

Natural abundances of stable isotopes trace anthropogenic N and C in an urban stream

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Abstract. Important ecological services of low-order streams are greatly affected by urbanization. North Buffalo Creek, in the headwaters of the Cape Fear River basin in Greensboro, North Carolina, receives point- and nonpoint-source pollutants. Natural abundances of the stable isotopes of C (^{13}C) and N (^{15}N) were used to determine the influence of anthropogenic nutrients on seston $\delta^{15}\text{N}$, nutrient concentrations, C/N ratios, and patterns of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in foodweb components in North Buffalo Creek during different hydrological conditions. Baseflow seston $\delta^{15}\text{N}$ varied significantly among sampling sites. Baseflow seston $\delta^{15}\text{N}$ ranged from 3.7‰ to 4.6‰ at forested sites and was slightly enriched at open sites, and probably reflected nonpoint sources of N in North Buffalo Creek. Seston $\delta^{15}\text{N}$ also reflected point sources of N in North Buffalo Creek. The most enriched seston $\delta^{15}\text{N}$ values (8.4‰) were found directly downstream of the Waste Water Treatment Plant (WWTP). Seston $\delta^{15}\text{N}$ values at the Rankin Mill Road (Rankin) site, several km downstream of the WWTP, also were strongly influenced by effluent from the WWTP. The Summit Avenue site (Summit) received textile effluent until June 2001. Before June 2001, seston ^{15}N at Summit was depleted compared to seston ^{15}N at sites upstream of the plant, probably because the textile effluent was depleted in ^{15}N . During storms, seston $\delta^{15}\text{N}$ was negatively correlated with nutrient concentrations upstream of the WWTP. However, at Rankin Mill Road, seston $\delta^{15}\text{N}$ was positively correlated with NO_3^- flux, which explained 54% of the variation in seston $\delta^{15}\text{N}$. $\delta^{15}\text{N}$ was not correlated with NH_4^+ and PO_4^{3-} fluxes at Rankin Mill Road. During storms, seston $\delta^{15}\text{N}$ was influenced partially by nonpoint sources of N, a pattern consistent with observed C/N ratios. $\delta^{13}\text{C}$ values for most foodweb components and $\delta^{15}\text{N}$ values for all foodweb components varied significantly between sites, suggesting that sources of C and N differed among sites. Foodweb components at Rankin were enriched in ^{15}N from incorporation of sewage-derived N from the WWTP effluent, whereas, prior to June 2001, foodweb components at Summit appeared to be depleted in ^{15}N from incorporation of textile effluent. Thus, specific influences of point sources of N could be distinguished in foodweb components. Nonpoint sources and stormwater influenced seston $\delta^{15}\text{N}$ during storm events, but these sources could not be distinguished in consumers by using natural abundances of stable isotopes.

Key words: urban streams, $\delta^{15}\text{N}$, $\delta^{13}\text{C}$, wastewater, storm events, food webs, anthropogenic nutrients, C/N ratio.

Low-order streams are at risk of disturbance from urban landuse practices (Resh et al. 1988, Meyer and Wallace 2001, Peterson et al. 2001). This risk is a significant concern because low-order streams are spatially and functionally important in watersheds, they constitute most stream miles (Hynes 1975), and they serve as sites of N processing and retention (Meyer et al. 1988, Meyer 1997, Peterson et al. 2001). Disruption of the nutrient dynamics of low-order streams may have downstream repercussions in

streams, lakes, and estuaries, where water-quality problems are often very serious (Vitousek et al. 1997).

Streams are often nutrient-limited, and addition of nutrients may alter stream community structure (Dodds and Welch 2000). Urbanization of watersheds increases delivery of all forms of N from impervious surfaces (Characklis et al. 1979, Soranno et al. 1996, Corbett et al. 1997, Albanese and Matlack 1998), lawn-fertilizer runoff (Wahl et al. 1997, Carpenter et al. 1998), pet waste (NCDENR 2004), construction sites (Carpenter et al. 1998), nutrients in precipitation (Silva et al. 2002), stormwater runoff (Silva et al. 2002), leakage or overflow of wastewater sewers (Seager and Abrahams 1990, City of Greensboro Storm Water Services 2000), and sewage effluent

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(e.g., Hershey et al. 2004). For example, Wahl et al. (1997) found $11\times$ more $\text{NO}_x\text{-N}$ loading into a stream from an urbanized watershed than into a stream from a forested watershed. Increased delivery of P from sewage effluent and runoff of construction-derived sediment also is a problem in urban watersheds (Carpenter et al. 1998, Paul and Meyer 2001).

Stable isotopes of N have been used successfully to detect anthropogenic N in aquatic systems. Different pools of N can be identified and traced because they have distinct $^{15}\text{N}/^{14}\text{N}$ ratios (Peterson and Fry 1987, McClelland et al. 1997, Robinson 2001). Natural abundances of ^{15}N have been used to trace wastewater influence in aquifers (Kreitler and Browning 1983, Aravena et al. 1993) and in marine (Rau et al. 1981, Valiela et al. 1992, Van Dover et al. 1992, McClelland and Valiela 1998a, b), lotic (Wayland and Hobson 2001, deBruyn and Rasmussen 2002), and lentic (Hansson et al. 1997) systems. Natural abundances of ^{15}N also have been used to trace N fertilizer in streams (Kohl et al. 1971) and animal wastes in groundwater (Karr et al. 2001, 2002). Differing isotopic signatures in aquatic systems can be caused by a number of different disturbances and, therefore, may be useful for evaluating how these disturbances have altered ecosystem processes and pathways of material through the system (Peterson 1999).

Stable isotopes also are useful tools for foodweb analysis. $\delta^{15}\text{N}$ can be used to determine trophic positions of foodweb components (Peterson and Fry 1987, Fry 1991, Robinson 2001) because $\delta^{15}\text{N}$ of each trophic level is typically ~ 3 to 5‰ higher than its source of N (Minigawa and Wada 1984, Peterson and Fry 1987, Fry 1991, Robinson 2001). ^{15}N -tracer experiments (Mulholland et al. 2000) and the natural abundance of ^{15}N (e.g., Richey et al. 1975, Mathisen et al. 1988, Kline et al. 1990) have been used successfully in studies of trophic interactions. $\delta^{13}\text{C}$ also can be used to trace C pathways in food webs because the $\delta^{13}\text{C}$ of each component of a food web is 0‰ to 1‰ higher than its source of C (DeNiro and Epstein 1978, Rounick and Winterbourn 1986, Peterson and Fry 1987, Michener and Schell 1994, McCutchan et al. 2003).

Seston is an important component of stream systems. Seston is a food source for filter-feeding organisms (Cummins 1974), plays a role in nutrient processing (Wallace et al. 1991), and is

a major pathway of organic matter transport and export throughout the watershed (Wallace and Grubaugh 1996). Seston is derived from a variety of sources, including terrestrial organic matter, autochthonous production, and fecal and animal detritus (Cummins 1974). Thus, seston $\delta^{15}\text{N}$ should integrate the ^{15}N signature of several sources of N, including anthropogenic sources.

The 1st objective of our study was to determine if the natural abundance of seston ^{15}N could be used to detect anthropogenic sources of N in an urban stream. Two questions were of particular interest: 1) How does seston $\delta^{15}\text{N}$ change as seston moves through a city, i.e., upstream to downstream and through areas of different land use? 2) How does seston $\delta^{15}\text{N}$ fluctuate with discharge (i.e., during storms)? We hypothesized that $\delta^{15}\text{N}$ of seston would reflect $\delta^{15}\text{N}$ of anthropogenic point sources of N and could be used as a tool to identify those sources. We also expected seston $\delta^{15}\text{N}$ signatures to reflect $\delta^{15}\text{N}$ signatures of anthropogenic, nonpoint sources of N, but that it might be difficult to determine the sources given the complexity and number of anthropogenic nonpoint inputs in urban streams (Duda et al. 1982). Nutrient concentrations tend to reflect point sources at base-flow conditions and nonpoint sources during storms (Carpenter et al. 1998). Therefore, we hypothesized that the $\delta^{15}\text{N}$ signature of the seston would fluctuate with discharge. To test these hypotheses, we examined nutrient concentrations and the $\delta^{15}\text{N}$ signature of the seston along a transect of changing land use during base flow, and during ascending and descending limbs of the stream hydrograph.

The 2nd objective of our study was to determine which foodweb components of an urban stream assimilated anthropogenic sources of N and to determine how foodweb dynamics differed among sites. We hypothesized that anthropogenic inputs would alter the flow of energy and nutrients through the food web and cause foodweb components from different sites to reflect the C and N signatures of the anthropogenic sources. To test this hypothesis, we examined changes in, and distribution of, stable isotopes of N and C in the food web of an urban stream at sites that differed in point and nonpoint sources of pollution.

