2010 South Fork Holston River

Environmental Monitoring Studies

THE ACADEMY OF NATURAL SCIENCES

of DREXEL UNIVERSITY

Patrick Center for Environmental Research

2010 South Fork Holston River Environmental Monitoring Studies

Report No. 10-04F

Submitted to:

Eastman Chemical Company
Tennessee Operations

Submitted by:

THE ACADEMY OF NATURAL SCIENCES
of DREXEL UNIVERSITY

Patrick Center for Environmental Research

1900 Benjamin Franklin Parkway
Philadelphia, PA 19103-1195

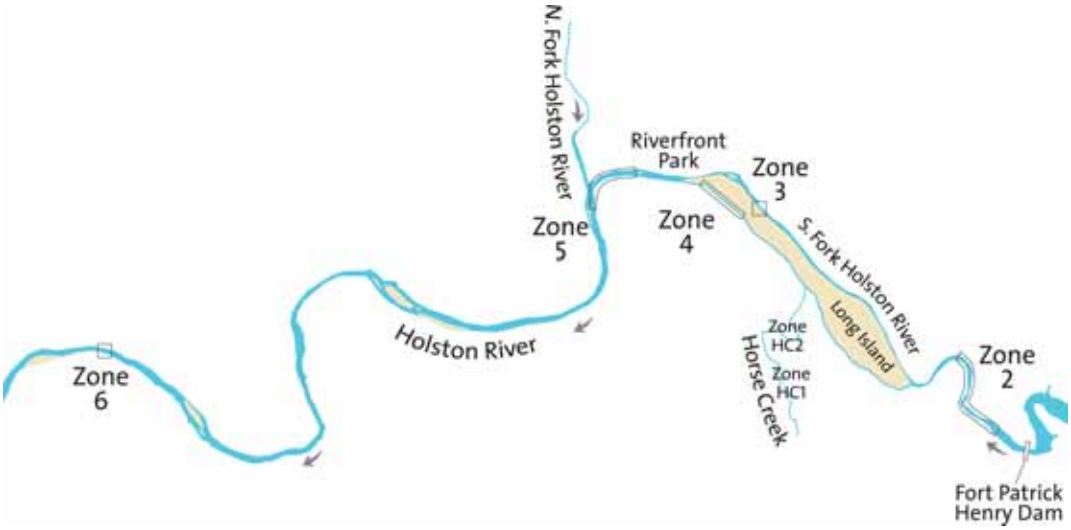
April 20, 2012

Executive Summary

The 2010 study was the seventh in a series of comprehensive studies of aquatic biota and water chemistry conducted by the Academy of Natural Sciences of Drexel University in the vicinity of Kingsport, TN. Previous studies were conducted in 1965, 1967 (cursory study, primarily focusing on algae), 1974, 1977, 1980, 1990 and 1997. Elements of the 2010 study included analysis of land cover, basic environmental water chemistry, attached algae and aquatic macrophytes, aquatic insects, non-insect macroinvertebrates, and fish. For each study element, field samples were collected and analyzed from zones located on the South Fork Holston River (Zones 2, 3 and 5), Big Sluice (Zone 4), mainstem Holston River (Zone 6), and Horse Creek (Zones HC1and HC2), the approximate locations of which are shown below. The design of the 2010 study was very similar to that of previous surveys, allowing comparisons among surveys. In addition, two areas of potential local impacts were assessed for the first time: Big Tree Spring (BTS, located on the South Fork within Zone 2) and Kit Bottom (KU and KL in the Big Sluice, upstream of Zone 4). The field sampling was conducted from 11-17 July 2010.

Scientists from the Academy's Patrick Center for Environmental Research have conducted seven major environmental monitoring studies on the South Fork Holston River since 1965.

The primary objectives for each element were 1) assessment of differences among zones as indicators of potential stressors, such as flow regulation by the Fort Patrick Henry Dam, various municipal and industrial activities in Kingsport, and effects of watershed development; and 2) assessment of temporal trends, as indicators of changes in local conditions. Results of the study will fulfill requirements of Eastman's hazardous waste management permit, aid Eastman's evaluation of its efforts to protect the



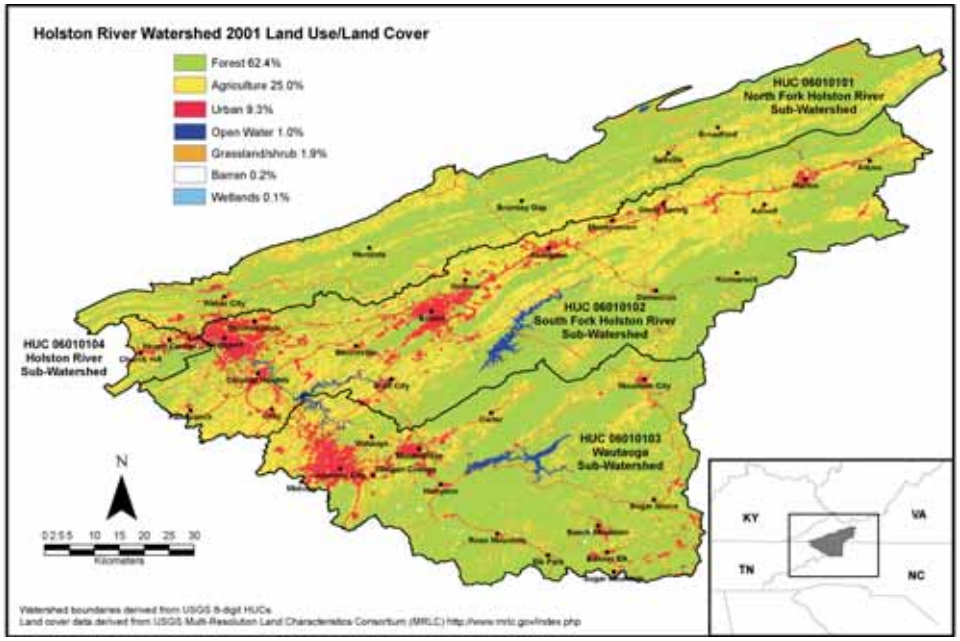
Map of the 2010 South Fork Holston River environmental monitoring studies zones.

On the cover: *Dr. Raymond W. Bouchard Jr. and Sylvan Klein (left to right) collecting macroinvertebrates at Zone 6, and examples of the many aquatic plants, macroinvertebrates and fishes recorded during the 2010 survey (from left to right; a hellgrammite, American Waterweed and a redear sunfish).*

environment, and help to fulfill the company's Responsible Care® goals of understanding and communicating environmental issues. The main findings of each study element are summarized below.

Land Cover

An analysis of land cover in the subwatersheds of the study area showed that the area was predominantly forested (62.4%), with significant amounts of agricultural (25.0%) and urban (9.3%) lands in 2001 (the most recent data available). There were relatively small changes in land cover between 1991 and 2001, with decreases in forested area (1.4%) and increases in agricultural (0.45%), grassland/scrub (0.49%), and urban lands (0.40%).



The watersheds that flow into the Academy's study area contain a wide variety of land uses, with each potentially impacting downstream water quality.

Environmental Geochemistry

Trends of increasing concentrations in the downstream direction were noted for several parameters along the South Fork and mainstem Holston rivers, including total alkalinity, total hardness, dissolved major ions, total nitrogen, total phosphorus, ortho-phosphorus, and dissolved organic carbon (DOC). Dissolved oxygen concentrations (DO), % DO saturation, and water temperature were lowest at Zone 2,

Senior Chemist, Paul Kiry, compositing a water sample from Zone 2.

near the bottom-release Fort Patrick Henry Dam. Among South Fork and mainstem Holston rivers zones, dissolved ammonium+ammonia and total phosphorus concentrations were highest at Zone 5, while other parameters showed no other consistent spatial pattern within the river system. A few parameters showed elevated levels in Horse Creek (compared to most or all of the five river zones), including turbidity, conductivity, total solids, some dissolved major ions, soluble Kjeldahl nitrogen, total phosphorus, DOC and TOC. DO and % DO saturation were lower in Horse Creek than in the South Fork and mainstem Holston rivers zones. Biological oxygen demand (BOD) concentrations were low (< 1 mg O_2/L) across the study area, although slightly higher than seen in the 1997 survey. A high concentration of fecal coliforms was noted in one sample from Zone 3, presumably related to runoff containing animal wastes, leachate from failed septic systems, and/or point and non-point source runoff between Zones 2 and 3 following a recent rain event. In general, concentrations were similar to those of the 1997 survey.

Water quality at Big Tree Spring (BTS) differed from the adjacent mainstem in several parameters, such as lower water temperature, lower pH, higher turbidity, conductivity, total alkalinity, and total hardness, and higher concentrations of dissolved major ions, dissolved nitrate+nitrite, and total nitrogen. Differences between BTS and river water may derive from the nature of groundwater in areas with carbonate rocks (e.g., higher hardness), from landfill leachate, or from other point or non-point sources contributing water to the spring. However, the flow of BTS was small relative to the river, and the area of mixing encompassed only a small area around the mouth of the spring.

Data from all ANS Holston River surveys (1965-2010) were standardized and compared among surveys. Concentrations of selected parameters show an improvement from the earlier surveys. This is evident for BOD, fecal coliform and possibly dissolved ammonium+ammonia concentrations. The largest improvements occurred from the late 1970s to the mid-1980s. Over the past 20 years water quality appears to be stable with no substantial change in the parameters examined. A few parameters, such as dissolved nitrate+nitrite and dissolved chloride, show recent slight increases (following earlier declines), although current values are similar or lower than those during the earliest surveys. These increases may come from watershed sources, related to land development and population growth in the region.

Phycologist, Frank Acker, collecting an algae sample from Horse Creek.

Algae and Aquatic Macrophytes

Due to modern taxonomic techniques, there has been considerable recent change in algal taxonomy, especially for diatoms and blue-green algae (cyanobacteria). The number of taxonomic changes since the previous survey (1997)

A mixed stand of American waterweed, long-leafed pondweed and sago pondweed at Zone 3. Submerged aquatic vegetation provides food and shelter for a wide variety of macroinvertebrates and fishes.

is probably greater than the changes observed since studies of Holston River algae started in 1965. Compilation of changes since 1997 was done to allow comparison of results.

Filamentous algal forms (blue-greens, greens and yellow-greens), which can indicate enrichment when abundant, were found consistently throughout the study area, probably more so in 2010 than the previous study (1997) when the most pronounced forms were diatoms. The largest algal growths in the 2010 studies were small "streamers" of green algae from moderate- to heavily-sedimented rocks in slow to moderate flow. Similar to the previous four surveys (1977, 1980, 1990 and 1997), the abundant diatom species were indicative of waters with high nutrients.

The major differences in diatom communities during the 2010 Holston River studies were between the Horse Creek zones and Zone 2, below the Fort Patrick Henry Dam, and the other Holston River zones. The diatom communities in the Horse Creek zones were influenced by higher turbidity and alkalinity. Influences to the periphyton communities in Zone 2 were dam-related. There were more planktonic algae than in other downstream Holston River zones and, related to larger water level fluctuations, there were greater amounts of diatoms that could withstand daily desiccation.

The algal community in the area of the Big Tree Spring was essentially a very large mat of filamentous green algae with epiphytic diatoms and several populations of blue-green algae near the interface between water and muddy sand.

Beds of submerged aquatic vegetation (SAV) were observed throughout the study area, consisting of *Heteranthera dubia*, *Elodea canadensis*, and several species of *Potamogeton*. SAV was particularly extensive at Zones 3 and 6 and appeared more extensive than in previous surveys at several zones, e.g., Zone 2, part of Zone 3 and at Zone 6. Although there are many factors that explain the presence and absence of aquatic plants, the amount of plant material at several of the zones, especially Zones 2 and 3, was indicative of organic enrichment. However, there has been substantial variation in the amount of SAV among years, making it difficult to ascribe patterns in a single year to trends in water quality.

Overall comparisons of the algal and aquatic plant communities in 2010 with previous studies reveal few differences from the 1990 and 1997 studies. However, there was much improvement from conditions observed in the 1960s and early 1970s. The algal communities observed in the general vicinity of Kingsport during the first studies in 1965 and 1967 were a result of conditions

where pollutants had not been broken down into their inorganic constituents. Large growths of the sewage bacterium *Sphaerotilus* out-competed all but the most tolerant algal forms. Where algae grew, there were very few species, and only those considered tolerant of the most polluted condition. Improved conditions were noted in 1974 with little *Sphaerotilus*, but large aquatic plant and filamentous algal growth was noted in Zones 3 and 5. In 1974, there was less organic enrichment in Zones 4 and 6; the conditions in Zone 2 were, as previously observed, affected by its close proximity to the Fort Patrick Henry Dam. Subsequent studies have revealed organic enrichment in all zones above and below the Kingsport area, although not as much as above Kingsport; algal species considered tolerant of severe pollution conditions were not observed. In some years, algal communities in Zone 4, and to an extent Zone 6, differed from Zones 3 and 5, especially in the amount of plant material observed.

Didymosphenia geminata, an invasive diatom species that was reported to be found in the tailwaters of the Fort Patrick Henry Dam above Zone 2 (http://www.tva.gov/river/neighbors/aug05/algae.htm), was not observed during this survey.

Non-Insect Macroinvertebrates

Five groups of non-insect macroinvertebrates have dominated the faunal surveys in the study area. In 2010, the 39 taxa collected included 10 snail, 7 crustacean, 8 leech, 4 worm and 5 clam taxa. These five groups constituted 87% of the non-insect macroinvertebrate taxa from the Holston River, and it is in these larger groups that changes in fauna among the years can often be observed. In 2010, the remaining groups were either widely collected (e.g., planarians) or spotty in distribution (e.g., sponges, ectoprocts and water mites). Of the 34 (number adjusted for taxonomic changes) taxa collected in 1997, the dominant groups (88%) were snails (10 species), crustaceans (6 taxa), leeches (6 taxa), worms (4 taxa) and clams (4 taxa). In 1990, 89% of the fauna consisted of snails (9 taxa), clams (5 taxa), crustaceans (6 taxa), worms (2 taxa) and leeches (3 taxa). In 1980, these 5 groups out of a total of 27 taxa (78%) consisted of 7 kinds of snails, 4 kinds of crustaceans and 5 kinds of leeches, 2 kinds of worm and 3 kinds of clams. A lower number of taxa was collected in 1977 (23 taxa), 1974 (25 taxa), and 1965 (20 taxa).

Dr. Raymond W. Bouchard collecting non-insect macroinvertebrates (crayfish, worms, etc.) from Zone 6.

An analysis of species composition using data from all seven surveys indicated several patterns. Some of the largest differences between samples were among years with many of the samples from the 1965, 1974, 1977 and 1980 surveys more similar to each other than zones across years. An exception to this was Zone 2 samples, which clustered together across survey years 1965-1980. The 2010, 1997 and 1990 surveys grouped together and indicated that the non-insect macroinvertebrate communities have been similar during this period. During the three most

An Appalachian Brook Crayfish, one of the many species of non-insect macroinvertebrates collected during the 2010 South Fork Holston River Monitoring Studies.

recent surveys, it is also apparent that zones are more similar to each other across years, which suggests that the differences among the zones have been maintained during these years. In the years when the Horse Creek zones were sampled (i.e., 1990, 1997 and 2010), these zones clustered apart from the Holston River zones, indicating that the non-insect macroinvertebrate communities in Horse Creek and Holston River zones were less similar.

The non-insect macroinvertebrates from Zones 2 through 6 in 2010 indicate impacts at Zones 2, 3 and 4 compared to Zones 5 and 6. No rare or endangered species were collected. Most taxa collected during the survey were classified as tolerant to pollution (59%) and only two taxa (5%) were classified as sensitive. The remaining taxa were classified as having moderate tolerance to pollution (26%) or were not classified (10%) due to a lack of information on those taxa.

The 39 taxa collected in the 2010 study is the highest total ever collected, after adjusting for changes in taxonomic resolution since 1965. Despite an increase in the number of taxa collected in 2010, there were only three taxa (*Piscicolaria reducta*, *Helisoma anceps* and *Hydrachna* sp.) new to the Holston River surveys. The increases in taxa richness in the 1990, 1997 and 2010 surveys have been largely a result of collecting more of the taxa historically found in the Holston River. There was an increase in species richness between the 1997 and 2010 surveys at all the zones except Zone 4. The number of taxa at Zone 4 dropped from 21 in 1997 to 17 in 2010. With the exception of Zone 4, the 2010 survey of non-insect macroinvertebrates in the Holston River indicated similar conditions (Zone 6) or improvements (Zones 2, 3 and 5) compared to the 1997 survey. Both Horse Creek zones indicated similar conditions compared to 1997. These findings indicate a continued and marked improvement in water quality since surveys began in 1965.

Aquatic Insects

Qualitative Assessment

Qualitative sampling augmented quantitative sampling from Holston River Zones 2-6, and HC1 (including both HC1U and HC1L) and HC2. The standardized effort included two observers for about 2-3 hours of searching at each zone. Data from quantitative and qualitative collections were pooled to form a comprehensive list of all species found at each zone. While taxa lists are more difficult to analyze statistically, they provide more comprehensive information on the range of taxa present at each zone. The combined list includes very small insects

found in careful laboratory sorting, uncommon species not collected by the PIBS, species collected from riffles (from PIBS sampling) and species from pools, backwaters, vegetation, sand, mud, and snags (from qualitative sampling).

South Fork and mainstem Holston rivers

Qualitative collections in 2010 were markedly different from those in 1997 over most of the river zones because most zones were inhabited by more species than in previous years; these changes were especially noteworthy at Zone 3. In 1997, flatworms, midges and blackflies were virtually the only invertebrates found under rocks in open riffle habitat; there were no mayflies or caddisflies. The 1965 and 1974 surveys found even more depauperate aquatic insect communities (0-8 taxa) at Zone 3 than were observed in 1997 (16 taxa). In 2010, flatworms were scarce, and mayflies and caddisflies were abundant. Although the diversity of these orders was lower at Zone 3 than observed at Zone 6, it represents a localized and marked improvement in aquatic insect diversity. Moreover, the presence of these organisms in large numbers suggests drastic improvements in water quality.

Aquatic insects, like damselflies (left) and hellgrammites, are key elements of the benthic macroinvertebrate fauna of the Holston River.

In 1997, extensive sampling of Zone 3 in macrophyte beds (mainly along the "river-right" bank) yielded only two damselflies. In 2010, damselflies and dragonflies were extremely abundant in macrophyte beds. Many common species of both dragonflies and damselflies are moderately tolerant to a variety of pollutants, so their presence often reveals more about habitat structure than about water quality. However, their previous rarity among these ideal habitats was disconcerting, and thus their abundance in 2010 represents both a substantial improvement in water quality and a substantial improvement to the structure and function of aquatic insect communities of the zone.

The change over the years was also apparent at Zone 2. Academy surveys from 1965-1980 found 6-14 aquatic insect taxa at Zone 2, and each year Zone 2 included more taxa than Zone 3. In 1990, 35 taxa were collected from Zone 2 and only 17 from Zone 3. 2010 is the first year in which more taxa were collected from Zone 3 than from Zone 2. The richness of the sites was very high (Zone 2: 30, Zone 3: 59). There were several mayfly and caddisfly taxa present at Zone 2, but they were more difficult to find because their relative abundance was lower. Habitat in the river channel was lacking for many native aquatic insects. Most specimens were collected in backwaters, which is a rare habitat within the zone. Especially noteworthy was the

lack of hellgrammite larvae (*Corydalus cornutus*) in Zone 2. Hellgrammite larvae are ubiquitous in Appalachian rivers and are large predators that hunt among the interstices of benthic substrata. Interstitial spaces have been filled with gravel and sand at Zone 2, as a typical effect of dam operations.

As in previous years, the aquatic insect composition generally showed an increase in diversity downstream from Fort Patrick Henry Dam. Historically, Zone 5 has shown a partial recovery in aquatic insect diversity relative to Zone 3; the two zones supported relatively congruent communities in 2010. The comprehensive taxa list suggests that Zone 3 actually supported more taxa than Zone 5. This difference resulted from the rarity of low velocity-macrophyte bed habitat in Zone 5 and the abundance of such habitat in Zone 3.

There was a shift in the relative abundance of the two dragonfly species *Boyeria vinosa* and *Basiaeschna janata* between 1997 and 2010. Both of these aeshnid species inhabit similar habitats among root wads and branches of undercut banks near flowing water. In 1997, *Boyeria vinosa* was nearly ubiquitous in this habitat, while *Basiaeschna janata* was absent or uncommon. In 2010, *Basiaeschna janata* was ubiquitous, and *Boyeria vinosa* was uncommon. This difference occurred throughout all zones and is probably due to factors other than the operation of the Eastman facility.

Horse Creek

In 2010, the comprehensive list of taxa developed for Horse Creek was similar to the one developed in 1997. Horse Creek was the only location in the 1997 survey to support stoneflies (Plecoptera), although they were not especially abundant in either year. The common Appalachian genus *Sweltsa* was most abundant in 1997, but was not collected in 2010. The larvae were quite mature in 1997 and they may have emerged as winged adults before 2010 sampling.

One rare species of stonefly was collected in 2010 that had not been collected in previous surveys. A PIBS sample from HC1 (upper) contained *Hansonoperla appalachia*. The species is not listed as federally threatened or endangered. The species is known to occur only rarely through its range, which extends through the Appalachian Mountains from New Hampshire to northern Georgia. It has been ranked as Globally Vulnerable (G3) to extirpation by NatureServe, and its state conservation status in West Virginia is "Imperiled" (S2). Its status in Tennessee is "vulnerable" (S3), but the Tennessee Department of Environment and Conservation does not include *Hansonoperla* on its list of rare species.

Brett Marshall using a Portable Invertebrate Box Sampler (PIBS) to collect aquatic insects at Zone 6.

Quantitative Assessment

Most of the effort to describe and compare aquatic insect assemblages was drawn from the collection of quantitative samples collected by means of a Portable Invertebrate Box Sampler (PIBS; 0.05 m^2, 500-µm mesh netting). This device and standardized laboratory procedures ensure that all samples comprise a standard unit of effort and allow more statistical comparisons than are otherwise possible.

From all zones, just under 106,000 macroinvertebrate specimens were collected, composed primarily of aquatic insects and a few non-insect taxa (mostly mites and worms). A total of 135 distinct taxa were found, of which 121 taxa were aquatic insects. The average density was greatest at Zone 2 (about 82,000 organisms per square meter, reflecting high densities of midges). Densities in the other zones were lower (8,000-37,000 organisms per square meter) but more diverse.

These data were used to calculate several ecological summary measures (commonly called metrics) for each sample. The metrics were used to statistically compare the structure of benthic assemblages among zones on the Holston River and Horse Creek, and also to contrast changes along a gradient near Kit Bottom on the Big Sluice. Sample-specific covariates were used to ensure that the influence of habitat was accounted for in statistical comparisons when necessary.

South Fork and mainstem Holston rivers

Fort Patrick Henry Dam was by far the most important anthropogenic influence on the South Fork and mainstem Holston River zones. Its influence on benthic communities was manifested as several distinct longitudinal gradients in the biological metrics. Specifically, several metrics (taxa richness, diversity, community evenness, EPT richness) steadily increased (suggesting improved water quality) moving downstream from Fort Patrick Henry Dam. The relative abundance of midges showed a dramatic decreasing gradient as distance from the dam increased. Dams are known to have strong influences on aquatic insect community structure and function and the data showcase those effects above all else. Although there were minor differences among zones, none of these differences suggested impairment of Zone 3 relative to other zones.

The dam's influence on aquatic insect assemblages of the Holston River appeared to be more extreme than observed in 1997. The reason for this is two-fold. First, routine dam operation has changed since 1997, and the current regime appears to limit physical habitat for invertebrate colonization near the dam. Second, there are many more aquatic insects living in Zone 3 than there were in 1997. Sometimes, one impairment can obscure another. Quantitative sampling collected significantly more species at Zone 3 than previous surveys. Moreover, the aquatic insect assemblage at Zone 3 included several mayflies and caddisflies in 2010; these taxa were not present at Zone 3 in 1997.

Horse Creek

In 2010, an additional reference zone was added to account for the growth and expansion of a golf course near the original quantitative sampling reach of upper Horse Creek (HC1). Golf courses can affect the abundance of aquatic insects, through pesticide and fertilizer runoff. The new zone (called HC1 upper) appended the traditional HC1 zone (now called HC1 lower), allowing sampling in a riffle that was at the upstream boundary of the earlier HC1 zone. The communities of the 1997 zones (HC1L and HC2) were compared with those of a new riffle farther upstream (HC1U).

Three of the four metrics based on the relative abundance of aquatic insect functional feeding groups exhibited a statistically significant difference among the HC zones. This seems to have been mainly influenced by the four-lane bridge that shaded much of HC1U and reduced the relative abundance (proportion among all insects) of scrapers, which resulted in a corresponding increase in one or more of the other metrics (e.g., % collectors, % shredders). The metric % shredders is generally considered an indicator of natural ecosystem function for small Appalachian streams. None of the significant differences indicated significant impairment of HC2 relative to the other HC zones.

Kit Bottom

There were several significant differences among the three zones used to evaluate the ecological condition of the Big Sluice near Kit Bottom. Most of these differentiated both the zone above Kit Bottom (KBU) and the zone adjacent to Kit Bottom (KBL) from the farthest downstream zone (Zone 4). These differences were likely due to the proximity of the KBU and KBL samples to the bank, where they would have been more shaded, and would be more influenced by riparian vegetation. Samples were collected very close to the bank to maximize any effects of trace leachates on the benthic community. Zone 4 samples have traditionally spanned the entire Big Sluice. Efforts to make Zone 4 samples comparable with other Holston River samples with respect to depth and velocity required sampling around and below mid-channel bars. The only significant difference that suggested an impact was the relative abundance of non-insect taxa at KBL. This metric often increases in places where conditions become inhospitable to aquatic insects. A re-evaluation of these data indicated that the higher average relative abundance of non-insects was due to one sample from KBL, which exhibited elevated oligochaete worm density of 1,444 worms/m^2, whereas most of the other samples from KBU, KBL and Zone 4 were below 50 worms/m^2. One of the reasons 10-16 samples are collected from a zone is to minimize the influence of a single aberrant sample. We collected fewer samples from the Kit Bottom sites, which allowed a single outlier to have a greater influence on the site average for this metric. When this single sample was removed, the difference among zones was no longer statistically significant. Kit Bottom samples were collected very close to the bank, where physical characteristics can cause localized high concentrations of some small worm species. Therefore, we do not believe this metric provides evidence that Kit Bottom significantly altered the benthic community structure of the Big Sluice.

Overall Summary

- The most pervasive impairment to development of natural aquatic insect communities in the Holston River is the hydrological regime imposed by Fort Patrick Henry Dam.
- The aquatic insect communities at Zone 3 were more diverse than in any of our previous Holston River surveys and now include relatively sensitive orders of aquatic insects (mayflies and caddisflies).
- No relevant changes in the community structure of Horse Creek or in the Big Sluice near Kit Bottom were observed.
- A species of conservation concern was collected, but it was not federally listed as rare, threatened or endangered. Only a single specimen was collected, and there is no evidence of its survival being affected by operation of the Eastman facility.
- The comprehensive taxa list for each Holston River zone was equal to or greater than in previous years, while the quantitative assessment indicated that abundance and diversity in riffles were similar to previous surveys.

Fish

A total of 3,948 individuals of 47 species was collected in the 2010 survey, including 17 species of carp and minnow, 5 species of sucker, 8 species of centrarchid (bass and sunfish) and 8 species of darter. Mimic shiner, mountain madtom, speckled darter, striped bass, and shorthead redhorse have not been reported in previous Academy Holston River surveys. Overall, the most widespread species were the Tennessee snubnose darter, telescope shiner (all zones), central stoneroller (all zones except 2), greenside darter and smallmouth bass (all major zones except 2), and rock bass, banded sculpin, northern hog sucker, redline darter, and redbreast sunfish (collected at 8 of the 11 zones). The Tennessee snubnose darter was the most abundant species overall. The banded sculpin was the second-most common, but it was found in greatest abundance at Zones HC1 and HC2.

For the South Fork and mainstem Holston rivers zones, there was a general pattern of increasing richness and abundance from Zone 2 through Zone 6. There were no significant differences in fish assemblages among Zones KU, KL and 4, the Big Sluice zones. The abundance of the Tennessee snubnose darter was highest in the three Big Sluice zones, with no clear differences among these zones. Analyses of individual fish condition and growth showed somewhat different patterns. For stoneroller, condition was highest at the Big Sluice zones and possibly at Zone 3L. Condition of Tennessee snubnose darters was highest at Zones HC1 and KU, and lowest at Zone 3R. However, the daily growth rate of the darter was somewhat higher at Zone 3R than at the other zones. Zone 2 showed the most extreme growth conditions, with later hatching dates and lower daily growth rates. Together, these conditions resulted in very small young-of-year fish at Zone 2 compared to the other zones.

A variety of techniques are used to sample all available fish habitats. Dave Keller and Paul Overbeck (left; front to back, respectively) are using a boat and electrofishing equipment to sample large fishes at Zone 5. Dr. Richard Horwitz (right; center) with Corey Click (left) and Michelle Brannin (right) are sampling smaller fishes with a backpack electrofishing unit.

The observed pattern in fish communities parallels the pattern of hydrological impacts of the dam on the river, the two most obvious effects being variation in temperature and flow (water depth and current speed). Given the gradient in hydrological conditions downstream from Zone 2, it is difficult to separate out possible impacts of stressors in the vicinity of Kingsport.

Few lesions, deformities or other abnormalities were noted. There was no spatial pattern in anomalies, except for greater frequency of mouth lesions (presumably hook wounds) at Zone 5. In 1997, a few fish were noted at Zone 3L with significant amounts of lesions or skin thickening of the tail and caudal peduncle. No such anomalies were noted in 2010.

Total species richness in 2010 was higher than in any previous survey. Comparisons among surveys and zones need to account for differences in effort and changes in sampling techniques. For example, more species were caught in Zone 2 than in previous surveys, mainly because of capture of large fish (e.g., trout, suckers, and sunfishes) by boat electrofishing. Except for this, collections at Zone 2 were similar or worse than in 1997. Fish collections at Zones 3L and 3R were more diverse than in previous surveys. Species richness at Zones 5, 6 and the two Horse Creek zones were similar to those in 1997, although there were some differences in species occurrence. Several species generally intolerant of habitat and water quality degradation were caught for the first time. Several species which were relatively frequent in the most recent surveys were not caught or were less common in 2010. These include some intolerant species, as well as some species, such as mosquitofish, indicative of poor conditions. In 1997, green sunfish was common and a number of hybrids of green sunfish and other sunfishes were noted, suggesting recent introduction. The abundance of green sunfish was much lower in 2010, and only one hybrid was caught.

The fish surveys indicate a significant improvement in the fish communities since the earlier surveys. Over the entire survey record, improvements have been seen throughout the South Fork and mainstem Holston rivers. In more recent surveys, conditions at the lowest zones (5 and 6) appear to be stable or changing slowly. Improvements appear ongoing at Zone 3, which had the lowest quality in the earliest surveys. Zone 2 continues to be affected by upstream dam releases. While the tailwater supports sport fish, such as trout, and some native species, the abundance and diversity of many fish taxa continues to be low.

