sup>1</sup>

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<sup>1</sup>Rosi-Marshall, E. J., J. L. Meyer, K. Neumann, E. Y. Graham, and W. B. Lyons. To be submitted to Ecological Applications.

### Abstract

Metal contamination can pose serious threats to urban river food webs and humans who consume fishes. The concentrations of mercury (Hg), arsenic (As), cadmium (Cd) and copper (Cu) were measured in components of the snag food web at four sites along the Chattahoochee River, which flows through metropolitan Atlanta. Stable isotopes of C and N were measured to indicate food web relationships. We hypothesized that an essential metal (Cu) would not biomagnify and non-essential metals (As, Cd and Hg) would. The food web components measured were suspended fine particulate matter (SFPM), collector/gathering chironomids, scraping mayflies, filtering caddisflies, catfish, carp, shad and largemouth bass. Cd and Cu concentrations were 100 and 30X lower, respectively, in fishes than in macroinvertebrates. Hg and As concentrations were 6 and 2X higher, respectively, in fishes than in macroinvertebrates. The  $\delta^{13}\text{C}$  values were within a limited range (-23 to -29‰) which is consistent with the carbon source for the higher trophic levels being predominantly derived from SFPM. Hg and As concentrations were significantly related to  $\delta^{15}\text{N}$  (an indicator of trophic position) while Cu and Cd were not. Hg and As move in similar ways in the Chattahoochee River food web. Diminution of Cu and As, and biomagnification of Cd occurred from SFPM to macroinvertebrates. Diminution of Cu and Cd occurred from macroinvertebrates to fishes. Biomagnification of Hg and As occurred from macroinvertebrates to fishes. Arsenic biomagnification does not typically occur; we attribute As biomagnification to food web structure, exposure route and source dynamics. The concentrations of As and Hg in fish muscle tissues were high enough (As ranged from 0.2-2.0 mg/kg wet wt. and Hg ranged from 0.01-0.6 mg/kg

wet wt.) to warrant concern for human consumption, especially in high risk populations such as young children and women of reproductive age.

Key words: trophic transfer, stable isotopes, seston, macroinvertebrates, fishes

### **Introduction**

Contaminants in food webs can pose serious threats to higher trophic levels, including humans (Newman 1998). When mercury (Hg) biomagnifies, concentrations in higher trophic levels can be high enough to pose a threat to humans who consume them (Newman 1998, Mason et al. 2000, US EPA 2001). Other metals such as arsenic (As) (a metalloid but hereafter referred to as a metal), copper (Cu) and cadmium (Cd) are also harmful to aquatic organisms and humans, but these metals do not typically biomagnify in freshwater ecosystems (Chen and Folt 2000, Chen et al. 2000, Mason et al. 2000).

Metals can enter food webs via several routes. Direct exposure to dissolved metals can occur to gills and tissues in all trophic levels, i.e. fishes, macroinvertebrates, and algae. Bioaccumulation from the dissolved phase has been widely examined and well modeled for some aquatic species (mainly fishes) (Playle and Dixon 1993, Hollis et al. 1999). Metals sorbed to suspended fine particles and sediments can enter the food web through trophic exposure, i.e., ingestion of contaminated particles. Metals associated with particles can enter aquatic food webs through ingestion in concentrations much greater than through gill exposure (Roditi and Fisher 1999, Schlegel et al. 1999, Wang and Fisher 1999, Lee et al. 2000).

Our main objective was to determine the extent of metal contamination in an urban river food web. The extensive point and non-point sources of metals associated with urbanization may lead to contamination of the food web; however, these systems have not been well studied (Paul and Meyer 2001). In large southeastern rivers, urbanization can lead to a high concentration of suspended solids, both organic and inorganic (Paul and Meyer 2001); therefore metals in the sorbed phase may pose a greater risk to biota than dissolved metals. In large southeastern rivers, shifting bed sediments inhibit macroinvertebrates from dwelling in the benthos and their major habitat is snags (Wallace et al. 1987, Benke and Wallace 1997). The dominant food resource for snag-dwelling organisms is suspended fine particulate matter (SFPM) (Wallace et al. 1987, Benke and Wallace 1997). Three hypotheses structured our research: (1) Organisms which rely on SFPM will be contaminated with metals in this urban river. (2) Non-essential metals (such as Hg, As and Cd) will biomagnify in higher trophic levels, specifically fishes. (3) In contrast, essential metals (such as Cu) will not biomagnify because of the ability of organisms to regulate the concentrations of essential metals.

### **Methods and Study sites**

#### Study sites

The Chattahoochee River flows through metropolitan Atlanta, and point and non-point source pollution contribute to the problem of inorganic and organic pollution throughout the basin (Frick et al. 1998). Metal concentrations in sediments and Corbicula tissues pose an ecological risk to native fauna (<http://wwwga.usgs.gov/nawqa> Jan. 2002). We sampled water column metals and collected macroinvertebrates and fishes

within and below the city of Atlanta on an unimpounded section of the river between two reservoirs (Table 5.1). The first site (MF) is in a northern section of the city of Atlanta, just downstream of the Morgan Falls impoundment, which is used for hydroelectric power generation. While this site is upstream of most of the city, it is downstream of suburban wastewater treatment plants and suburban runoff; hence, it is not an upstream reference site. The second site (ATL) is located at the Atlanta Road crossing of the Chattahoochee River. This site is just downstream of Peachtree Creek, the tributary that drains downtown Atlanta and is downstream of a major wastewater treatment facility. The third site (166) is located at the Georgia Route 166 crossing of the Chattahoochee River. This site is downstream of most of the urban runoff, a number of industrialized tributaries, and a majority of the point sources in the major metropolitan area, including municipal and industrial facilities. The final site (FRA) is at the GA Route 27 crossing of the Chattahoochee River. This site is approximately 160 km downstream of Atlanta, in Franklin, GA, just upstream of the next impoundment on the river, West Point Reservoir. This site was chosen to determine the extent of contamination downstream.

#### Collection of food web components

Three major trophic levels were chosen for analysis based on previous research of large southern riverine food webs (Wallace et al. 1987, Benke and Wallace 1997, Rosi-Marshall and Wallace 2002). The basal resource that we measured was suspended fine particulate matter (SFPM), the intermediate trophic level measured was macroinvertebrates in three functional feeding groups (filterer, scraper, and collector/gatherer), and the highest trophic level was fishes.