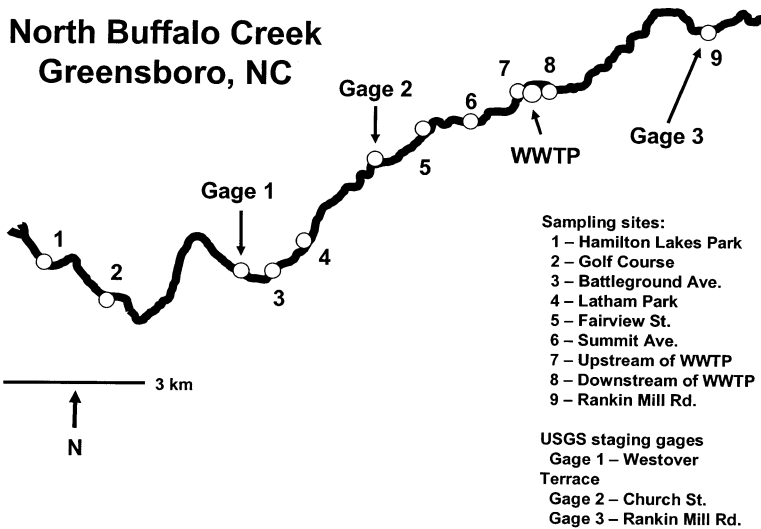


FIG. 1. Sampling sites on North Buffalo Creek, Greensboro, North Carolina. WWTP = Waste Water Treatment Plant, USGS = US Geological Survey.

Methods

Site description

Our research was conducted in a 4th-order section of North Buffalo Creek in Greensboro, North Carolina. Greensboro is a growing city with a population of 224,000. The city spans 296 km² and is located in the originating headwaters of the Cape Fear River Basin. North Buffalo Creek is a typical urban stream. It was recognized as impaired in the Cape Fear Basinwide Water Quality Management Plan (NCDENR 2000) based on instream habitat degradation, impaired biological communities, and the presence of fecal coliform bacteria.

Nine sampling sites, distributed along the length of the stream (Fig. 1), were chosen based on land use adjacent to the stream. Average discharge during sampling ranged from 0.67 m³/s to 1.97 m³/s at the 3 US Geological Survey

(USGS) staging gages along the stream (Table 1). Site 1, the most upstream site on the stream transect was Hamilton Lakes Park (Hamilton), a headwater tributary of North Buffalo Creek. This site was in a residential area and had a mixed conifer–hardwood riparian zone. Hamilton was considered the most pristine site, relative to other sites, because the channel meandered, large woody debris was present, and the riparian vegetation consisted of intact forest. However, the watershed above Hamilton was entirely within an upper-middle-class residential area in the City of Greensboro. Thus, the site received stormwater runoff, which could have been contaminated with lawn fertilizer, pet waste, and other materials. Contamination from leaky sanitary sewer pipes also may have been present. Hamilton was not sampled during dry periods of the year because of no or low flow. Site 2, Golf Course, was downstream from a golf course. The site had little shading from riparian vegetation. Sites 3 and 4, Battleground and Latham, were along Greensboro’s greenway park system, which follows a power-line corridor. These sites were maintained by regular mowing, had few trees, and were often subject to full sun. Site 5, Fairview, was in an industrial section of Greensboro, 3.39 stream km downstream of Latham. This site was downstream of a section of stream that ran under a building, and it was upstream of Cone Mills textile plant. This site

TABLE 1. Daily mean and maximum discharge at 3 US Geological Survey gages along the North Buffalo Creek transect during sampling events.

Gage location	Mean discharge (m ³ /s)	Maximum discharge (m ³ /s)
Westover	0.67	3.59
Church	0.89	4.73
Rankin	1.97	9.40

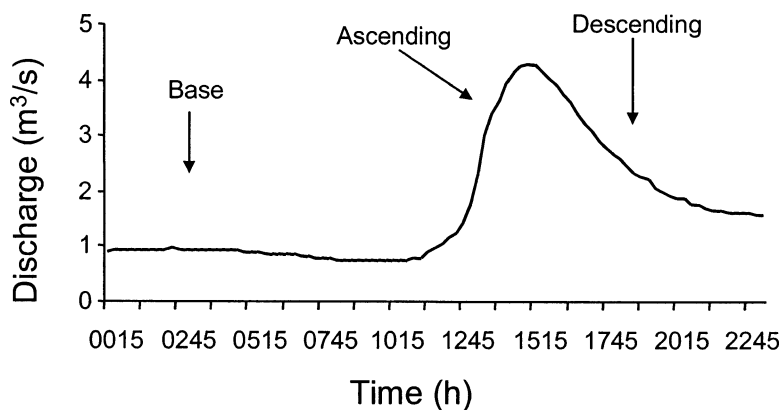


FIG. 2. Sample hydrographic data from the US Geological Survey staging gage located at Rankin Mill Road outside Greensboro, North Carolina. Arrows indicate possible sampling times during baseflow, and ascending and descending portions of the hydrograph.

had some shade, but part of the site was adjacent to a baseball field, so sections of the stream were subject to full sun. Site 6, Summit Avenue (Summit), was just downstream of Fairview and Cone Mills textile plant. This site was forested. The Waste Water Treatment Plant (WWTP) was ~1.48 stream km downstream of Summit. Sites 7 and 8 were immediately upstream and downstream, respectively, of the WWTP. Samples at these sites were taken directly upstream and downstream of the WWTP from the facility bridges, which were 0.58 stream km apart. WWTP effluent also was sampled. Site 9, Rankin Mill Road (Rankin), was ~5.94 stream km downstream from the WWTP, and a distinct odor of WWTP effluent was present. Except for a landfill immediately downstream of the WWTP, the stream was forested between the WWTP and Rankin.

Influence of Cone Mills textile effluent on Summit

Until June 2001, Summit received industrial effluent from Cone Mills textile plant. The presence of industrial discharge was apparent as far downstream as the WWTP (WWTP personnel, North Buffalo Creek WWTP, personal communication) because the effluent often contained dye that colored the streamwater. Cone Mills was cited repeatedly for violating effluent toxicity limits (NCDENR 2000) until it was connected to a WWTP facility in June 2001. Data collected from Summit before June 2001 are presented separately in our study. Data collected

from Summit after June 2001, are presented as part of the stream transect through the city.

Seston stable isotope and nutrient sampling

Seston was sampled during baseflow (defined as no rain for 72 h prior to sampling) (8 dates), and the ascending (5 dates) and descending (5 dates) limbs of the stream hydrograph (Fig. 2) during rainfall from October 2000 to June 2002. Baseflow samples were collected from all 9 sites so that variation among seasons and sites could be analyzed. Sites 7 and 8 (upstream and downstream of the WWTP) were not sampled on ascending or descending limbs of the hydrograph because it was difficult to sample at the WWTP on short notice.

Water for seston samples was collected in 4-L cubitainers, returned to the lab, and filtered onto precombusted glass-fiber filters (Gelman Sciences, Ann Arbor, Michigan). The filters were dried at 50°C for ≥ 48 h, and the seston was scraped from the filter with a stainless steel blade for stable isotope analysis.

Nutrient samples were collected concurrently with seston sampling. NO_3^- (Cd-reduction method), NH_4^+ (sodium salicylate and dichloro isocyanuric method), and PO_4^{3-} (ascorbic acid method) concentrations (APHA 1989) were measured using a Bran+Luebbe TRAACS 2000 autoanalyzer. Nutrient flux (mg/s) was calculated from discharge (L/s) and nutrient concentration (mg/L). Discharge was calculated using data from the 3 USGS gages located on Westov-

er Terrace, Church Street, and Rankin Mill Road (Rankin sampling site) along North Buffalo Creek. Distances of USGS gages and sampling sites from the headwater (Hamilton) were determined by digitizing images of North Buffalo Creek from 7.5-min series USGS topographic maps (1:24,000; Greensboro and McCleansville quadrangles, Guilford County, North Carolina). Daily average discharge from each gage and stream distance from the headwater were fit to a regression model to estimate discharge for each sampling site and date for those sites located between USGS gages (Ulseth 2003). Nutrient flux was calculated for each sampling date by multiplying discharge by concentration for each nutrient species at each site.

Foodweb sampling

Sampling was done approximately every 2 mo from June 2001 to June 2002 during baseflow. Hamilton, Latham, Fairview, Summit, and Rankin (Fig. 1) were selected for foodweb sampling on the basis of land use adjacent to the site or because the site was known to receive urban point-source discharges. Key components of the food web sampled for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses included seston, algae, moss, conditioned leaves, hydropterygids caddisflies, 2 crayfish species (*Cambarus hobbsorum* and *Procambarus acutus*), and Asian clams (*Corbicula fluminea*). Seston was expected to be formed from a combination of sources, especially leaf detritus and algae (e.g., Allan 1995) (see below). Therefore, algae, moss, and conditioned leaves were collected from several patches within a site to provide composite samples that would be as representative as possible of source materials of seston for the stream food web. These samples were checked for macroinvertebrates, which were removed before the samples were rinsed with deionized (DI) water and immediately dried. Hydropterygids caddisflies and Asian clams were kept in filtered stream water for 24 h to allow gut contents to clear before the animals were prepared for isotope analysis. Asian clams were removed from their shells prior to preparation. Crayfish were frozen (-15°C) as soon as they were brought in from the field; the tail muscle was dissected later and prepared for isotope analysis.

Stable isotope analyses

All samples for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses were dried at 50°C for ≥ 48 h. Samples were homogenized, weighed, and placed in 4×6 -mm tin capsules. Several samples were checked for the presence of carbonates by looking for bubbles following the addition of a drop of 10% HCl. No bubbles were seen, so samples were not acidified. Macroinvertebrate samples consisted of ~ 1 to 7 individuals to ensure enough dry mass for isotope analysis.