Forty-seven species of fishes, representing a wide variety of functional feeding groups, were recorded during the 2010 South Fork Holston River studies including (clockwise from left) channel catfish, redline darter and redear sunfish.

General Conclusions

Four main general conclusions can be drawn from the 2010 study of aquatic communities in the vicinity of Kingsport, TN.

- A major stressor affecting biological communities of the South Fork Holston River in the vicinity of Kingsport continues to be the Fort Patrick Henry Dam, with communities being most disturbed at Zone 2 and improving downstream.
- Analyses of chemistry and biological communities also show effects of nutrient enrichment downstream of the dam, which may come from a variety of industrial, municipal and watershed sources.
- The status of biological communities is similar to that observed in the Academy's 1990 and 1997 studies, with evidence of some improvement after 1997 in parts of the study area, e.g., Zone 3.
- No evidence of biological impacts from Eastman operations was found at the Horse Creek zones.

PERSONNEL AND ACKNOWLEDGMENTS

These studies were performed under the supervision of Dr. David Velinsky, Senior Scientist and Vice President of the Patrick Center for Environmental Research of The Academy of Natural Sciences of Drexel University. Dr. Richard Horwitz, Project Leader, was responsible for the professional quality of all field and laboratory work. Ms. Robin S. Davis, Director of Scientific Communications, edited and produced the report for this program. The direction and implementation of individual project elements were the responsibility of the Principal Scientific Investigators. The following are the personnel who participated in the 2010 South Fork Holston River Environmental Monitoring studies.

Watershed Geography and GIS

Principal Scientific Investigator: Jerry Mead, Ph.D.
Cartography: Roger L. Thomas and Jerry Mead, Ph.D.

Environmental Geochemistry

Principal Scientific Investigator: David Velinsky, Ph.D.
Field and Laboratory Chemist: Paul Kiry, M.S., Paula Zelanko, M.S.

Algae and Aquatic Macrophytes

Principal Scientific Investigator: Donald F. Charles, Ph.D.
Field Biologist and Report: Frank W. Acker, M.S.
Diatom Analyst: Eduardo Morales, Ph.D.
Laboratory Biologists: Melanie Mills, M.S., Zoe Ruge and Sylvan Klein
Database and Programming: Pat Palmer and Patrick Boylan

Non-Insect Macroinvertebrates

Principal Scientific Investigator and
Field and Laboratory Biologist: Raymond W. Bouchard, Jr., Ph.D.
Field Biologist: Sylvan Klein

Aquatic Insects

Principal Scientific Investigator,
Field and Laboratory Biologist: Brett Marshall, M.S.
Field Biologist: Roger L. Thomas
Laboratory Technician: Ezmeralda Ortiz

Fish

Principal Scientific Investigator
and Field Biologist: Richard Horwitz, Ph.D.
Field and Laboratory Biologists: Paul Overbeck, David Keller, M.S., Michelle Brannin, M.S.
Data Entry: Andrea Kreit

Quality Assurance

Quality Assurance Officer: Robin S. Davis

The following Eastman personnel also contributed to the success of the 2010 South Fork Holston River Environmental Monitoring Studies:

Vice President and General Manager, Worldwide Manufacturing Support and Quality

J. Parker Smith

Director, Operations Support Services and Global Quality

Linda Lewis

Manager, Environmental Affairs

Richard Strang

Environmental Affairs

Marsha Edwards, Janet Evans, Stacey Griffith, Keith Harris, Keri Hochstetler, Brenda Lowder, Tim Musick, Daniel Robertson, Jane Welch, Sharon Williams, Justin Crawford, Avery Yu

Superintendent, Utilities Division

John G. Perdue

Superintendent, Waste Disposal Services Department

John B. Barber

Utilities Distribution Services

Allen Clem, Tim Presley, Larry Patrick, David Light, Scott Tucker

Environmental Services Laboratory

Richard Grese, Darrell P. Murphy, Jim Milhorn, Brenda Woods

Eastman personnel also contributing were:

Richard Guinn	Product Safety and Health
Wanda Valentine	Corporate Communications
CeeGee McCord	Manager, State Government Relations

Corey Click	Government Relations
Kelly Hammonds	Senior Photographer
Ben Dowdy	Photographic Services
Harry Watts	Creative Services
Brenda Mercer	Manager, Logistics Services
Tim Price	Supply Chain Supervisor, Sample Central
John Cradic	Sample Central
Martin Roberts	Sample Central
Roslyn Robinson	Sample Central
Nancy Gilley	Indirect Procurement

We would also like to thank the following for their assistance during the 2010 South Fork Holston River Environmental Monitoring Studies:

David McNutt	Brock Services, Ltd.
Mike Frazier	Brock Services, Ltd.
Mike Quillen	Brock Services, Ltd.
Charlie Floyd	Domtar Industries, Inc.
Chelsey Holmes	Domtar Industries, Inc.
Anthony Robinson	Domtar Industries, Inc.
A. H. "Dusty" Parsons	Domtar Industries, Inc.
Claudia Leonard	President, Board of Directors, Ridgefields Country Club
Nodjya Cook	Head Golf Professional, Ridgefields Country Club
Andy King	General Manager, Meadowview Conference Resort & Convention Center
Debbie Wilson	Meadowview Conference Resort & Convention Center
Molly Carpenter	Meadowview Conference Resort & Convention Center
Pete deBraal	Director of Golf, Cattails at Meadowview Golf Club
Susan Jacks	TVA Special Operations
John Hoover	TVA Special Operations
Janice G. Pinkston	TVA River Scheduling
Ronny Hammonds	City of Kingsport Public Works
Ryan McReynolds	Director, City of Kingsport Public Works

Mr. & Mrs. Dean and Heather Greer	Kingsport property owners
Mr. Bob Glover	Kingsport property owner
Ms. Sandra Click	Hawkins County property owner
Mr. & Mrs. Wayne Lingerfelt	Hawkins County property owner
Mr. Claude Begley	Hawkins County property owner

QUALITY ASSURANCE STATEMENT

Study Number: 582

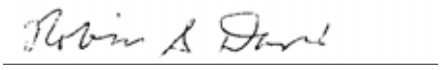
Study Title: 2010 South Fork Holston River Environmental Monitoring Studies

This study was performed under the general provisions of the Patrick Center's Quality Assurance Implementation Plan (Rev. 1, June 1999). The final report will be reviewed to determine that it is an accurate reflection of the data obtained.

The dates of Quality Assurance activities on this study are listed below.

TYPE OF AUDIT	DATE AUDIT COMPLETED
Environmental Geochemistry	2/15/2011
Algae and Aquatic Macrophytes	2/10/2011
Non-Insect Macroinvertebrates	1/19/2011
Aquatic Insects	1/13/2012
Fish	3/3/2011
Report	3/21/2012

ARCHIVING: Raw data and the final report will be filed in the Patrick Center's archives.



Robin S. Davis Date: 3/21/2012

Quality Assurance Unit
Patrick Center for Environmental Research
The Academy of Natural Sciences

TABLE OF CONTENTS

Page

1. INTRODUCTION

Since 1965, the Academy of Natural Sciences of Drexel University has conducted a series of aquatic field studies in the South Fork and mainstem Holston rivers for Eastman Chemical Company's Tennessee Operations. The purpose of these studies has been to augment existing data and monitoring programs, and to evaluate potential biological impacts of multiple stressors along the South Fork and mainstem Holston rivers in the vicinity of Kingsport, TN. These stressors include not only the Eastman facility, but also several other major municipal and industrial dischargers and TVA's Fort Patrick Henry Dam.

Previous comprehensive biological and chemical studies were conducted by the Academy in 1965, 1974, 1977, 1980, 1990 and 1997. All of these studies have examined the same key biological groups, using similar sampling methods and sampling locations. As a result, they have proved to be a valuable monitoring tool for assessing long-term patterns of change in the study area. These studies have documented substantial improvements in various characteristics of biological communities in the South Fork and mainstem Holston rivers since 1965.

The objectives of the 2010 study were to assess potential biological differences among zones along the South Fork Holston River, mainstem Holston River and Horse Creek, and to assess long-term temporal trends throughout the study area. Results of the study will fulfill requirements of Eastman's hazardous waste management permit, aid Eastman's evaluation of its efforts to protect the environment, and help to fulfill the company's Responsible Care© goals of understanding and communicating environmental issues.

The 2010 study includes the main elements of the 1997 study: basic environmental water chemistry, attached algae and aquatic macrophytes, aquatic insects, non-insect macroinvertebrates and fish. The purpose of the chemistry component is to support the biological elements; as in 1997, no special studies of sediment, water column, or tissue chemistry (as was conducted during the 1990 study) are included. In the 1997 study, a geographical information system (GIS) for the study area and the entire South Fork Holston watershed was constructed. This system was updated for the 2010 study. The GIS is used to document land-use patterns and locations of the numerous potential stressors (e.g., industrial and municipal point-sources, non-point sources of sediment and nutrients, Fort Patrick Henry Dam), and recent changes in these patterns.

The 2010 study also addresses two potential point-source issues associated with shallow groundwater discharged through Big Tree Spring (into the South Fork Holston within Zone 2) and from Eastman's historical Kit Bottom landfill into the Big Sluice upstream of Zone 4. Potential effects of Big Tree Spring (BTS) are addressed by selected analyses at the mouth of the spring, in comparison with Zone 2 conditions. Potential Kit Bottom groundwater effects are addressed by sampling two new zones in Big Sluice, one (KU) between the mouth of Horse

Creek and the Kit Bottom landfill site, and the other within the potential groundwater discharge area (KL).

The report is organized as follows: Executive Summary, Introduction, a brief description of the study area in Section 2, an overview of the study design and its rationale in Section 3, field and laboratory methods in Section 4, results of the various study elements in Section 5, Literature Cited in Section 6, and Appendices in Section 7.

2. STUDY AREA

2.1 Location of Sampling Zones

The study area consists of four parts: the South Fork Holston River between the Fort Patrick Henry Dam and its confluence with the North Fork Holston River, Big Sluice, the segment of the mainstem Holston River between the confluence of the North and South Fork Holston rivers and Goshen Valley Road, and the segment of Horse Creek between the vicinity of Meadowview Parkway and Big Sluice.

Within the study area, sampling was conducted in nine main zones plus BTS, located within Zone 2 (Fig. 2.1): three on the South Fork Holston River (Zones 2, 3 and 5; part of Zone 5 lies in the mainstem Holston River, but is entirely within the plume of the South Fork Holston, immediately downstream from its confluence with the North Fork Holston), one on the Holston River (Zone 6), two on Horse Creek (Zones HC1 and HC2), and three on the Big Sluice (KU, KL and Zone 4), downstream from Horse Creek. (Note: historical Zone 1 is located upstream from the Fort Patrick Henry Dam, but this zone has been discontinued.) Sub-zones within the main zones were employed for some of the biological groups; e.g., fish sampling was conducted separately near the left bank (sub-zone 3L) and right bank (sub-zone 3R) in Zone 3, with “left” and “right” assigned facing downstream. Specific sampling sites within zones differed among study elements, due to differing requirements for effective sampling. Locations of these sites are detailed in Section 4.

Zone 2 serves as a reference area on the South Fork Holston River for various potential impacts in Kingsport (including Eastman's Tennessee Operations discharges). It is upstream of all major NPDES dischargers in Kingsport. However, Zone 2 is strongly affected by releases from the Fort Patrick Henry Dam, and the zone reflects impacts of hydrological and water quality effects of the dam. Zone 3 is exposed to Tennessee Operation's cooling-water and waste-water effluents, to cooling-water effluent from the USA Holston Army Ammunition Plant Area A, and to urban runoff via Mad Branch, while Zone 5 is exposed to Eastman effluents as well as several other major NPDES discharges in the Kingsport area (e.g., the Kingsport Sewage Treatment Plant, Domtar) and urban and agricultural runoff via Reedy Creek. Zone 6 is located on the mainstem Holston River, approximately 10.7 mi downstream from the confluence of the North and South Fork Holston rivers. The purpose of Zone 6 is to determine whether biological communities well downstream from Fort Patrick Henry Dam and the Kingsport area show evidence of recovery from disturbance on the South Fork Holston, as well as disturbance from the North Fork Holston River.

Among the five remaining sampling zones, Zone HC1 serves as a reference area on Horse Creek for comparison with Zone HC2. Zone 4 is located on Big Sluice, just upstream from its

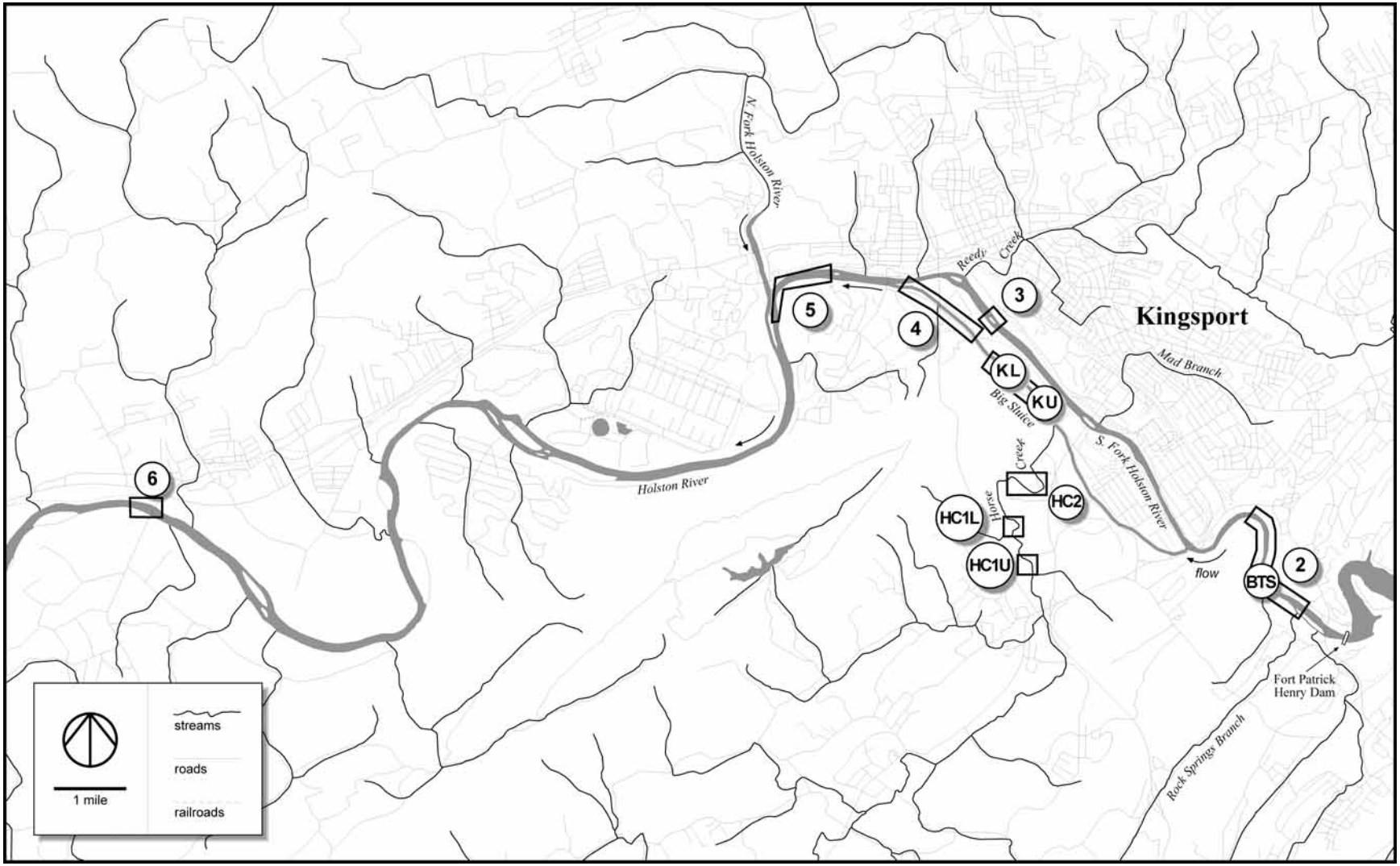


Figure 2.1. Sampling zones used in the 2010 South Fork Holston River study.

confluence with the South Fork Holston River. Zone KU is located between the mouth of Horse Creek and the potential groundwater discharge area, and Zone KL within the potential groundwater discharge area.

2.2 Watershed Geography

The main portion of the study area lies in the immediate vicinity of Kingsport, TN, in the South Fork Holston sub-basin (USGS Cataloguing Unit 06010102) (Fig. 2.2). The Watauga, North Carolina, Tennessee sub-basin (USGS Cataloguing Unit 06010103) drains into the South Fork sub-basin upstream of the study area. These two sub-basins have a combined area of approximately 2040 mi^2 and include portions of Tennessee, Virginia and North Carolina. Land use in these two sub-basins in 2001 was predominantly forest (60.6%), with 25.2% agriculture, and 11% being urban. The study area, however, has significant urban and agricultural influences.

Zone 6, the study zone farthest downstream of the study area, is used as a recovery zone (Fig. 2.2) and includes the same sub-basins as the main study area, plus the North Fork sub-basin of the Holston River (USGS Cataloguing Units 06010101, 06010102 and 06010103). The North Fork may improve the quality of river water because it has substantially less development than the South Fork sub-basin or the Watauga, North Carolina, Tennessee sub-basin. However, the North Fork has a history of point-source disturbance, which may affect the Holston River. Overall, the Zone 6 watershed has an area of approximately 2750 mi^2 and includes portions of Tennessee, Virginia and North Carolina. Land use in 2001 was predominantly forest (62.4%), with 9.3% of land cover being urban and 25.0% agriculture (see 2001 land use Fig. 2.3).

The current analyses used a 30-m resolution, digital elevation model (DEM) to compute slope and higher quality classifications of land cover (Fig. 2.3). The higher resolution DEM was also based on "real" data collected from the NASA's Space Shuttle radar topography mission, while the 250-m resolution DEM in the previous report used data from stereo optical analyses of photographs. Hence, slope and land cover data are much more accurate in this report than in the previous report.

Change in land cover was simulated from 1991 to 2041 using the GEOMOD land cover change model. GEOMOD creates a map of suitability for conversion of land cover and then uses this map to extrapolate a rate of change in land cover over time and space. GEOMOD created the suitability map as a function of maps of slope, elevation, distance to urban areas, and existing land cover. The observed rate of change in land cover from 1991 to 2001 was used to run GEOMOD from 1991 to 2041 at time steps of 10 years.

2.2.1 Change in Land Cover

Land cover in 1991 vs. 2001 was compared in order to determine if there were significant changes in land cover in the sub-basins described above. Between 1991 and 2001 there were only minor changes in land cover in the three sub-basins of the study area upstream of Zone 6 (Fig. 2.4 and Table 2.2.1). The Multi-Resolution Land Characteristics Consortium land

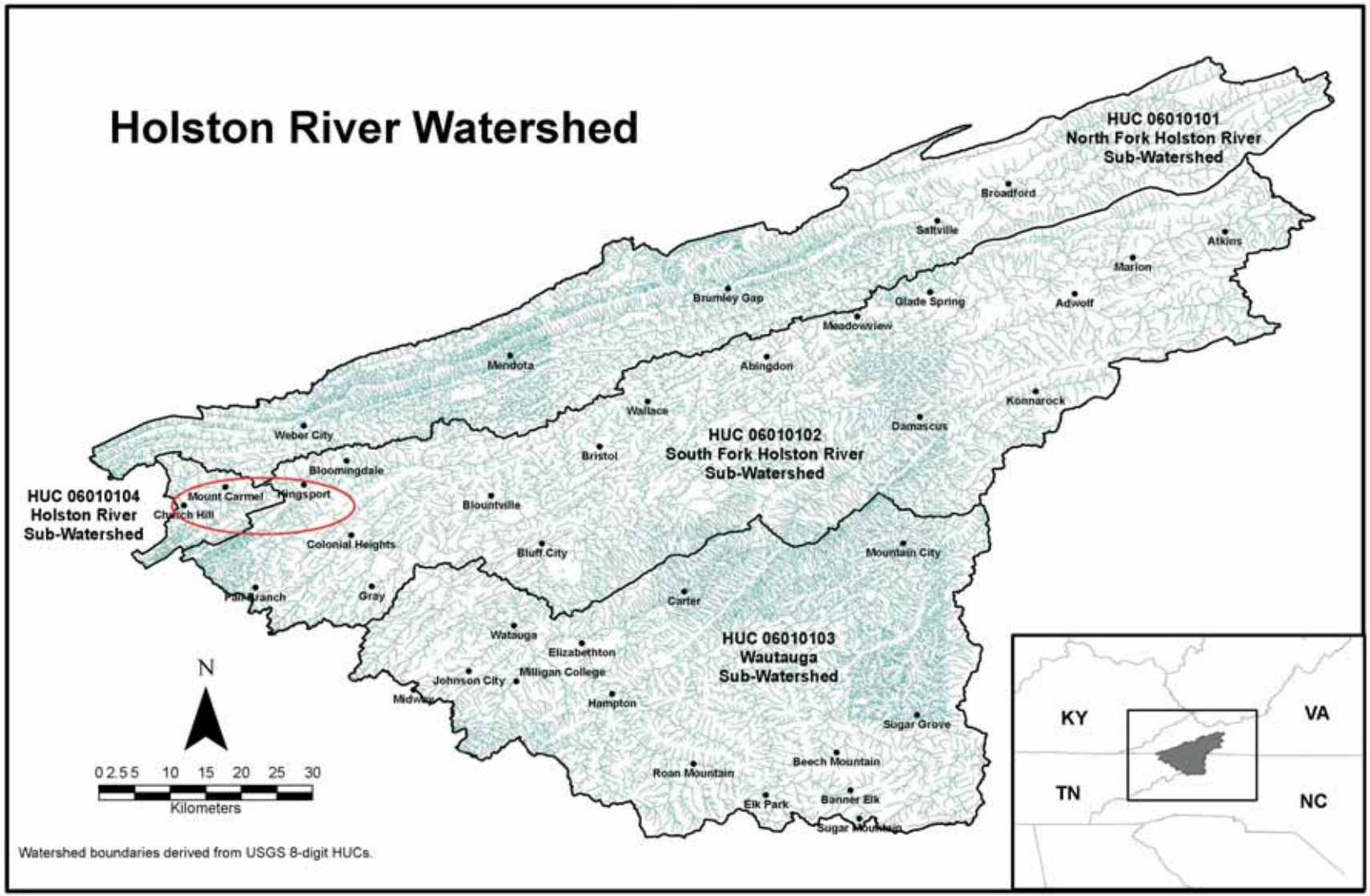


Figure 2.2. Subwatersheds draining into the 2010 South Fork Holston River study area (red oval).

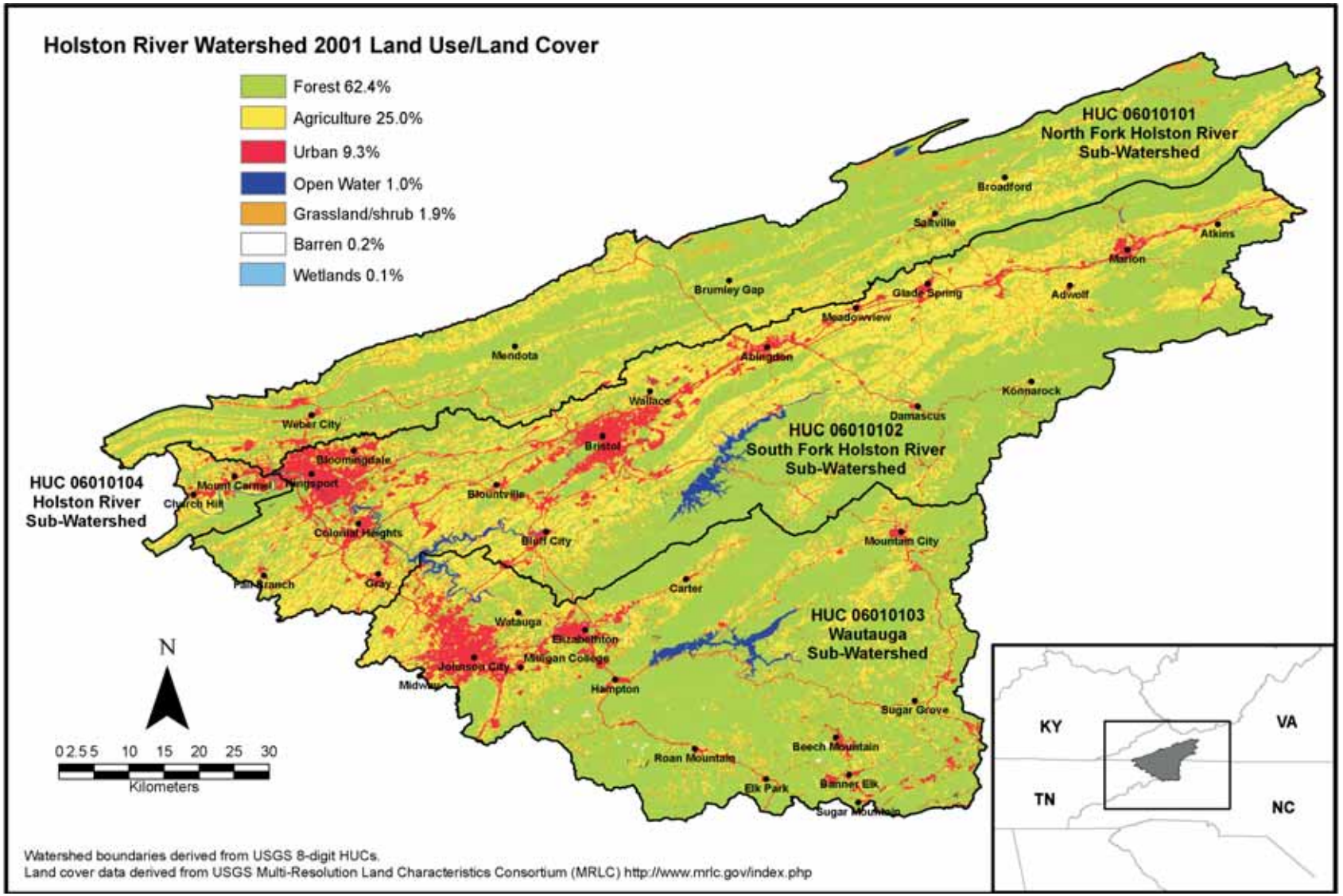


Figure 2.3. 2001 Land cover classifications used during the 2010 South Fork Holston River study.

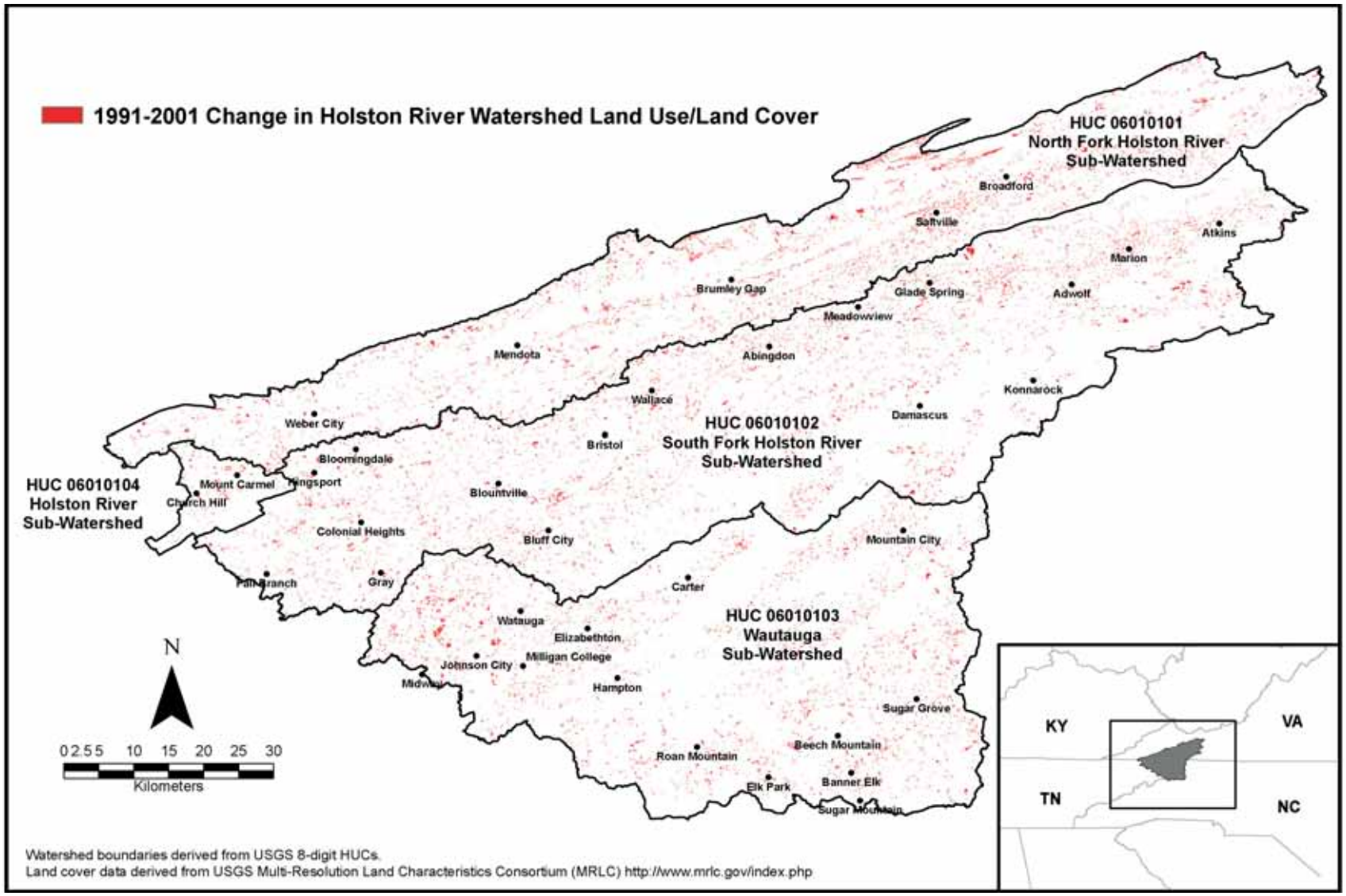


Figure 2.4. 1991-2001 change in land cover classifications.

Table 2.2.1. Land cover classifications and approximate 1991-2001 change in land cover classification area (percent and sq. mi.) for the subwatersheds in the study area.

Land Cover	% Change in Area	Change in Sq. Mi.
Urban	0.40	11.0
Barren	0.03	1.0
Forest	-1.41	-39.0
Grassland/Shrub	0.49	13.6
Agriculture	0.45	12.6
Wetlands	0.01	0.2

cover change dataset indicated there was a net loss of forest of 1.4% of the watershed or approximately 39 mi^2. Furthermore, there was a net increase in the area of the watershed used for agriculture (+0.45% or 12.6 mi^2), urban (+0.4% or 11.0 mi^2), grassland/shrub (+0.49% or 13.6 mi^2), barren (+0.03% or 1.0 mi^2), and wetlands (+0.01% or 0.2 mi^2).