Water samples and SFPM were collected during base flow conditions. We used a 20 $\mu$ m mesh net placed in the flow of the river to collect SFPM (>20 $\mu$ m) for stable isotope analysis from the four sites in Oct. 1998, Jan., Apr., and Aug. 1999. Water column samples were collected for metals analysis. The concentration of metals in the sorbed fraction of the water column (>0.4  $\mu$ m) was used to represent metals associated with SFPM. Teflon bottles (1000ml for Hg, 250ml for other trace metals) were soaked in 10% HNO<sub>3</sub> for a week, then filled with fresh acid solution and kept at a sub-boiling temperature overnight. Finally, they were rinsed with DI water, filled with DI water and acidified to a pH < 2 with Optima<sup>®</sup> HNO<sub>3</sub>. The bottles were double-bagged in the lab and stored until sampling. In the field, the trace element samples were collected using the clean hand/dirty hand technique, i.e., one person (dirty hand) opening only the outer bag, and the second person (clean hand) opening the inner bag, taking the sample, putting the bottle back in the inner bag, and the first person closing the outer bag. Samples were stored on ice and transported to the laboratory for analysis.

Macroinvertebrates were collected from woody debris (snags) during October 1998, January, April, and July 1999. Individuals of the same taxon were pooled, placed in pre-acid washed HDPE bottles, allowed to depurate for 24 hours and frozen for metals analysis. A second set was collected during Jan., April, and July 1999 and frozen for stable isotope analysis. Three macroinvertebrate taxa (which consistently dominated biomass (E. J. Rosi-Marshall personal observation)) were collected: hydropsyhid caddisflies (filterers) (Hydropsyche and Cheumatopsyche), snag-dwelling chironomids (collector/gatherers), and heptageniid mayflies (scrapers)(Stenonema). These

macroinvertebrates were found at all sites and were abundant in some fish guts. No macroinvertebrate predators were selected as they were rarely found in the field.

Fishes were collected during July and August 1999 by electro-fishing and were placed on ice in the field. Upon returning to the lab, the fishes were frozen for analysis of tissue stable isotopes and metals. Fishes were much less abundant than expected at all sites, so replication was limited. The fish species that we used for analysis include carp (Cyprinus carpio (L.)), an omnivore; gizzard shad (Dorosoma cepedianum (Lesueur)), a planktivore; largemouth bass (Micropterus salmoides (Lacepede)), a piscivore; and channel catfish (Ictalurus punctatus (Rafinesque)), an insectivore and piscivore. These species were chosen because they are fished and consumed by humans in the area.

#### Stable isotope analysis

Stable isotope analysis ( $^{15}\text{N}$  and  $^{13}\text{C}$ ) was conducted on SFPM, macroinvertebrates, and fishes. Macroinvertebrate guts were removed prior to measurement, and only fish muscle tissue was analyzed. Macroinvertebrates were pooled by season for analysis. When available, more than one sample of each fish species was analyzed for each site for stable isotopes; means are reported. Stable isotope analysis was done by high temperature direct combustion and continuous flow analysis using a Finnigan Delta C Stable Isotope Ratio Mass Spectrometer (manufactured by Finnigan, Brehaven, Germany) at the Institute of Ecology Analytical Chemistry Laboratory, University of Georgia, Athens, GA.

## Metals analysis

One aliquot of each water sample was filtered through acid-cleaned 0.4 $\mu$ m Nucleopore filters and then acidified to a pH < 2 with Optima<sup>®</sup> HNO<sub>3</sub>, while the other aliquot was acidified without filtration. The filtered aliquots were analyzed for dissolved metal concentrations ( $M_{\text{diss}}$ ). The unfiltered/acidified samples were filtered just before analysis ( $M_{\text{tot}}$ ). The concentrations of metals ( $\mu\text{g/g}$ ) on the suspended particles ( $M_{\text{part}}$ ) can be calculated  $((M_{\text{tot}} - M_{\text{diss}}) / \text{TSS})$ , where TSS = total suspended solids. TSS was determined by filtering 1L of water through a pre-weighed nucleopore filter, drying and re-weighing the filter. Mercury samples were treated the same way as the trace metal samples. They were analyzed using cold vapor atomic fluorescence spectrometry (CV-AFS). Although water samples were collected at all sites during four seasons, some samples were not used because high field blanks and low recovery rates indicated the results were not reliable.

Macroinvertebrate and fish muscle tissues were placed in pre-weighed Teflon-containers, freeze-dried, and weighed again. Then 5ml Optima HNO<sub>3</sub> was added, the sample was covered and heated to sub-boiling temperatures for 1 to 2 days. A sub-sample was diluted to 2% acid using DI water and analyzed using Inductively Coupled Plasma (ICP) to obtain concentrations of metals (As, Cu and Cd). A portion of the sample material was used for Hg measurement, using a Brooks-Rand cold-vapor atomic fluorescence spectrometer (CV-AFS). We used Student's t-test to determine if the concentrations of Hg, As, Cu and Cd were different between macroinvertebrates and fishes.

## Biomagnification

The relationship of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  was used to determine the trophic interactions among taxa (Peterson and Fry 1987). To determine if metal concentrations were biomagnifying in the food web, we related the  $\delta^{15}\text{N}$ , an indicator of trophic position, to the concentrations of the various elements measured. Regression analysis was used to determine if there were any significant relationships between  $\delta^{15}\text{N}$  and metal concentrations. Secondly, we calculated the biomagnification factor (BMF) of each trophic relationship (Newman 1998, Chen et al. 2000) for As, Hg, Cu and Cd at each site. Macroinvertebrates consume primarily SFPM (Chapter 4), so we used the concentration of metals sorbed to particles as their food source to calculate BMFs [BMF = (metal concentration in macroinvertebrate) / (metal concentration in SFPM)]. Based on limited gut content analysis, fishes consumed macroinvertebrates so we used the concentration of metals sorbed to particles as their food source to calculate BMFs [BMF = (metal concentration in fish) / (average metal concentration in macroinvertebrates)]. Therefore, BMFs represent the biomagnification from SFPM to macroinvertebrates and from macroinvertebrates to fishes. BMF >1 indicates biomagnification and BMF <1 indicates diminution.

## Results

### Metal concentrations

The concentrations of As, Hg, Cu and Cd were measured in the components of the food web at sites along the Chattahoochee River (Table 5.2). Insufficient replication

prevented comparison of fishes among sites or for examining relationships between length and tissue metal concentrations. The number of analyses was limited due to the low abundance of fishes caught. The concentrations of Hg and As were significantly higher in fishes than macroinvertebrates (Student's t-test,  $p < 0.001$  and  $p < 0.03$ , respectively). Conversely, the concentrations of Cu and Cd were significantly lower in fishes than macroinvertebrates (Student's t-test,  $p < 0.0001$  and  $p < 0.002$ , respectively).