Stable isotopes of C and N were analyzed using a Thermo-Finnigan DELTA-plus Advantage Mass Spectrometer coupled to a Carlo-Erba NC2100 Elemental Analyzer (EA) at the Colorado Plateau Stable Isotope Lab (Northern Arizona University, Flagstaff, Arizona). Ratios of $^{13}\text{C}/^{12}\text{C}$ and $^{15}\text{N}/^{14}\text{N}$ were expressed relative to known standards (VPDB and atmospheric N, respectively) in per mil (‰) notation (e.g., $\delta^{13}\text{C}_{\text{sample}} = [(^{13}\text{C}/^{12}\text{C}_{\text{sample}})/(^{13}\text{C}/^{12}\text{C}_{\text{standard}}) - 1] \times 1000$). Sample data were normalized using 4 International Atomic Energy Association (IAEA) reference standards (CH6, CH7, N1, and N2). An internal laboratory standard (National Institute of Standards and Technology, NIST 1547—peach leaves) was run every 10 samples. Measurement errors (1 SD) on the laboratory standard were ~ 0.05 ‰ ($\delta^{13}\text{C}$) and ~ 0.11 ‰ ($\delta^{15}\text{N}$). The laboratory reported measurement error on duplicate samples as ~ 0.15 ‰ ($\delta^{13}\text{C}$) and ~ 0.19 ‰ ($\delta^{15}\text{N}$). Duplicates of field samples were not analyzed because of this high level of precision and because internal laboratory standards were used.

C/N ratios

C/N ratios were determined from the ‰C and ‰N data obtained as part of the stable isotope analyses for all foodweb components. Many of the samples were scraped from filters, so absolute ‰C and ‰N values may not have been accurate, but the C/N ratios should not have been affected. C/N ratios were used to assist in interpretation of foodweb sources.

Statistical analyses

All statistical analyses were run using JMP IN 4.0 (SAS Institute, Cary, North Carolina), and all data sets were examined for normality and

transformed as needed using either $\log_{10}(x)$, $\log_e(x)$, or x^2 prior to analyses. Statistical significance of all tests was judged at $p < 0.05$. Analysis of variance (ANOVA) was used to evaluate the effect of sampling date on baseflow seston $\delta^{15}\text{N}$. Baseflow seston $\delta^{15}\text{N}$ did not differ seasonally (see Results). Therefore, sampling dates were used as replicates in two 1-way ANOVAs to evaluate the effects of site and flow condition on seston $\delta^{15}\text{N}$. Sites 7 and 8 were excluded from the analysis of the effect of flow condition on seston $\delta^{15}\text{N}$ values because no samples were taken at these 2 sites during storms. After exclusion of these sites, remaining samples were categorized as base, ascending, and descending stream-flow conditions, and 1-way ANOVA was used to evaluate the effect of flow condition on seston $\delta^{15}\text{N}$ (G. Kissling, Department of Mathematical Sciences, University of North Carolina Greensboro, personal communication).

One-way ANOVAs were used to evaluate the effects of flow condition on nutrient concentrations in the stream water. Sites 7 and 8 were excluded from the analysis (see above), and nutrient data from Rankin were analyzed separately because of elevated nutrient concentrations from the influence of WWTP effluent. Regressions were used to determine the relationships between seston $\delta^{15}\text{N}$ and flux of NO_3^- , NH_4^+ , and PO_4^{3-} .

Multivariate ANOVA (MANOVA) was used to evaluate the effects of season on $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in foodweb components. No seasonal patterns were detected (see Results), so sampling dates were used as replicates (Ulseth 2003) in a MANOVA to evaluate the effects of site and isotope species on changes in baseflow enrichment values of the foodweb components. No clams and only one caddisfly were found at Hamilton; therefore, Hamilton was excluded from comparisons among sites for these 2 foodweb components. MANOVA was done to reduce the chance of making a type I error while determining whether enrichment values for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in foodweb components differed among sites. If MANOVA results for a foodweb component indicated that enrichment values differed depending on isotope species, then separate 1-way ANOVAs (1 for each isotope) were used to identify differences in $\delta^{15}\text{N}$ or $\delta^{13}\text{C}$ values among sites.

Separate 2-way ANOVAs were used to evaluate the effects of site and date on the C/N ratios of each foodweb component. Replication

was not sufficient to permit analysis of interactions between components. Separate 1-way ANOVAs were used to evaluate the effect of flow condition on C/N ratios of foodweb components at combined sites upstream of the WWTP and at Rankin.

Results

Seston $\delta^{15}\text{N}$ and nutrient concentrations

Baseflow seston $\delta^{15}\text{N}$ did not differ with respect to sampling date (ANOVA, $p = 0.62$, $\delta^{15}\text{N} = 5.6 \pm 0.4$ in autumn, 4.5 ± 0.8 in spring, 5.2 ± 0.3 in summer, 4.9 ± 0.7 in winter). Baseflow seston $\delta^{15}\text{N}$ values differed significantly among sampling sites (Fig. 3). Seston $\delta^{15}\text{N}$ values were lowest at Hamilton and highest at site 8, directly downstream of the WWTP. Seston $\delta^{15}\text{N}$ values at site 8 were significantly more enriched than all of the sites upstream of the WWTP (Tukey–Kramer Honestly Significant Difference [TK HSD]). Seston $\delta^{15}\text{N}$ at Rankin was significantly more enriched than seston $\delta^{15}\text{N}$ at Hamilton, Golf Course, Fairview, Summit, and upstream of the WWTP (TK HSD). Seston $\delta^{15}\text{N}$ values differed significantly among flow conditions (Table 2). Seston $\delta^{15}\text{N}$ values did not differ between ascending and descending conditions, but values were significantly more depleted under ascending and descending conditions than during baseflow (TK HSD).

Nutrient concentrations differed significantly among flow conditions. NO_3^- , NH_4^+ , and PO_4^{3-} concentrations at sites upstream of the WWTP were significantly lower during baseflow than during ascending and descending conditions (Fig. 4), but concentrations did not differ between ascending and descending conditions (TK HSD). Nutrient concentrations at Rankin did not differ significantly among flow conditions (Fig. 5), although NO_3^- and PO_4^{3-} concentrations appeared higher during baseflow than during ascending and descending conditions, and NH_4^+ concentrations appeared lower during baseflow than during ascending and descending conditions (Fig. 5). Overall, responses of nutrients to flow conditions at Rankin were not similar to responses of nutrients at sites upstream of the WWTP.

Seston $\delta^{15}\text{N}$ at sites above the WWTP was diluted as nutrient flux increased (Table 3). Seston $\delta^{15}\text{N}$ upstream of the WWTP was significantly

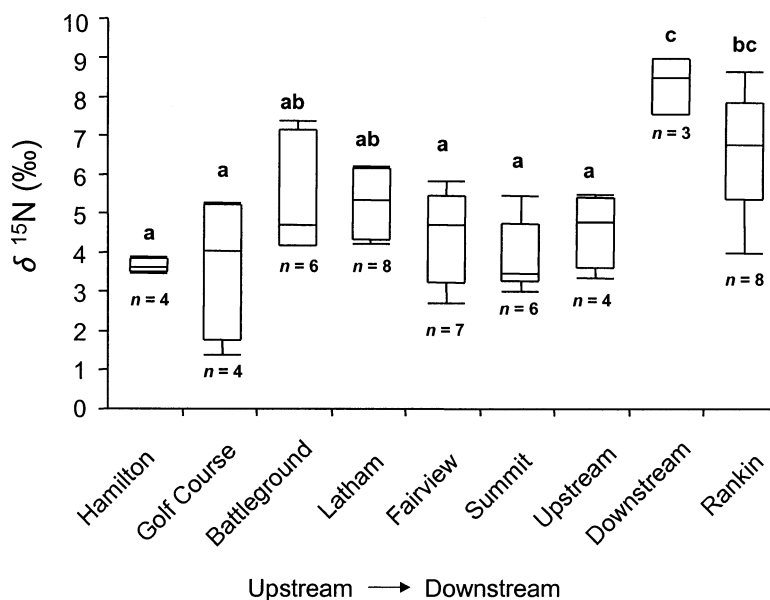


FIG. 3. Box plots of seston $\delta^{15}\text{N}$ for samples taken during baseflow at sites on North Buffalo Creek from October 2000 to April 2002. Plots show medians (horizontal bars), interquartile ranges (rectangles), and ranges (vertical bars extending from rectangles). Means with the same letters are not significantly different. Arrow indicates upstream–downstream direction. Upstream = upstream of Waste Water Treatment Plant (WWTP), Downstream = downstream of WWTP.

and negatively correlated with $\ln(\text{NH}_4^+)$, NO_3^- , and $\ln(\text{PO}_4^{-3})$ (Table 3), and changes in these nutrient fluxes explained 22%, 27%, and 16%, respectively, of the variation in $\delta^{15}\text{N}$ at sites above the plant (Table 3). At Rankin, 6 km below the plant, $\delta^{15}\text{N}$ was not significantly correlated with $\ln(\text{NH}_4^+)$ or $\ln(\text{PO}_4^{-3})$, but $\delta^{15}\text{N}$ increased with increasing $\ln(\text{NO}_3^-)$ ($R^2 = 0.54$; Table 3).

Site and seasonal variation in $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of foodweb components

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ did not differ among seasons for any component (Table 4), but did differ among sites for all foodweb components except

TABLE 2. Mean (+1 SE) seston $\delta^{15}\text{N}$ across all sites during baseflow, and ascending and descending portions of the hydrograph. * indicates values significantly different from baseflow ($p < 0.05$).