2.2.2 Simulated Change in Land Cover (1991-2041)

Simulations of change in land cover were broken out into three sub-basins (Holston, North Fork and South Fork/Watauga). The Holston sub-basin is the section of the watershed contributing to study zones downstream of Zone 5 to Zone 6 and has a watershed area of 55 mi^2. The Holston sub-basin represents only a small portion of the watershed area draining to Zone 6, but is a good indicator of changes in land cover near to the study area.

Suitability maps for conversion of land cover from non-developed to developed (Fig. 2.5) shows a preference for conversion in areas with low slopes closest to urban areas. Similar suitability maps were generated for agriculture and forest cover. Rate of change in the Holston sub-basin in land cover for forest was 2.9 times higher, urban was 6.0 times higher, and agriculture was about 2.5 times higher than rates of change in the North Fork and the South Fork/Watauga sub-basins (Table 2.2.2). Hence, change in percent coverage was minimal in the North Fork and the South Fork/Watauga sub-basins, with most of the changes occurring near urban areas in the downstream portions of the river. The North Fork and the South Fork/Watauga sub-basins have similar rates of change in land cover except that agriculture is expanding a little faster in the South Fork/Watauga sub-basin than in the North Fork sub-basin (Table 2.2.2).

2.3 Potential Stressors

Numerous potential environmental stressors (e.g., NPDES dischargers, and Toxic Release Inventory, Superfund and hazardous waste sites) are situated in the watershed (Fig. 2.6). The predominant number of stressors occurs in the South Fork and Watauga sub-basins.

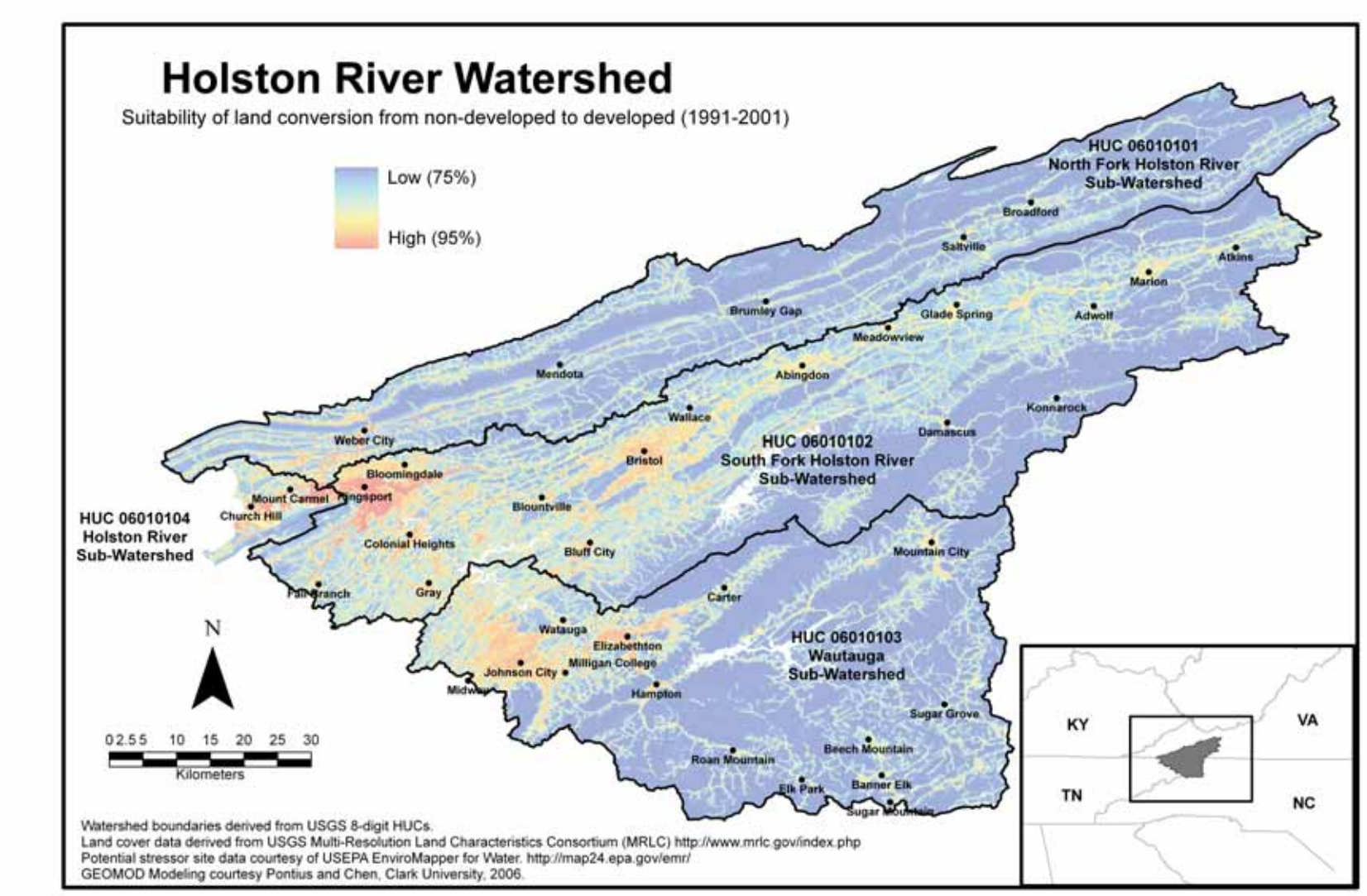


Figure. 2.5. Map of suitability of land for conversion from non-developed to developed land. GEOMOD derives the suitability maps by relating observed land cover of non-developed and developed land in 1991 to potential predictors or drivers of land cover. Potential drivers of land use change include urban areas, slope of land, elevation, forest cover and agriculture cover. Areas in the suitability map with higher values have a higher suitability for conversion.

Table 2.2.2. Simulated change in land cover classifications from 1991 to 2041 by subwatershed.

	Forest (%)			Urban (%)			Agriculture (%)		
Time	Holston	N. Fork	S. Fork and Watauga	Holston	N. Fork	S. Fork and Watauga	Holston	N. Fork	S. Fork and Watauga
1991	48	70	61	16	4	11	32	24	25
2001	44	69	61	23	4	11	33	24	26
2011	39	68	60	25	5	11	35	24	26
2021	37	67	58	25	5	12	36	25	27
2031	34	66	57	27	5	12	37	25	27
2041	32	64	56	28	6	13	37	25	28

Several of these dischargers are classified as major by the USEPA, including the Abingdon Sewage Treatment Plant, Johnson City Regional Sewage Treatment Plant, Bristol Sewage Treatment Plant #2, Kingsport Wastewater Treatment Plant, USA Holston Army Ammunition Plant, Domtar Paper and Eastman's Tennessee Operations. Many of the dischargers are located various distances upstream from Fort Patrick Henry Dam; several are located in Kingsport, within the Academy's study area. Other potential stressors in the Kingsport area include several tributary streams that carry urban runoff, exfiltration (leakage) from the city sewer system, pollutants from rural septic tanks, and runoff from cattle farms and other agricultural operations.

For example, Horse Creek, a study area since 1990, has experienced the development of an expanded golf course and construction of two fairly large parking/building complexes in the area around Zone HC1. Such development may change the quantity, pattern and quality of runoff, due to changes in the amount of impervious cover, lawns and fertilizers. In addition, during the 2010 survey, grass clippings originating from maintenance of the golf course were observed on the banks of Horse Creek, as well as in the stream. These could have physical effects relating to reduction of riparian vegetation, as well as effects on water quality relating to nutrients and BOD.

A substantial portion of the agricultural lands near the study area occurs on slopes of greater than 3% (Fig. 2.7). These lands have high erosion potential, and tributaries to the South Fork Holston that originate in such areas (e.g., Horse Creek) are expected to carry high loads of suspended sediment, as well as particle-bound nutrients and contaminants of agricultural origin.

Releases of water from the Fort Patrick Henry Dam are a major stressor on biological communities in the South Fork Holston River and Big Sluice. This is particularly true during the warmer or rainier months of the year, when dam discharge oscillates between 0 and 2000-8000 cfs several times each day (Fig. 2.8). Fort Patrick Henry Dam discharge data for July 2010 (including the week of the Academy study) are presented in Figure 2.9. Each release cycle produces substantial fluctuations in water depth and current speed in both the South Fork Holston River and the Big Sluice. Although dam releases of 0 cfs occur throughout each day, neither 0 river discharge nor channel dewatering occur downstream of the dam, because of the time lag in river drainage and other downstream water inputs.

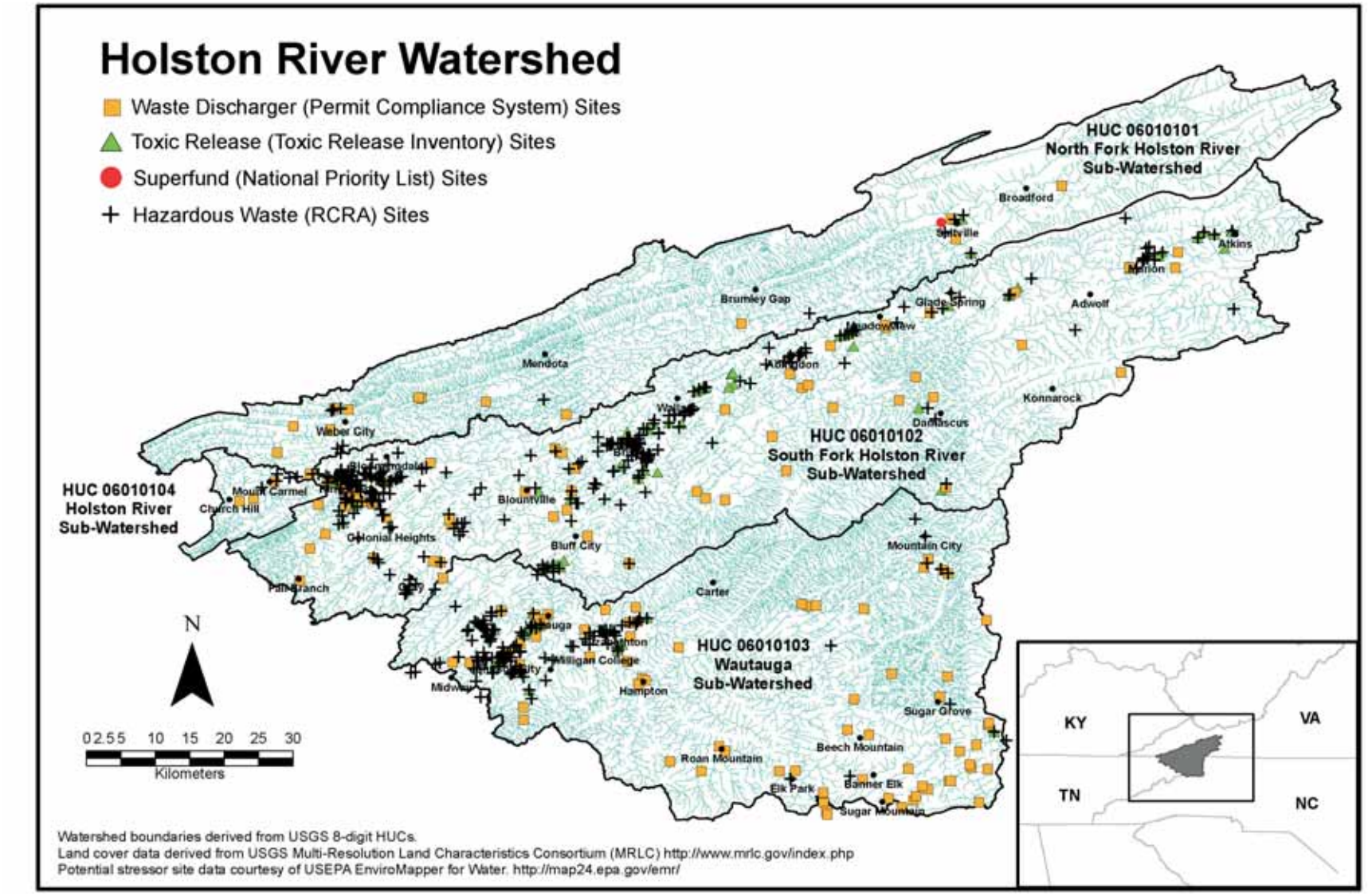


Figure 2.6. 2001 Potential stressors that may affect the water quality at zones within the 2010 Holston River study area.

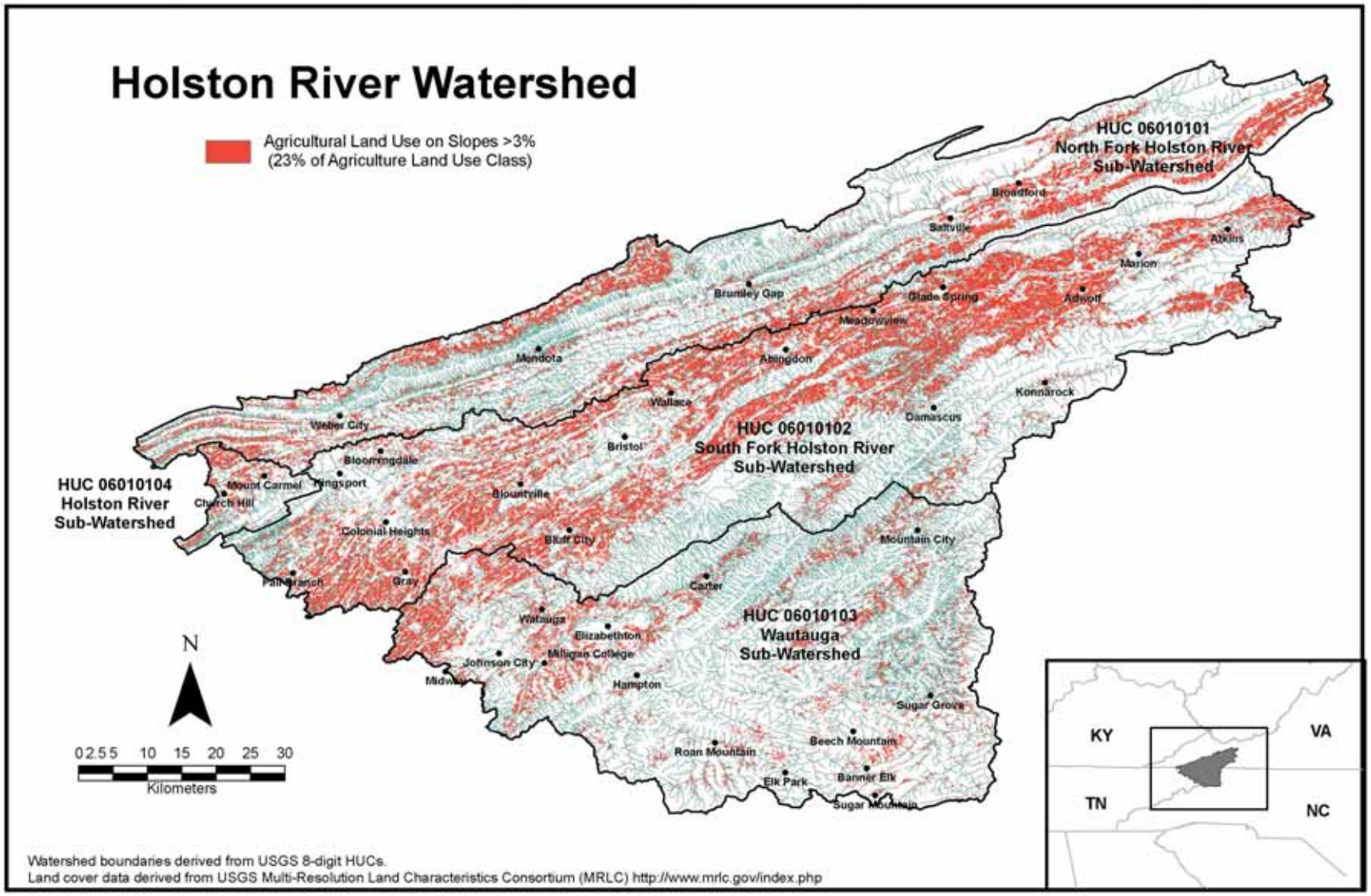


Figure 2.7. 2001 Agricultural land use on slopes with >3% slope within the 2010 Holston River study area.

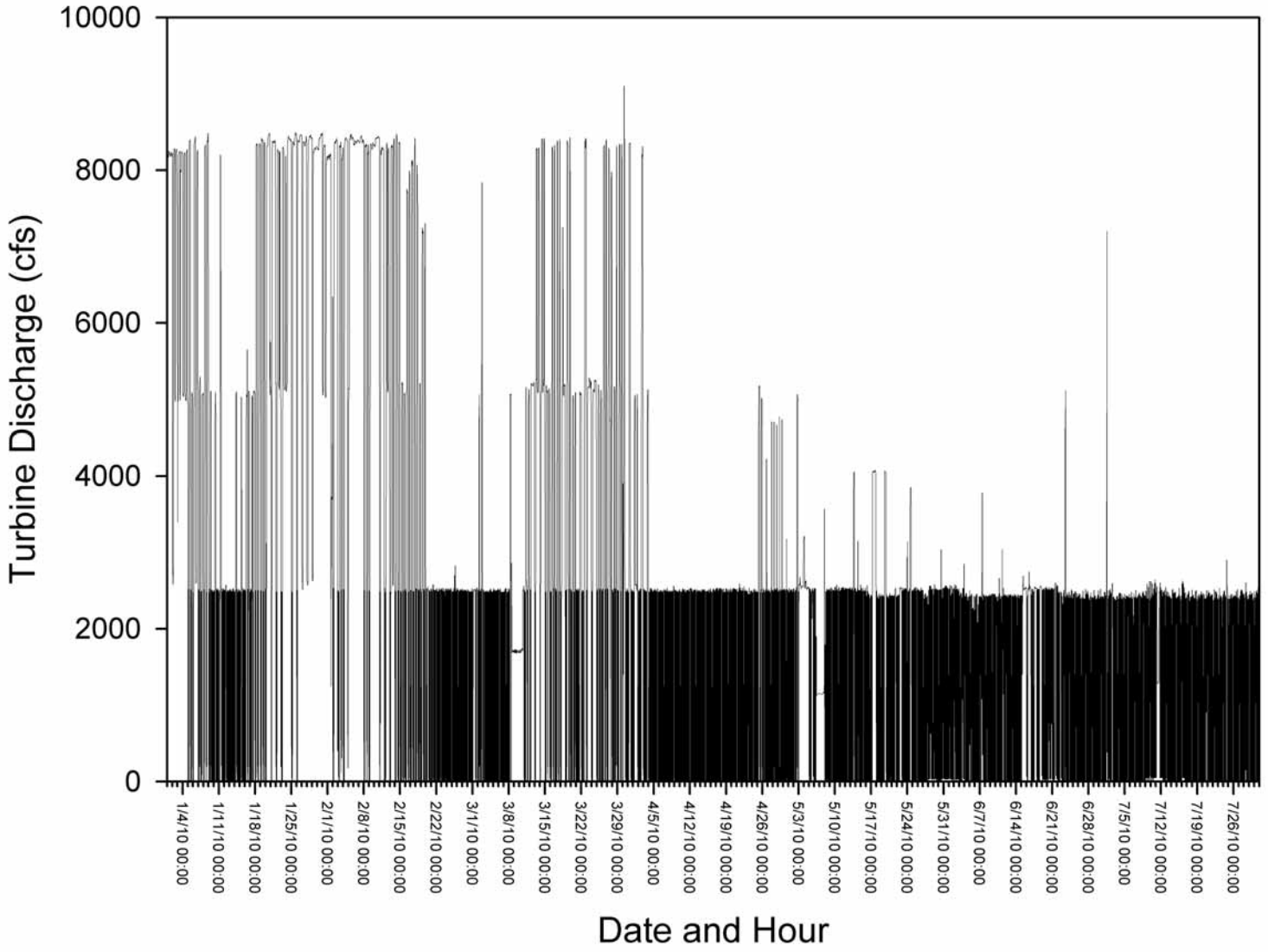


Figure 2.8. Hourly discharge through turbines at the Fort Patrick Henry Dam for the period 1 January though 31 July 2010.

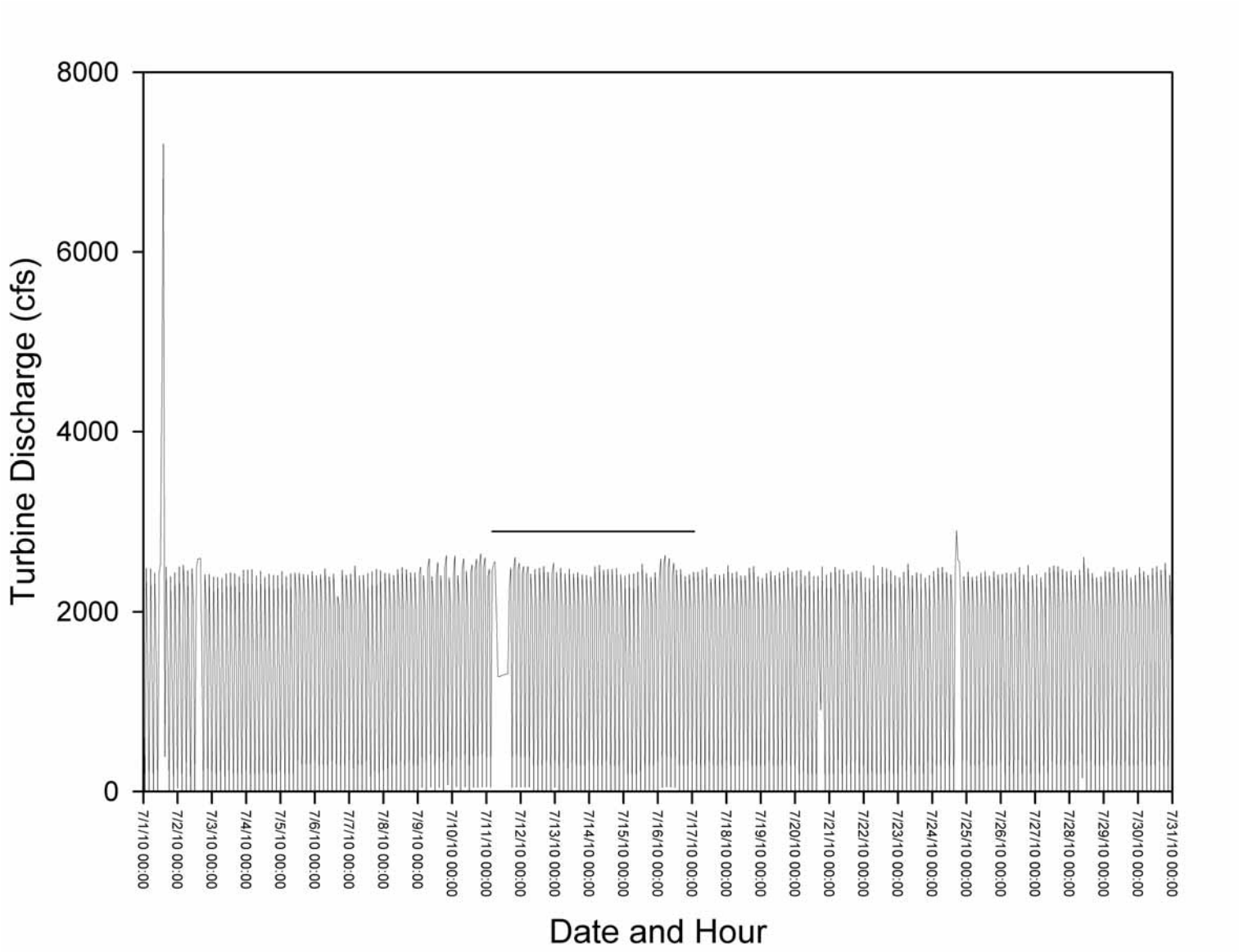


Figure 2.9. Hourly discharge through turbines at the Fort Patrick Henry Dam for the period 1-31 July 2010 (the horizontal line indicates the days of the Academy's 2010 Holston River monitoring study).

3. STUDY DESIGN

3.1 Study Components

Five main components were included in the 2010 South Fork and mainstem Holston rivers study: environmental geochemistry (basic water chemistry), attached algae, benthic insects, non-insect benthic macroinvertebrates and fish. The biological groups chosen for study were selected because they are important components of the river ecosystem, are sensitive to changes in water quality, and span the aquatic food web from top to bottom (Fig. 3.1). In addition, most of the groups can be sampled quantitatively, permitting rigorous statistical analysis of the data. The environmental geochemistry component provides important supporting information about the chemical and physical environment (e.g., nutrient levels, turbidity).

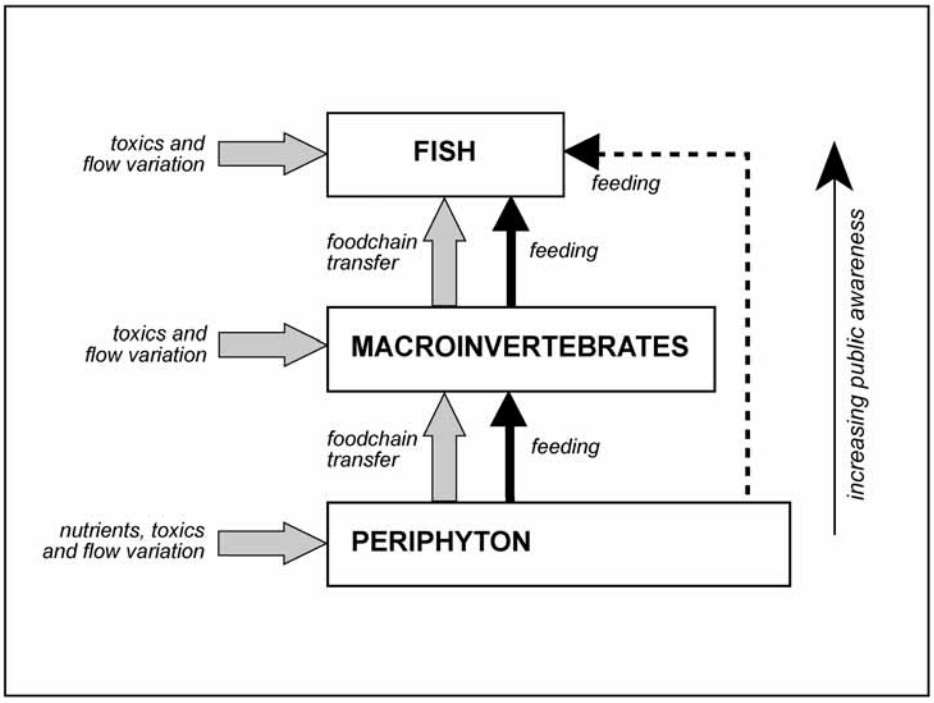


Figure 3.1. Basic relationships among the major biological groups assessed in the Academy's Holston River studies. Periphyton (attached algae) are eaten mainly by benthic macroinvertebrates, which are eaten in turn by fish. Flow variation can affect all three groups by impacting available habitat. Pollutants released into the river can affect these groups by both direct and indirect routes. Substances acting by the direct route include toxics (affecting all three major groups) and nutrients (affecting periphyton). Toxics can also act by an indirect route via food chain transfer.

3.2 Rationale

The 2010 study assessed potential biological impacts of stressors (e.g., industrial and municipal effluents, dam releases, non-point sources) originating in two main areas: the Kingsport area along the South Fork and mainstem Holston rivers and Horse Creek (a tributary to the Big Sluice). In each case, the biological studies were designed to determine whether there is statistically sound evidence that properties of biological assemblages located upstream and downstream from the stressor area differ in ways unlikely to reflect simply natural variation along the river. To accomplish this goal, sampling was conducted in zones upstream and downstream from the Kingsport area and on Horse Creek (see Section 2.1). The study design allows two main types of variation to be quantified (Fig. 3.2):

- Variation among replicate samples collected within a sampling zone
- Variation among different sampling zones

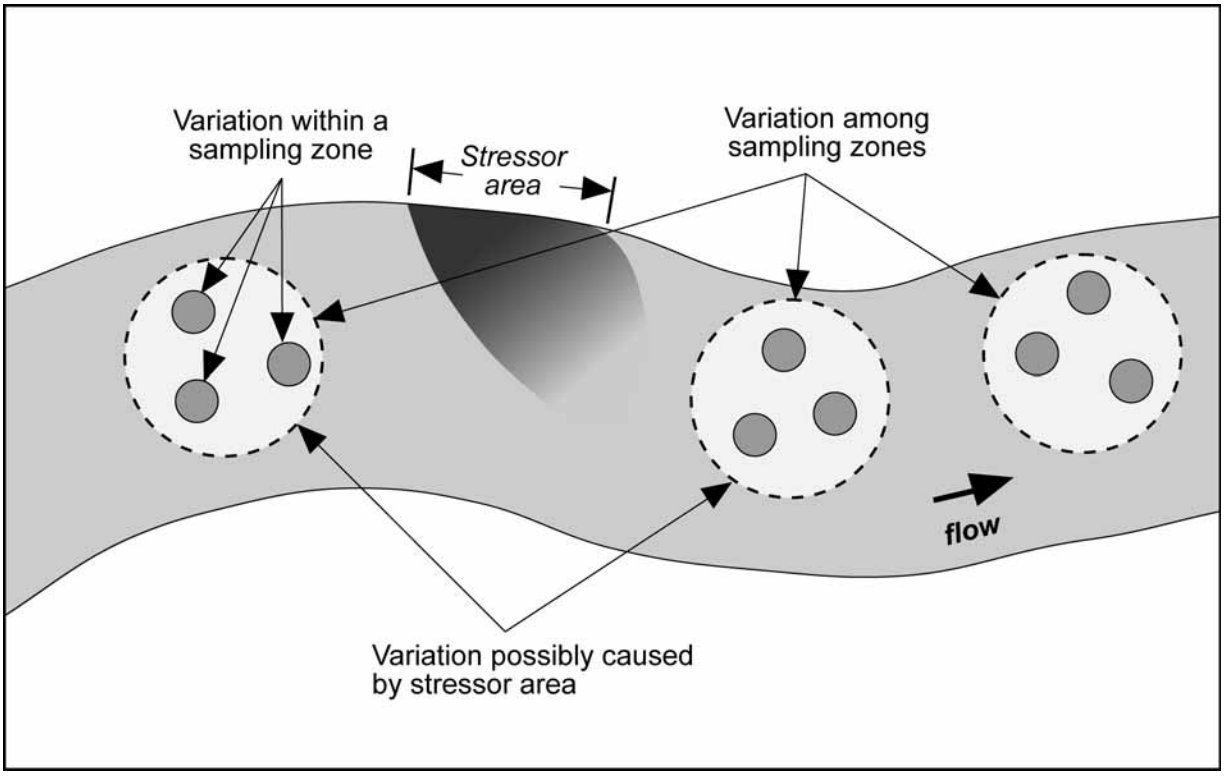


Figure 3.2. Major sources of variation addressed by the Academy's Holston River studies. Replicate samples (shown as small disks) are collected within each sampling zone (large disks), providing an estimate of within-zone variation. Multiple sampling zones are employed to estimate among-zone variation. (The number of samples and zones shown is for purposes of illustration only.) Analysis of variance uses the ratio of average among-zone variation to average within-zone variation as the basis for deciding whether there are significant differences among zones: if this ratio is sufficiently large, it is concluded that real differences exist.