#### Stable isotopes and biomagnification

The  $\delta^{13}\text{C}$  values were within a limited range (-23 to -29‰) which is consistent with the carbon source for the higher trophic levels being predominantly derived from SFPM (Table 5.3). Gut content analysis of macroinvertebrates indicated that they consumed primarily SFPM (Chapter 4). Limited gut content analysis of fishes and  $\delta^{15}\text{N}$  values indicates that fishes consume macroinvertebrates from snags, although this is not their only food resource. Fishes are not exclusively insectivorous and have other potential trophic routes of metal exposure. Crayfishes may be an important food resource for piscivorous fishes in the Chattahoochee River (Hess 1999) and may be an important route of exposure for largemouth bass and carp. Though oversimplified, we only analyzed the biomagnification from SFPM to macroinvertebrates to fishes. In these Chattahoochee River food webs, Cu and Cd concentrations were unrelated to trophic position ( $\delta^{15}\text{N}$  values) (Figures 5.1a and b). Arsenic and Hg concentrations were positively related to  $\delta^{15}\text{N}$  (Figures 5.1c and d). The concentrations of Hg and As in the taxa of the food web of the Chattahoochee were significantly positively related to each other (Figure 5.2),

although Hg concentrations ranged over 3 orders of magnitude while As only ranged from 0.2 to 6 mg/kg DM.

Arsenic and Cu BMFs for macroinvertebrates were less than one indicating diminution (Figure 5.3). Cd BMFs for insects were generally around one; however, at the most downstream site they were all greater than one. Hg concentrations in macroinvertebrates were much greater than concentrations in the SFPM, with large BMFs. BMFs of As and Hg for fishes were usually greater than one, indicating biomagnification; conversely BMFs of Cu and Cd were less than one for fishes, indicating diminution.

### **Discussion**

The macroinvertebrates which inhabit the Chattahoochee River rely on SFPM as a major food resource (Chapter 4), which is similar to patterns observed in other southeastern rivers and streams (Wallace et al 1987, Couch et al. 1996, Benke and Wallace 1997, Hall and Meyer 1998, Rosi-Marshall and Wallace 2002). Some fishes can consume macroinvertebrates from the snags. This food web structure allowed examination of metal movement from SFPM to macroinvertebrates to fishes in the Chattahoochee River.

Biomagnification of As and Cu from SFPM to macroinvertebrates did not occur; in most cases, diminution occurred. Biomagnification of Cd from SFPM to macroinvertebrates occurred at the most downstream site, and biomagnification of Hg was very high from SFPM to macroinvertebrates at all sites. The concentration of metals in SFPM represents the metals associated with particles greater than 0.4 $\mu$ m in a water sample. These particles were acidified and the metals released were measured. However,

the macroinvertebrates that consume SFPM may not consume all particle size fractions. In addition, all of the metals associated with these particles are not necessarily in a bioavailable form. The diminution of elements from SFPM to macroinvertebrates indicates that only a fraction of the metals associated with SFPM are bioavailable. Biomagnification of Cd at the most downstream site suggest that the Cd associated with SFPM at this site is bioavailable. The high degree of Hg biomagnification indicates that Hg associated with SFPM is highly bioavailable and that macroinvertebrates do not have the means to eliminate Hg.

The concentrations of As and Hg were significantly higher in fishes than in macroinvertebrates, and the concentrations of Cu and Cd were significantly lower in fishes than macroinvertebrates. These data partly support our original hypothesis for fishes, namely that essential metals (Cu) would not biomagnify, but non-essential metals (As, Hg) would. Diminution of Cu concentrations occurred from macroinvertebrates to fishes. As and Hg biomagnified from macroinvertebrates to fishes. Cd, however, is a non-essential metal that did not biomagnify. This is most likely due to the ability of fishes to sequester metals in their liver and kidneys as a detoxification and elimination mechanism. Although we did not measure metal concentrations in livers or kidneys, Cd can be highly elevated in fish liver tissues (Mason et al. 2000, Besser et al. 2001). Liver bile can provide a route for contaminant elimination; metals such as Cd and As can be incorporated into bile and eliminated in the feces (Newman 1998). However, re-absorption of contaminants from the bile in the small intestines can lead to contaminant persistence and may lead to liver damage. Cd bound to metallothionein can be resorbed in the kidneys that can lead to increased persistence (Newman 1998). The Cd in the fishes

diets in the Chattahoochee River may lead to lower growth rates and physiological effects (Woodward et al. 1995).

Arsenic can biomagnify in aquatic systems (Mance 1987); however, As diminution occurred in lake and stream systems that received atmospheric additions of As (Chen et al. 2001, Mason et al. 2000). In coastal Maryland streams, low particulate load resulted in As predominantly found in the dissolved phase (Mason et al. 2000) and the major transfer route of As was from the water to algae. In numerous lakes throughout the northeast, As biodiminished from zooplankton to fishes (Chen et al. 2001).

In contrast, As did slightly biomagnify in the food web of the Chattahoochee River. Food web structure may be the reason for the difference in As dynamics. Arsenic is predominantly in the sorbed phase in the Chattahoochee River because of the high particulate load (Table 5.1). SFPM to which As is sorbed is the dominant food resource and is the primary route of As exposure to macroinvertebrates. In other studies the dominant food resource was algae (Mason et al. 2000, Chen et al. 2001) and the primary route of exposure is from dissolved As. The As which is taken in by algae may not be bioavailable to consumers (Mason et al. 2000), while the As sorbed to particles is bioavailable (this study). Exposure to dissolved As, leads to sorption of As to macroinvertebrate exoskeletons which is not bioavailable to fishes (Mason et al. 2000). Arsenic in macroinvertebrate tissues from ingestion of SFPM is bioavailable to fishes. Individual metal assimilation efficiencies of prey may influence the bioavailability of prey tissue metals to their predators (Reinfelder et al. 1998). Consumption of SFPM by macroinvertebrates may lead to more bioavailable As to their fish predators than metals sorbed to exoskeletons.

The source of As may also influence the As dynamics in the food web. Coal-fired power plants in the Atlanta metropolitan area contribute large amounts of As to the Chattahoochee River; there is a ten-fold increase in dissolved As concentrations below power plants (Froelich and Lesley 2001, Lesley and Froelich 2001). Arsenic from these point sources may be more bioavailable to the food web than atmospherically deposited As.

The concentrations of Hg and As in the muscle tissue of fishes pose a threat to humans consuming them, especially higher risk segments of the population such as children and women of reproductive age (Table 5.4). The US EPA recommends limiting consumption of fishes which have concentrations of As and Hg in the range measured in the fishes from the Chattahoochee River (US EPA 2000). The metal concentrations measured were in muscle tissues only, and consumption of the liver or kidneys of fishes (e.g. if whole fish were used in stews) may increase exposure to toxins (Mason et al. 2000, Besser et al. 2001).