Condition	<i>n</i>	$\delta^{15}\text{N}$	SE
Baseflow	43	4.99	0.24
Ascending	29	3.50*	0.40
Descending	25	4.07*	0.21

Procambarus (see below). A significant interaction between isotope and site for algae, leaves, moss, and seston indicated that the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was not consistent among sites for these foodweb components. The nonsignificant interaction between isotope and site for hydropsychid caddisflies, *Procambarus*, and clams indicated that the relationship between $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ was consistent among sites for these foodweb components. Significant differences among sites for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values justified analyses of the foodweb components by 1-way ANOVAs to determine where the variance occurred.

$\delta^{13}\text{C}$ varied among sites for most foodweb components. $\delta^{13}\text{C}$ of algae at Rankin and Summit was significantly depleted relative to $\delta^{13}\text{C}$ of algae at Hamilton and Latham (Fig. 6A). $\delta^{13}\text{C}$ of algae at Fairview did not differ significantly from other sites (Fig. 6A). $\delta^{13}\text{C}$ of hydropsychid caddisflies at Fairview and Summit was significantly depleted relative to $\delta^{13}\text{C}$ of hydropsychid caddisflies at Latham and Rankin (Fig. 6B). $\delta^{13}\text{C}$ of *Cambarus* at Summit was significantly depleted relative to $\delta^{13}\text{C}$ of *Cambarus* at Latham, Fairview, and Rankin (Fig. 6C). *Cambarus* at

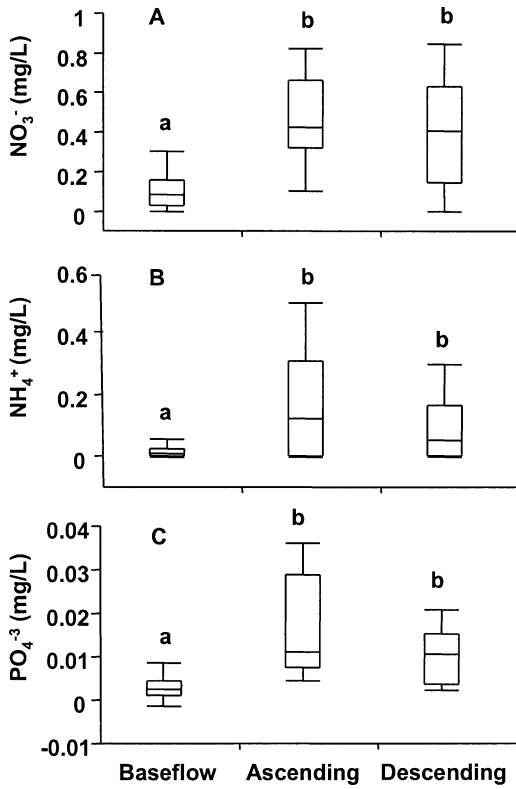


FIG. 4. Box plots of NO_3^- (A), NH_4^+ (B), and PO_4^{-3} (C) concentrations for samples collected during different flow conditions at sites upstream of the Waste Water Treatment Plant. Box plots as described in Fig. 3. NH_4^+ data were log transformed for analyses, but untransformed data are shown. Means with the same letters are not significantly different.

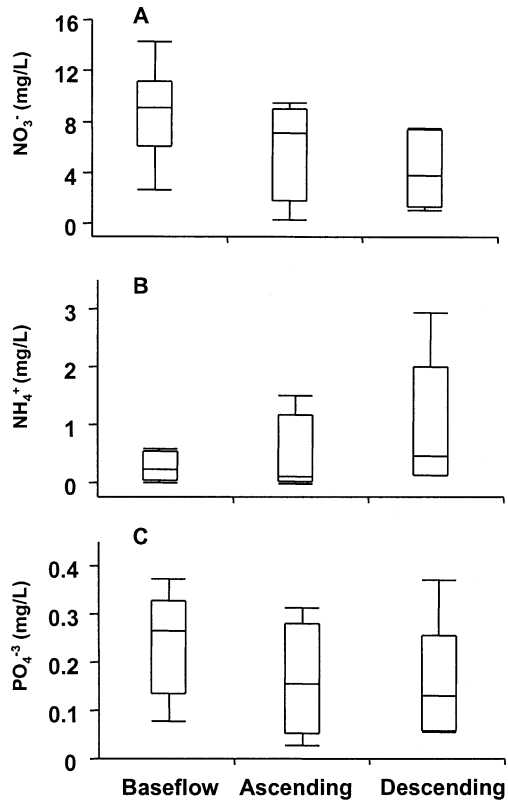


FIG. 5. Box plots of NO_3^- (A), NH_4^+ (B), and PO_4^{-3} (C) concentrations for samples collected during different hydrograph conditions at Rankin downstream of the Waste Water Treatment Plant. Box plots as described in Fig. 3. NH_4^+ data were log transformed for analyses, but untransformed data are shown.

Fairview was significantly depleted in $\delta^{13}\text{C}$ compared to *Cambarus* at Latham and Rankin (Fig. 6C). $\delta^{13}\text{C}$ of *Procambarus* did not vary significantly among sites (Fig. 6D). $\delta^{13}\text{C}$ of clams at

Rankin was significantly enriched relative to $\delta^{13}\text{C}$ of clams at Latham, Fairview, and Summit (Fig. 6E). $\delta^{13}\text{C}$ of clams at Latham was significantly enriched relative to $\delta^{13}\text{C}$ of clams at Sum-

TABLE 3. Regression parameters for linear regression analyses of $\delta^{15}\text{N}$ vs nutrient flux at pooled sites upstream and at the Rankin site downstream of the Waste Water Treatment Plant. * = $p < 0.01$, ** = $p < 0.001$, *** = $p < 0.001$.

	Nutrient flux (mg/s)	n	Intercept $\delta^{15}\text{N}$	Slope	R^2
Pooled upstream sites	$\ln(\text{NH}_4^+)$	51	4.60***	-0.24***	0.22
	NO_3^-	51	5.73***	-0.37***	0.27
	$\ln(\text{PO}_4^{-3})$	54	4.23**	-0.25*	0.16
Rankin	$\ln(\text{NH}_4^+)$	16	6.31***	-0.25	0.06
	$\ln(\text{NO}_3^-)$	17	2.92*	1.83**	0.54
	$\ln(\text{PO}_4^{-3})$	17	7.42***	0.65	0.06

TABLE 4. Seasonal mean (\pm SE) $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ for each foodweb component by season, and results of multiple analysis of variance (MANOVA) used to analyze isotope values by season.

Component	$\delta^{15}\text{N}$				$\delta^{13}\text{C}$				MANOVA results		
	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	n	df	p
Algae	9.4 \pm 0.7	3.5	6.8 \pm 1.5	6.8 \pm 1.1	-30.5 \pm 1.6	-33.5	-29.8 \pm 3.1	-31.7 \pm 1.6	21	3,17	0.4
Hydropsychidae	10.5 \pm 1.5	10.0 \pm 1.6	9.2 \pm 1.1	8.9 \pm 1.3	-28.5 \pm 0.9	-31.1 \pm 1.3	-28.6 \pm 1.1	-28.4 \pm 0.7	21	3,17	0.8
Cambarus	9.3 \pm 0.9	9.6 \pm 0.7	9.0 \pm 0.9	8.3 \pm 0.2	-26.4 \pm 0.9	-25.3 \pm 0.3	-25.6 \pm 0.3	-25.6 \pm 0.3	44	3,40	0.5
Procambarus	6.6	8.4 \pm 0.6	8.4 \pm 0.4	7.8 \pm 0.3	-23.7	-26.1 \pm 0.9	-25.2 \pm 0.9	-23.9 \pm 0.3	15	3,11	0.3
Clams	7.4 \pm 0.7	8.3 \pm 0.9	7.9 \pm 0.9	6.3 \pm 1.0	-25.6 \pm 0.9	-25.4 \pm 0.9	-26.4 \pm 0.9	-26.8 \pm 0.6	28	3,24	0.5
Leaves	2.7 \pm 1.5	2.1 \pm 0.5	2.3 \pm 0.7	2.6 \pm 0.4	-28.5 \pm 0.6	-28.6 \pm 0.6	-29.0 \pm 0.3	-28.4 \pm 0.3	27	3,22	0.5
Moss	10.0 \pm 2.0	4.1 \pm 1.2	7.3 \pm 2.4	8.4 \pm 1.4	-33.7 \pm 2.6	-36.7 \pm 1.6	-33.6 \pm 2.2	-34.2 \pm 2.1	22	3,18	0.9
Seston	5.3 \pm 0.4	4.2 \pm 0.7	5.1 \pm 0.9	5.0 \pm 0.4	-27.4 \pm 0.8	-29.3 \pm 1.3	-27.0 \pm 0.5	-26.8 \pm 0.2	33	3,29	0.4

TABLE 5. Mean (\pm 1 SE) C/N ratios of foodweb components from North Buffalo Creek by season and site. Data were analyzed using 2-way analysis of variance (ANOVA). Within-column means with the same letters are not significantly different (Tukey's Honestly Significant Differences). WWTP = Waste Water Treatment Plant, - = foodweb component not analyzed.