Evidence for impact requires that variation among sampling zones be large compared to variation within zones (indicating that real differences among zones exist), and that differences between zones upstream and downstream from a stressor area be of such a nature that they cannot reasonably be attributed to natural differences in habitat (e.g., different water depths, current speeds, or substrate types). For example, a statistically significant increase in the abundance of pollution-tolerant species and decrease in the abundance of pollution-sensitive species downstream from a stressor area compared to upstream would be strong evidence for impact.

Several properties of the biological assemblages were quantified in the 2010 studies. These include taxa abundance, taxa richness, taxa diversity and pollution tolerance. A taxon is simply a taxonomic group; e.g., a species or genus. Taxa abundance is the number of individuals of a taxon per sample. Taxa diversity measures both the number of taxa (taxa richness) and the similarity or evenness of their proportionate representations in samples (taxa evenness); it is highest when many taxa are present and all are similar in abundance. In the case of benthic insects, pollution tolerance was measured by Hilsenhoff's procedure, in which each taxon is assigned a standard pollution-tolerance score and an overall index for the assemblage is computed. All of these properties are known to be sensitive to the effects of pollution.

Sampling zones were compared by several methods. A presence-absence table was prepared for each major biological group, listing all taxa collected in the study and showing which were present in, and which were absent from, collections at each zone. In the case of algae, benthic insects and fish, quantitative estimates of abundance at each zone were also obtained. Statistical comparisons among zones were used to determine whether there is evidence of impact between various pairs of sampling zones. The methods employed include Cochran's Q test (which looks for evidence that the pattern of taxa presence-absence among zones is nonrandom; if not, there is no evidence that the overall taxa composition differs among zones), cluster analysis (which produces a diagram that shows whether the exposed zones tend to be similar to one another but different from the upstream reference zone), detrended and canonical correspondence analyses (which, like cluster analysis, produce diagrams that make it possible to visually assess similarities and differences among sampling zones), and analyses of variance and covariance (which rigorously determine whether differences among samples from different zones are significantly greater, on average, than differences among samples from the same zone; if not, there is no evidence of among-zone differences). (For an overview of these methods see Appendix 7.1.)

4. METHODS

4.1 Timing of Sampling

Field sampling was done during a period of warm ambient temperature and low river flow, since impacts of stressors are expected to be most pronounced under such conditions. Sampling for all groups was conducted between 11-17 July 2010. There had been relatively low rainfall prior to the sampling period. However, a substantial rainfall event occurred on 12 July 2010.

4.2 Environmental Geochemistry

4.2.1. Introduction

As part of a series of biological and chemical studies on the Holston River in the vicinity of Eastman's Tennessee Operations facility, water chemistry and bacteriological data were obtained on 12-13 July 2010. The purpose of the environmental geochemical component of the study was to provide data on selected chemical parameters in support of the biological survey. Overall, the data are used to assist in determining whether patterns observed in the biological components of the study indicate effluent impacts in the river.

4.2.2. Water Samples

Water samples were collected from two zones on Horse Creek (Zones HC1 and HC2), two on the Big Sluice (Zones 4 and Kit Bottom (KL)), three on the South Fork Holston River (Zones 2, 3 and 5), one at the mouth of Big Tree Spring (BTS) where it flows into Zone 2, and one on the mainstem of the Holston River below Kingsport (Zone 6). Samples for fecal coliform and water quality chemistry were taken on separate days. Each sample was time-composited over an approximately 5-min period by hand dipping a polyethylene container below the water's surface. Samples were collected by wading in the river as far from the bank as was safe. In each zone, three samples were taken within 5 to 10 ft of one another. Specific sampling locations were as follows:

South Fork Holston River

Zone 2: three samples taken by boat, center channel, within a 1- to 3-m reach, 2.2 km downstream of Fort Patrick Henry Dam.

BTS: three samples were collected from this small spring that flows into the South Fork of the River within Zone 2. One sample was collected from each of three separate outwelling areas, about 2 to 5 m downstream from the large tree roots.

Zone 3: three samples within a 1- to 3-m reach, downstream of Eastman's Tennessee Operations facility about 25 m from the right bank and about 100 m upstream of the former Willamette cooling water discharge and dam (now Domtar).

Zone 5: three samples within 1 to 3 m of each other, downstream of Zones 3 and 4, approximately 300 m above the confluence with the Holston River, between the island and Ridgefields Country Club.

Mainstem Holston River

Zone 6: three samples within 1 to 3 m of each other, center channel, 20 m downstream of Goshen Valley Road Bridge and approximately 10.7 mi downstream from the confluence of the North Fork and South Fork Holston rivers.

Big Sluice

Zone 4: three samples within 1 to 3 m of each other (on the opposite side of Long Island from Zone 3 at Domtar Park) about 20 m downstream of the bridge to the park, center channel.

Zone KL: three samples from the left bank, the first sample taken about 10 m below the well of interest and 3 m from shore, the second sample 10 m below the first sample and 10 m toward center channel and the third sample 10 m below the second sample in center channel.

Horse Creek

Zone HC1: three samples within 1 to 3 m of each other, 10 m downstream of the Meadowview Golf Course cart bridge.

Zone HC2: downstream of Zone HC1; three samples within 1 to 3 m of each other, about 10 m above the Meadowview Golf Course bridge near the firefighting training center.

The composited samples were placed on ice in a cooler and brought to the field laboratory for sample splitting, filtration and preservation. Aliquots of each sample were shipped via overnight express to the Environmental Geochemistry Laboratory at the Patrick Center for Environmental Research for filtration, titrations and final preservation. Dependent on the parameter, samples were filtered using either a 0.45-µm membrane filter or a 0.7-µm glass fiber filter. Filtered or unfiltered samples were placed in the appropriate pre-cleaned container: high density polyethylene plastic, Teflon or glass. Samples that required long-term storage (i.e., nutrients, dissolved organic carbon, etc.) were immediately frozen to -20°C until analysis, while other samples such as dissolved chlorides and other major ions were kept at 4°C. The samples for

fecal coliform analysis were collected in sterile containers directly from the river or creek and analyzed the same day. During field sampling, readings were taken near the surface at each zone for dissolved oxygen, conductivity, pH and temperature using a pre-calibrated YSI Model 556 multi-probe meter. Turbidity measurements were taken in the field laboratory with a HACH 2100P Turbidimeter. All water samples were analyzed for the parameters given in Table 4.2.1. Algal rock scraping and surficial sediment/algal samples were analyzed for benthic chl *a* and ash-free dry mass (AFDM). Sample collection, preparation and analyses followed Academy standard operating procedures and the protocols given in Table 4.2.2.

In addition, three water samples were obtained from Zone KL for specific organic compound analyses (benzene, aniline and 1,4-dioxane). Samples were shipped overnight to Lancaster Laboratories (Lancaster, PA) for volatile and semi-volatile analysis. Split samples were sent to Eastman's Environmental Services Laboratory. Samples were collected in pre-cleaned brown amber bottles or vials and shipped cold (4^{o}C) with wet ice.

Table 4.2.1. Parameters determined in sub-surface water or benthic algal samples collected from the 2010 South Fork Holston River, Holston River, Big Sluice and Horse Creek study sites.

Field	
Dissolved Oxygen	Temperature
pH	
Specific Conductance	
Field Laboratory	
Fecal Coliform Bacteria	
Turbidity	
Philadelphia Laboratory	
Dissolved Ammonia+Ammonium	Dissolved Potassium
Dissolved Chlorides	Dissolved Sodium
Total Alkalinity	Total Hardness
Dissolved Nitrate+Nitrite	Particulate Carbon
Particulate Nitrogen	Dissolved Organic Nitrogen
Total Nitrogen (calculated)	Dissolved Orthophosphate
Total Organic Carbon	Dissolved Organic Carbon
Soluable Kjeldahl Nitrogen*	Dissolved Magnesium
Total Phosphorus	Dissolved Sulfate
Total Suspended Solids	Dissolved Calcium
Total Solids	Suspended Chlorophyll *a*
Biochemical Oxygen Demand	Benthic Chl *a* and ash-free dry mass (AFDM)

* Replaces Total Kjeldahl Nitrogen as a method to determine Total Nitrogen.

Table 4.2.2. Analytical methods and procedures for water and benthic algal sample analyses.

Dissolved Oxygen: Reported as mg/L and % or percent dissolved oxygen; determined by membrane electrode method using a YSI Oxygen meter. U.S. EPA, 1983; Method 360.1.

pH: Reported as standard pH units; determined by electrometric method with a YSI pH meter. APHA, AWWA, WEF, 1998; Method 4500-H B.

Specific Conductance: Reported as μS/cm; determined by YSI SCT meter. U.S. EPA, 1983; Method 120.1.

Temperature: Reported as degrees Celsius; determined by thermometer or thermistor method, pre-calibrated using a YSI meter. U.S. EPA, 1983; Method 170.1.

Turbidity: Reported as NTU (nephelometric turbidity units); determined by nephelometric method using 2100P turbidimeter. U.S. EPA, 1993; Method 180.1 (Rev. 2.0).

Total Alkalinity: Reported as mg/L $CaCO_3$; APHA, AWWA, WEF, 1998; Method 2320 B.

Dissolved Chlorides: Reported as mg/L Cl; APHA, AWWA, WEF, 1998; Method 4500-Cl C.

Total Hardness: Reported as mg/L $CaCO_3$; APHA, AWWA, WEF, 1998; Method 2340 C.

Dissolved Sulfate: Reported as mg/L SO_4; Determined by the turbidimetric method. APHA, AWWA, WPCF, 1981; Method 426 C.

Calcium, Magnesium, Sodium and Potassium: Reported as mg/L; determined by FAA U.S. EPA, 1983; Methods 215.1, 242.1, 258.1 and 273.1, respectively.

Dissolved Ammonia+Ammonium: Reported as μg/L NH_3-N; determined by a Alpkem Autoanalyzer (RFA 300), utilizing the colorimetric phenate method. U.S. EPA, 1993; Method 350.1 (Rev. 2.0).

Soluble (filtered) Kjeldahl Nitrogen: Reported as μg/L NH_3-N; determined by Alpkem Autoanalyzer (RFA 300), utilizing semi-automated block digester and colorimetric phenate method. U.S. EPA, 1993; Method 351.2 (Rev. 2.0).

Dissolved Nitrate and Nitrite: Reported as μg/L NO_3-N + NO_2-N; determined by an Alpkem Autoanalyzer (RFA 300), utilizing cadmium reduction of nitrate to nitrite, followed by diazotization. U.S. EPA, 1993; Method 353.2 (Rev. 2.0).

Total Phosphorus: Reported as μg/L PO_4-P; determined by persulfate digestion. The resulting orthophosphate concentration was measured on the Alpkem Auto-analyzer (RFA 300) by the ascorbic acid colorimetric method. U.S. EPA, 1993; Method 365.1 (Rev. 2.0).

Dissolved Orthophosphate: Reported as μg/L PO_4-P; determined as above, except with the elimination of the block digestion step. U.S. EPA, 1993; Method 365.1 (Rev. 2.0).

Table 4.2.2 (continued). Analytical methods and procedures for water and benthic algal sample analyses.

Fecal Coliform: Reported as colonies per 100 ml; determined by membrane filtration, incubated at 44.5°C for 24±2 hrs. Millipore, 1972.

Total Solids: Reported as mg/L; determined gravimetrically after drying at 103-105°C. APHA, AWWA, WEF, 1998; Method 2540 B.

Total Suspended Solids: Reported as mg/L; the residue retained on a glass fiber filter is determined gravimetrically after drying at 103-105°C. APHA, AWWA, WEF, 1998; Method 2540 D.

Dissolved (<0.7 pm Filtered) Organic Carbon (DOC): Reported as µg/L C; determined on a Shimadzu TOC – 5000A. APHA, AWWA, WEF, 1998; Method 5310 B.

Total Organic Carbon (TOC): Reported as µg/L C; determined as the sum of DOC and PC.

Dissolved Organic Nitrogen (DON): Reported as µg/L N; calculated by the difference of soluble kjeldahl nitrogen (SKN) and ammonia-ammonium nitrogen.

Particulate N and C (PN and PC): Reported as µg/L C or N; determined on an elemental analyzer at 975°C as molecular N and carbon dioxide. U.S. EPA, 1992: Method 440.0.

Biochemical Oxygen Demand (5-day): BODs were determined on untreated river water by recording the decrease in dissolved oxygen after 5 days of incubation at 20°C in the dark. APHA, AWWA, WEF, 1998; Method 5210 B.

Suspended Chlorophyll *a*: Reported as µg/L Chlor *a*: determined on a Turner Design fluorometer after extraction with acetone:water. APHA, AWWA and WEF, 1992.

Benthic Chlorophyll *a* and Ash Free Dry Mass (AFDM or %Organic Matter): Benthic algal chl *a* and % organic matter were determined following Patrick Center procedures modified from Standard Methods (APHA, AWWA and WEF 1992). Benthic algae were analyzed by weighing approximately 0.2 g of algal material from thawed samples and extracting in 10 ml 90% acetone at 4°C for 48 h. All samples were extracted in duplicate and phaeophyton-corrected chl *a* abundance determined using a Turner Designs TD-700 fluorometer.

Percent solids and percent organic matter were determined using Standard Methods (APHA, AWWA and WEF 1992). A minimum of 1 g algal material from each sample was placed in a pre-weighed crucible, dried at 105°C for 24 h, weighed to a constant weight (mass of solids), and ashed in a muffle oven at 550°C for 1 h. Samples were not re-hydrated following combustion at 550°C. Percent organic matter was calculated as the difference between the dried and ashed weights.

Organic Chemicals (benzene, aniline, and 1,4-dioxane)*: Volatile organic compounds (VOCs; benzene) were analyzed using a purge and trap method following the procedures outlined in EPA Method 8260, while semivolatile organic compounds (SVOCs; aniline and 1,4-dioxane) were analyzed by solvent extraction and GC-MS using EPA Method 8270.

*These analytes were measured at the Kit Bottom sites only.

4.3 Attached Algae and Aquatic Macrophytes

Methods for collection and analysis of algae and aquatic macrophytes were designed to be comparable with previous Holston River surveys (1965, 1974, 1977, 1980, 1990 and 1997). In addition to sampling zone changes in previous surveys (elimination of Zone 1 above Fort Patrick Henry Lake after the 1980 survey and addition of Horse Creek sampling in the 1990 survey), there was, during the 2010 survey, additional algal sampling at Big Tree Spring in Zone 2. The additional quantitative sampling, initiated in the 1997 survey, was continued in 2010. Diatom enumeration procedures have been exactly the same since a modification for the 1977 survey (see below) and are comparable to the earlier comprehensive surveys (1965 and 1974).

4.3.1 Field Procedures

The number of microhabitats and substrates which support algal growth require a variety of collection methods and techniques. Uniform, flat algal communities on solid substrates (e.g., rocks, logs and twigs) were scraped and lifted with a pocket knife or scalpel. Forceps were used to collect filamentous algae which formed small "streamers" on various substrates. Algal communities on unstable substrates (e.g., sand and mud) were collected with a small glass or plastic pipette. Filamentous algae, aquatic moss, tree roots and rootlets were placed in a vial and shaken to separate epiphytic forms. Collections were put into vials labeled with zone, collection number and project code. Field notes included specific habitat data, general overall abundance and observations of hydrological conditions, especially flow.

Following methods of a large, national sampling program (Porter et al. 1993), a procedure to collect algae quantitatively was used to sample two common substrates, rocks and sandy mud and water interfaces. A sampler (termed SG90 by Porter et al.) was modified to form a template on the surface of a rock. Triplicate samples were taken from different rocks by compositing several of the template areas on each rock. Similarly, a 47-mm petri dish template (see Porter et al. 1993) was used to collect triplicate algal samples from the sandy mud and water interface. Samples were immediately cooled and taken out of the sunlight.

In the field laboratory, preliminary observations on untreated hand collections were made to establish which species, especially diatoms, were actually living at the collection site when the samples were collected. Important diagnostic characteristics in filamentous and fragile forms are sometimes lost through preservation. Diatom collections were split from samples with abundant diatoms and preserved with 1-2 drops of formaldehyde. The remaining collections were preserved with formaldehyde (final concentration was 3-5%) and transferred to the Academy. Quantitative samples were measured (weight) and split for separate analyses of community composition and algal biomass (as chlorophyll *a*). The chlorophyll *a* samples were frozen prior to transfer to the Academy.

Rooted aquatic plants, aquatic mosses and macroscopic algae were carefully collected by hand, so that important diagnostic characteristics such as root and fruit would be preserved.

Representative specimens were moistened, put in clear plastic bags and stored in a dark and cool place until transfer to the field laboratory. Field notes on substrate type, water depth and relative size of stands were made for each zone. At the field laboratory, specimens were floated, lifted onto newspaper, tentatively identified and dried in a plant press.

4.3.2 Laboratory Analyses

At the Academy, diatom collections were prepared for identification by cleaning the siliceous frustules of organic material and mounting them on glass slides. The samples were digested with nitric acid in a microwave apparatus (ANSP Protocol P-13-42 "Diatom Cleaning by Nitric Acid Digestion With a Microwave Apparatus"). After washing samples of digestion salts by rinsing and decanting with distilled water, permanent diatom slides were made by mounting the cleaned frustules on glass microscope slides using Naphrax mounting medium (ANSP Protocol P-13-49 "Preparation of Diatom Slides Using Naphrax Mounting Medium"). This procedure more clearly exposes the diagnostic characteristics of the diatom cell walls.

Diatom enumeration techniques were the same as those used in the previous four surveys (1977, 1980, 1990 and 1997) in which detailed analyses of individual collections were made. Diatom enumeration during early surveys (1965, 1967 and 1969) consisted of the analysis of one composite slide at each zone. Five hundred diatom frustules were identified and enumerated from each collection. This procedure gives information on diatom communities in the various microhabitats found within each zone. A diatom species was defined as being established within a zone (and thus listed on the species list) if it was found a total of eight times in the enumeration of the collections from that zone. Because of the change in enumeration techniques, comparisons of species numbers could not be made between the earlier (1965, 1967 and 1974) and later (1977, 1980, 1990, 1997 and 2010) surveys.

The collections of algae other than diatoms were re-examined on wet mounts at 400x and 1000x. Further identifications were made by comparison with previous voucher collections and specimens in the Academy herbarium. The most abundant species were determined, and the samples were cataloged and saved as voucher specimens. Aquatic macrophytes were identified and voucher specimens were saved.

In addition to diatom counts (500 frustules), the quantitative samples were analyzed for total algal cells and biomass. Three hundred algal counting units were enumerated in a Palmer-Maloney Cell (ANSP Protocol P-13-63 "Analysis of Soft Algae and Enumeration of Total Number of Diatoms in USGS NAWQA Program Quantitative Targeted-Habitat (RTH and DTH) Samples"); the total number of algal cells was determined along with the relative contributions of non-diatom, soft-bodied algal forms. Algal biomass, as chlorophyll *a*, was determined as described in Section 4.2.

At the Academy, an expert made final identifications of rooted aquatic plants, aquatic mosses and macroscopic algae, and voucher specimens were saved.

4.3.3 Multivariate Analyses

The multivariate procedure of Canonical Correspondence Analysis (CCA; ter Braak 1987) has proven very useful for understanding relationships among diatom assemblages as they vary with time, zone and environmental variables (e.g., Jongman et al. 1987, Leland 1995). This technique is widely used for analysis of community data (ter Braak and Verdonschot 1995). Analyses were performed using the CANOCO program (version 4.5 CANOCO for Windows; ter Braak and Smilauer 2002).

There were two sets of data files: 1) quantitative samples of rocks and sediments and; 2) qualitative samples which included rocks, epiphytes of moss, tree roots and sediment and samples from either logs or filamentous algae (both of these substrates were associated with heavy sedimentation). For the quantitative data set, there were three of each substrate from seven zones (2, 3, 4, 5, 6, HC1 and HC2); quantitative samples from BTS were not analyzed. For the qualitative data set, there was one sample from each of the five substrates with samples from eight sampling areas (BTS was included). Prior to analysis with the above programs, all diatom count data and environmental data, except pH, were log transformed (log+1). Analyses were run using both CCA and Detrended CCA. Results of both methods were very similar and only CCA results are included in this report.

Canonical Correspondence Analysis is an ordination technique (see Appendix 7.1). It seeks to order samples and taxa along axes in such a way that those most different from each other are located at opposite ends of the axes, and all the others are arranged in between according to the relative difference and similarity with those at either end. The analysis first determines locations for each sample and taxon on Axis 1, using the major differences in taxonomic composition to calculate axis scores. It then calculates scores for Axes 2 and higher, using differences in taxonomic composition not used for calculations of previous axes. Plots with one axis oriented horizontally and another vertically can then be constructed that show locations of samples and taxa in two dimensions. The value of this type of plot is that the difference between the composition of one sample and all other samples is shown by the distance between their respective points. Thus, two samples similar in composition will be located near each other, and very different samples will be located far from each other. On graphs showing ordinations of samples, points near each other represent samples that have similar diatom communities.

One of the major advantages of CCA is that it quantifies relationships among individual samples, taxa and environmental variables. Environmental variables, or axes, are represented on CCA diagrams as arrows. All arrows emanate from the origin. The arrow points in the direction of the highest values. In general, the length of the axis indicates the relative strength of the relationships with samples or taxa. Longer arrows represent variables that explain most of the variation among samples. Distance between arrows represents the relative correlation between environmental variables. The angle between an arrow and an axis indicates how closely scores along that axis and an environmental variable are correlated. (See Appendix 7.1 for an overview of CCA and other multivariate techniques used in this report.)

4.4 Non-Insect Macroinvertebrates

4.4.1 Description of Sample Zones

Sampling of non-insect macroinvertebrates was conducted in seven zones: South Fork Holston River (three zones), Big Sluice (one zone), mainstem Holston River (one zone) and Horse Creek (two zones) (Fig. 2.1).

Two areas were sampled in Zone 2: 1) near the Cliffside put-in approximately 2.4 km (1.5 mi) downriver from Fort Patrick Henry Dam and 2) a riffle approximately 1.1 km (0.7 mi) downriver from the Fort Patrick Henry Dam. This second area was not sampled in 1997. Both areas in Zone 2 are subject to large and rapid releases of cool, less oxygenated waters from the use of electric generating turbines, which alter water levels, currents, water temperatures and dissolved oxygen levels in a manner that does not reflect the typical seasonal levels and cycles to which the native fauna was historically exposed. The majority of sampling was done during periods of lower water levels when the permanently wetted zones were accessible. Sampling of the area near the Cliffside put-in was conducted along the right bank (downstream orientation) and included a short, rocky peninsula (at low water levels) and a nearby riffle and shoreline. Downriver from the peninsula the shore area bears a steep mud bank and a backwater. Upriver of the peninsula several snags were also sampled. The dominant submerged aquatic vegetation was *Elodea* with beds that occurred downriver from the peninsula and along the right bank. Sampling of the riffle approximately 1.1 km downriver from the dam was conducted on both banks although most sampling was performed on the left bank. A small shoal covered with smartweed (*Polygonum* sp.) was located near the left bank. Upriver from this shoal was a deep backwater with a mixed soft sediment, organic debris and cobble substrate. The main channel substrate was largely gravel, cobble and boulders which were covered in aquatic mosses. The right bank consisted of boulders and bedrock with some snags.

Zone 3 was located approximately 2.4 km (1.5 mi) downriver from the Wilcox Drive bridge crossing over the South Fork of the Holston River. A low dam created a pool of water at the lower end of the zone. Sampling was conducted along the left bank, along the banks of the upriver end of a midriver island, and in macrophyte beds on the right bank. The left bank had a small backwater and several fallen trees that created small slack water areas. Several riffle areas were present in this zone including to the left of the midriver island and in small breaks in the island. Downriver of a clay outcrop on the right bank, dense beds of macrophytes were present (*Potamogeton* sp. and *Elodea* sp.). The 1990 and earlier surveys concentrated on collecting immediately downriver from the low dam along the right shore to a small island near the left shore.

Zone 4 was on the Big Sluice, a canal bypass that diverts part of the flow of the Holston River. Two areas in this zone were sampled. The first was located approximately 75-175 m downriver from the Riverport Road bridge crossing. Bedrock was common in this area of reduced flow and shallow waters. Collecting was conducted along both banks and along a small, vegetated (*Polygonum* sp.) island that lies along the left shoreline. Some root mats were present along both banks and several small backwaters were present along the right bank. The second area sampled

in this zone was located approximately 600 m upriver, from the main channel to the Interstate 26 bridge crossing. Habitat in this area largely consisted of bedrock and other coarse substrates. Some limited root mats and small backwaters were also present along the left bank.

The Zone 5 area sampled for non-insect macroinvertebrates was located between the Ridgefields Golf and Country Club and Phipps Island near the confluence of the north and South Forks of the Holston River. Collecting was conducted along the left bank near Phipps Island and areas around the island. Root mats and some backwaters around logs were present along the left bank. The riffles around the island had substrates ranging from gravel to boulders. Several small backwaters were present on the left side of the island.

Zone 6 was located in the Holston River downriver from the confluence of the north and south forks of the river. The area sampled included habitats approximately 175 m downriver and habitats approximately 50 m upriver of the Goshen Valley Road bridge crossing. Zone 6 was referred to as Zone 6A in surveys conducted in 1974 and 1977 and was approximately 4.8 km (3 mi) downriver from the original Zone 6 sampled in 1965. As in the 1980, 1990 and 1997 surveys, this Goshen Valley Road locality will be referred to as Zone 6. Dense beds of macrophytes (*Potamogeton* spp. and *Elodea* sp.) were present downriver from the bridge along the left bank. Water stargrass (*Zosterella dubia*) was also present in the zone. Snags, root mats and a backwater were present near the left bank upriver of the bridge. A broad riffle was present in the main channel with substrates ranging in size from gravel to boulder.

Horse Creek, a small stream that flows into Big Sluice, was sampled at two locations. Zone HC1 was collected ~200 m upstream and ~160 m downstream of Meadowview Parkway. Habitats in this stream zone consisted of glides with sand and soft sediment substrates and a riffle of gravel to boulder substrates. Large numbers of root mats were present along both banks. Zone HC2 was collected upriver and downriver of a metal bridge on Eastman's Tennessee Operation's property. This latter locality was reached from South Wilcox Drive by Horse Creek Lane. The creek at Zone HC2 was shaded by riparian trees and because of the shallow nature of the stream all available habitats could be sampled. The stream was silty with riffles and runs of gravel, rocks of shale and limestone and tilted outcrops of shale crossing the stream. Water willows (*Justicia americana*) were abundant at Zone HC2 lining the gravel and bedrock runs. Root mats were also common in this zone. A side channel that was apparently part of the main channel during the 1997 survey contained several pools.

4.4.2 Field Sampling and Laboratory Methods

Approximately 4-5 hours were spent at each sampling zone, including time spent to survey the area by foot to identify habitats that differ in substrate type, current velocity, water depth and composition and patterns of aquatic and riparian vegetation hanging into the water. At every river zone, all available habitats outside the swiftest currents were sampled.

Non-insect macroinvertebrates were sampled in a number of ways because they exhibit numerous morphologies and behaviors. Slow moving and sedentary forms were usually best collected by hand, with smaller species more easily removed from the substrate with small

forceps. In deeper water these animals were collected with a Wildco bottom aquatic dip net (#425-K52) with a mesh size of 500 µm. More mobile animals were taken by a dip net which was swept through debris, patches of submerged aquatic vascular plants, leaf litter, exposed root masses of riparian trees, and leaves and stems of herbaceous riparian vegetation hanging into the river. Vegetation and soft-bottomed sandy, silty, or muddy substrates were also sampled with a dip net or Needham scraper. Harder substrates of rock or logs were collected with a dip net placed perpendicular to the river flow and then upriver substrates were agitated using the feet or hands to dislodge animals. The rocks were examined for smaller organisms that were removed with forceps. Woody deadfalls and debris trapped in shallows along the shore were examined and organisms removed with forceps. Shallow sandy to muddy backwater areas were sampled using a dip net or Needham scraper to collect bivalve molluscs and other macroinvertebrates. Leeches were also picked from fish that were collected by the fish sampling crew. Areas in and at the heads of riffles were examined for mussels. In addition, relic mussel shells were collected when present.

The contents of the dip net or scraper were rinsed in the river to remove sediment and were then placed into a shallow tray. Small animals were removed from debris and some common species were immediately identified, recorded and released. Reference material and taxa which could not be identified with certainty in the field were preserved in 80% ethyl alcohol and taken to the laboratory for identification. Before storage in alcohol, highly contractile organisms (i.e., turbellarians, oligochaete worms and leeches) were relaxed in water with menthol crystals until movement stopped; they then were fixed in 10% formalin solution. The habitat and relative abundance of all the taxa were noted, and those brought to the laboratory were later identified to the lowest practical taxon using a dissecting microscope. Some oligochaetes and water mites were cleared using 10% potassium hydroxide (KOH) and slide mounted in Euparal. Slide mounted oligochaetes and water mites were identified using a compound microscope. Identifications were made using the following resources: Burch (1982), Clarke (1981), Hobbs (1989), Klemm (1985), Parmalee and Bogan (1998), Peckarsky et al. (1990), Smith (2001) and Thorp and Covich (2001). Taxonomy followed Turgeon et al. (1998) for molluscs and the Integrated Taxonomic Information System (ITIS; http://www.itis.gov/) was used for other non-insect macroinvertebrates. Relative abundances were categorized as rare (1 animal), uncommon (2 to 3 animals), moderately common (4 to 15 animals), common (16 to 30 animals) and abundant (31 or more animals).

4.4.3 Analyses

The sensitivity or tolerance to pollution for the taxa collected during the study was determined from Barbour et al. (1999) (Table 4.4.1). Tolerance values (TV) ranged from 0-10 with 0 representing very sensitive and 10 very tolerant. Most of the tolerance values used were developed for the southeastern United States (NC), but data from the Mid-Atlantic Coastal Plain (NJ, DE, MD, VA, NC and SC), Midwest (OH), or Northwest (ID) were used when TVs from this region were not available. In some cases, species level or genus level TVs were not available and TVs for higher taxa (e.g., family or genus) were used. Taxa were placed into three broad categories: Tolerant (TV>7), Moderately Tolerant (TV 3-7) and Sensitive (TV<3) (Table 4.4.1).

Table 4.4.1. Tolerance values from Barbour et al. (1999) for non-insect macroinvertebrate taxa collected from Holston River and Horse Creek during the July 2010 survey, Hawkins and Sullivan counties, TN (T= Tolerant, M = Moderate, S = Sensitive). Unless noted, tolerance values are from work in the southeast (NC). Other tolerance values are derived from the Mid-Atlantic Coastal Plain (MACS [NJ, DE, MD, VA, NC, SC]), Midwest (OH), and Northwest (ID) regions.