In a survey conducted by the Upper Chattahoochee Riverkeeper at these sites on the Chattahoochee River in Atlanta, fishers upstream (at Morgan Falls) believed the fish were contaminated and did not consume fishes they caught. Downstream (Atlanta Road, Route 166 and in Franklin), most people fishing consumed the fishes they caught, at times with very high frequency (1-2 times per week). Fish consumption guidelines are published for the Chattahoochee River; however, the guidelines published in Georgia are for adult males (Georgia DNR 1999). These guidelines are available where fishing licenses are purchased, although they are somewhat complicated to interpret. The number of people surveyed who are consuming fish illustrates that these guidelines are not

effective. The Atlanta metropolitan area is populated by numerous immigrants, and the effectiveness of guidelines would greatly increase if they were available in languages other than English. The Chattahoochee River's proximity to densely populated areas and the number of people using this resource, increases the need to more adequately publicize the threat which consumption of fishes poses to humans.

The numerous point and non-point sources of Cu, Cd, Hg and As in metropolitan Atlanta (Frick et al. 1998) are most likely the dominant factor influencing the concentrations of metals in the food web of the Chattahoochee River. Throughout the world, many rivers drain urban areas (Paul and Meyer 2001), and the potential for biomagnification of metals in these systems may be similar to what we observed in the Chattahoochee River. Contamination by metals can be harmful to the species within the food web (Woodward et al. 1995) and terrestrial consumers that rely upon them, such as birds and mammals. Humans also rely on fishes from urban rivers as a food resource. The risk that these resources pose to all segments of society must be measured, and the public should be adequately informed.

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Table 5.1. Characteristics of the four study sites along the Chattahoochee River. The four sites include: Morgan Falls (MF), Atlanta Road crossing (ATL), Route 166 crossing (166) and Franklin, GA (FRA).

Variable	MF	ATL	166	FRA
Drainage area (km <sup>2</sup> )*	4110	4121	5325	6941
Mean annual flow (m <sup>3</sup> s <sup>-1</sup> )*	71.47	72.04	100.9	115.6
Runoff (cm <sup>yr</sup> <sup>-1</sup> )*	60.7	48.78	59.72	57.99
Temperature (C°)*				
Oct. 2 1998	17.3	19.6	22.2	23.4
Jan. 22, 1999	11.2	12.9	15.9	14.9
May 4, 1999	16.9	18.1	21.6	23.0
Aug. 17, 1999	20.8	24.4	29.6	32.7
Permitted Wastewater Discharge (PWD) (10 <sup>2</sup> Ls <sup>-1</sup> )	7.58	38.76	72.05	91.9

\*From Frick et al.1996

★ Measured by Neumann, K. pers.comm.

Table 5.2. Metal concentrations ( $\mu\text{g g}^{-1}$  DM) in macroinvertebrates and fishes and the sorbed concentrations ( $\mu\text{g/g}$ ) measured at four sites along the Chattahoochee River. Values are the mean of sorbed sample from the number of seasons (N) mean of pooled macroinvertebrate samples from the number of seasons (N) and the mean of the number of fishes (N) analyzed. Standard error (SE) of the means is also provided. NA indicates where samples were not available for analysis. The number of sorbed samples varies because some samples were not used because the concentrations measured were not reliable.

Taxa	MF			ATL			166			FRA		
	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
<b>Arsenic</b>												
Sorbed	2	6.6	0.56	2	15.1	10.2	2	8.7	0.33	2	23.5	0.41
Chironomids	1	0.22	--	2	1.82	0.6	1	7.73	--	2	1.38	0.8
<u>Stenonema</u>	2	1.65	0.86	3	1.94	0.83	3	1.98	1.4	2	1.2	0.7
Hydropsychids	2	2	0.39	4	1.7	0.4	4	1.68	0.4	1	3.80	--
Bass	2	4.54	0.28	1	2.94	--	1	4.26	--	2	3.44	0.45
Carp	1	2.90	0.81	0	NA	--	1	0.69	--	2	5.28	1.5
Catfish	1	4.31	--	1	4.20	--	1	6.11	--	0	NA	--
Shad	0	NA	--	3	5.02	0.31	1	6.10	--	0	NA	--
<b>Mercury</b>												
Sorbed	4	0.0002	0.0002	4	0.0002	0.0001	4	0.0003	0.0001	4	0.0003	0.0002
Chironomids	4	0.06	0.02	2	0.12	0.08	1	0.35	NA	1	0.08	--
<u>Stenonema</u>	2	0.05	0.03	3	0.08	0.03	1	0.13	NA	2	0.01	0.001
Hydropsychids	2	0.14	0.12	3	0.16	0.06	2	0.06	0.02	1	0.02	--
Bass	4	1.59	0.65	1	0.38	--	2	0.88	0.29	1	1.15	--
Carp	3	1.42	0.21	2	0.37	0.07	1	0.29	--	2	0.45	0.1
Catfish	1	0.62	--	1	0.48	--	1	0.33	--	0	NA	--
Shad	0	NA	--	2	0.12	0.03	1	0.09	--	0	NA	--

## Copper

Sorbed	1	75.4	--	1	122.2	--	1	209.8	--	1	149.7	--
Chironomids	3	5.1	0.9	2	34.4	7.2	3	35.4	27	2	21.3	16
<u>Stenonema</u>	2	13.6	9.3	3	30.0	8.9	3	36.3	19	2	16.0	7.5
Hydropsychids	3	43.7	9.6	4	38.3	8.3	4	39	7.4	2	48.1	1.2
Bass	2	0.73	0.09	1	0.9	--	1	0.8	--	2	0.5	0.03
Carp	1	0.9	--	0	NA	--	1	1.3	--	2	1.0	0.11
Catfish	1	0.8	--	1	1.1	--	1	0.9	--	0	NA	--
Shad	0	NA	--	3	1.1	0.4	1	1.7	--	0	NA	--

## Cadmium

Sorbed	0	NA	--	1	0.23	--	4	10.0	8.1	3	0.79	0.66
Chironomids	1	0.09	--	2	0.30	0.3	3	0.66	0.5	2	2.19	1.9
<u>Stenonema</u>	2	0.52	0.2	3	0.34	0.12	3	3.62	2.5	2	2.61	2.2
Hydropsychids	3	0.3	0.06	2	0.13	0.02	4	0.55	0.16	2	2.00	1.8
Bass	2	0.01	0.001	1	0.01	--	1	0.01	--	2	0.01	0.002
Carp	1	0.01	--	0	NA	--	1	0.01	--	2	0.03	0.01
Catfish	1	0.01	--	1	0.01	--	1	0.02	--	0	NA	--
Shad	0	NA	--	3	0.01	0.001	1	0.01	--	0	NA	--

Table 5.3. Mean stable isotope values of nitrogen and carbon ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) for taxa measured at four sites along the Chattahoochee River during 3 seasons. Standard errors are also provided (SE). SFPM are particles  $> 20\mu\text{m}$ .