Season	Seston				Leaves	Moss	Clams	Hydropsychidae	Cambarus	Procambarus
	Algae	Algae	Algae	Algae						
Spring	7.8 \pm 0.4	8.8 \pm 0.7 ^b	34.5 \pm 2.1 ^b	9.1 \pm 1.1	9.1 \pm 1.1	4.7 \pm 0.3 ^a	5.2 \pm 0.2 ^a	3.2 \pm 0.02 ^{ab}	3.1 \pm 0.05	3.1 \pm 0.05
Summer	12.8 \pm 2.0	7.9 \pm 0.8 ^{ab}	20.6 \pm 2.2 ^a	9.0 \pm 0.3	9.0 \pm 0.3	4.7 \pm 0.2 ^a	4.4 \pm 0.1 ^b	3.1 \pm 0.03 ^{ab}	3.2 \pm 0.08	3.2 \pm 0.08
Autumn	8.6 \pm 1.0	6.7 \pm 0.3 ^a	28.5 \pm 0.8 ^{ab}	7.5 \pm 0.3	7.5 \pm 0.3	4.0 \pm 0.1 ^b	4.3 \pm 0.2 ^b	3.1 \pm 0.02 ^a	3.1	3.1
Winter	8.7 \pm 1.0	6.8 ^{ab}	34.5 \pm 4.4 ^b	9.9 \pm 0.6	9.9 \pm 0.6	3.8 \pm 0.1 ^b	5.1 \pm 0.1 ^a	3.2 \pm 0.01 ^b	3.1 \pm 0.02	3.1 \pm 0.02
Site										
Hamilton	10.2 \pm 2.4	8.1 \pm 1.8	39.0 \pm 1.6	11.3 \pm 0.5	11.3 \pm 0.5	-	-	3.2 \pm 0.04	-	-
Golf Course	7.3 \pm 1.0	-	-	-	-	-	-	-	-	-
Battleground	8.1 \pm 0.9	-	-	-	-	-	-	-	-	-
Latham	8.2 \pm 0.8	7.7 \pm 0.5	29.4 \pm 5.0	8.0 \pm 0.5	8.0 \pm 0.5	4.5 \pm 0.3	4.6 \pm 0.3	3.2 \pm 0.02	3.2 \pm 0.04	3.2 \pm 0.04
Fairview	13.3 \pm 4.6	7.1 \pm 0.5	22.1 \pm 3.5	8.7 \pm 0.4	8.7 \pm 0.4	4.3 \pm 0.2	4.8 \pm 0.3	3.2 \pm 0.02	3.1 \pm 0.05	3.1 \pm 0.05
Summit	15.2 \pm 5.4	7.7 \pm 0.4	27.9 \pm 2.6	8.6 \pm 0.8	8.6 \pm 0.8	4.5 \pm 0.4	5.0 \pm 0.2	3.2 \pm 0.03	3.1 \pm 0.04	3.1 \pm 0.04
Upstream WWTP	14.9 \pm 3.9	-	-	-	-	-	-	-	-	-
Downstream WWTP	8.5 \pm 1.2	-	-	-	-	-	-	-	-	-
Rankin	10.5 \pm 2.5	8.3 \pm 0.8	23.1 \pm 2.5	9.3 \pm 0.5	9.3 \pm 0.5	4.0 \pm 0.1	4.6 \pm 0.2	3.2 \pm 0.03	-	-

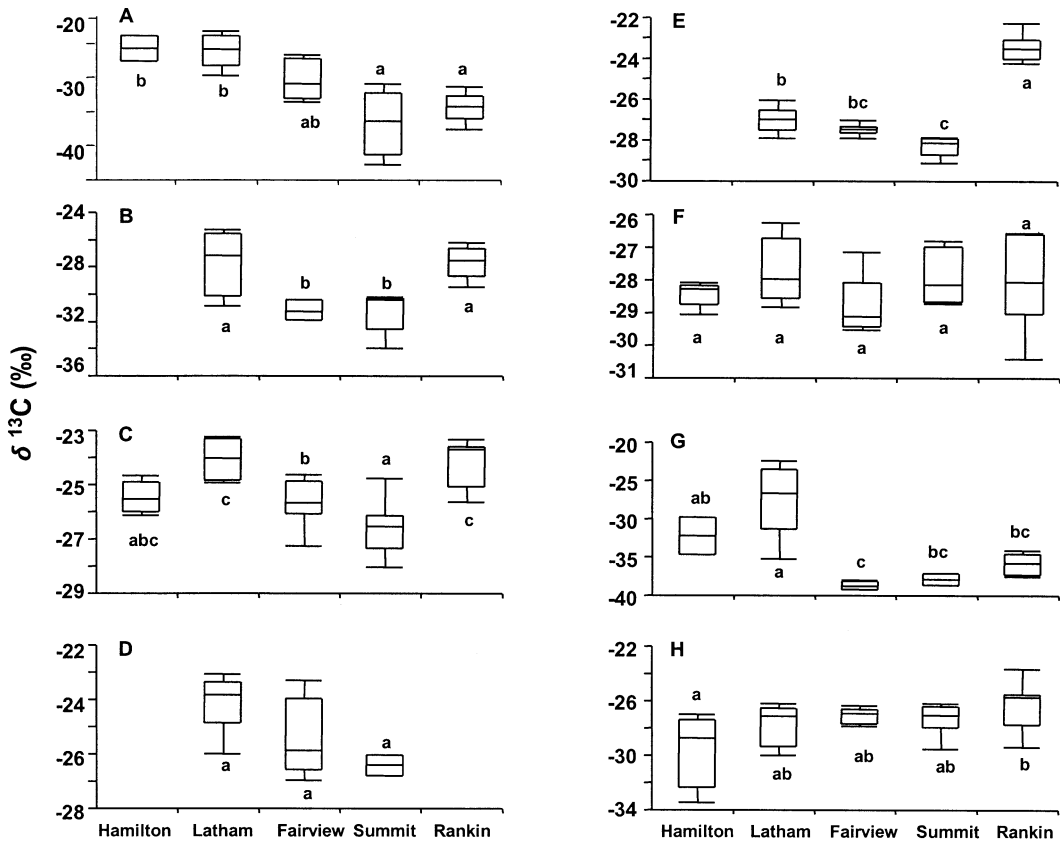


FIG. 6. Box plots of $\delta^{13}\text{C}$ values for algae (A), caddisflies (B), *Cambarus* (C), *Procambarus* (D), clams (E), leaves (F), moss (G), and seston (H) from sampling sites along North Buffalo Creek. Box plots as described in Fig. 3. Sites with the same letters are not significantly different.

mit (Fig. 6E). $\delta^{13}\text{C}$ of leaves did not vary significantly among sites (Fig. 6F). $\delta^{13}\text{C}$ of moss at Latham was significantly enriched relative to $\delta^{13}\text{C}$ of moss at Fairview, Summit, and Rankin. $\delta^{13}\text{C}$ of moss at Fairview was significantly depleted relative to $\delta^{13}\text{C}$ of moss at Hamilton (Fig. 6G). $\delta^{13}\text{C}$ of seston at Rankin was significantly enriched relative to $\delta^{13}\text{C}$ of seston at Hamilton (Fig. 6H).

$\delta^{15}\text{N}$ also varied among sites for most foodweb components. $\delta^{15}\text{N}$ of algae at Rankin was significantly enriched relative to $\delta^{15}\text{N}$ of algae at Hamilton, Fairview, and Summit (Fig. 7A). $\delta^{15}\text{N}$ of hydropsychid caddisflies and *Cambarus* at Rankin was significantly enriched relative to $\delta^{15}\text{N}$ of hydropsychid caddisflies and *Cambarus* at all other sites (Fig. 7B, C). $\delta^{15}\text{N}$ of *Cambarus* at Summit and Fairview was significantly enriched relative to $\delta^{15}\text{N}$ of *Cambarus* at Hamilton (Fig. 7C). $\delta^{15}\text{N}$ of *Procambarus* did not vary sig-

nificantly among sites, but *Procambarus* were not collected at Rankin or Hamilton (Fig. 7D). $\delta^{15}\text{N}$ of clams at Rankin was significantly enriched relative to $\delta^{15}\text{N}$ of clams at all other sites (Fig. 7E). $\delta^{15}\text{N}$ of leaves at Hamilton was significantly depleted relative to $\delta^{15}\text{N}$ of leaves at Latham, Fairview, Summit, and Rankin (Fig. 7F). $\delta^{15}\text{N}$ of moss at Summit and Latham was significantly enriched relative to $\delta^{15}\text{N}$ of moss at Hamilton (Fig. 7G). $\delta^{15}\text{N}$ of moss at Rankin was significantly enriched relative to $\delta^{15}\text{N}$ of moss at Latham, Fairview, and Hamilton (Fig. 7G). $\delta^{15}\text{N}$ of seston at Rankin was significantly enriched relative to $\delta^{15}\text{N}$ of seston at Fairview, Summit, and Hamilton, but not Latham (Fig. 7H).