Taxon	Tolerance	Notes	Category
Spongillidae	unknown		
Dugesia tigrina	7.5		T
Plumatella repens	unknown		
Branchiura sowerbyi	8.4		T
Tubificidae	10	MACS	T
Stylaria lacustris	8.5		T
Eiseniella cf. *tetraedra*	10	MACS	T
Erpobdella punctata	8	based on family tolerance value	T
Mooreobdella microstoma	7.8	used genus level tolerance value	T
Helobdella triserialis	8.9		T
Gloiobdella elongata	9.9		T
Helobdella stagnalis	6.7		M
Placobdella papillifera	9		T
Placobdella parasitica	6.6		M
Piscicolaria reducta	10	Northwest; family level tolerance value	T
Campeloma decisum	6.7		M
Pleurocera uncialis	2.5	used closely related *Elimia*	S
Leptoxis praerosa	1.9		S
Fossaria obrussa	8	Midwest	T
Gyraulus parvus	5.5	Midwest	M
Micromenetus dilatatus	8.4		T
Helisoma anceps	6.5	Midwest	M
Physella heterostropha	9.1	used genus level tolerance value	T
Laevapex diaphanus	7.3	based on *L. fuscus*	T
Ferrissia rivularis	6.9		M
Pisidium sp.	6.8		M
Musculium securis	5	MACS	M
Sphaerium fabale	7.7	used genus level tolerance value	T
Sphaerium striatinum	7.7	used genus level tolerance value	T
Corbicula fluminea	6.3		M
Caecidotea sp.	6	MACS	M
Hyalella azteca	7.9		T
Crangonyx sp.	8		T
Orconectes rusticus	unknown		
Cambarus bartonii cavatus	8.1	used genus level tolerance value	T
Cambarus girardianus	8.1	used genus level tolerance value	T
Cambarus striatus	8.1	used genus level tolerance value	T
Lebertia sp.	8	Northwest	T
Hydrachna sp.	unknown		

Several statistical analyses were used to determine if there were differences between the non-insect macroinvertebrate communities from the zones sampled on the Holston River and on Horse Creek. In order to identify if differences in non-insect macroinvertebrate communities were statistically significant among zones, Cochran's Q-test (Sokal and Rohlf 1995) was used. This test is a randomized-block analysis of variance for presence-absence data. Species are treated like blocks, with differences among the zones as the main effect. Cochran's Q test was calculated using the following equation:

$$Q = \frac{c(c-1)\sum_{j=1}^{c}\left(X_j - \frac{N}{c}\right)^2}{\sum_{i=1}^{b} X_i\left(c - X_i\right)}$$

where c = number of treatments (or zones); X_j = column total for j^{th} treatment; b = number of blocks or taxa; X_i = row total for the i^{th} block; N = grand total. Calculation of Cochran's Q test was performed in Excel. This test was run two ways with different datasets: 1) all seven zones and 2) with only the five Holston River zones. Significance at the $\alpha = 0.05$ level was tested using a Chi-square distribution table where the degrees of freedom (df) were equal to k-1. In the case of all seven zones, the df = 6 and a Cochran's Q statistic of greater than 12.59 was needed to be considered significant (i.e., the pattern of presence/absence was nonrandom). For a comparison of the five Holston River zones only, the df = 4 and a Cochran's Q statistic of greater than 9.49 was needed to be considered significant at the $\alpha = 0.05$ level. Non-metric multidimensional scaling (NMDS) was also performed to look for differences among zones and survey years. This analysis was performed in R (R Development Core Team 2010) using the "ecodist" package (Goslee and Urban 2007). For this analysis the Bray-Curtis dissimilarity statistic was used as the distance measure. NMDS analysis was performed both with only 2010 data and then with data from all seven surveys. Plots of the NMDS as well as the Bray-Curtis dissimilarity matrix were examined to identify patterns of community similarity between zones and across years.

4.5 Aquatic Insects

4.5.1 Study Design

The aquatic insect portion of this survey is divided into three separate study components. The first component was to evaluate changes in the South Fork and mainstem Holston rivers insect community structure related to industrial effluents. This study component uses Zones 2-6. The expected response of a significantly disturbed community would show a "healthy" benthic community at Zone 2, followed by a significant decline (see specific endpoints (metrics) below) in condition at Zone 3 and a recovery which could span Zones 5 and 6. The statistical methods described below (section 4.5.4.2) were applied to test the null hypothesis that there were no significant differences in the assemblages sampled at any zones. In the event that significant differences were detected, the methods described below were used to assess specifically which zones were significantly different from each other; then the implications and likely causes are discussed.

Second, the aquatic insect communities of Horse Creek were assessed for longitudinal changes which could suggest downstream ecological impairment. Zone HC2 is sited to allow assessment of potential impacts from a historic Eastman landfill adjacent to Horse Creek. In addition, this survey also assessed the integrity of the study zone HC1 to serve as a true reference; Zone HC1 may have been compromised by development (golf course, urbanization). Therefore, a new upper limit for the study area designated HC1U (Horse Creek 1, upper) was set. The Horse Creek assessment had two goals for the 2010 survey: to describe downstream changes in community structure and to specifically assess HC1U as a comparable replacement of HC1L. Development in the area is likely to continue to increase and future assessments may rely upon HC1U as a permanent replacement of HC1L.

The third study component was to evaluate the benthic community structure of Big Sluice, a perennially flowing Holston River side-channel, for potential impacts from a historical Eastman landfill located adjacent to the Big Sluice. This study component uses Zone 4 and two new zones, KL and KU (Fig. 2.1), to assess longitudinal changes in benthic community structure. Samples were collected very close to the river-left bank, in the perennially wetted channel to maximize the contact-potential of insects to toxicants if they are released from Kit Bottom.

4.5.2 Field

Qualitative and quantitative aquatic insect samples were collected from all seven sampling zones during the period of 11-17 July 2010 (Fig. 2.1). Quantitative methods were used to allow statistical hypothesis testing to describe any deleterious effects of potential stressors on assemblages of aquatic insects. Qualitative collections were used to ensure that most species not sufficiently represented in quantitative samples were accounted for.

4.5.2.1 Qualitative Collections

Aquatic insects were collected by hand from as many habitats of the study area as possible. The purpose of these collections is to document taxa which potentially could have been omitted by the quantitative methodology. Qualitative samples were collected throughout the entire field sampling period.

Various aquatic habitats were sampled with an aquatic dip net and by hand. The dip net was swept through floating debris and along the substrata of both swift and slow flowing reaches within the study area. Roots from undercut stream banks, submerged woody debris, macrophytes, inorganic substrata and overhanging riparian vegetation represented the diverse habitats sampled. Since the quantitative samples in 2010 were collected from the slower margins of riffles (q.v., section 4.5.2.2), it was also important for the qualitative sampling to include some collection from high-velocity, high-turbulence patches within riffles.

All debris removed from the river was placed in trays and examined carefully for insects. Specimens were removed and placed in bottles containing 95% ethanol and a label. The labels (hereafter, standard labels) included the following information: unique sample code, date, zone

and collector's name. The samples were topped off with 95% ethanol and processed by Eastman's Sample Central facility for transport.

4.5.2.2 Quantitative Collections

The substrata of riffle areas of the Holston River were composed primarily of a heterogeneous mix of cobbles, pebbles and gravel. Horse Creek zones, as well as Zone 4, had similar substrata, but also had reaches of scoured bedrock and shale (i.e., flat, wide pebbles and cobble) mixed with gravel. Flow measurements were taken at each zone with a Marsh McBirney digital flow meter to determine a consistent range of flows for sampling at all study zones. These velocity readings were measured as close to the substrata as the equipment would allow (~3 cm), and suggested that samples could be collected consistently from habitats with flows between 9-30 cm/s (0.3-1 ft/sec). Thus, the samples collected in 2010 represent benthic assemblages from slower water than was sampled in the 1997 survey (~30-60 cm/s [1-2 ft/sec]) because the fastest near-substrata flows in sampleable[1] habitat at Zone 2 consisted of this range, which was then used to determine the acceptable range for collecting quantitative samples from all other zones. Water velocity (near the substrata) can account for much, if not most, of the variation in aquatic insect community structure (e.g., Fonseca and Hart 1996, Hart and Finelli 1999). The highly variable flow regimes in this reach of the river are the result of releases from TVA's Fort Patrick Henry Dam and it is especially important to control quantitative sampling for this highly influential variable to prevent spurious results.

Depth is a field measure that can also be a useful covariate to explain some variation in aquatic insect assemblages. However, since river levels fluctuated continuously while sampling, a simple point measure of depth is of little use. The measured depth was used as a covariate, but the measure probably reflects maximum depth fluctuation more than actual depth *per se*. At Zone 2, the first samples were collected from about 45 cm depth, but by the time the water began to fall, the area from which these samples were collected was about 10 cm deep. The locations sampled were marked to ensure that they remained submerged for an entire generation cycle. All samples were collected as deeply as possible where the flow fluctuated significantly (i.e., 30-40 cm) to ensure ephemeral aquatic habitats were not sampled. Locations that were obviously in the variable zone were avoided (these locations were evident because they often had a crust of dried and re-wetted algae or other detritus). During field reconnaissance at Zone 2, it was found that perennial habitat could be reliably sampled with the sampling device (described below) for periods of about 1 h at a time, after which 2 h of high flow prevented quantitative sample collection. This was primarily an issue only at Zone 2 as the depth fluctuation at other locations was much more moderate.

A Portable Invertebrate Box Sampler (PIBS; Sample area =0.05 m^2) was used to collect 10 samples from "riffle" areas within each of the 5 South Fork and mainstem Holston rivers zones (Zones 2-6), and the 3 Horse Creek zones (HC1U, HC1L, HC2). Each of the new Kit Bottom

1 Sampleable habitat was defined by changing river conditions, which left an especially large portion of Zone 2 riffles periodically inundated and desiccated. The maximum effective depth of the sampling gear is <40 cm. Thus, perennially inundated river bottom had to be accessed when it was covered with less than 40 cm of water.

zones (KL, KU) was represented by five PIBS samples. The sampler was equipped with a standard, removable 500-μm mesh net. Initial samples were collected from the downstream end of each sampling zone, with successive samples collected upstream to prevent the collector's movement from altering the abundance of insects in the samples. Before the sampler was placed, a velocity measurement was recorded from the substratum-water interface at the downstream edge of the sample area. If the flow was not within the 9-30 cm/s range, another sample location was selected. After the sampler was firmly placed, and the polyfoam seal verified, the relative proportion of particle size classes was estimated using the classes sand, gravel, pebble, cobble and shale. All large (≥50 mm) substrata were scrubbed with a vegetable brush and washed into the sample net (along with detritus) to dislodge attached insects. Any remaining specimens were removed with forceps and added to the sample. After the large particles were scrubbed, removed and discarded, the remaining substrata were scrubbed vigorously and stirred with the brush, and the remaining insects and organic debris were washed into the sample net. On the shore, the net was removed and all clinging invertebrates and detritus were rinsed into the end of the net with river water. The net was then carefully reversed to expel the sample into a 500-ml wide-mouth polymethylpentene jar containing 95% ethanol and standard labels. Specimens clinging to the net were removed by hand and added to the sample. Each sample jar was then filled to volume with 95% ethanol and tightly sealed. Information from standard labels was inscribed on the lids of jars with solvent-resistant ink.

4.5.3 Laboratory

4.5.3.1 Qualitative Collections

All qualitatively collected aquatic insects from a given zone were pooled for sorting and identification. The chironomid midge larvae were separated from all other taxa and slide-mounted for taxonomic determination. All taxa were identified to the lowest practical taxon considering specimen maturity, specimen condition, and, because the larvae of many aquatic insect species remain undescribed, the availability of appropriate taxonomic keys. The most reliable method of species taxonomy involves rearing the larvae to adulthood which may take several months to a year. Thus, to keep the project on budget and schedule, it was impossible to identify all specimens to species. However, this should not hinder the value of the study since: (1) the taxa were described with the same resolution as in 1990 and 1997; and (2) the taxa were described with the same level of taxonomic resolution in all treatments (zones). No unfamiliar species were encountered and vouchers were added to the Holston River reference collection for future use.

Taxa lists generated from both qualitative and quantitative collections were combined, thereby yielding a comprehensive summary of all taxa collected from each zone. Since the taxa list generated by the hand collections may not represent all taxa actually occurring at each zone, the comprehensive taxa list is probably the best representation of the benthic community composition at each zone. This is particularly important since in pre-1990 Holston River surveys, investigators spent an entire day at each zone collecting qualitative samples. Furthermore, before 1997 sorting was done under a microscope, allowing greater recovery of small taxa. The addition of quantitative samples to the study design in 1990 left much less field time for all qualitative samples involved. If the hand collection list were used singularly to

represent the presence or absence of taxa, it could appear to describe a loss of taxa in 2010. By pooling the sampling effort into one comprehensive taxa list, small taxa (e.g., Chironomidae) can be accounted for, providing more accurate description of the aquatic biota of the Holston River.

4.5.3.2 Quantitative Collections

Although the study was not designed to quantify the amount of algae or moss occurring at the zones, it became apparent that some samples contained more moss than others. Aquatic insects do not eat significant amounts of moss, but moss increases the surface area of substrata, creates flow refugia and traps fine organic detritus. This makes moss especially suitable for some insects (e.g., certain collectors) and less suitable for others (e.g., scrapers). It was difficult to find stones in the proper flow-range which were not moss covered at Zone 2, but mossy stones were rare at Zone 6. Since this might be correlated with the density or species composition of insects in the samples, a "greenness" index was used, similar to the one used for earlier Eastman surveys of the Holston River (ANSP 1998) and White River, AR (ANSP 1997). The index was used as a covariate in analysis of covariance (GREEN in the covariance model). All sample containers were filled to capacity with 95% ethanol for >48 h and then a 20-ml volume of the preservative was transferred to scintillation vials. The pigment of each sample (all samples inclusive) was then ranked in the order of greenness. The Rank-score for all samples was then divided into classes of five samples from the least green to the most green. Thus the covariate GREEN was represented by values ranging from 1-10 (the Horse Creek, KL and KU samples were excluded from this analysis), with values of 10 representing the 5 greenest samples, and values of 1 representing the 5 least-green samples. It was assumed that variable GREEN is proportional to the relative amount of chlorophyll in each sample, which in turn is proportional to the amount of moss, algae, macrophytes, or riparian vegetation; all of which could influence the density of macroinvertebrates. The assumption is reasonable since commonly used methods of determining algal biomass also extract chlorophyll with organic solvents.

Although this section is entitled "Aquatic Insects," non-insect macroinvertebrates are often constituents of benthic communities and may contribute measurably to the functioning of benthic food webs. Therefore, an analysis of non-insect taxa was included in the quantitative aspects of the study. In North American freshwater ecosystems, aquatic insects are usually dominant—in terms of diversity, abundance and production. High relative abundance of non-insects is often a signal that something is different about the ecosystem. Anthropogenic stressors, geothermal influences, sedimentation, and salinity can cause a reduction in the success of insects and an increase in some non-insect groups.

Each quantitative sample was rinsed from its bottle into a quadrant Petri dish for sorting under 9-12x magnification. If invertebrate density was low (less than about 125 individuals), the entire sample was sorted. If density was greater, the sample was subsampled to an organism count of 100-150 individuals. To subsample, a quadrant was selected randomly and transferred to a second quadrant Petri dish. Insects and associated debris were mixed to homogenize the sample, and random quadrants of the second Petri dish were processed consecutively until the goal of 100-150 individuals was reached.

Non-chironomid invertebrates were separated from chironomid midges as they were picked from debris, because chironomid taxonomy requires slide mounting of head capsules. All non-chironomid specimens were identified to the lowest practical taxonomic level, enumerated and stored in scintillation vials containing 75% ethanol and standard labels.

Additional subsampling was performed on Chironomidae because of the number of specimens and the time required to mount their head capsules. There are two commonly used laboratory methods for subsampling Chironomidae. One method is to randomly select a fixed number of specimens (a subset of the total number of midges in the sample) to mount and then to assign taxonomic abundance according to proportional abundance of mounted specimens. The more labor-intensive method is to first divide all the midges into recognizable morphospecies, and to mount several specimens of each morphospecies for detailed taxonomy. Both methods are commonly used in ecological assessments, but the labor-intensive morphospecies technique is favored because it reduces the chance of omitting unusual or rare midge taxa from the analyses. The midges of each sample were divided into as many morphospecies as required to account for morphological variation observed at 80x magnification. Five to ten individual midges of each morphospecies from each sample were mounted. If there were fewer than five specimens of each morphospecies, all were mounted. Multiple specimens of each morph were mounted to ensure that all the taxonomic characters required to properly identify the genus/species were visible. This also helped to validate the morphs by allowing identification of more cryptic species that could have been accidentally grouped together during the initial division (80x) of morphospecies. Morphospecies were not used as taxonomic units of analysis; all mounted midges were identified to the lowest practical taxon, usually genus. The number of each midge taxon in the sample was estimated based on the total number of midges found in the sample, and the number of each taxon identified. For example, to determine the abundance of each midge genus in a subsample[2] if 10 of the type that was identified as *Rheotanytarsus* were found, and 10 of the type later identified as *Tanytarsus* were found, and the number of midges in the subsample was 40, then the genera *Rheotanytarsus* and *Tanytarsus* were represented in the subsample with an abundance of 20 each. These data were then added to the Taxonomic abundance matrix before the samples were corrected for subsampling efforts and area to calculate density. Actual identification of midge larvae was performed with a phase contrast compound microscope at 400-1000x. Specimens were mounted in PVA mounting medium (Bioquip, Inc.). PVA is not as permanent as Euparal (used in 1997), which may provide museum quality mounts for nearly 20 years. PVA is less toxic, easier to work with, and mounts can last for several years after preparation.

No unfamiliar taxa were encountered in the course of this survey and there was no need to consult with external taxonomists or specialists. A voucher collection was assembled and will be kept at the Academy.

2 Recall that all invertebrates were systematically subsampled to 100-300 individuals often using about ¼ of the sample.

4.5.4 Data Analysis

4.5.4.1 Overview

A range of biological metrics was used, as well as several multivariate community approaches, to describe ecological trends in the study area. The biological metrics used were: total number of individuals per sample; the number of taxa per sample (taxa richness); Community Evenness (J'); Shannon-Wiener diversity (H'); the number of taxa within the orders Ephemeroptera, Plecoptera and Trichoptera (EPT Index); the proportional dominance of Chironomidae; proportional dominance of non-insect taxa; modified Hilsenhoff biotic index; and the proportional contribution of several functional feeding groups.

Three types of biological metrics were examined as experimental endpoints for all hypothesis testing. The first metrics (total abundance, taxa richness, evenness and diversity) are descriptive measures commonly used by ecologists to describe biological communities. For discussion, these metrics are called Descriptive Community Metrics. Environmental disturbances may cause high mortality or emigration and thus reduce the total abundance of organisms collected. Alternatively, total abundance may actually increase in response to disturbances when they provide resources for tolerant organisms. Taxa richness is typically reduced by environmental disturbances. Community Evenness (J') and Shannon-Wiener (H') diversity were calculated according to Rosenberg and Resh (1993), using natural logarithms. Community evenness describes the relative equality of representation of all taxa in a community and ranges between zero and one, with one indicating equal abundance of all taxa. Diversity integrates taxa richness and evenness, with high values reflective of both high richness and evenness.

The second group of metrics, Community Stress Metrics, is based upon the hypothesized response of benthic assemblages specifically to anthropogenic disturbances. These metrics include measures based on the abundance and diversity of sensitive and tolerant taxa. EPT richness (EPT; Lenat and Penrose 1996) is expected to decline for anthropogenically impacted communities because many members of these orders are sensitive to many types of pollution. The relative abundance, or percent abundance, of chironomid larvae (CHIRO) or non-insect taxa (NONI), is based on the observation that many members of these groups are relatively tolerant of organic pollution and often show an increase in relative abundance in disturbed streams. Hilsenhoff's biotic index (HBI; Hilsenhoff 1987) measures the relative abundance (percent abundance) of tolerant and sensitive taxa by assigning "tolerance values" to each taxon and weighting the index according to the abundance of each taxon. Tolerance values range from 0 to 10, with higher values assigned to more tolerant taxa and lower values to more sensitive taxa. The HBI's range is identical to the range of the tolerance values and the index score can be thought of as the mean pollution tolerance of the organisms collected. The specific tolerance values provided by Lenat (1993) from the North Carolina Biotic Index (NCBI) were used rather than those of the original HBI (Wisconsin) because the NCBI is regionally more relevant. In the event that a taxon was identified to genus, and the NCBI provided tolerance values for species, that species was usually assigned an average NCBI tolerance for that genus. The actual tolerance values used for this survey are reported in Appendix 7.2.

The third group of metrics is specifically meant to use community structure as a surrogate for a community function study. The Community Function Metrics were calculated as the relative abundance of each of the five major functional feeding groups (Cummins 1973, 1974, Merritt et al. 2008) based on their primary method of subsistence—their role in processing organic material in river systems. The primary source of this information was Merritt et al. (2008), but information from other sources (Pennak 1989, Smith 2003, Wiederholm 1983, Peckarsky 1996 and Barbour et al. 1999) was also used. Changes in the abundance of insects from different functional feeding groups are often an indication of an alteration in community function (Rosenburg and Resh 1993, Resh and Rosenberg 1984). The actual functional feeding group classifications used for this survey are presented in Appendix 7.2.

When differences in metric scores or functional feeding groups are observed, it is often useful to examine which specific taxon or group of taxa contributed to the observed difference. The percent abundances of the 10 most abundant taxa at each Holston River zone were compared. Likewise, dominance of the 10 most abundant taxa from the three Horse Creek and two Kit Bottom zones were compared.

4.5.4.2 Analyses

Occasionally, condition or maturity of some specimens prevented exact taxonomic determinations, and only family level identifications were possible. This may cause a problem with data by artificially inflating some of the metric scores. For example, if three genera from a family are collected and a few individuals are too damaged to allow generic determination, the family-level determination alone would be assigned to those specimens. Thus, while only three taxa actually occurred within the family, four would be reported and analyzed. To correct for metric inflation, the abundances of coarse taxonomic determinations were apportioned throughout the composite taxa according to their proportional abundance. Thus, if 10 individuals were identified to family, and the 3 composite genera of that family had 50, 30 and 20 individuals, respectively, then 5, 3 and 2 individuals would be added to the respective genera, and the family abundance would be reduced to zero. This procedure was performed for the 1997 Holston River Survey, but was not mentioned for the 1990 survey. The procedure has been common in ANSP benthic surveys since about 1994 and presents the most conservative estimate of taxa richness that can be attained from a given data set. Thus it cannot cause artificial increases in taxa richness.

The relative contribution of substrata from different size classes was summarized by generating a PARTICLE index based on the Wentworth (1922) scale of particle size. This index assumes that aquatic insects prefer larger and more stable substrata (e.g., Minshall 1984). Likewise, when shale occurred in the samples, it was pebble sized but more prone to shift than pebbles. Therefore, the various particle sizes were weighted to generate the index as follows:

PARTICLE = 8•(Cobble) + 4•(Pebble) + 2•(Gravel) +2•(Shale) + 1•(Sand)

where: PARTICLE = the particle index

Cobble = the percentage of cobble substrata in the sample

Pebble = the percentage of pebble substrata in the sample

Gravel = the percentage of gravel substrata in the sample

Shale = the percentage of shale substrata in the sample

Sand = the percentage of sand substrata in the sample.

If trace amounts of a substrate class occurred (usually sand), it was assigned a value of 1%, and the contribution of the next largest class was reduced by 1%. Thus, in calculations, substrata estimations of 19% and 1% may be used although this precision could not be made in the field. These are the same procedures used to describe particle size in 1997.

All metrics and habitat descriptors were calculated in Microsoft Excel after data were proofed to ensure accuracy. Before metric calculation, the taxa abundance matrix was corrected for different levels of subsampling. Thus, samples that were subsampled by ½ had the abundance of each taxon multiplied by 2, samples that were subsampled by ¼ had the abundance of each taxon multiplied by 4, and so forth. Since this scalar is applied across all taxa in a given sample, only the total density of insects should be affected by this transformation. The results of metric calculation were compiled into a metric matrix, in Microsoft Excel, for export to SYSTAT 13 statistical software (SYSTAT Software, Inc.).

A General Linear Model algorithm (GLM) was used to test the significant differences among zones once the variation related to significant covariates was accounted for. The Tukey's Honestly Significant Difference test (Tukey's HSD) was used to diagnose which specific zones were significantly different from each other, when the GLM model indicated that the zones were significantly different from each other. This procedure is mathematically equivalent to the Covariance models used in 1990 and 1997, but is more resilient to violations of certain assumptions of independence, missing data and normality.

There are several ways to assemble GLM models. The approach for this study was to start the procedure using the metrics as the response variables, with zones, covariates and interaction terms all in the model as predictors. Then, a backwards stepwise modeling algorithm was used to produce a final model in which only significant model terms ($P<0.15$) remained. Note that this probability criterion was only used for parameter selection of the model; the critical P-value for hypothesis tests remained at < 0.05. This process was favored over "forward" or "progressive" modeling because it begins with the premise *a priori* that the benthos usually responds to these variables; the reason these variables were measured was because other studies have indicated invertebrate communities are responsive to these factors (e.g., Resh and Rosenberg 1984, Merritt et al. 2008). Therefore, it makes sense to begin with all of them in the model, then systematically remove variables that fail to explain a significant portion of variance.

When spurious terms remained in the model, they were removed and the procedure was repeated. For example, if the "final model" only accounted for variation due to the terms ZONE and the interaction term ZONE*PARTICLE, the interaction term is considered to be spurious because it refers to a variable that is not in the model. In this case, the interaction term

ZONE*PARTICLE is removed, and the modeling procedure reinitiated from the beginning. Often, but not always, this would result in a model where the parent variables were retained in the final model (i.e., ZONE, PARTICLE). Note that this procedure did not ultimately change the findings of significant differences among zones because, in one case, part of the variation was explained by an interaction effect, and in the subsequent model approximately the same amount of variation was explained by another variable. In all cases, this procedure resulted in only subtle changes in average metric values (usually < 1%) and did not obfuscate any ecologically relevant differences among zones.

Although the GLM procedure is more robust than the standard ANOVA model, it is not immune to violation of assumptions. Levene's test (Wilkinson 2009) of equal variances and the Kolmorogov-Smirnov test of normality were conducted in conjunction with the GLM analysis. When significant violations of these assumptions were detected, the data were transformed as appropriate (Zar 1999, Krebs 1999). If transformations failed to correct the violation, a non-parametric equivalent test was used to test differences among zones only (Kruskal-Wallis, followed by a Bonferroni corrected Dwass-Steel-Critchlow-Fligner pairwise comparison; Wilkinson 2009).

Only the biological metric values and functional feeding groups were evaluated with the GLM procedure. Conducting similar analyses on each of the quantitatively-collected taxa could take months to fully interpret and would be of limited utility in addressing the concerns of this study. For example, if a 5% type-I statistical error rate were used, identical communities will produce significant differences due to chance alone nearly five times for analyses of 100 species. Species abundance matrices are more suited for statistical description by multivariate analysis rather than for hypothesis testing using ANOVA, ANCOVA, or GLM analyses. Readers are cautioned that although the biological metrics and the contribution of functional feeding groups are adjusted for the influence of habitat variables, no such adjustment has been made in the case of dominant individual taxa.

In the Results and Discussion, the term “significant” refers to the statistical probability that two or more mean values differ from one another more than would be expected by chance. Unless otherwise stated, a difference among means was termed significant whenever the probability that the means differed by chance was less than 5% (i.e., Pcrit ≤ 0.05), but a Pcrit of 15% is used for model selection in the GLM model. As such, a significant difference among means does not necessarily indicate a large absolute difference in their values, nor does it always mean that the differences are ecologically important.

Multivariate Analyses. Cluster analysis was performed on the natural logarithm-transformed taxa abundance matrix using the Bray-Curtis index of community dissimilarity (or distance) (see Appendix 7.1 for an explanation of cluster analysis). Bray-Curtis dissimilarity uses abundance data rather than presence-absence data and ranges between zero and one, with zero indicating identical communities, and values near one indicating very different communities. PC ORD Software (MjM Software Design) was used to generate a cluster diagram (called a “dendrogram” because of its branched appearance) using a hierarchical, agglomerative algorithm (the unweighted pair groups method, UPGMA).

The taxa abundance data were also analyzed using detrended correspondence analysis (DCA, Hill and Gauch 1980). DCA examines the among-sample variation in taxa composition and produces an ordination diagram in which samples with similar taxonomic abundances are plotted near each other (see Appendix 7.1 for an explanation of detrended correspondence analysis). DCA was performed using SYSTAT 13 (SYSTAT Software, Inc.).

4.5.4.3 Synthesis

Because the South Fork and mainstem Holston rivers and Horse Creek represent two functionally different aspects of the River Continuum (Vannote et al. 1980), they were examined separately in all analyses. Thus, the principal South Fork and mainstem Holston rivers survey used Zones 2-6. The Horse Creek assessment considered a gradient from HC1U through HC1L and HC2. The Kit Bottom assessment focused on describing changes along a gradient from KU, through KL and Zone 4.

4.6 Fish

4.6.1 Water Levels During the Sampling Period and Relation to Fish Sampling

Water levels in each of the river and Big Sluice zones (i.e., Zones 2-6) are regulated by flow from the Fort Patrick Henry Dam. A new flow regime has been instituted since the 1997 study. The new regime maintains about 3-h cycles throughout the day, e.g., with one or two turbines running for 1-2 h, followed by no turbine flow for 1-2 h. For the first day of the 2010 survey, a different flow regime was instituted at the request of Eastman's Tennessee Operations. This regime involved daily cycles, with flow maintained at a constant “low flow” condition. However, the “low flow” condition for this release was too high to allow successful sampling of many groups. Therefore, the typical pattern of 3-h cycles was re-instituted for the remainder of the study. This contrasts with conditions during the 1997 survey, when water levels typically alternated between low and medium in the morning, with an increase from medium to high flows in the afternoon. The 1997 morning low flows were typically lower than 2010 flows during the low-flow portion of each cycle.

The timing and magnitude of water level fluctuations varied with distance below the dam. Fluctuations were greatest at Zone 2, where water levels varied about 1 m during each cycle. At the lowest levels, some rocks in riffles were exposed, although there was algal growth over the exposed rocks. Riffles were submerged at the higher levels. All backpack sampling at Zone 2 was done at low-flow levels.

The amplitude of fluctuation was smaller at the downstream zones, typically less than 1 m. Water level fluctuations constrained sampling at Zone 3. While noticeable at Zones 4 and 5, and to a lesser extent Zone 6, fluctuations did not greatly affect sampling at these zones.