Taxa	$\delta^{15}\text{N}$ (‰)		$\delta^{13}\text{C}$ (‰)	
	Mean	SE	Mean	SE
SFPM	8.0	0.7	-26.7	0.4
Chironomids	11.1	0.7	-26.8	1.0
<u>Stenonema</u>	12.6	1.1	-28.2	0.5
Hydropsychids	13.6	1.4	-29.3	2.2
Bass	15.7	1.2	-23.9	0.6
Carp	15.5	0.8	-23.6	0.9
Catfish	14.3	1.4	-23.6	1.0
Shad	14.7	0.7	-23.2	0.3

Table 5.4. Fish consumption guidelines for As and Hg (EPA 2001) and the proportion of fishes measured with those concentrations in the Chattahoochee River.

Concentration in fish mg/kg wet wt.	Proportion	Number of 8 oz. meals per month		
		Children	Women of reproductive age	Adults
<b>Arsenic</b>				
<0.2	0.04	2	NA	28
0.2-0.4	0.08	1	NA	14
0.4-0.6	0.13	1	NA	11
0.6-0.8	0.20	6/y	NA	8
0.8-1.0	0.29	6/y	NA	6
1.0-2.0	0.24	None	NA	5
<b>Mercury</b>				
0.01-0.03	0.13	9	30	30
0.03-0.05	0.04	4	23	28
0.05-0.08	0.17	3	13	15
0.08-0.2	0.08	1	10	10
0.2-0.4	0.47	6/y	3	3
0.4-0.6	0.12	None	1	1

### Figure Legends

Figure 5.1. The relationship of  $\delta^{15}\text{N}$  and metal concentrations ( $\mu\text{g/g DM}$ ) in the tissues of taxa collected along the Chattahoochee River: Chironomids ( $\square$ ), Stenonema ( $\diamond$ ), hydropsychids( $\circ$ ), bass ( $\blacksquare$ ), carp ( $\blacktriangle$ ), shad ( $\bullet$ ) and channel catfish ( $\blacklozenge$ ).

a) Cu ( $r^2 = 0.04$ ,  $p = 0.39$ )

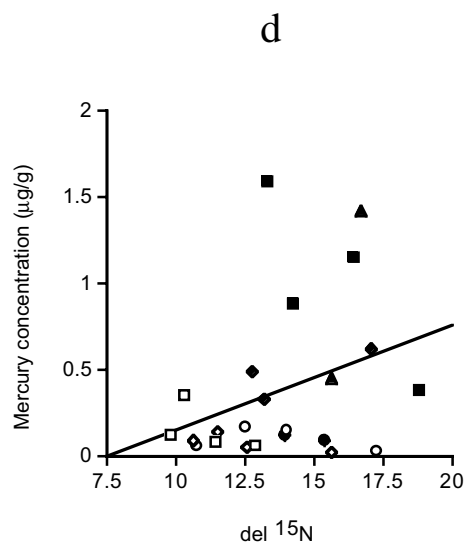
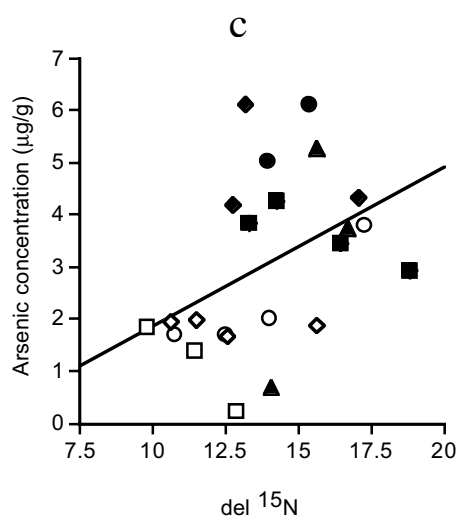
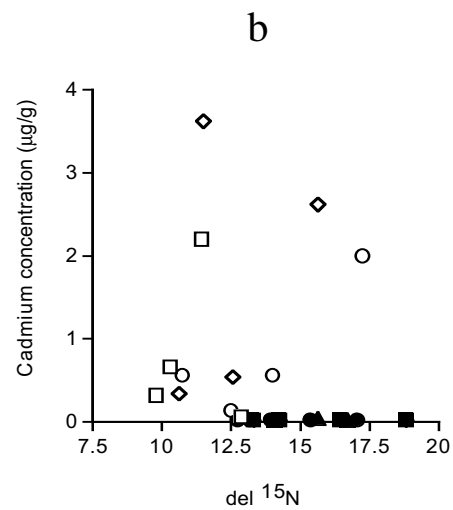
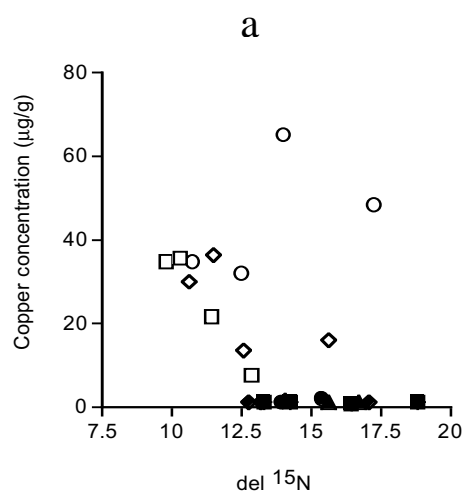
b) Cd ( $r^2 = 0.0$ ,  $p = 0.98$ )

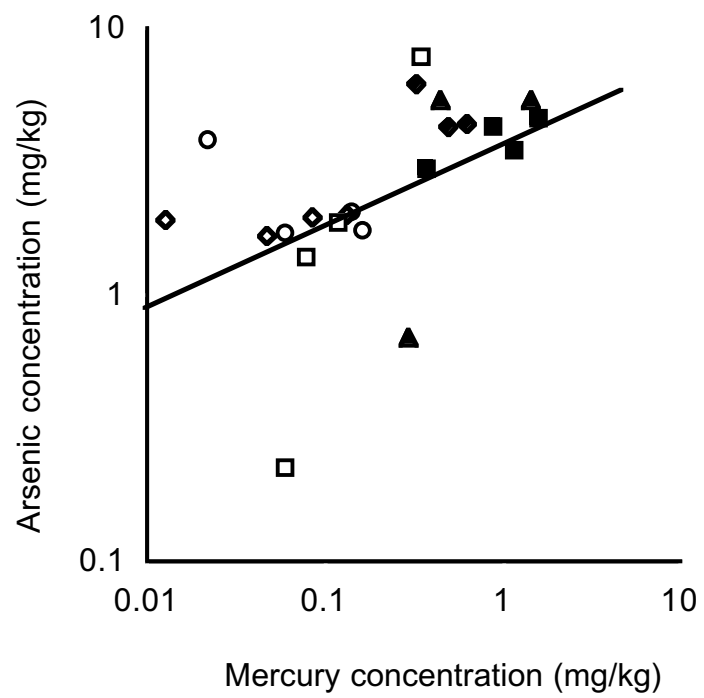
c) As ( $r^2 = 0.18$ ,  $p = 0.04$ ,  $\text{As} = -1.17 + 0.31 \delta^{15}\text{N}$ )