$\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of foodweb components

Seston $\delta^{15}\text{N}$ signatures generally were intermediate between $\delta^{15}\text{N}$ signatures of leaves and

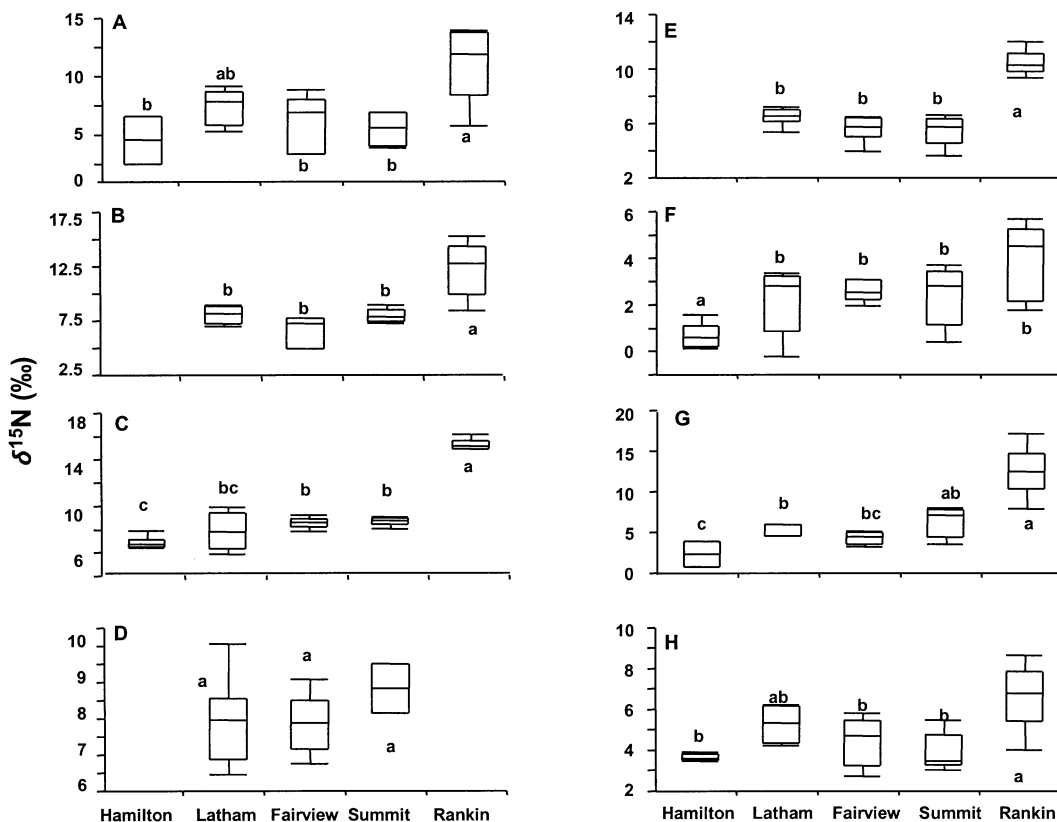


FIG. 7. Box plots of $\delta^{15}\text{N}$ values for algae (A), caddisflies (B), *Cambarus* (C), *Procamburus* (D), clams (E), leaves (F), moss (G), and seston (H) from sampling sites along North Buffalo Creek. Box plots as described in Fig. 3. Sites with the same letters are not significantly different.

algae, a pattern that is consistent with the presumption that seston was derived largely from leaves and algae (Fig. 8A–E). However, at Fairview, Summit, and Rankin, $\delta^{13}\text{C}$ of seston was more enriched than $\delta^{13}\text{C}$ of both leaves and algae, indicating the presence of other important sources of seston at these sites (Fig. 8C–E). At Hamilton, seston $\delta^{13}\text{C}$ was more depleted than $\delta^{13}\text{C}$ of both algae and leaves, also indicating the presence of other sources of seston at this site (Fig. 8A). At Rankin, $\delta^{13}\text{C}$ of seston was $>8\%$ enriched relative to $\delta^{13}\text{C}$ of algae and was intermediate between $\delta^{13}\text{C}$ of WWTP effluent and $\delta^{13}\text{C}$ of leaves (Fig. 8E), indicating that seston at this site was derived largely from WWTP effluent and leaves.

Hydropsychid caddisflies appeared to be assimilating seston, algae, and moss but the relative importance of these sources was not consistent among sites. At most sites, $\delta^{15}\text{N}$ of hy-

dropsychids was enriched ~ 3 to 5% relative to $\delta^{15}\text{N}$ of seston, a pattern consistent with the trophic shift expected if the diet consisted of seston. However, $\delta^{13}\text{C}$ values of hydropsychids did not track $\delta^{13}\text{C}$ values of seston at all of the sites. At Hamilton, incorporation of moss C is the most likely explanation for the observed hydropsychid $\delta^{13}\text{C}$ value (Fig. 8A), although we had only one hydropsychid $\delta^{13}\text{C}$ observation at that site. At Latham, hydropsychids appeared to be assimilating ^{15}N and ^{13}C from either seston or moss or both (Fig. 8B). At Fairview, hydropsychids appeared to be assimilating ^{15}N from seston, but the ^{13}C signature was consistent with assimilation of a considerable amount of moss (Fig. 8C). At Summit, hydropsychids appeared to be assimilating C from either moss or algae or both (Fig. 8D). At Rankin, hydropsychid $\delta^{13}\text{C}$ was slightly depleted compared to seston $\delta^{13}\text{C}$, and the depletion could be accounted for by in-

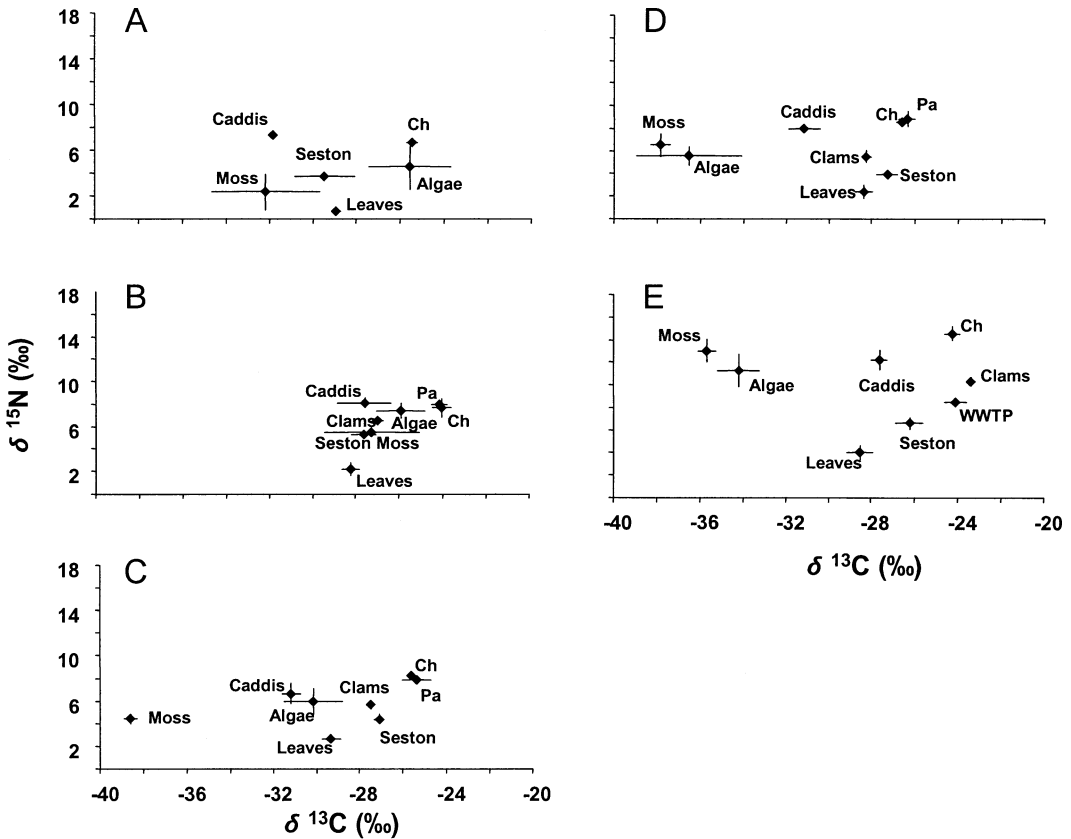


FIG. 8. $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ biplots (mean \pm SE) of foodweb components for Hamilton (A), Latham (B), Fairview (C), Summit (D), and Rankin (E). WWTP = Waste Water Treatment Plant, caddis = caddisflies, Ch = *Cambarus*, Pa = *Procambarus*.

clusion of ^{13}C from algae and moss (Fig. 8E). However, hydropsychids appeared to be assimilating ^{15}N from seston (Fig. 8E). Therefore, hydropsychid caddisflies appeared to be assimilating C and N from different combinations of source components at the various sites.

Clams appeared to be deriving their C and N from different sources at different sites. At Latham, clams appeared to be assimilating ^{13}C largely from seston (Fig. 8B). However, at Fairview and Summit, clam $\delta^{13}\text{C}$ was slightly depleted relative to seston $\delta^{13}\text{C}$, and this pattern indicated that clams may have been assimilating some ^{13}C from leaves or algae (Fig. 8C, D). At Fairview and Summit, clam $\delta^{15}\text{N}$ was enriched $\sim 1.5\text{‰}$ compared to seston $\delta^{15}\text{N}$, a pattern that does not reflect a diet composed entirely of seston. At Rankin, clam $\delta^{13}\text{C}$ appeared to be strongly influenced by effluent from the WWTP (Fig. 8E), but clam $\delta^{15}\text{N}$ was enriched only 1‰

compared to WWTP effluent $\delta^{15}\text{N}$. However, at Rankin, clam $\delta^{15}\text{N}$ was enriched $\sim 3.7\text{‰}$ compared to seston $\delta^{15}\text{N}$ (Fig. 8E). These results suggest that WWTP effluent was important in clam diets, and this effluent was a major component of the seston at the Rankin site.