Water levels have varied among surveys, which could affect the efficiency of different sampling techniques. The 1990 survey was conducted under generally uniform flows near the medium release levels of 1997. Like the 1997 survey, the 1980 survey was performed under conditions of

fluctuating flows, but with lower minimum flows. ANSP (1981) described riffles at Zones 2 and 5 as being reduced to isolated pools at the minimal flows, which was not observed in 1997 or 2010, although edge habitats were exposed at the minimal 1997 and 2010 releases. In 1980, Zone 3 was sampled below the dam on the right bank, in slow current areas with macrophytes. Little similar habitat was noted in this area in 1997 and 2010, possibly due to higher minimum flows during sampling.

4.6.2 Collecting Zones

Fishes were collected at Zones 2, 3 and 5 on the South Fork Holston River, Zone 4 on Big Sluice, Zone 6 on the mainstem Holston River and Zones HC1 and HC2 on Horse Creek (Fig. 2.1). In 2010, two additional zones were collected within the Big Sluice in the vicinity of Kit Bottom discharge between the Horse Creek confluence and Zone 4. Kit Bottom Upper (KU) was located about 0.45 km downstream of the Horse Creek confluence. Kit Bottom Lower (KL) was within the area of potential groundwater discharge from the historic landfill and was located about 1.25 km downstream of the Horse Creek confluence.

Within the sampling zones, sampling locations were chosen to:

- match sites of previous sampling;
- sample representative habitats accessible to the sampling techniques used; and
- sample similar habitats across zones, to allow better zone comparisons; in particular, riffles and associated eddy habitats were targeted for sampling.

Most backpack electrofishing sampling at Zone 2 was in the area at and below the mouth of Rock Springs Branch, a left bank (facing downstream) tributary about 1.3 km below the Fort Patrick Henry Dam. This area contains a cobble riffle extending across the main channel. Block backpack electrofishing samples were taken in this riffle. The edges of these riffles were exposed at minimal flows. Sampling was done in nearshore areas which were exposed and near the center of the channel, in areas which were not exposed at the minimal release levels. Shore backpack electrofishing was done in a silt-boulder backwater at the mouth of Rock Springs Branch. This area was reduced to a narrow, slender channel at the minimal release, but was flooded at the medium and high release levels. This area was sampled in 1990 by backpack, as well, although the 1990 sampling covered greater amounts of shallow, marginal riffle areas and did not sample the deeper, mid-channel riffle. A single electrofishing sample was taken in the mouth of Rock Springs (called T2), the tributary creating the bar where riffle samples were taken. The tributary was sampled during high water, just after a significant rainfall earlier in the day. The mouth of the tributary may have been used as a refuge for river fish during periods of high river flow. The tributary may also be a source of fish to the river, e.g., species typical of small streams. The area sampled in Rock Springs tributary included a large pool just upstream of the junction with the river, and shallow riffles and pools upstream of the large pool.

Boat electrofishing at Zone 2 was done along one or both banks from about 0.42 km upstream of the railroad bridge (i.e., 0.45 km downstream of Cliffside Landing) upstream to the base of the Rock Springs riffle. In 1997, Zone 2 sampling was concentrated in similar areas as 2010,

although individual samples were not matched. These areas were sampled by gill netting and trapping in 1990.

Some exploratory sampling was done with dip nets at Cliffside Landing, at the mouth of a small, unnamed creek. This area has relatively poor habitat for fish and few fish were observed. Some backpack electrofishing was done at Cliffside in 1990. Prior to 1990, most or all fish sampling at Zone 2 was done at Cliffside.

Because of differences in point-source mixing, samples from the left bank at Zone 3 (3L) were separated from right bank (3R) samples. Sampling at Zone 3L was done at the left bank of the main channel, in the same place sampling was done in 1997 and 1990. This area has a shallow run with fallen tree and snag cover at the top, a high velocity, boulder-cobble riffle and a small eddy in a cove alongside the riffle. Shore sampling was done in the cove, edge of the riffle and the run. Block sampling was done in the riffle. The left bank also had a narrow backwater, ending in a small, shallow, macrophyte-filled pond. At high water levels, this backwater re-enters the river downstream of the pond, but there was no downstream connection at the time of sampling. This backwater and pond were sampled by dipnetting.

Block backpack electrofishing was also done along the left side of the island in the zone (i.e., the right side of the left channel); this area was called Zone 3LR. Sampling was done in riffles alongside a narrow wall forming the top of the island and along the island downstream of a breach in the wall. This area was sampled by shore backpack electrofishing in 1997. Notes suggest that the upper part of this area contained more pool habitat and less riffle habitat in 1997 than 2010. This area has been sampled by other faunal elements of the ANSP survey and may have been sampled in some previous fish surveys as well. In 1997, this area was treated as part of Zone 3L. However, measurements of conductivity across the river at the top of the island and in the sampling area and observation of flow patterns indicate that flow in 3LR derives from the right bank of the river. Therefore, this area is treated as part of Zone 3R in this report. In comparisons with the 1997 study, the 1997 data summaries have been modified to include 3LR with 3R.

Shore sampling at Zone 3R was conducted along the side of the pool formed by the small dam at the Domtar cooling-water discharge. This area contained a mix of gravel, boulders and concrete debris. There were extensive macrophytes with submerged logs and woody debris in this area. Silt covered some of the nearshore areas sampled. These habitat conditions differ from those in 1997 when more extensive fine substrates (silt and clay) were noted, likely resulting from deposition from upstream construction activity. This area was sampled in 1990, as well. Sampling was also done below the dam (designated 3Rlower), since this area contained riffle and run habitats more similar to those at the other zones. In 2010, one block backpack sample was taken in the bedrock riffle below the dam, and a seine sample was taken in the run along the right bank below the dam. In 1997, a shore backpack sample was taken in this run. The reports of the 1974 and 1980 surveys (ANSP 1975, 1981) indicate that sampling was done below the dam in those surveys, as well. However, part of the area is described as more similar in habitat to the pool habitat above the dam, with moderate flow and macrophytes.

Sampling at Zone 4 (Big Sluice) was done at two sites. One was the area at the steep ledges below the Domtar Park access road. This area has been sampled in all previous surveys. The ledges consist of almost-vertically tilted rock strata, with fast chutes where the water drops over the ledges, and long, narrow eddies in the troughs. The zone also has a large, deep pool at the bottom of the ledges. In the 2010 survey, three shore electrofishing samples were taken in the ledge area, one along the right bank and another across the upstream face of the ledges from the left to right bank. An additional area along the left bank was sampled along the edge of a cobble riffle with some snags adjacent to the ledge area. In the 1997 survey, shore sampling was done along the right bank of this area, from the pool at the base of the ledges up through the right side of the ledges.

Sampling was also done in cobble-gravel-bedrock riffles downstream of the Riverport Road crossing of the Sluice. This area has riffles more similar to those at the other zones. This area also has riffles formed by bedrock ledges, but the ledges are lower and the gradient less steep, and large parts of the bedrock are covered with cobble and gravel. There are several islands in the channel below the road crossing. Block sampling was done in the riffle upstream and downstream of the railroad bridge located downstream of the Riverport Road crossing. No shore sampling was done in this upper area, as was done in 1997. This area was also sampled in the 1990 survey.

Backpack samples at Zone 5 were taken along the left bank adjacent to the Ridgefields Country Club. In 2010, the majority of the electrofishing samples were taken in a secondary channel flowing between the island and the left bank. As in 1997, block electrofishing samples were taken in the cobble riffle at the top of the island, adjacent to a boulder/cobble bar, along the left bank of the river. The edges of this riffle are slightly exposed at minimal and medium flows, and inundated at the high release level. Additional block samples were taken further downstream on the left inside of the island in the secondary channel and in the main channel flow off the right side of the island. Because of swift flow, no shore sampling was done in 2010 along the outer right side of the island (adjacent to the main riffle), as was done in 1997. A shore electrofishing sample was taken along the left bank of the river (left side of the secondary channel) in 2010. The same area was sampled in 1997 and 1990. Prior to the 1990 survey, sampling was done along the right side of the river, but this area has not been sampled in subsequent surveys, since it may now receive water from the North Fork of the Holston from leaks in the levee separating the two channels.

At Zone 5, boat electrofishing was done along both banks of the pool upstream of the main riffle near the junction of the North Fork Holston to about 0.2 km upstream of the Riverfront Park boat ramp located on the right bank. The right bank area included boulder/concrete riprap placed along the bank to prevent erosion, a large macrophyte bed (downstream sample) and downed submerged trees and snag piles. The left bank was shallower with minimal structure. The same general areas were sampled by boat electrofishing in 1997, and by gill netting, trapping and boat-mounted backpack electrofisher in 1990. However, the rock riprap was not present in 1990.

Sampling at Zone 6 was done upstream and downstream of the Goshen Valley Road bridge. Block electrofishing was done in the riffle downstream of the road bridge, mainly from the left bank to the center of the channel. Shore electrofishing was done along the left bank from just

downstream of the bridge to upstream of the bridge. This area contained mainly gravel substrates with submerged logs and very extensive macrophytes. These areas were sampled in previous surveys as well. In 1997, a shore electrofishing sample was taken downstream of the riffle, in a shallow run in a narrow cove formed by bank slumping. In 2010, block sampling was done in the main riffle. A range of microhabitats was sampled in the riffle, ranging from shallow cobble-gravel areas to fast, boulder-cobble riffle areas. The same general area has been sampled in the 1974, 1980, 1990 and 1997 surveys. The 1965 survey sampled at a different location farther upstream.

Boat electrofishing was done along both banks below and adjacent to the riffle, in eddies below the riffle, and in eddies around the bridge abutments. Similar areas were sampled in 1997.

Zone HC1 was sampled by backpack electrofishing at the Meadowview Parkway bridge crossing, within the Meadowview Golf Course. The sample area started about 70 m downstream of the lower end of the road bridge and continued under the bridge ending at a narrow cobble riffle in the creek about 15 m upstream of the bridge. The 2010 sampling area covered shallow bedrock riffle/runs and a deeper pool with snags and submerged logs at the downstream end. The upstream portion was wider and shallower with a gravel riffle under the bridge and a flooded grassy island at the top. A golf cart walkbridge within the sampling area had rock riprap along the shoreline on both banks. Edge habitats contained shallow bedrock with minimal water, root masses with woody snags and some flooded grasses. The same area was included in the 1997 and 1990 surveys of this zone, although the 1990 sample started farther downstream (the 1990 sample also differed in that no block was used at the lower end, only one electrofishing unit was used, and only one sampling pass was made). However, the area has been modified by road and other construction activities since the 1990 survey. The 1997 sampling area had a mix of steep bedrock ledges (at the top), cobble and gravel riffles, shallow gravel riffles, run and shallow edge habitats and a pool (about 1.0 m deep) at the base of the ledge.

Zone HC2 was sampled by backpack electrofishing at Eastman's Tennessee Operations service bridge in the vicinity of the Eastman fire training facility. The sample encompassed an area from about 3 m below the bridge to a large downed tree blocking the creek 84.2 m upstream. Sampling was done in the same area in 1997, except the sample area also included a 17-m side channel pool, which was almost dry in 2010. In 1990, the sampling area went from the bridge downstream. The 2010 sampling area covered shallow gravel riffles; faster, deeper cobble boulder riffles; cobble-gravel runs, large emergent weed beds of *Justicia americana* (river willow), and two pool areas (at the upper end of the side channel and the area around the downed tree block) with silt and clay substrates. Edge habitats varied and included shallow gravel cobble banks, steep shale ledges, weed beds (river willow), large root masses and woody snags.

Two zones were sampled in the Big Sluice in the vicinity of Kit Bottom between the Horse Creek confluence and Zone 4. Kit Bottom Upper (KU) is located approximately 0.8 km upstream of Eastman's Monitoring Well RO071 at Kit Bottom and about 0.45 km downstream of the mouth of Horse Creek. Kit Bottom Lower (KL) is located approximately 25-m upstream and 25-m downstream of Monitoring Well RO071. Two shore electrofishing samples were taken at KL while three were taken at KU. The sample lengths were all 50-m except a short 15-m sample

taken at KU in a hole with deeper water and a snag pile. Samples were completed at similar habitats at both zones and included riffle areas with small side pools and snag piles.

In the initial proposal, electrofishing was proposed for the area around Big Tree Spring. However, on examination, the area of concern is small and unlikely to contain enough fish to allow any type of comparison with other areas. Furthermore, because of higher conductivity in the mixing area of the spring and river, it would be difficult to separate effects of conductivity on sampling efficiency from any real difference. Therefore, no special fish sampling was done at Big Tree Spring. One of the boat electrofishing samples covered the Big Tree Spring mixing zone, as well as areas upstream and downstream.

4.6.3 Sampling Techniques

Previous surveys used several techniques to sample small fishes, including rotenone, seining and backpack electrofishing. Backpack electrofishing was the primary technique in the 2010 survey. Backpack electrofishing was used in the 1997 and recent surveys (1980 and 1990) because it is effective in a variety of habitat conditions and water level (i.e., it is not constrained to backwaters or very low flows, as is rotenone), including areas with rocky substrates. It has been effective in riffles and other shallow-water habitats at all Holston River zones.

4.6.3.1 Backpack Electrofishing

Backpack electrofishing was done using a gas generator-powered Smith-Root electrofisher. A Smith Root battery-powered electrofisher was used in previous surveys. The two types do not differ markedly in sampling effectiveness; the difference does not affect comparison of results. Two types of backpack sampling designs were used for the 2010 survey:

1) Block-net electrofishing. Electrofishing was done in riffles in 5- x 5-m (16.4- x 16.4-ft) blocks, with a 6.1-m (20-ft) long, 0.32-cm (1/8-in) mesh extra-weighted (chain) block net at the lower end (because of bowing of the net, the block net effectively covers a 5-m wide band). Fish were collected by a 0.32-cm (1/8-in) mesh dip net by the operator and from the block net at the end of sampling. Depth, current velocity and substrate type were measured or noted at five points within each sampling zone. Water chemistry parameters were recorded at each sample location including dissolved oxygen (mg/L), conductivity (µS/cm), temperature (ºC) and pH. Depending on available habitat, two to nine sites were sampled at the larger river zones with riffle habitat (i.e., Zones 2, 3L, 3R and 3LR, 5L and 6). Seven samples were taken at Zone 4. One block sample was taken at Zone 3R in a riffle-lateral eddy at the base of the dam within the zone. This technique was not used at the Horse Creek zones, where shore electrofishing (see below) covered appropriate habitat. This technique was used to statistically compare fish densities among the zones, using individual samples as pseudo-replicates to estimate within-zone variability. The technique also provided primary information on occurrence of species in riffle habitats.
2) Shore electrofishing. Measured lengths of shoreline (20-95 m; 66-312 ft) were electrofished. Shocking was done in a single pass along one shore (i.e., in a band typically 3-m wide), except

at Horse Creek, where the entire stream within each zone was sampled. No block nets were used in the river and Sluice zones. The main river and Big Sluice electrofishing was done with a backpack electrofisher, with fish collected by dip net by the operator and 3-6 other people. Notes on habitat (substrate types, range of depths, notable habitat features, etc.) were taken. One to three sites were sampled at each zone (Appendix 7.3), depending on the availability of appropriate habitat.

In general, samples were taken mainly in runs or eddies along shore, often in small coves or backwaters formed by creek mouths (Zone 2), or areas of bank slumping and erosion (Zone 6). Many areas had cover formed by trees and snags in the water (especially sites at Zones 3L, 5 and 6), and undercut banks and eroded root mats (especially sites at Zones 3L and 4). The site at Zone 2 was in a side channel at the mouth of Rock Springs Creek. The side channel contained a mix of soft substrates and bedrock ledges and was bordered by emergent plants. The site at Zone 3R had a mix of boulders, concrete debris and soft substrate, with large patches of submerged macrophytes. Several of the sites with runs and fallen trees (e.g., sites at Zones 3L, 5 and 6) or in eddies (e.g., Zone 3LR) had patches of sandy substrates, and runs or pools with coarse substrates (gravel, cobble and boulders) were present in most areas (especially sites at Zones 3LR, 3Rlower, 4 and 5). Many of the sites (e.g., at Zones 2, 3L, 4 and 5) were adjacent to riffles. There was limited sampling of these riffles as part of the shore electrofishing, but these riffles were not extensively sampled, since this habitat was effectively sampled by the block electrofishing. Many of the areas sampled had also been sampled in 1997, although there were some differences in the precise location of samples between 2010 and 1997. Many of these sites were sampled in 1990 as well, but the 1990 collections included more riffle sampling, since there was no other riffle-sampling component to that survey.

A modified method of shore sampling was used at the Horse Creek zones. Block nets of 0.32-cm (1/8-in) mesh (or a natural block at the top of the Zone HC1 sampling site) were used at the two Horse Creek zones. Each site was sampled simultaneously by two crews (operators and netters) with backpack electrofishers. Two sampling passes were made. Collected fish were identified, counted and measured at the end of each pass. The fish were held live in tubs between passes. The entire widths of the creek zones were sampled. At Zone HC2, fish were collected by the operator and two to three other people with dip nets per crew. The Zone HC1 site was collected by the operator and two other persons with dip nets per crew.

This technique provided a valuable complement to the block sampling for several reasons. It covered larger areas more efficiently than the block electrofishing; the coverage of more area is useful in monitoring fish in areas of low fish abundance. It also allowed sampling of a variety of microhabitats, including nearshore eddies, undercut banks and irregularly shaped areas. This technique is similar to that used in the 1980, 1990 and 1997 surveys. However, there was less coverage of riffle habitats at some zones in the 1997 shore electrofishing (especially Zones 2, 3L and 6), since these areas were effectively sampled by the block electrofishing samples.

4.6.3.2 Boat Electrofishing

The rotenone, seining and backpack electrofishing sampling techniques used in surveys before 1997 primarily collected smaller fishes. Other techniques (gill netting, trapping, trotlines, angling) were used to collect larger fish in the 1990 survey. While this sampling was done largely to collect specimens for chemical analysis, it was also useful in documenting a portion of the fish community poorly sampled in previous surveys. In 2010 and 1997, boat electrofishing was used to sample three zones (2, 5 and 6) which contained accessible pool habitat. The boat electrofishing gear and technique of collection used in 2010 was different from that used in 1997 and was probably more effective. This difference is important in comparing results of the two surveys.

Boat electrofishing was used (in place of the other techniques used in 1990), because of several advantages:

1) it was useful in a variety of shallow-water habitats;

2) it was effective on a variety of sizes and species of fish; and,

3) most fish recover from electrofishing, allowing field identification and release.

Boat electrofishing was done using a Smith-Root 5.0 GPP (Gas Powered Pulsator) powered by a Honda GX 160 5.5 HP generator mounted in a 14-ft Jon boat, with current applied to the water through dropper electrodes mounted on booms from the bow and wires dangling from the side of the boat. The current was controlled by the Smith-Root 5.0 GPP controller, in place of the Coffelt VVP controller used in 1997. Affected fish were collected with dip nets along measured reaches of shoreline by one netter in the electrofishing boat. Each sample typically lasted 15 min. In addition to the main electrofishing boat, a second "chase" boat was used, and affected fish that escaped initial capture by the main boat were sampled by one or two netters in the chase boat. Attempts were made to capture all fish observed (i.e., including smaller individuals). Fish catch from both boats was tabulated separately. Measurement of sample length was done on Google maps, using latitude and longitude of starting and end points, as well as landmarks.

4.6.3.3 Other Techniques

Dipnetting was done during the fish sampling effort at Zones 2, 3L and 6. Specimens of fish were also obtained by dipnetting as part of the macroinvertebrate sampling program and by the PIBS benthic insect sampling. These were tabulated where they document a species occurrence at a zone not collected by other techniques. In general, most fish specimens caught during macroinvertebrate collecting were not enumerated and were released, so that the numbers of specimens reported for this technique were minimal.

4.6.4 Specimen Handling

All fishes captured were identified to species, except for a sunfish. One sunfish was considered as a hybrid, since it possessed characteristics of several species. Likely ancestral species were identified, based on the mix of characteristics of the species. However, these designations are

tentative, since there are no diagnostic characteristics for such designations. Hybrids may represent F-1 crosses (crosses between pure individuals of two species), backcrosses (crosses between a hybrid and a parent species) or F-2 hybrids (offspring of hybrids).

Many specimens observed but not captured (e.g., during electrofishing) were identifiable to species, but others (e.g., redhorse and minnows) were identifiable only to genus or family. One specimen of genus *Morone* was observed at Zone 2. This specimen was probably a striped bass (*M. saxatilis*), based on size and known occurrence of striped bass in Fort Patrick Henry Reservoir. However, it could have been a hybrid striped bass-white bass, which is an artificially-produced hybrid stocked in some areas. Striped bass are stocked in both Cherokee Lake (downstream of the study area and Boone Lake (upstream of the Fort Patrick Henry Reservoir). Striped bass are not stocked in Fort Patrick Henry Reservoir, but occur in the reservoir via passage through the Boone Dam. Hybrid striped bass are stocked in Boone Lake as well, but are not listed as occurring in Fort Patrick Henry Reservoir (information on fish occurrence in the reservoirs is from the Tennessee Wildlife Resources Agency, www.tnfish.org/ReservoirLakeInformation_TWRA). The specimen is referred to as striped bass in this report.

In this report, the Tennessee snubnose darter and central stoneroller are sometimes referred to as "snubnose darter" and "stoneroller," since they are the only snubnose darter (subgenus *Ulocentra*) and stoneroller (*Campostoma*) in the study area.

Specimens were either identified, measured and released in the field, or preserved for laboratory identification. Fish were either preserved in 10% buffered formalin (most fish) or in 70-95% ethanol (selected young-of-year fish for otolith analysis). Most fish were identified in the field and released, to comply with collecting permit requirements, which were more restrictive during this study than during previous studies. Lab and field specimens were examined and recorded for external abnormalities (e.g., fin wear, lesions, leeches, etc.).

4.6.5 Laboratory Analyses

4.6.5.1 Fish Abundance and Density

Catch rates were standardized to number of fish per unit area. For the block backpack electrofishing samples, catch rates among zones were compared using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The ANCOVAs used habitat variables (depth, current velocity and substrate) as covariates to analyze and adjust for differences in microhabitat among sampling sites. The habitat variables were average values of the five measurement points within each sample area. Substrate was codified as a numerical value ranging from coarse substrates (low values) to fine substrates (high values). The substrate score used both primary and secondary substrate types, weighting the primary type by two-thirds and the secondary type by one-third. Some of the habitat variables are typically intercorrelated, making it hard to select the best habitat variable for the ANCOVA analyses. To deal with this, a principal component analysis (PCA) was run on the average habitat values. The PCA calculates new, independent (i.e., non-correlated) variables which are linear combinations of the original habitat values. Correlations

of the original habitat values and the PCA axes can be used to interpret the axes. The first three axes were used as covariates in the analyses of abundance, as well as the original habitat values.

Two types of zone comparisons were done. *A priori* comparisons are those which are specified prior to analysis, while *post hoc* comparisons involve comparisons based on results without *a priori* specification. *Post hoc* multiple pairwise comparisons among zones were done where there were significant zone differences in the ANOVAs, using the Tukey's Honestly Significant Difference (HSD) test. Because these tests involve all possible pairwise comparisons, statistical power is reduced to lower the risk of false positives (i.e., determining a non-significant difference as significant). These tests are not meaningful in ANCOVAs, since they do not control for covariate effects. *A priori* linear contrasts were done for both ANOVAs and ANCOVAs. These tests involve specification of comparisons of interest. Based on the study design, linear contrasts were: 1) Zone 2 versus other zones; 2) Zone 3L versus Zone 3R; and 3) Zone 6 versus Zone 5. For ANCOVAs, the linear contrasts are comparisons of the Least Squares Means (LSMs), which are adjusted for covariate effects.

4.6.5.2 Condition Study

During identification and enumeration, notes were made on the occurrence of external signs of disease (including lesions), fin wear, morphological anomalies and parasitism.

In the field, measurements of length and weight were made on two species: stoneroller (*Campostoma anomalum*) and Tennessee snubnose darter (*Etheostoma simoterum*). Only specimens greater than 3.5 cm total length were used for these comparisons, since there is relatively large error in weights for smaller fish. Condition (weight at length relationships) was compared using the slopes and intercepts of length-weight regressions and analyzed using ANCOVA, performed using General Linear Models in Statistica. Condition analyses were based on field measurements in order to minimize the number of fish collected, in accord with collecting permit restrictions.

4.6.5.3 Age-growth Rate Analysis

Enough specimens of Tennessee snubnose darter were caught to allow comparison of growth rates across zones. While stonerollers were analyzed in 1997, too few individuals were caught to permit meaningful analysis.

Growth rates (in length) were estimated by comparisons of the size distribution of young-of-year Tennessee snubnose darters. The distributions were based on field measurements of length. This differs from past surveys, when analyses were based on measurements of preserved fish.

Age (days since hatching) was determined for selected individuals of Tennessee snubnose darter using otolith banding patterns. This allowed calculation of average daily growth rates and correction for differences in birth dates within or across zones. For growth analysis using otoliths, three to eight specimens of each species from each zone (treating 3L and 3R separately) were examined.

The length and weight of each specimen was measured and at least one otolith was dissected from the inner ear of each specimen. The larger sagitta was used in aging the snubnose darters. Otoliths were embedded in a drop of Epo-Kwick resin (Buehler Ltd.), measured using image analysis software and viewed with a compound microscope with a video camera attachment. Most otoliths were read under 1000x power with immersion oil. Where necessary, the otolith was hand polished using successive grades of aluminum oxide embedded lapping film sheets (12.0-0.3 μm) to reveal otolith ring structure. The age of the specimen (in days) was estimated as the number of putative daily rings from the inner kernel (or nucleus) to the outer growing edge of the otolith.

5. RESULTS AND DISCUSSION

5.1 Environmental Geochemistry

5.1.1 Results and Summary

Water chemistry results are presented in Tables 5.1.1-5.1.3. The data presented in these tables conform to QA/QC data requirements as stated for the project goals. In each zone, three separate samples were taken within 1.7-3.4 m (5-10 ft) of each other and analyzed. The average, standard deviation, minimum and maximum for each parameter are presented in Table 5.1.4. In Figures 5.1.1-5.1.7, mean values for each parameter are presented graphically with the different zones separated with regard to their location in the river.

A brief description of the mean data is presented to help interpret the biological components of the river survey and observations are made concerning zone-related (spatial) trends for each parameter or parameter group. In Section 5.1.2, a comparison to the most recent survey is presented when appropriate, and in the following section (Section 5.1.3) a historical comparison is made for selected parameters and zones (ANSP 1966, 1975, 1978, 1981, 1992, 1998).

5.1.2 Differences Among Sampling Zones

5.1.2.1 Dissolved Oxygen and Oxygen Saturation

Concentrations of dissolved oxygen were corrected for the altitude of the river system (approx. 1200 ft above sea level) using published equations. Dissolved oxygen concentrations ranged from approximately 6.0 and 6.3 mg O_2/L in HC1 and HC2 to a high of 11.0 mg O_2/L in Zone 4 (Tables 5.1.1 and 5.1.4; Fig. 5.1.1). In the South Fork and mainstem locations, Zone 2 (below the dam) had the lowest oxygen values increasing downstream to 9.7 mg O_2/L at Zone 6. Overall, concentrations were similar to the previous ANSP study in 1997 (ANSP 1998).

Dissolved oxygen saturation is the ratio of the measured dissolved oxygen concentration to that concentration that would be present at saturation in the water at given temperature times 100. Oxygen saturation values ranged from approximately 70% at HC1, HC2 and Zone 2, to over 130% (super saturation) at Zone 4 on the Big Sluice. Water oxygen saturation generally increased from below the dam at Zone 2 (73%) to Zone 6 (115%). Again values are in general agreement to those measured in the 1997 survey.

5.1.2.2 Temperature

In the South Fork and mainstem Holston rivers, water temperatures ranged from 17.2 to 26.1°C with lowest values in Zone 2 and highest temperatures in Zone 3 (Tables 5.1.1 and 5.1.4; Fig.

Table 5.1.1. Water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek. (Page 1 of 2).

Station	Date Collected	Time Collected	Sample ID	DO mg/L	DO %	pH unitless	Temp °C	Sp.Cond µS/cm	Turbidty NTU	TSS mg/L	TS mg/L	BOD5 mg O_2/L	Fecal Coliform* Col/100 ml
Big Tree Spring (BTS) A	7/13/2010	1300	4525	8.66	82.5	6.50	12.97	1096	13.0	24.1	724.3	0.55	32 EC
Big Tree Spring (BTS) B	7/13/2010	1300	4526	8.62	82.1	6.52	12.98	1096	13.5	11.0	713.8	0.42	39 EC
Big Tree Spring (BTS) C	7/13/2010	1300	4527	8.60	81.8	6.49	12.96	1096	13.9	12.6	734.2	0.40	31 EC
2A	7/13/2010	1315	4528	7.08	73.5	7.44	17.26	237	3.8	4.2	133.5	0.71	14 EC
2B	7/13/2010	1315	4529	7.05	73.3	7.38	17.20	237	4.3	4.3	133.2	0.70	12 EC
2C	7/13/2010	1315	4530	7.02	72.8	7.36	17.17	237	3.7	6.0	130.3	0.80	10 EC
3A	7/13/2010	1420	4531	9.79	120.5	8.30	26.14	243	1.8	2.3	133.5	0.65	1500 EC
3B	7/13/2010	1420	4532	9.90	122.3	8.30	26.13	242	1.7	2.3	133.0	0.78	1400 EC
3C	7/13/2010	1420	4533	9.82	121.3	8.29	26.13	243	1.9	2.6	133.4	0.70	>3500
5A	7/13/2010	1540	4534	9.77	114.3	7.97	23.20	332	3.0	4.0	193.4	0.83	550
5B	7/13/2010	1540	4535	9.65	112.6	7.98	23.10	335	3.3	4.2	195.9	0.85	700
5C	7/13/2010	1540	4536	9.56	112.0	7.91	23.05	338	3.4	2.5	194.5	0.90	600 EC
6A	7/13/2010	1630	4537	9.57	113.8	8.28	24.14	334	3.2	3.5	193.0	0.70	86
6B	7/13/2010	1630	4538	9.76	115.7	8.28	24.16	337	2.9	3.6	193.1	0.80	76
6C	7/13/2010	1630	4539	9.69	116.0	8.31	24.16	337	3.5	3.7	194.4	0.78	78

Table 5.1.1 (continued). Water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek. (Page 2 of 2)

Station	Date Collected	Time Collected	Sample ID	DO mg/L	DO %	pH unitless	Temp °C	Sp.Cond µS/cm	Turbidty NTU	TSS mg/L	TS mg/L	BOD5 mg O_2/L	Fecal Coliform* Col/100 ml
Kit Bottom (KBA)	7/13/2010	1100	4540	8.20	94.9	7.82	22.52	296	6.8	6.2	167.7	0.58	1200 EC
Kit Bottom (KBB)	7/13/2010	1100	4541	8.59	99.8	7.90	22.74	291	6.1	6.9	164.6	0.65	900 EC
Kit Bottom (KBC)	7/13/2010	1100	4542	8.71	101.7	7.92	23.11	279	5.3	4.5	125.3	0.60	500 EC
4A	7/13/2010	1450	4544	11.22	135.4	8.45	24.88	248	4.2	3.7	143.4	0.60	420
4B	7/13/2010	1450	4545	10.83	131.2	8.44	24.85	250	3.9	3.6	142.2	0.52	550 EC
4C	7/13/2010	1450	4546	10.96	132.2	8.44	24.77	253	4.0	3.9	167.9	0.60	400
HC1A	7/13/2010	0902	4547	6.03	68.4	7.88	21.46	462	23.4	22.4	290.2	0.82	200 EC
HC1B	7/13/2010	0902	4548	5.96	67.6	7.87	21.45	459	23.7	21.7	292.0	0.87	158 EC
HC1C	7/13/2010	0902	4549	6.01	68.2	7.88	21.45	458	24.5	20.7	294.6	0.92	158 EC
HC2A	7/13/2010	0940	4550	6.48	73.8	7.87	21.60	455	22.4	20.4	288.0	1.02	258 EC
HC2B	7/13/2010	0940	4551	6.38	72.4	7.90	21.59	455	23.6	19.9	289.0	1.05	167 EC
HC2C	7/13/2010	0940	4552	5.89	66.9	7.90	21.61	455	23.0	21.3	292.6	1.05	158 EC
Rinseate Blank	7/13/2010	1745	4524	NA	NA	NA	NA	NA	NA	0.25	1.5	0.15	0
Rinseate Blank	7/13/2010	1200	4543	NA	NA	NA	NA	NA	NA	0.01	41.8	0.08	0
Detection Limits (MDL)				0.5	NA	0.0	NA	1.0	3.0	3.0	4.0	0.5	0.0

*Fecal Coliform Samples collected on July 12, 2010.