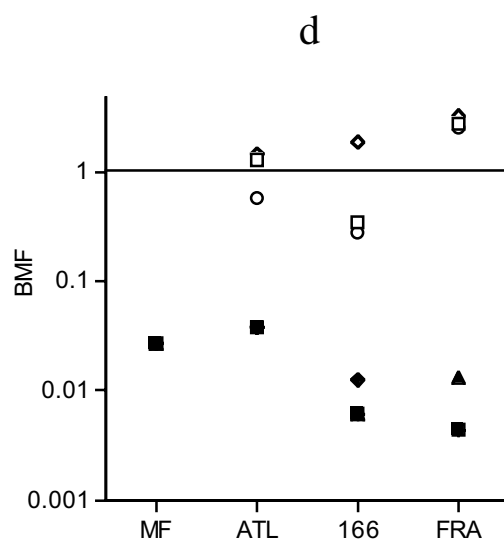
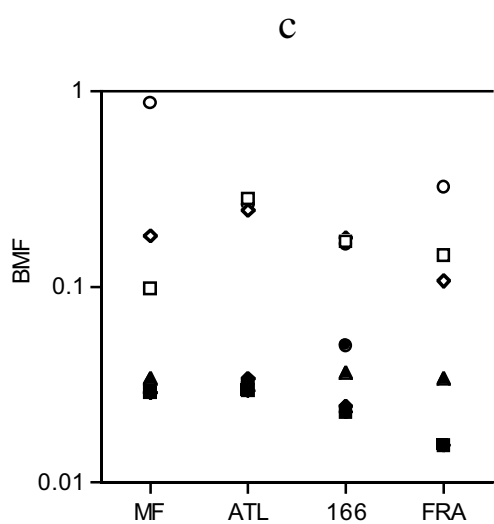
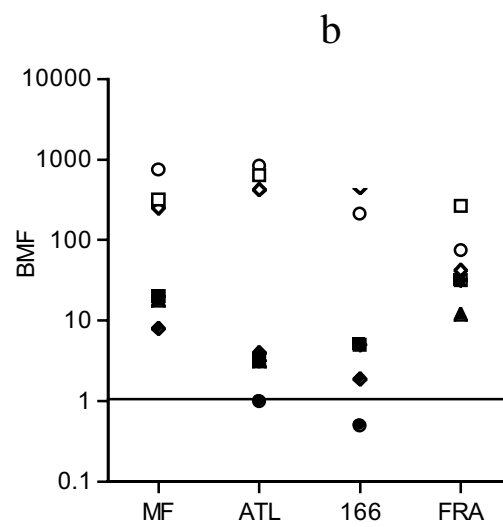
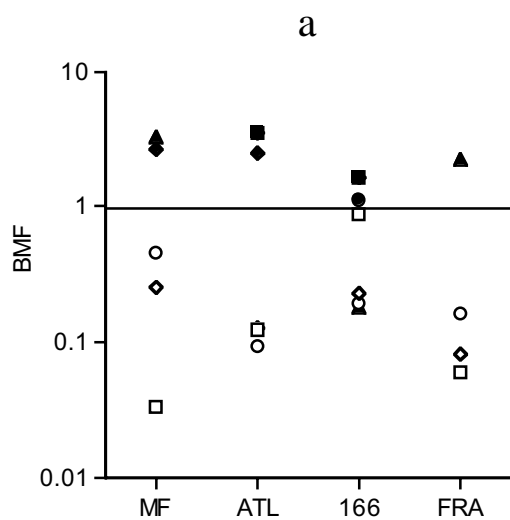
d) Hg ( $r^2 = 0.11$ ,  $p = 0.10$ ,  $\text{Hg} = -0.45 + 0.06 \delta^{15}\text{N}$ ).

Figure 5.2. The relationship of annual mean Hg and As concentrations for all sites for chironomids ( $\square$ ), Stenonema ( $\diamond$ ), hydropsychids( $\circ$ ), bass ( $\blacksquare$ ), carp ( $\blacktriangle$ ), shad ( $\bullet$ ) and channel catfish ( $\blacklozenge$ ) ( $r^2 = 0.21$ ,  $p = 0.03$ ;  $\text{Log}(\text{As}) = 1.42 + 0.28 \text{Log}(\text{Hg})$ )

Figure 5.3. The biomagnification factors (BMFs) for a) As, b) Hg, c) Cu and d) Cd in Chironomids ( $\square$ ), Stenonema ( $\diamond$ ), hydropsychids( $\circ$ ), bass ( $\blacksquare$ ), carp ( $\blacktriangle$ ), shad ( $\bullet$ ) and channel catfish ( $\blacklozenge$ ) along the Chattahoochee River. A line at BMF 1 is provided to indicate if biomagnification or diminution occurred.







## CHAPTER 6

### CONCLUSIONS AND LEGAL IMPLICATIONS

The quality of suspended fine particulate matter (SFPM) can vary naturally along a river continuum (Chapter 2). Using the predictions of the RCC (Vannote et al. 1980), I hypothesized that SFPM quality would decline along a river continuum as labile fractions were used. However, as measured by instantaneous growth rate (IGR) of chironomids, SFPM quality was significantly higher at the most downstream site than the most upstream site along the Little Tennessee River continuum. Traditional measurements of SFPM quality (N/C, calories, lipids, % diatoms, and % inorganic) were not related to instantaneous growth rates of chironomids fed SFPM. High secondary production of filter feeders measured at the downstream site of the Little Tennessee River (Grubaugh et al 1997) can be attributed to high quality SFPM and high quality stable habitat.

These results led me to predict that SFPM collected from an urban river, with numerous point and non-point sources of contaminants, would be of decreased quality (Chapter 3). I hypothesized that SFPM samples collected downstream of a major metropolitan area would be of reduced food quality for aquatic insects. SFPM collected during high flow should have higher concentrations of contaminants from non-point source run-off; hence, I hypothesized that the quality of SFPM collected during high flow would decrease downstream of the city more than that collected during base flow. Using permitted waste water discharge as a correlate of urbanization and IGR of chironomids

fed SFPM as a bioassay of SFPM quality, the quality of SFPM collected during base flow steadily declined with increasing urbanization. In contrast, the quality of SFPM collected during high flow did not correlate with urbanization; however, SFPM was of consistently low quality. In comparison to IGRs of chironomids fed SFPM collected from the Little Tennessee River, IGRs of chironomids fed SFPM collected from the Chattahoochee River were 5X lower. Although, bioassay measures of SFPM quality in the Chattahoochee River could not be attributed to traditional measures of quality or metal contamination, some aspect of urbanization appears to reduce the food quality of SFPM.