C/N ratios of foodweb components

Baseflow seston C/N ratios did not differ among seasons or sites (Table 5). At sites upstream of WWTP, seston C/N ratios were significantly higher during the ascending limb of the hydrograph than during baseflow or the descending limb (Table 6). At Rankin, seston C/N ratios did not differ significantly among flow conditions (Table 6). Algal C/N ratios were similar among sites but were significantly higher during spring than during autumn (Table 5). Moss C/N ratios did not differ among seasons

TABLE 6. Mean (± 1 SE) C/N ratios of seston under different hydrological conditions at pooled sites upstream and at the Rankin site downstream of the Waste Water Treatment Plant. Means with the same letter are not significantly different. Number in parentheses indicate the sample size used to calculate means.

Condition	Pooled upstream sites	Rankin
Baseflow	10.5 \pm 1.4 ^b (35)	10.2 \pm 2.5 (8)
Ascending	13.7 \pm 0.8 ^a (24)	9.8 \pm 1.3 (5)
Descending	9.1 \pm 0.5 ^b (18)	7.8 \pm 1.1 (4)

or sites. Clam C/N ratios were similar among sites, but were significantly higher during spring and summer than during autumn and winter (Table 5). Leaf C/N ratios were significantly lower during summer than during winter and spring (Table 5). Hydropsychid C/N ratios did not differ among sites but were significantly higher during winter and spring than during summer and autumn (Table 5). *Cambarus* and *Procambarus* C/N ratios did not differ among sites (Table 5). *Procambarus* C/N ratios did not differ among seasons, but *Cambarus* C/N ratios were higher during winter than during autumn. However, the variance for *Cambarus* C/N ratios was extremely low, and the observed seasonal difference reflected low variance rather than a large difference among means (Table 5).

Influence of Cone Mills textile effluent at Summit on foodweb components

$\delta^{15}\text{N}$ of foodweb components sampled at Summit while the site was receiving textile effluent from Cone Mills were depleted compared to $\delta^{15}\text{N}$ of foodweb components sampled after the plant stopped discharging into the stream (Fig. 9). The organisms probably were assimilating N from Cone Mills effluent because Cone Mills $\delta^{15}\text{N}$ signatures were consistently depleted. No statistical analyses were done because the number of samples collected before June 2001 was too low, but the data in Fig. 9 give some insight into the site-specific effects of industrial effluent on the natural abundance of $\delta^{15}\text{N}$ of some of the foodweb components in North Buffalo Creek.

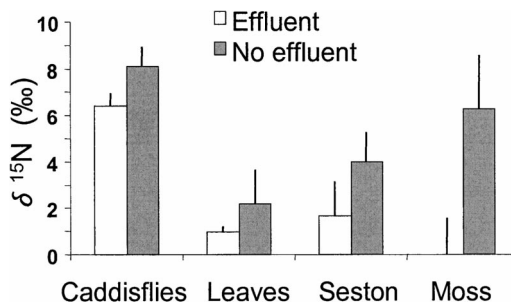


FIG. 9. $\delta^{15}\text{N}$ values (mean \pm SE) of foodweb components sampled at Summit. Effluent samples were taken from autumn 2000 to June 2001 while Cone Mills textile plant was discharging industrial effluent into North Buffalo Creek. No-effluent samples were taken from June 2001 to June 2002 after the discharge from Cone Mills textile plant was diverted.

Discussion

Baseflow seston $\delta^{15}\text{N}$

Baseflow seston $\delta^{15}\text{N}$ values varied significantly between sites in North Buffalo Creek, and 3 sites showed evidence of point-source inputs (Figs 3, 9). Site 8, directly below the WWTP, was most enriched in ^{15}N . This result was not surprising because the WWTP discharges 45,000 to 60,000 m^3/d of ^{15}N -enriched treated effluent into North Buffalo Creek during dry conditions (WWTP personnel, North Buffalo Creek WWTP, personal communication). At Rankin, seston $\delta^{15}\text{N}$ appeared to be influenced primarily by WWTP effluent, but it also may have been influenced partially by discharge from the city. In the past, nonpoint sources upstream of the WWTP have caused water-quality problems at Rankin (NCDENR 2000). At Summit, seston $\delta^{15}\text{N}$ was depleted while Cone Mills was discharging ^{15}N -depleted textile effluent into the stream. Thus, for each of the 3 sampling sites that clearly were receiving point-source pollutants, the $\delta^{15}\text{N}$ data suggested that seston $\delta^{15}\text{N}$ was sensitive to those point-source inputs. Moreover, during baseflow, the seston response was site-specific rather than cumulative along the transect.

At baseflow, seston $\delta^{15}\text{N}$ values at every site, including Hamilton ($\delta^{15}\text{N} = 3.7\text{‰}$), were more enriched than $\delta^{15}\text{N}$ values reported for pristine sites elsewhere ($\delta^{15}\text{N} \cong 0\text{‰}$; Fry 1991, Salas and Dudgeon 2001). However, this result was expected given the urban setting of North Buffalo

Creek. Salas and Dudgeon (2001) compared forested, shaded, and open streams in Hong Kong, and found that seston $\delta^{15}\text{N}$ was 6.4‰ in open sites and ~ 0 ‰ in forested sites. They attributed this difference to the higher proportion of autochthonous material contributing to seston flux at open sites than at forested sites. Seston $\delta^{15}\text{N}$ values from nonurban streams in the piedmont of North Carolina ranged from 3.6‰ to 8.1‰ (Ulseth 2003). Thus, the ~ 3 to 4‰ ^{15}N enrichment of seston at urban sites that were not receiving point-source inputs was generally consistent with values of seston $\delta^{15}\text{N}$ in nonurban streams in the region.

Seston $\delta^{15}\text{N}$ values in North Buffalo Creek were consistent with values that have been reported for soil $\delta^{15}\text{N}\text{-NO}_3^-$ in the region. Hübner (1986) reported soil $\delta^{15}\text{N}\text{-NO}_3^-$ values from +3‰ to +8‰, and Kendall (1998) reported $\delta^{15}\text{N}\text{-NO}_3^-$ values from -2 ‰ to +14‰ in natural soils and from -2 ‰ to +12‰ in agricultural soils. At 17 Long Term Ecological Research (LTER) sites, most of which are more pristine than many other locations, Fry (1991) reported mineral soil- $\delta^{15}\text{N}$ values that ranged from 0‰ to +8‰ and organic soil- $\delta^{15}\text{N}$ values that ranged from -3 ‰ to +2‰.

Historical land use also influences piedmont streams. Humans have been altering the landscape for centuries, but understanding these historical impacts in relation to the life histories of aquatic organisms and systems we study has not always been emphasized (Meyer and Wallace 2001). For instance, Harding et al. (1998) found that macroinvertebrate and fish community structure was better explained by 1950s watershed land use than by current riparian and watershed land use. Very little of the North Carolina piedmont has not been disturbed (Mulholland and Lenat 1992). The southeastern piedmont was heavily affected by European settlers. Historical landuse practices included forest removal and agriculture (Mulholland and Lenat 1992). Soil organic matter is oxidized during cultivation, and this oxidation causes increases in $\delta^{15}\text{N}\text{-NO}_3^-$ (Heaton 1986). In addition, several studies have attributed high $\delta^{15}\text{N}$ values of stream food webs to agricultural land use within the watershed (Bunn et al. 1997, Harrington et al. 1998). Therefore, seston $\delta^{15}\text{N}$ signatures may reflect historical as well as current landuse practices.

Seston $\delta^{15}\text{N}$ during storms

During ascending and descending limbs of the stream hydrograph at sites upstream of the WWTP, nutrient concentrations were elevated and seston $\delta^{15}\text{N}$ was depleted relative to values during baseflow. These results were consistent with the observed decline in seston $\delta^{15}\text{N}$ with increasing nutrient flux. Stormwater inputs influence nutrient concentrations in streams (Carpenter et al. 1998). Seston sampled from storms and a leaky sewer pipe along North Buffalo Creek during storms was depleted in $\delta^{15}\text{N}$ ($\delta^{15}\text{N} = 1.4\text{‰} \pm 0.3$, $n = 10$, and $\delta^{15}\text{N} = -1.6\text{‰}$, $n = 1$, respectively). WWTP influent, which should be representative of sewage leaks, also was depleted in $\delta^{15}\text{N}$ ($\delta^{15}\text{N} = -0.3\text{‰} \pm 0.7$, $n = 7$; Ulseth 2003). These depleted sources of ^{15}N could partially explain the depletion of seston $\delta^{15}\text{N}$ observed during storms. However, if the proportion of allochthonous contribution (leaves) to seston increased during storms, then the ^{15}N signal in seston would have been diluted because leaf $\delta^{15}\text{N}$ was depleted compared to algal $\delta^{15}\text{N}$. This hypothesis was only partially supported by C/N ratios. The C/N ratios of seston were higher during the ascending portion of the hydrograph than during baseflow, and leaves had higher C/N ratios than other foodweb components (Table 5). However, the C/N ratio increased from 10.5 ± 1.4 at baseflow to 13.7 ± 0.8 during the ascending limb, and this small increase was consistent with only a slightly higher-than-baseflow contribution of allochthonous detritus to the seston. However, the depleted $\delta^{15}\text{N}$ values of seston during the ascending portion of the hydrograph may have been caused by changing proportions of a combination of terrestrial detritus (high C/N ratio and low $\delta^{15}\text{N}$), autochthonous material (low C/N ratio and high $\delta^{15}\text{N}$), and anthropogenic inputs (low $\delta^{15}\text{N}$ and intermediate C/N ratio; C/N ratio of storm pipe seston = 17 ± 1.3 , $n = 10$, C/N ratio of leaky sewer seston = 12.3 , $n = 1$; and C/N ratio of WWTP influent = 10 ± 0.1 , $n = 6$; Ulseth 2003). All of these sources would be expected to increase during high discharge, and a combination of them could have caused the observed C/N ratios and $\delta^{15}\text{N}$ signatures during ascending conditions. Nevertheless, seston $\delta^{15}\text{N}$ and C/N ratios during storm events were most similar to those of anthropogenic

sources, and could not be accounted for strictly by allochthonous detritus.