EC - estimated counts.

NA - not applicable

Table 5.1.2. Water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek. (Page 1 of 2)

Station	Date Collected	Time Collected	Sample ID	T. Alkalinity mg/L	T. Hardness mg/L	Chloride mg Cl/L	SO_4 mg SO4/L	Mg mg Mg/L	Ca mg Ca/L	Na mg Na/L	K mg K/L
Big Tree Spring (BTS) A	7/13/2010	1300	4525	272.4	533	64.2	175.9	87.3	138	32.0	19.1
Big Tree Spring (BTS) B	7/13/2010	1300	4526	274.8	553	64.6	178.2	92.6	146	34.0	20.3
Big Tree Spring (BTS) C	7/13/2010	1300	4527	264.8	553	64.2	178.6	91.3	145	34.0	20.0
2A	7/13/2010	1315	4528	86.6	113	11.2	8.9	7.5	27.4	6.5	2.1
2B	7/13/2010	1315	4529	86.3	108	10.6	8.7	7.5	27.3	6.4	2.0
2C	7/13/2010	1315	4530	88.1	110	10.3	8.8	7.6	27.8	6.3	2.1
3A	7/13/2010	1420	4531	88.2	109	10.6	8.8	7.7	28.1	6.9	2.1
3B	7/13/2010	1420	4532	87.0	112	10.6	8.8	7.6	28.1	7.0	2.1
3C	7/13/2010	1420	4533	87.4	113	11.0	9.8	7.6	28.1	7.0	2.1
5A	7/13/2010	1540	4534	91.8	121	13.0	45.3	9.3	30.8	20.1	2.5
5B	7/13/2010	1540	4535	89.4	123	12.9	22.5	9.1	30.4	20.4	2.4
5C	7/13/2010	1540	4536	92.0	124	13.5	21.1	9.0	30.0	20.9	2.5
6A	7/13/2010	1630	4537	95.1	130	17.1	37.4	9.1	31.2	19.6	2.5
6B	7/13/2010	1630	4538	94.2	124	17.3	37.2	9.3	31.4	19.4	2.5
6C	7/13/2010	1630	4539	94.7	120	17.5	37.2	9.2	31.3	20.1	2.5

Table 5.1.2 (continued). Water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek. (Page 2 of 2)

Station	Date Collected	Time Collected	Sample ID	T. Alkalinity mg/L	T. Hardness mg/L	Chloride mg Cl/L	SO_4 mg SO4/L	Mg mg Mg/L	Ca mg Ca/L	Na mg Na/L	K mg K/L
Kit Bottom (KBA)	7/13/2010	1100	4540	103.5	134	12.1	15.1	9.3	33.1	7.7	2.2
Kit Bottom (KBB)	7/13/2010	1100	4541	105.0	133	11.7	15.8	9.3	33.0	7.7	2.2
Kit Bottom (KBC)	7/13/2010	1100	4542	96.5	121	11.3	13.0	8.6	30.8	7.2	2.2
4A	7/13/2010	1450	4544	89.9	118	10.9	12.0	8.0	28.5	7.2	2.1
4B	7/13/2010	1450	4545	89.6	115	10.8	12.0	8.0	28.7	7.2	2.1
4C	7/13/2010	1450	4546	89.9	115	11.2	12.0	7.9	28.7	7.1	2.1
HC1A	7/13/2010	0902	4547	174.0	248	15.1	30.5	17.1	55.7	10.5	3.1
HC1B	7/13/2010	0902	4548	174.7	249	15.3	29.6	17.2	55.3	10.5	3.1
HC1C	7/13/2010	0902	4549	172.8	232	15.7	29.2	17.3	56.7	10.5	3.1
HC2A	7/13/2010	0940	4550	168.6	242	15.7	32.8	16.5	55.1	10.0	3.0
HC2B	7/13/2010	0940	4551	165.0	243	15.9	32.8	16.9	55.9	10.2	3.1
HC2C	7/13/2010	0940	4552	167.0	241	16.0	33.6	16.9	55.3	10.1	3.0
Rinseate Blank	7/13/2010	1745	4524	0.1	2	1.0	0.8	0.01	ND	0.1	0.1
Rinseate Blank	7/13/2010	1200	4543	0.1	2	NM	0.9	ND	0.0	0.3	0.1
Detection Limits (MDL)				2.0	2.0	1.0	1.2	0.03	0.1	0.1	0.2

ND - not determined

NM -not measured

Table 5.1.3. Water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek. (Page 1 of 2)

Station	Date Collected	Time Collected	Sample ID	NO_3+NO_2-N µg N/L	NH_3-N µg N/L	SKN µg N/L	PN µg N/L	TN µg N/L	O-P µg P/L	TP µg P/L	DOC µg C/L	DON µg N/L	PC µg C/L	TOC µg C/L	Chlor *a* µg/L
Big Tree Spring (BTS) A	7/13/2010	1300	4525	3881	77.8	234	99	4215	4.4	19.9	1042	157	450	1492	0.24
Big Tree Spring (BTS) B	7/13/2010	1300	4526	3913	72.2	239	88	4241	3.7	14.9	1017	167	397	1414	0.07
Big Tree Spring (BTS) C	7/13/2010	1300	4527	3980	77.4	211	95	4286	3.0	22.4	1153	133	521	1674	0.85
2A	7/13/2010	1315	4528	728	38.4	198	122	1047	1.7	17.9	1359	159	650	2009	4.13
2B	7/13/2010	1315	4529	725	39.6	207	115	1047	1.7	19.2	1380	167	582	1962	3.93
2C	7/13/2010	1315	4530	725	40.8	190	98	1012	1.7	19.4	1351	149	445	1796	3.61
3A	7/13/2010	1420	4531	704	25.7	216	86	1006	6.4	23.6	1337	191	309	1646	1.38
3B	7/13/2010	1420	4532	708	25.4	221	91	1020	6.7	23.4	1486	196	319	1805	1.21
3C	7/13/2010	1420	4533	715	26.5	220	89	1024	7.0	25.3	1470	194	342	1812	1.04
5A	7/13/2010	1540	4534	753	56.9	279	114	1146	38.4	79.8	1969	222	564	2533	1.69
5B	7/13/2010	1540	4535	745	64.1	245	113	1103	31.6	76.0	1906	181	571	2477	1.88
5C	7/13/2010	1540	4536	745	54.0	291	106	1142	35.7	85.4	1887	237	539	2426	1.75
6A	7/13/2010	1630	4537	790	35.9	281	97	1167	28.7	73.7	2136	245	471	2607	1.48
6B	7/13/2010	1630	4538	803	35.2	255	104	1162	29.4	67.7	2054	220	487	2541	1.34
6C	7/13/2010	1630	4539	774	34.7	270	ND	1044	30.8	68.5	2236	236	ND	2236	1.15

Table 5.1.3 (continued). Water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek. (Page 2 of 2)

Station	Date Collected	Time Collected	Sample ID	NO_3+NO_2-N µg N/L	NH_3-N µg N/L	SKN µg N/L	PN µg N/L	TN µg N/L	O-P µg P/L	TP µg P/L	DOC µg C/L	DON µg N/L	PC µg C/L	TOC µg C/L	Chlor *a* µg/L
Kit Bottom (KBA)	7/13/2010	1100	4540	651	53.9	244	107	1002	8.6	34.6	1673	190	612	2285	2.33
Kit Bottom (KBB)	7/13/2010	1100	4541	651	37.7	198	110	958	9.3	32.8	1765	160	630	2395	2.89
Kit Bottom (KBC)	7/13/2010	1100	4542	670	24.3	172	97	939	8.3	30.8	1505	148	464	1969	3.39
4A	7/13/2010	1450	4544	641	27.6	214	96	960	8.5	30.6	1422	186	463	1885	1.20
4B	7/13/2010	1450	4545	643	27.0	206	102	959	9.9	28.9	1483	179	498	1981	1.35
4C	7/13/2010	1450	4546	632	27.8	211	94	974	10.6	29.4	1526	183	414	1940	1.74
HC1A	7/13/2010	0902	4547	661	33.7	284	138	1083	13.2	37.3	3242	250	915	4157	1.73
HC1B	7/13/2010	0902	4548	623	34.1	317	146	1085	11.9	58.8	3293	283	924	4217	1.46
HC1C	7/13/2010	0902	4549	660	34.5	367	ND	1027	14.4	63.9	3383	332	ND	3383	1.60
HC2A	7/13/2010	0940	4550	626	29.2	297	152	1075	13.1	62.6	3344	268	903	4247	1.92
HC2B	7/13/2010	0940	4551	626	26.4	327	159	1112	11.5	50.9	3526	301	978	4504	1.99
HC2C	7/13/2010	0940	4552	630	29.2	313	146	1088	13.0	55.8	3585	284	838	4423	1.94
Rinseate Blank	7/13/2010	1745	4524	9	5.2	ND	ND	ND	1.0	2.3	ND	ND	172	ND	ND
Rinseate Blank	7/13/2010	1200	4543	7	3.0	ND	ND	ND	0.3	2.3	ND	ND	234	ND	0.02

ND - not determined

Table 5.1.4. Mean, SD (± standard deviation), minimum and maximum values from water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek (Page 1 of 3).

		BTS				Zone 2				Zone 3			
Parameter	**Units**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**	**Mean**	**SD**	**Min**	**Max**
DO	mg/L	8.63	0.03	8.60	8.66	7.05	0.03	7.02	7.08	9.84	0.06	9.79	9.90
pH	unitless	6.50	0.02	6.49	6.52	7.39	0.04	7.36	7.44	8.30	0.01	8.29	8.30
Temp	^{o}C	12.97	0.01	12.96	12.98	17.21	0.05	17.17	17.26	26.13	0.01	26.13	26.14
Sp.Cond	µS/cm	1096	0	1096	1096	237	0	237	237	243	1	242	243
Turbidty	NTU	13.5	0.5	13.0	13.9	3.9	0.3	3.7	4.3	1.8	0.1	1.7	1.9
TSS	mg/L	15.9	7.1	11.0	24.1	4.9	1.0	4.2	6.0	2.4	0.2	2.3	2.6
BOD5	mg O_2/L	0.46	0.08	0.40	0.55	0.74	0.06	0.70	0.80	0.71	0.07	0.65	0.78
Fecal Coliform	Colonies/100 mL	34	4	31	39	12	2	10	14	2133	1185	1400	3500 >
TS	mg/L	724	10	714	734	132	2	130	134	133	0	133	133
T. Alkalinity	mg/L	270.7	5.2	264.8	274.8	87.0	1.0	86.3	88.1	87.5	0.6	87.0	88.2
T. Hardness	mg/L	546.3	11.5	533.0	553.0	110.2	2.2	108.2	112.6	111.5	2.2	109.0	113.2
Chloride	mg Cl/L	64.3	0.2	64.2	64.6	10.7	0.5	10.3	11.2	10.7	0.2	10.6	11.0
SO_4	mg SO_4/L	177.6	1.5	175.9	178.6	8.8	0.1	8.7	8.9	9.1	0.6	8.8	9.8
Mg	mg Mg/L	90.4	2.7	87.3	92.6	7.5	0.1	7.5	7.6	7.6	0.0	7.6	7.7
Ca	mg Ca/L	143.2	4.3	138.3	146.2	27.5	0.3	27.3	27.8	28.1	0.0	28.1	28.1
Na	mg Na/L	33.4	1.1	32.0	34.0	6.4	0.1	6.3	6.5	6.9	0.1	6.9	7.0
K	mg K/L	19.8	0.6	19.1	20.3	2.0	0.0	2.0	2.1	2.1	0.0	2.1	2.1
NO_3+NO_2-N	µg N/L	3925	50	3881	3980	726	1	725	728	709	6	704	715
NH_3-N	µg N/L	75.8	3.1	72.2	77.8	39.6	1.2	38.4	40.8	25.9	0.6	25.4	26.5
SKN	µg N/L	228	15	211	239	198	9	190	207	219	2	216	221
PN	µg N/L	94	6	88	99	112	13	98	122	89	3	86	91
TN	µg N/L	4247	36	4215	4286	1036	20	1012	1047	1017	10	1006	1024
DON	µg N/L	152	17	134	167	159	9	149	167	193	3	191	196
O-P	µg P/L	3.70	0.70	3.00	4.40	1.70	0.00	1.70	1.70	6.70	0.30	6.40	7.00
TP	µg P/L	19.1	3.8	14.9	22.4	18.8	0.8	17.9	19.4	24.1	1.0	23.4	25.3
DOC	µg C/L	1071	72	1017	1153	1363	15	1351	1380	1431	82	1337	1486
PC	µg C/L	456	62	397	521	559	104	445	650	323	17	309	342
TOC	µg C/L	1527	133	1414	1674	1922	112	1796	2009	1754	94	1646	1812
Chlor *a*	µg/L	0.39	0.41	0.07	0.85	3.89	0.26	3.61	4.13	1.21	0.17	1.04	1.38

Table 5.1.4 (continued). Mean, SD (± standard deviation), minimum and maximum values from water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek (Page 2 of 3).

		Zone 5				Zone 6				Kit Bottom			
Parameter	Units	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
DO	mg/L	9.66	0.11	9.56	9.77	9.67	0.10	9.57	9.76	8.50	0.27	8.20	8.71
pH	unitless	7.95	0.04	7.91	7.98	8.29	0.02	8.28	8.31	7.88	0.05	7.82	7.92
Temp	^{o}C	23.12	0.08	23.05	23.20	24.15	0.01	24.14	24.16	22.79	0.30	22.52	23.11
Sp.Cond	µS/cm	335	3	332	338	336	2	334	337	289	9	279	296
Turbidty	NTU	3.2	0.2	3.0	3.4	3.2	0.3	2.9	3.5	6.0	0.8	5.3	6.8
TSS	mg/L	3.6	0.9	2.5	4.2	3.6	0.1	3.5	3.7	5.9	1.2	4.5	6.9
BOD5	mg O_2/L	0.86	0.04	0.83	0.90	0.76	0.05	0.70	0.80	0.61	0.04	0.58	0.65
Fecal Coliform	Colonies/100 mL	617	76	550	700	80	5	76	86	867	351	500	1200
TS	mg/L	195	1	193	196	193	1	193	194	153	24	125	168
T. Alkalinity	mg/L	91.1	1.4	89.4	92.0	94.7	0.5	94.2	95.1	101.7	4.5	96.5	105.0
T. Hardness	mg/L	122.7	1.5	121.2	124.2	124.7	5.1	120.0	130.2	129.5	7.2	121.2	134.0
Chloride	mg Cl/L	13.1	0.3	12.9	13.5	17.3	0.2	17.1	17.5	11.7	0.4	11.3	12.1
SO_4	mg SO_4/L	29.6	13.6	21.1	45.3	37.3	0.1	37.2	37.4	14.6	1.5	13.0	15.8
Mg	mg Mg/L	9.2	0.1	9.0	9.3	9.2	0.1	9.1	9.3	9.1	0.4	8.6	9.3
Ca	mg Ca/L	30.4	0.4	30.0	30.8	31.3	0.1	31.2	31.4	32.3	1.3	30.8	33.1
Na	mg Na/L	20.5	0.4	20.1	20.9	19.7	0.3	19.4	20.1	7.5	0.3	7.2	7.7
K	mg K/L	2.5	0.0	2.4	2.5	2.5	0.0	2.5	2.5	2.2	0.0	2.2	2.2
NO_3+NO_2-N	µg N/L	748	4	745	753	789	14	774	803	657	11	651	670
NH_3-N	µg N/L	58.3	5.2	54.0	64.1	35.3	0.6	34.7	35.9	38.6	14.8	24.3	53.9
SKN	µg N/L	272	24	245	291	269	13	255	281	204	36	172	244
PN	µg N/L	111	5	106	114	100	5	97	104	105	7	97	110
TN	µg N/L	1130	24	1103	1146	1158	69	1044	1167	966	32	939	1002
DON	µg N/L	213	29	181	237	233	13	220	245	166	22	148	190
O-P	µg P/L	35.23	3.42	31.60	38.40	29.63	1.07	28.70	30.80	8.73	0.51	8.30	9.30
TP	µg P/L	80.4	4.7	76.0	85.4	70.0	3.3	67.7	73.7	32.7	1.9	30.8	34.6
DOC	µg C/L	1921	43	1887	1969	2142	91	2054	2236	1648	132	1505	1765
PC	µg C/L	558	17	539	571	479	12	471	487	568	91	464	630
TOC	µg C/L	2479	54	2426	2533	2621	198	2236	2607	2216	221	1969	2395
Chlor *a*	µg/L	1.77	0.10	1.69	1.88	1.32	0.17	1.15	1.48	2.87	0.53	2.33	3.39

Table 5.1.4 (continued). Mean, SD (± standard deviation), minimum and maximum values from water chemistry analyses of samples collected July 2010 from the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek (Page 3 of 3).

		Zone 4				Zone HC1				Zone HC2			
Parameter	Units	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
DO	mg/L	11.00	0.20	10.83	11.22	6.00	0.04	5.96	6.03	6.25	0.32	5.89	6.48
pH	unitless	8.44	0.01	8.44	8.45	7.88	0.01	7.87	7.88	7.89	0.02	7.87	7.90
Temp	^{o}C	24.83	0.06	24.77	24.88	21.45	0.01	21.45	21.46	21.60	0.01	21.59	21.61
Sp.Cond	µS/cm	250	3	248	253	460	2	458	462	455	0	455	455
Turbidty	NTU	4.1	0.1	3.9	4.2	23.9	0.6	23.4	24.5	23.0	0.6	22.4	23.6
TSS	mg/L	3.8	0.1	3.6	3.9	21.6	0.9	20.7	22.4	20.5	0.7	19.9	21.3
BOD5	mg O_2/L	0.57	0.05	0.52	0.60	0.87	0.05	0.82	0.92	1.04	0.02	1.02	1.05
Fecal Coliform	Colonies/100 mL	457	81	400	550	172	24	158	200	194	55	158	258
TS	mg/L	151	15	142	168	292	2	290	295	290	2	288	293
T. Alkalinity	mg/L	89.8	0.2	89.6	89.9	173.8	1.0	172.8	174.7	166.9	1.8	165.0	168.6
T. Hardness	mg/L	115.9	2.0	114.6	118.2	243.0	9.5	232.0	249.0	242.0	1.0	241.0	243.0
Chloride	mg Cl/L	11.0	0.2	10.8	11.2	15.4	0.3	15.1	15.7	15.9	0.2	15.7	16.0
SO_4	mg SO_4/L	12.0	0.0	12.0	12.0	29.8	0.7	29.2	30.5	33.1	0.5	32.8	33.6
Mg	mg Mg/L	8.0	0.0	7.9	8.0	17.2	0.1	17.1	17.3	16.8	0.2	16.5	16.9
Ca	mg Ca/L	28.6	0.1	28.5	28.7	55.9	0.7	55.3	56.7	55.4	0.4	55.1	55.9
Na	mg Na/L	7.2	0.0	7.1	7.2	10.5	0.0	10.5	10.5	10.1	0.1	10.0	10.2
K	mg K/L	2.1	0.0	2.1	2.1	3.1	0.0	3.1	3.1	3.1	0.0	3.0	3.1
NO_3+NO_2-N	µg N/L	657	11	651	670	648	22	623	661	627	2	626	630
NH_3-N	µg N/L	27.5	0.4	27.0	27.8	34.1	0.4	33.7	34.5	28.3	1.6	26.4	29.2
SKN	µg N/L	210	4	206	214	322	42	284	367	312	15	297	327
PN	µg N/L	97	4	94	102	142	6	138	146	152	7	146	159
TN	µg N/L	964	9	959	974	1112	33	1027	1085	1092	19	1075	1112
DON	µg N/L	183	3	179	186	288	41	250	332	284	17	268	301
O-P	µg P/L	9.77	0.91	8.80	10.60	13.13	1.25	11.90	14.40	12.53	0.90	11.50	13.10
TP	µg P/L	29.6	0.9	28.9	30.6	53.3	14.1	37.3	63.9	56.4	5.9	50.9	62.6
DOC	µg C/L	1477	52	1422	1526	3306	71	3242	3383	3485	126	3344	3585
PC	µg C/L	459	42	414	498	919	7	915	924	906	70	838	978
TOC	µg C/L	1936	48	1885	1981	4225	465	3383	4217	4391	131	4247	4504
Chlor *a*	µg/L	1.43	0.28	1.20	1.74	1.60	0.14	1.46	1.73	1.95	0.04	1.92	1.99

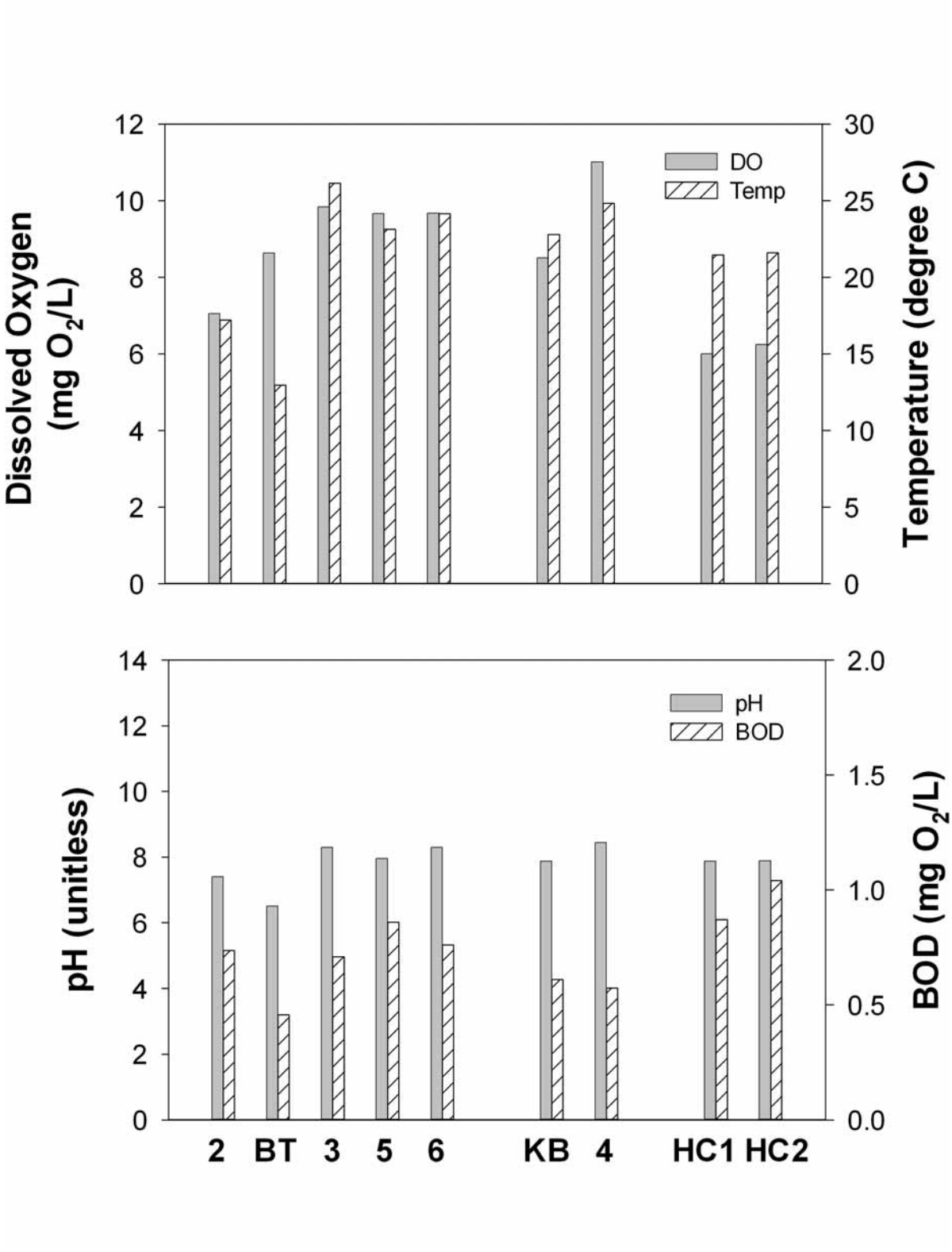


Figure 5.1.1. Mean values for a) dissolved oxygen and temperature and b) pH and biological oxygen demand in water samples collected on the South Fork and mainstem Holston rivers (Zones 2, 3, 5 and 6 and Big Tree Spring), Big Sluice (Zone 4; Kit Bottom) and Horse Creek (HC) in July 2010.

5.1.1). Temperature generally increased downstream to Zone 6 to 24.2°C. Big Tree Spring (BTS), which flows into Zone 2, had the lowest temperature of 13.0°C. The other sampling zones, including Horse Creek, had temperatures ranging from 21.5 (HC1) to 24.8°C (Zone 4) with no distinct spatial trend. This distribution and magnitude of these temperatures are similar to those measured in 1997 (ANSP 1998).

5.1.2.3 pH

The pH of the river water ranged from a low of approximately 6.5 in Big Tree Spring (BTS) to 8.44 in Zone 4 (Tables 5.1.1 and 5.1.4; Fig. 5.1.1) with no distinct trend from upstream to downstream. In the 1997 survey, Zone 4 also had the highest pH (8.6; ANSP 1998). These values are similar to or slightly higher than those obtained during previous ANSP study (ANSP 1998).

5.1.2.4 Biological Oxygen Demand 5-day (BOD_5)

The BOD_5 ranged from 0.5 mg O_2/L at BTS to 1.0 mg O_2/L in HC2 (Tables 5.1.1and 5.1.4; Fig. 5.1.1). Zones 5 and 6, located downstream of the facility in the South Fork and mainstem Holston rivers exhibited BOD_5 of <1 mg O_2/L. These values are slightly higher than those measured in 1997 (ANSP 1998), but overall are very low.

5.1.2.5 Turbidity

Turbidity values were lowest in Zone 3 at 1.8 NTU and highest in Horse Creek (HC1 and HC2) at approximately 23 NTU (Tables 5.1.1 and 5.1.4; Fig. 5.1.2). In BTS, turbidity was also elevated compared to the river at 13.5 NTU. On average, levels in 1997 were slightly higher than in 2010, and the magnitude of the turbidity levels were generally similar between studies (ANSP 1998). Turbidity may have been affected by high rainfall on July 12.

5.1.2.6 Total Suspended Solids (TSS)

The concentration of suspended solids ranged from 2.4 mg/L in Zone 3 to approximately 21-22 mg/L in Horse Creek (HC1 and HC2; Tables 5.1.1 and 5.1.4; Fig. 5.1.2). As with turbidity, BTS also had elevated TSS concentrations of 16 mg/L. The spatial distribution follows the turbidity distribution (Fig. 5.1.2), and there is a linear relationship between turbidity and TSS (r^2= 0.978; slope = 1.1). Concentrations were generally higher than those found in the 1997 survey.

5.1.2.7 Specific Conductivity

Specific conductivity values in the river ranged from approximately 240 µS/cm in Zones 2 and 3 to approximately 340 µS/cm in Zone 6, farther downstream (Tables 5.1.1 and 5.1.4; Fig. 5.1.2). Conductivity in Zone 4 and Kit Bottom were slightly lower than those determined in the South Fork and mainstem Holston rivers (Zones 5 and 6), while specific conductivity was elevated in BTS (~1100 µS/cm). Conductivity measurements were slightly higher than those measured in the 1990 and 1997 surveys (ANSP 1992, 1998).

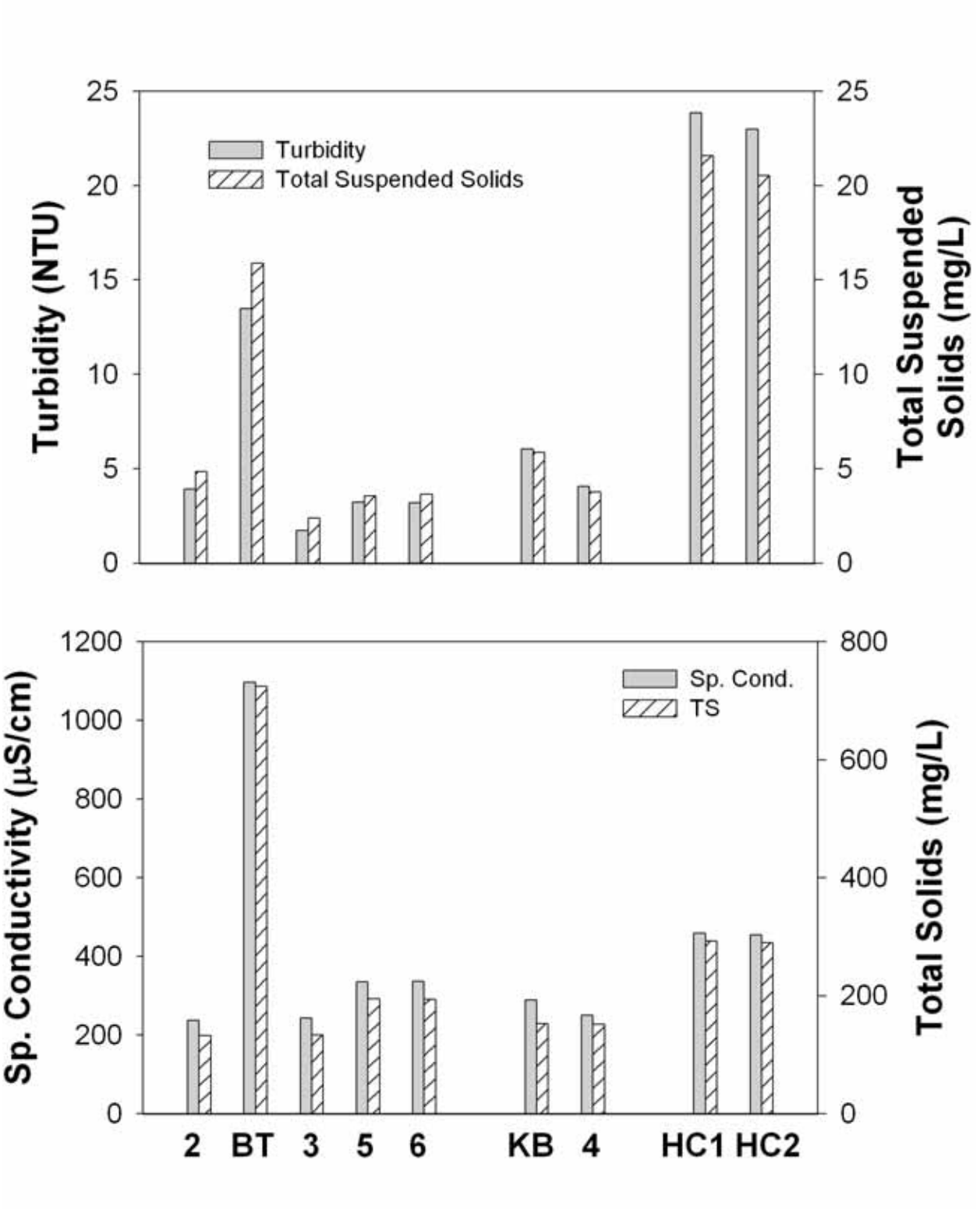


Figure 5.1.2. Mean values for a) turbidity and total suspended solids and b) specific conductivity and total solids in water samples collected on the South Fork and mainstem Holston rivers (Zones 2, 3, 5, 6 and Big Tree Spring), Big Sluice (Zone 4 and Kit Bottom) and Horse Creek (HC) in July 2010.