Because SFPM is an important food resource, it may also be a conduit of metals in the food web. I hypothesized that macroinvertebrate tissue concentrations of metals would increase downstream of Atlanta (Chapter 4). Macroinvertebrates from three functional feeding groups were collected during four seasons at four sites along the Chattahoochee River. Copper (Cu), cadmium (Cd), lead (Pb), arsenic (As) and mercury (Hg) concentrations were not significantly different among taxa or sites although temporal variability differed among taxa and sites. Factors such as diet, body size, dissolved and sorbed water column metal concentrations were not significantly related to tissue metal concentrations.

Finally, I examined the dynamics of Cu, Cd, As and Hg in the Chattahoochee River snag food web (Chapter 5). I hypothesized that an essential metal (Cu) associated with SFPM would not biomagnify in the food web and non-essential metals (Cd, As, and Hg) would. SFPM is an important food resource to the Chattahoochee River food web. Stable isotopes of  $^{15}\text{N}$  and  $^{13}\text{C}$  indicated the trophic relationships among taxa and arsenic and mercury concentrations were related to  $\delta^{15}\text{N}$ , an indicator of trophic position.

Biomagnification of Hg occurred from SFPM to macroinvertebrates to fishes. Arsenic biomagnified from macroinvertebrates to fishes. Biomagnification of Cd and Hg and diminution of Cu and As occurred from SFPM to macroinvertebrates. Diminution of Cu and Cd occurred from macroinvertebrates to fishes. The concentration of As and Hg in fishes are high enough to warrant concern for humans consuming them.

These findings have implications for water quality regulations. To explore these implications, I will first summarize current criteria used to regulate metals and how changes in these criteria have affected water quality in Georgia. Combining this information with data from the Chattahoochee River, I then suggest some changes in policy that should reduce metal contamination in aquatic food webs.

#### Current Metals Criteria

Metal contamination of streams throughout the US poses a threat to water quality, ecosystem and human health; therefore, metals are regulated to protect water quality. The US Environmental Protection Agency (US EPA) sets minimum water quality standards as guidelines for state standards (US Federal Water Pollution Control Act § 307, 1997). These guidelines are used to develop state criteria and set permit limits. In 1995, the US EPA promulgated a change in metals criteria for surface waters (US FWPCA§ 307, 1997). This change allowed states to use dissolved metal concentrations rather than total recoverable metal concentrations. In Nov. 1998, Georgia incorporated these changes in the Georgia Rules for Water Quality Control (Chapter 391-3-6) (Table 6.1).

Dissolved concentrations are obtained by filtering river water through a 0.45 µm filter and then acidifying and measuring the metal concentrations in the filtrate. Total

recoverable concentrations are measured by acidifying an unfiltered water sample and then measuring the metals in the total sample. Metals readily sorb to fine particles (Rao et al. 1993 and references therein, Maki and Hermansson 1994) and in surface waters with high concentrations of suspended particles, such as clays and organic material, sorbed metals that are removed through filtration can be significant. Concentrations for the new criteria are similar to the concentrations for the previous criteria (Table 6.1). This means that the new criteria permit much higher concentrations of total recoverable metals and are less stringent than previous criteria.

The US EPA promulgated the criteria change to reflect scientific findings at the time. “It is now the policy of the Office of Water that the use of dissolved metal concentrations to set measurable compliance with water quality standards is the recommended approach, because dissolved metal more closely approximates the bioavailable fraction of metal in the water column than does total recoverable” (Office of Water Memorandum 1993). Essentially, in laboratory toxicity tests, the dissolved fraction was the only fraction bioavailable; therefore the criteria were changed. It is important to note that current water quality criteria are only designed to protect biota from levels of toxicity usually measured with endpoints such as lethality (Office of Water Memorandum 1993). Criteria are not designed to deal with chronic trophic exposure. The memorandum goes on to state that “The ambient water quality criteria are neither designed nor intended to protect sediment or to prevent effects due to food webs containing sediment dwelling organisms”.

To deal with multiple effects of metals, the US EPA is currently working towards an integrated approach to metals (Figure 6.1). The sorbed metals associated with

suspended particulates (SFPM) are currently considered not bioavailable and the US EPA views them as an ecological and toxicological dead end. The US EPA Science Advisory Board (SAB) reviewed the recommendations of the US EPA and stated:

...that the conceptual model presented (Figure 6.1) does not include some potentially important considerations. The labeling of suspended particles as “not bioavailable” raises particular concern because suspended solids in the water column bind metals and are a major source of food for filter feeders (SAB 2000).

In the US EPA's official response to the SAB (US EPA 2000), they stated that they would incorporate these recommendations in their integrated approach as their model matures.

While my data did not demonstrate that macroinvertebrate concentrations were related to sorbed concentrations, the data clearly showed that metal concentrations in macroinvertebrates were not related to dissolved concentrations (Chapter 4). Recent laboratory studies confirm that tissue metal concentrations in biota are most related to the concentration of metals associated with their food particles, not with dissolved metals (Roditi and Fisher 1999, Schlegel et al. 1999, Lee et al. 2000).

#### Effect of criteria change on Georgia water quality

When this change was made in 1998, I hypothesized that the change would have ramifications for Georgia water quality (Rosi 1999). I also predicted that dissolved metal concentrations in the Chattahoochee River would be below the new criteria limits, but the food web would be contaminated. To test these predictions, I examined the 1998 and 2000 Georgia 305B lists to determine the effect of the metals criteria change on the status of streams throughout Georgia. The Georgia 305B list is a comprehensive listing of stream miles in Georgia that are partially or not supporting their designated use and the reason for the violation. Numerous rivers throughout the state were affected by the

change in metals criteria (Rosi-Marshall et al. 2001) (Table 6.2). For streams partially meeting designated uses in 1998, 398 stream miles are no longer listed for violations of metals criteria and 46% of these stream miles were removed entirely from the list. For streams not meeting designated uses in 1998, 545 stream miles are no longer listed for violations of metals criteria and 42% of these stream miles were removed entirely from the list. In a matter of two years, 943 miles of Georgia streams are now defined as free of metal contamination. Stream miles that are no longer listed as being in violation of metals criteria may have changed status due to the regulation change or better data obtained using clean techniques.