At Rankin, the relationship between seston $\delta^{15}\text{N}$ and NO_3^- flux was positive and explained 54% of the variability in seston $\delta^{15}\text{N}$. Storms increased input from nonpoint sources, which increased nutrient concentrations and flux, at sites upstream of the WWTP, but storms diluted nutrient concentrations and flux at sites downstream of the WWTP. During baseflow at Rankin, WWTP effluent dominated flow and, at times, made up ~50% of the stream discharge. However, WWTP effluent was diluted during storms, and this dilution reduced seston $\delta^{15}\text{N}$, illustrating the contrast between the effects of storm runoff and sanitary sewer sources of seston $\delta^{15}\text{N}$.

Foodweb $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$

$\delta^{13}\text{C}$ values of algae and moss at Latham and algae at Fairview were enriched compared to $\delta^{13}\text{C}$ values of algae and moss at Summit and Rankin and moss at Fairview. Overall, primary producers are variable with respect to their isotopic signatures (Boon and Bunn 1994), and we see several potential explanations for the differences among sites in our study. 1) Biomass of filamentous algae was much higher at Latham than at other sites. If the higher algal biomass at Latham was accompanied by higher productivity, then C could have been limiting at Latham, thereby increasing $\delta^{13}\text{C}$ values at that site (Hicks 1997). 2) Moss and algae at Summit and Rankin and moss at Fairview were collected from riffles, whereas moss and algae were collected from runs at Latham, because the Latham site had no riffles. The riffle at Fairview was fully covered with moss, so algae from Fairview was collected from a run. Differences in current velocity can affect moss and algae fractionation of ^{13}C (Finlay et al. 1999). 3) Species composition of moss and algae may have differed among sites. 4) $\delta^{13}\text{C}$ of dissolved inorganic C (DIC) may have differed among sites, thereby affecting moss and algal $\delta^{13}\text{C}$ at the sites (Rosenfield and Roff 1992).

Isotopic signatures of hydroptychid caddisflies, which are generally considered filter-feeders (Merritt and Cummins 1996), were not fully consistent with a diet primarily of seston across sites. For example, hydroptychid caddisfly $\delta^{13}\text{C}$ was ~4‰ depleted relative to seston at Fairview

and Summit. Moreover, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of hydroptychid caddisflies were inconsistent with those of their presumed food sources and, at some sites, the 2 isotope signatures suggested different food sources. These $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ results could have been the consequences of: 1) a seasonal shift in isotopic signatures of food sources (Hart and Lovvorn 2002, McCutchan and Lewis 2002), 2) failure to measure all possible food sources, or 3) use of >1 food source by caddisflies (Mihuc and Toetz 1994, Hicks 1997).

McCutchan and Lewis (2002) and Hart and Lovvorn (2002) suggested that seasonal shifts in isotopic signatures of food sources may cause time lags between assimilation of C and assimilation of N in consumers, resulting in disparate consumer $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values relative to their food sources. However, isotopic signatures for foodweb components in North Buffalo Creek did not vary seasonally (Table 4), nor did seston C/N ratios (Table 5), so this explanation is unlikely. Hydroptychid caddisflies are omnivores that feed on a variety of suspended organic materials, which may include detritus, invertebrates, and algae (Fuller and Mackay 1980). Larvae collect particles from the water column by using a silken net (Scheffer and Wiggins 1986). Thus, hydroptychid caddisflies may have selected particles with $\delta^{13}\text{C}$ values that were depleted relative to the $\delta^{13}\text{C}$ of the seston particles in our water column samples. However, we think it is most likely that the apparent mismatch between the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ signatures of hydroptychid caddisflies and those of their food sources reflected differential assimilation of ^{13}C and ^{15}N from the various components of a mixed diet. Unfortunately, the relative contributions of various potential food sources are difficult to infer from mixing models when >2 food sources are available (e.g., Kline et al. 1990). Moreover, the approach developed by Phillips and Koch (2002) for a 3-source mixing model is restricted to n isotopes with $\leq(n + 1)$ sources, and >3 food sources probably contributed to both seston and animal diets (e.g., algae, leaf detritus, moss, animal feces, animal detritus, and sewage).

$\delta^{13}\text{C}$ of clams was similar to $\delta^{13}\text{C}$ of seston, and clams appeared to be assimilating some ^{13}C from seston at most sites. However, at Rankin, $\delta^{13}\text{C}$ of clams was more similar to $\delta^{13}\text{C}$ of WWTP effluent than of seston. $\delta^{15}\text{N}$ of clams

was consistently enriched $\sim 1.5\%$ above the food sources suggested by the $\delta^{13}\text{C}$ values. Average trophic shifts for $\delta^{15}\text{N}$ are $\sim 3.4\%$ (Vander Zanden and Rasmussen 2001), but trophic shifts can range from 1% to 5% (Minigawa and Wada 1984, Mihuc and Toetz 1994, McCutchan et al. 2003). In a broad comparative study, McCutchan et al. (2003) noted that trophic shifts for $\delta^{13}\text{C}$ depended on the type of tissue analyzed and whether the sample was acidified, whereas trophic shifts for $\delta^{15}\text{N}$ depended on whether a consumer fed on invertebrates, other high-protein foods, or plant tissue. When the C/N ratio of the food source is high, less fractionation of ^{15}N occurs between trophic levels (Adams and Sterner 2000, Huryn et al. 2001, Hart and Lovvorn 2002). In our study, seston C/N ratios were only slightly higher than those of algae and much lower than those of leaves. Thus, clams probably were assimilating C and N from a variety of sources. For instance, clams at Fairview and Summit sites appeared to be assimilating some C and N from leaf detritus. Some clams, especially at Latham, were collected from the stream bank or from below the surface of the stream substrate. Therefore, clams may have been pedal-feeding on food sources that were not collected for stable isotope analysis.

$\delta^{15}\text{N}$ of nearly all foodweb components at Rankin was enriched relative to $\delta^{15}\text{N}$ of foodweb components at sites upstream of the WWTP. This result suggested that organisms at Rankin were assimilating WWTP effluent-derived N, despite the fact that Rankin was 6 km downstream of the point of WWTP discharge. The enrichment may have been the result of assimilation of sewage-derived $^{15}\text{N-NO}_3^-$ (which we did not measure) by algae or the incorporation of sewage-derived particulate organic matter (SDPOM) in the diets of consumers. Valiela et al. (1992) found that sewage-derived N was assimilated by primary producers as $^{15}\text{N-NO}_3^-$, and deBruyn and Rasmussen (2002) and Van Dover et al. (1992) found that consumers assimilated SDPOM. At Rankin, a combination of these 2 pathways seems likely because the degree of ^{15}N enrichment was as high for algae and moss as it was for primary and secondary consumers. deBruyn and Rasmussen (2002) found that algal $\delta^{15}\text{N}$ was higher downstream of a WWTP because adhered particles of SDPOM could not be washed from algae prior to anal-

ysis. However, moss and algae samples in our study were rinsed with DI water before analysis, and little or no adhered SDPOM was observed. Wayland and Hobson (2001) found that $\delta^{15}\text{N}$ values of algae and suspended sediment (seston) were enriched, a pattern they attributed to assimilation of N from sewage, which accounted for 42% of the algal N. Therefore, the enriched $\delta^{15}\text{N}$ of moss and algae at Rankin probably was caused by assimilation of $^{15}\text{N-NO}_3^-$ from the WWTP effluent rather than by contamination of samples with adhered SDPOM.

At Rankin, hydropsychid caddisflies appeared to be assimilating ^{15}N from WWTP effluent via seston and some algae or other enriched source. Clams, on the other hand, appeared to be assimilating ^{15}N from WWTP effluent as SDPOM. SDPOM is a mix of organic detritus and microorganisms. It is a high-quality food source and, therefore, it is often the preferred food source for macroinvertebrate consumers (deBruyn and Rasmussen 2002). As such, it may act as a vector for transfer of sewage-associated contaminants to aquatic consumers (deBruyn and Rasmussen 2002).

Overall, baseflow seston $\delta^{15}\text{N}$ in North Buffalo Creek reflected the $\delta^{15}\text{N}$ signatures of point-source inputs and the soil of the Piedmont region. Foodweb components at 3 sites along North Buffalo Creek were assimilating N and C from anthropogenic point-source effluents. Natural abundance of $\delta^{15}\text{N}$ was a useful tool for examining the contribution of these point sources to foodweb components and to seston. During storms, the importance of anthropogenic sources, including storm water and sewage leaks, to seston increased, but these various anthropogenic inputs could not be discriminated in consumer components of the food web.

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