Streams listed on the 305b list require total maximum daily loads (TMDLs) to be calculated for them. The stream miles which have been removed from the list no longer require TMDLs. Calculating and implementing TMDL is a costly and scientifically challenging endeavor; however, the use of TMDLs should provide a means to adequately protect surface waters from receiving pollution at levels which are harmful to the system. The TMDL process was developed to link point and non-point sources of contaminants in a water body. The total maximum daily load (TMDL) of a contaminant that the receiving stream can withstand is calculated. A margin of safety is usually included in the TMDL; however, gaps in regulations and scientific understanding of the most effective methods to regulate metals suggest that the margin of safety should be increased. Each waste source is allocated a portion of the TMDL and permit limits are then established. Through implementation of the permit limits and non-point source run-off control, the receiving stream should support its designated use. Currently in Georgia, TMDLs for metals consider point and occasionally non-point sources. Implementing TMDLs that

include non-point sources of metals, such as urban run-off, is difficult. However, there are a number of technologies available for controlling non-point sources, such as run-off settling basins, riparian corridors as filters, and treatment of urban run-off by treatment facilities (Riley 1998). In metropolitan Atlanta, urban and suburban run-off drains directly into streams. If technological advances in non-point source run-off control devices were implemented, the amount of metals entering the Chattahoochee River would likely decrease.

#### The Chattahoochee River below Atlanta

In 1998-1999, the dissolved concentrations of Cu, Cd, As, and Pb were consistently lower than the new criteria limit in the Chattahoochee River (Neumann and Lyons, unpublished data). This is in contrast to the total recoverable concentrations which approached and at times exceeded the previous total recoverable limits for Cu, Cd and Pb (Neumann and Lyons, unpublished data); however, metals in the food web pose a threat to biota in the system. The concentrations of metals in macroinvertebrates were as high as measured at other contaminated sites (Chapter 4), and concentrations in macroinvertebrates were not related to the concentration of dissolved or sorbed metal concentrations in the water column. In addition, an exposure route of metals to macroinvertebrates is through consumption of contaminated SFPM (Chapters 4 and 5), which is not regulated by current criteria. Arsenic concentrations in fishes are high enough to warrant concern for humans consuming them (Chapter 5). Cu and Cd were low in the fishes, but metal concentrations in liver and kidneys were not measured. These

results clearly show that although metal concentrations in the Chattahoochee River are well below the dissolved criteria, the food web is contaminated.

#### Policy Recommendations

The findings of this study indicate that metals standards adopted in 1998 are not effective for protecting Georgia surface waters. The dramatic reduction in the number of stream miles listed in the 305B list implies that a large number of streams in Georgia are now free of metal contamination compared to 2 years ago. This was due to a change in the criteria and implementation of clean techniques and should not be viewed as a dramatic improvement in water quality in Georgia streams.

The criteria change resulted in the Chattahoochee River changing status from contaminated to clean. The dissolved concentrations of Pb, Cd and Cu are well below the new limits; however, the food web is contaminated at many levels (from SFPM to fishes). Metal concentrations in macroinvertebrates were not related to dissolved or sorbed concentrations in the water column, suggesting that dissolved or sorbed concentrations are not related to bioavailable metals. The Chattahoochee River exemplifies why the new criteria are not sufficiently protective of biota.

Currently, criteria exist for the dissolved fraction of metals in a surface water and sediment criteria are being developed by the US EPA (SAB 2000). Dissolved regulations ignore trophic routes of exposure to sorbed metals. The US EPA is developing an integrated approach to metals; it is crucial that suspended sediments be included.

Dissolved criteria have serious problems in addition to ignoring other routes of exposure. Synergistic effects of dissolved metals are currently not considered. Regulations should at least assume that additive effects of numerous metals in a water

body occur and criteria should regulate the suite of contaminants present. In addition, it is not technologically feasible to continuously monitor dissolved metal concentrations in the field, and dissolved samples are easily contaminated. Water chemistry can influence the fraction of dissolved metals, and developing permits that adequately reflect the natural variability in the receiving body is very difficult. Dissolved metals are not the sole route of exposure in aquatic ecosystems. Regulations limited to dissolved concentrations protect biota from only a fraction of available metals.

The US EPA's integrated approach to metals (Figure 6.1), recommends the development of a criterion based on tissue residue, which I support. There are a number of advantages to using biota: organisms continuously sample the exposure regime and contamination of samples is much less of a problem. Despite these advantages, development of criteria based on tissue concentrations will be difficult. Research must be done which establishes tissue residue concentrations that result in toxicity. The concentration of metals that cause negative effects is difficult to establish, especially when multiple metals are involved; however, dissolved and sediment criteria must contend with the same problems. To obtain an accurate assessment of the bioavailability of metals in a surface water, measurement of the biota is crucial.

I conclude that the current standards are not adequate to protect aquatic food webs from metal contamination. Regulating dissolved concentrations may be protective, if used in combination with sediment, suspended sediment, and biotic criteria. While the US EPA has developed an integrated approach to metals, only dissolved metals are currently regulated. Development of sediment criteria has taken years and if the same pace is applied to development of criteria based on tissue residue, they will not be in place for

decades. In the interim, the EPA should reevaluate its dissolved criteria concentrations. If the Chattahoochee River does not exceed criteria despite the fact that its fishes have concentrations at which the EPA recommends limiting human consumption, surely the current dissolved criteria are not sufficiently protective. In addition, increased margins of safety (MOS) should be incorporated into the TMDL process to adequately protect biota while more adequate criteria are being developed. In the meantime, metals continue to enter aquatic ecosystems, posing a threat to food webs and humans. The persistence of metals and the slow pace of regulatory reform may lead to a legacy of contaminated food webs.

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Table 6.1. The previous total recoverable criteria concentrations and the new 1998 dissolved criteria.

Metal	Previous total recoverable criteria ( $\mu\text{gL}^{-1}$ )	Current dissolved criteria ( $\mu\text{gL}^{-1}$ )
As	50	50
Cd	0.7	0.62
Cr VI	11	11
Cu	6.5	6.2
Pb	1.3	1.2
Ni	88	88
Se	5.0	5.0
Zn	60	60

Table 6.2. Changes in the listing of streams, by river basin, from 1998 to 2000. Number of stream miles that were listed as violating metals criteria in 1998 305B list and were not listed in the 2000 305B list and the number of streams that were completely removed from the 305B list are given for streams partially and not supporting designated uses.

Basin	Partially Supporting Designated Uses		Not Supporting Designated Uses	
	Stream miles no longer listed for metals	Stream miles de-listed	Stream miles no longer listed for metals	Stream miles de-listed
Altamaha	23	0	0	0
Chattahoochee	97	94	192	108
Coosa	3	0	64	1
Flint	0	0	55	0
Ochlockonee	0	0	5	0
Ocmulgee	17	2	105	64
Oconee	0	0	14	10
Ogeechee	61	2	1	0
Satilla	43	43	23	0
Savannah	142	40	9	2
Suwanee	12	5	77	40
Total	398	184 (46%)	545	225 (42%)

### Figure Legends

Figure 6.1. US EPA's integrative approach to metals (redrawn from SAB 2000).