5.1.2.8 Total Solids

Total solids concentrations followed the same distribution as specific conductivity (Fig. 5.1.2) and ranged from 132 mg/L in Zone 2 to approximately 290 mg/L in Horse Creek (Tables 5.1.1 and 5.1.4; Fig. 5.1.2), while the highest total solids was from BTS (720 mg/L). As with conductivity, total solids were slightly higher in 2010 than in the previous surveys (ANSP 1992, 1998).

5.1.2.9 Fecal Coliform

Fecal coliform abundances ranged from a low of 12 colonies/100 ml at Zone 2 to over 2100 colonies/100 ml in Zone 3 (Tables 5.1.1 and 5.1.4; Fig. 5.1.3). Additionally, high fecal coliform abundances were measured at Zone 5, and Kit Bottom and Zone 4 (on the Big Sluice). Further downstream at Zone 6, fecal coliform decreased to 80 colonies/100 ml. Previous studies also indicate high fecal coliform in Horse Creek and other areas (ANSP 1992, 1998) and in the general area. The high FC levels in Zone 3 may be due to stormwater runoff from a rain event on the day of sampling.

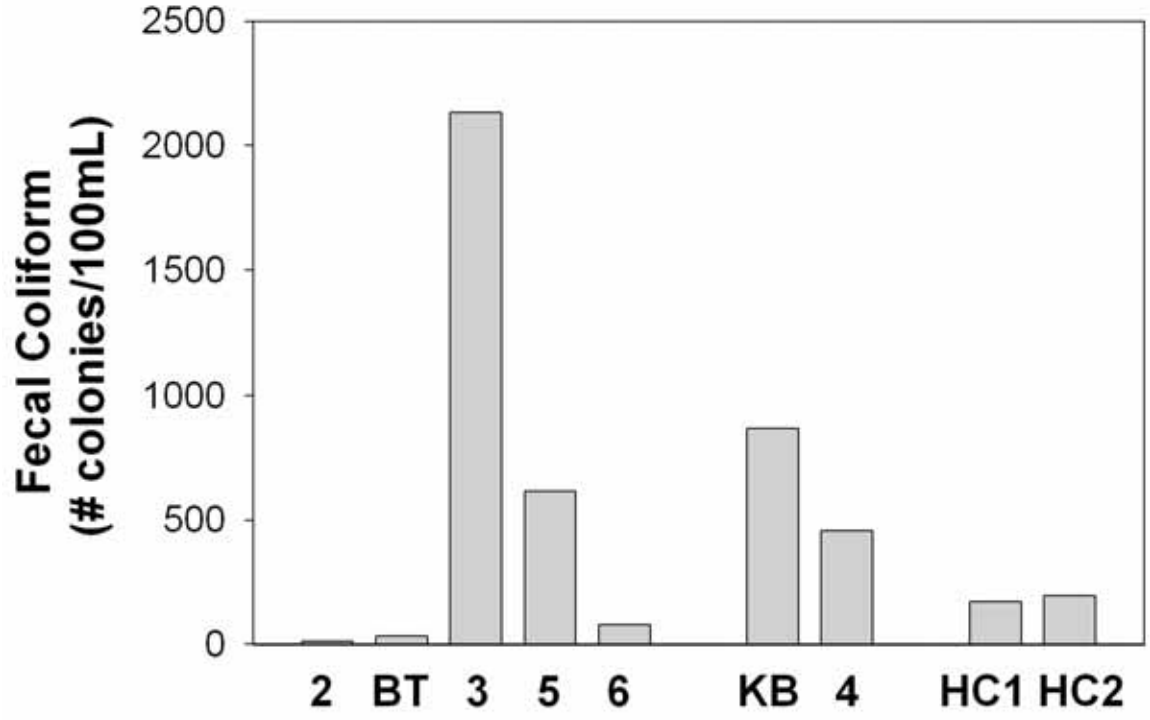


Figure 5.1.3. Mean values for fecal coliforms in water samples collected on the South Fork and mainstem Holston rivers (Zones 2, 3, 5, 6 and Big Tree Spring), Big Sluice (Zone 4 and Kit Bottom) and Horse Creek (HC) in July 2010.

5.1.2.10 Total Alkalinity and Total Hardness

Total alkalinity ranged from 87 mg/L in Zone 2 to over 271 mg/L in BTS (Tables 5.1.2 and 5.1.4; Fig. 5.1.4). Concentrations increased slightly from Zone 2 to Zone 6, with values in Zone 4 and Kit Bottom slightly higher than Zones 2 and 3. Horse Creek samples were also elevated compared to the river samples. Total hardness followed a similar distribution as total alkalinity and ranged from 110 mg/L at Zone 2 to approximately 550 mg/L in BTS and 240 mg/L in Horse Creek (Tables 5.1.2 and 5.1.4; Fig. 5.1.4). Concentrations in 2010 were generally similar or slightly higher for both alkalinity and hardness compared to previous studies (ANSP 1992, 1998).

5.1.2.11 Dissolved Magnesium (Mg) and Calcium (Ca)

Dissolved Mg and Ca concentrations increased slightly from Zones 2 through 6 and ranged from approximately 8 to 9 mg Mg/L and 28 to 31 mg Ca/L for magnesium and calcium, respectively (Tables 5.1.2 and 5.1.4; Fig. 5.1.4). Highest concentrations (over 90 mg Mg/L and 140 mg Ca/L) were measured at BTS, with intermediate concentrations in Horse Creek zones (HC1 and HC2; Fig. 5.1.4). Similar trends and concentrations were found during the 1997 survey (ANSP 1998) in the Holston River zones, but concentrations in the Horse Creek zones in 1997 were higher than in Zones 2-6. There was a linear trend ($r^2 = 0.978$) between the combined concentrations of Mg and Ca and total hardness suggesting that most of the hardness was composed of Mg and Ca carbonates.

5.1.2.12 Dissolved Chloride and Sulfate

Dissolved chloride concentrations ranged from approximately 11 mg Cl/L at Zones 2, 3 and 4 to over 64 mg Cl/L in BTS (Tables 5.1.2 and 5.1.4; Fig. 5.1.5). In general, concentrations increased from Zone 2 to Zone 6 (17 mg Cl/L). Concentrations were higher in 2010 than in the 1997 survey most likely due to the low rainfall in 2010. A similar spatial distribution was observed during the 1997 surveys (ANSP 1998).

Dissolved sulfate concentrations ranged from 8.8 to 9.1 mg SO_4/L in Zones 2 and 3 to between 30 and 37 mg SO_4/L in the downstream zones (Zones 5 and 6, respectively; Tables 5.1.2 and 5.1.4; Fig. 5.1.5). BTS had the highest concentrations in the 2010 survey of 178 mg SO_4/L. Concentrations were similar to or slightly higher than those measured in the 1990 and 1997 surveys (ANSP 1998).

5.1.2.13 Dissolved Sodium (Na) and Potassium (K)

Dissolved Na ranged from approximately 6.4 and 6.9 mg Na/L within Zones 2 and 3 on the South Fork to approximately 20 mg Na/L in Zones 5 and 6 (Tables 5.1.2 and 5.1.4; Fig. 5.1.5). Zones 4, Kit Bottom, HC1 and HC2 exhibited concentrations ranging from 7 to 11 mg Na/L. Highest concentrations were observed in BTS (33 mg Na/L). Sodium concentrations in this survey (excluding BTS) were similar to those in 1997 and reflect a similar downstream increase in concentration into Zones 5 and 6 (ANSP 1998).

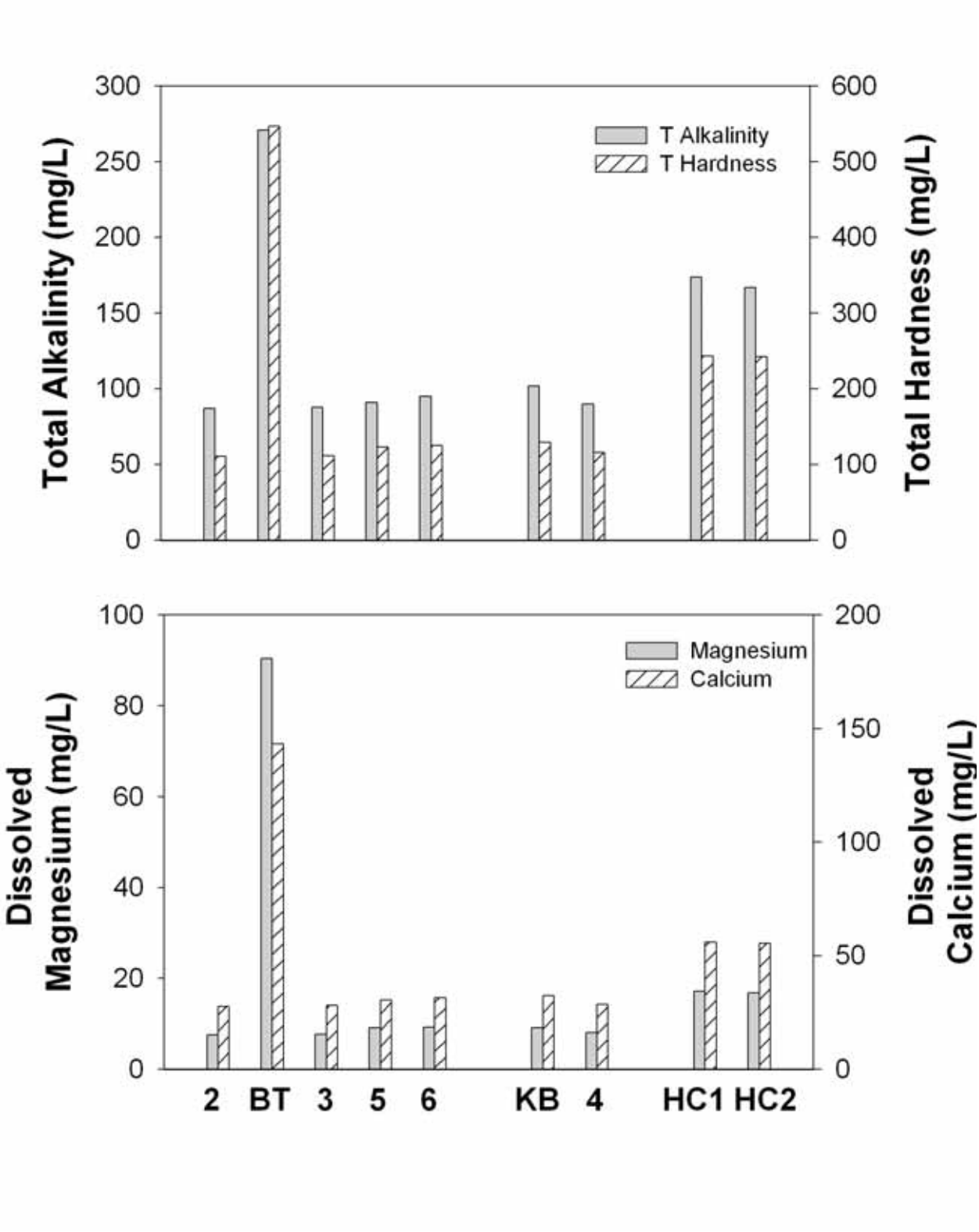


Figure 5.1.4. Mean values for a) total alkalinity and hardness and b) dissolved magnesium and calcium in water samples collected on the South Fork and mainstem Holston rivers (Zones 2, 3, 5, 6 and Big Tree Spring), Big Sluice (Zone 4 and Kit Bottom) and Horse Creek (HC) in July 2010.

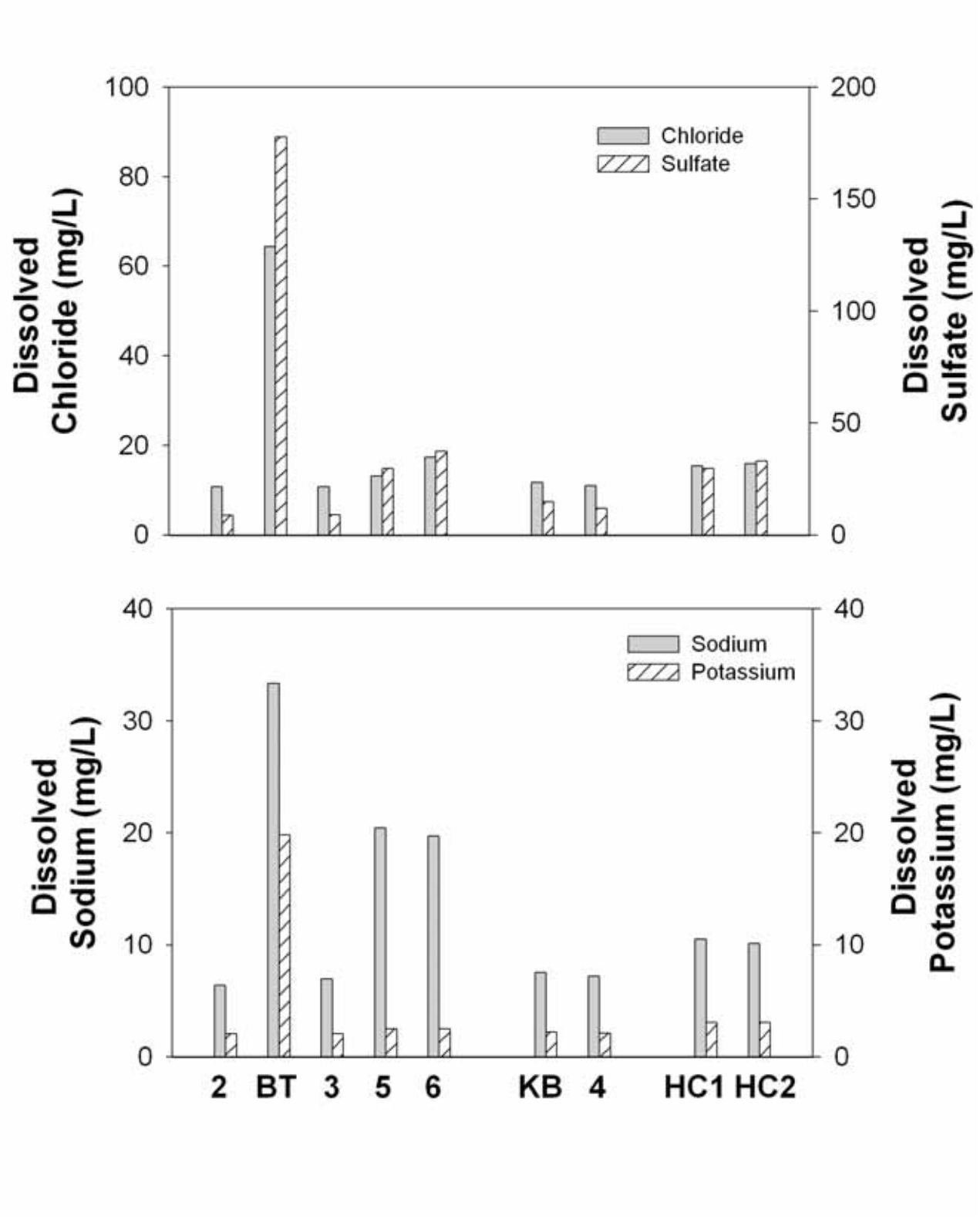


Figure 5.1.5. Mean values for a) dissolved chloride and sulfate and b) dissolved sodium and potassium in water samples collected on the South Fork and mainstem Holston rivers (Zones 2, 3, 5, 6 and Big Tree Spring), Big Sluice (Zone 4 and Kit Bottom) and Horse Creek (HC) in July 2010.

Dissolved K increased slightly downstream, ranging from 2.0 mg K/L at Zone 2 to 2.5 mg K/L in Zones 5 and 6 (Tables 5.1.2 and 5.1.4; Fig. 5.1.5). Potassium concentrations were fairly similar overall with highest concentrations at BTS (20 mg K/L). Concentrations of K were generally similar between 1997 and 2010 surveys and were slightly lower in 1990 (ANSP 1992, 1998).

5.1.2.14 Dissolved Nitrate+Nitrite

Dissolved nitrate+nitrite concentrations averaged 743±35 μg N/L for the South Fork and mainstem zones and increased only slightly downstream (Tables 5.1.3 and 5.1.4; Fig. 5.1.6). Concentrations in the Big Sluice and Horse Creek zones were generally similar (~630 to 660 μg N/L). Highest concentrations were from the BTS (approximately 3900 μg N/L), which flows into Zone 2. On average, concentrations were slightly higher in 1997 (750 versus 700 μg N/L on average without BTS) compared to the present survey (ANSP 1998) and followed a similar distribution in the South Fork and mainstem Holston rivers.

5.1.2.15 Dissolved Ammonium+Ammonia

Concentrations of dissolved ammonium+ammonia ranged from 26 μg N/L in Zone 3 to a high of 76 μg N/L in BTS (Tables 5.1.3 and 5.1.4; Fig. 5.1.6). As in 1997, concentrations were somewhat variable among the zones (Fig. 5.1.6). Concentrations in the previous survey were generally similar and averaged approximately 35 μg N/L in both 1997 and 2010 (ANSP 1998).

5.2.1.16 Total Nitrogen

Total nitrogen was calculated as the sum of SKN (dissolved ammonium + ammonia +DON), dissolved nitrate+nitrite and particulate nitrogen. Overall, concentrations ranged from approximately 965 μg N/L in Kit Bottom and Zone 4 to approximately 4250 μg N/L at BTS (Tables 5.1.3 and 5.1.4; Fig. 5.1.6). In the South Fork and mainstem Holston rivers, concentrations generally increased from upstream to downstream (1036 in Zone 2 to 1158 μg N/L in Zone 6). Dissolved nitrate+nitrite and organic nitrogen (as measured from SKN) were the major components of total nitrogen, with lesser amounts of dissolved ammonium+ammonia and particulate nitrogen; Table 5.1.3).

5.1.2.17 Dissolved Orthophosphate

Concentrations of dissolved orthophosphate ranged from 1.7 μg P/L at Zone 2 to over 35 μg P/L in Zone 5 (Tables 5.1.3 and 5.1.4; Fig. 5.1.7). Concentrations decreased to 30 μg P/L into Zone 6. The distribution is similar to that found in the 1997 survey. Highest concentrations were found in Zones 5 and 6 (Fig. 5.1.7). BTS and Horse Creek concentrations of dissolved ortho-P ranged from 3.7 to 13 μg P/L, respectively. Concentrations were generally somewhat lower in 2010 compared to the 1997 survey (ANSP 1998).

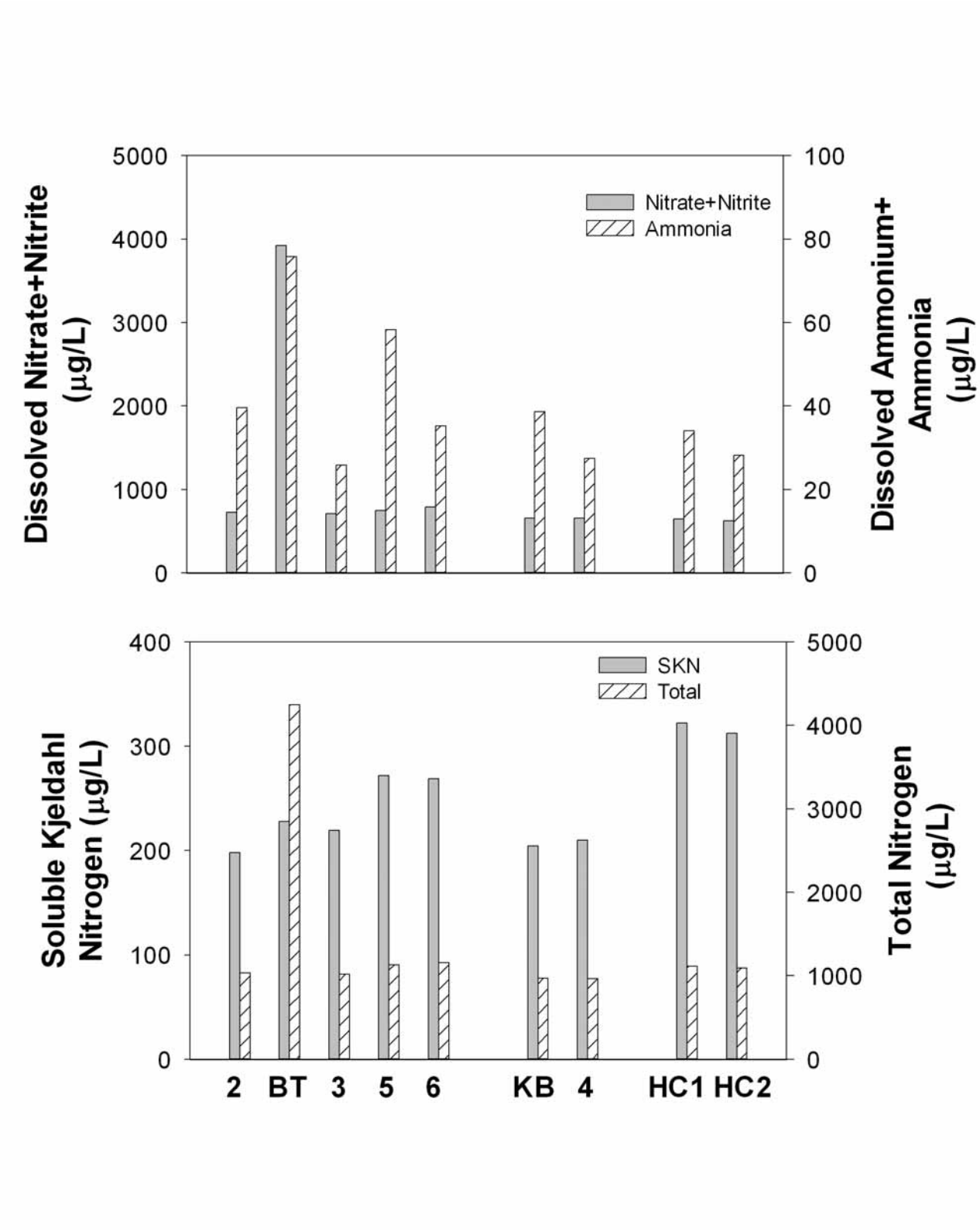


Figure 5.1.6. Mean values for dissolved nitrate+nitrite and ammonia (ammonia+ammonium) and soluble Kjeldahl nitrogen and total nitrogen in water samples collected on the South Fork and mainstem Holston rivers (Zones 2, 3, 5, 6 and Big Tree Spring), Big Sluice (Zone 4 and Kit Bottom) and Horse Creek (HC) in July 2010.

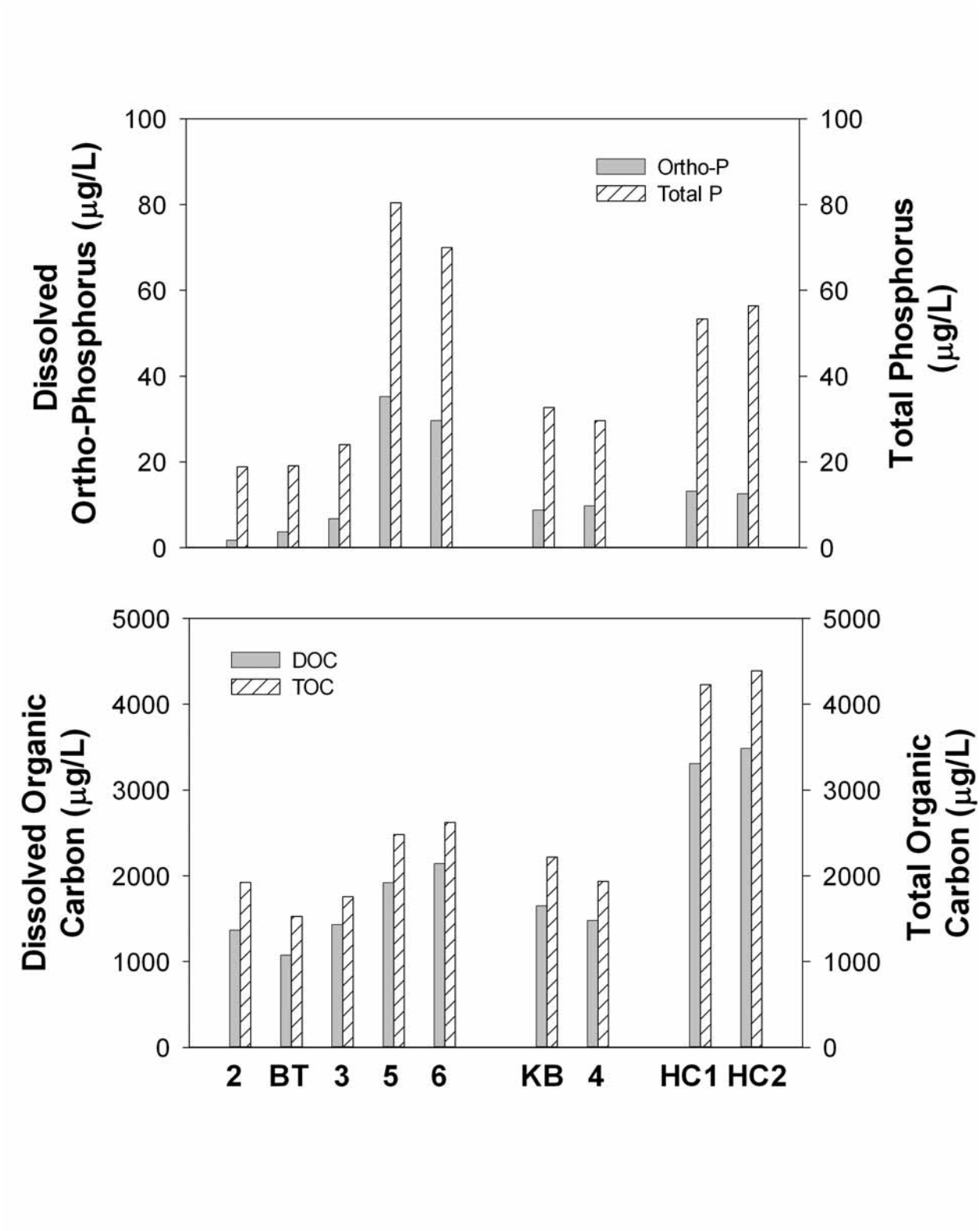


Figure 5.1.7. Mean values for dissolved ortho-phosphorus and total phosphorus and dissolved organic carbon and total organic carbon in water samples collected on the South Fork and mainstem Holston rivers (Zones 2, 3, 5, 6 and Big Tree Spring), Big Sluice (Zone 4 and Kit Bottom) and Horse Creek (HC) in July 2010.

5.1.2.18 Total Phosphorus

Total phosphorus concentrations ranged from 19 µg P/L at Zone 2 to 80 µg P/L in Zone 5 (Tables 5.1.3 and 5.1.4; Fig. 5.1.7). Concentrations in Zone 4 and Kit Bottom were 30 and 33 µg P/L, respectively. A similar spatial distribution was observed for dissolved orthophosphate as total phosphorus (Fig. 5.1.7). Concentrations were generally similar between the 1997 and the current survey (ANSP, 1998).

5.1.2.19 Dissolved Organic Carbon (DOC)

Concentrations of DOC ranged from approximately 1100 µg C/L in BTS to approximately 3400 µg C/L in HC1 and HC2 (Tables 5.1.3 and 5.1.4; Fig. 5.1.7). Intermediate concentrations were found at the other zones. Concentrations were generally similar in the 1997 and 1990 river surveys with a different spatial distribution (ANSP 1992, 1998).

5.1.2.20 Total Organic Carbon (TOC)

Concentrations of TOC (i.e., the sum of particulate organic carbon and DOC; Tables 5.1.3 and 5.1.4; Fig. 5.1.7) ranged from 1922 and 1754 µg C/L at Zones 2 and 3 to approximately 2480 µg C/L and 2620 µg C/L in the downstream areas (Zones 5 and 6). Highest concentrations of TOC were found in HC1 and HC2, while lowest overall concentrations were measured from BTS. The majority of the TOC is comprised of DOC with less than ~25% contributed by particulate carbon. Similar concentrations were observed during the 1997 river survey (ANSP 1998), with a similar upstream to downstream spatial distribution.

5.1.2.21 Organic Compounds: Aniline, Benzene and 1,4-dioxane

Surface water samples were collected from the Kit Bottom zone within the Big Sluice for the analysis of the volatile chemicals benzene and 1,4-dioxane, and the semi-volatile compound, aniline. Two independent laboratories conducted the analysis (Lancaster Laboratories, Lancaster, PA), with split samples going to Eastman's Environmental Services Laboratory (TN Eastman, Co).

For all samples (n=3), the three compounds were not detected with an MDL from Lancaster Laboratories of 0.5 µg/L, 1 µg/L and 1 µg/L for benzene, aniline and 1,4-dioxane, respectively. The split samples that were sent to the Environmental Services Laboratory were also reported as non-detected but the detection levels were somewhat higher (5 µg/L, 11 µg/L and 15 µg/L for benzene, aniline and 1,4-dioxane, respectively).

5.1.3 Historical Analysis: Academy Surveys from 1965 to 2010

The Academy of Natural Sciences conducted seven Holston River environmental monitoring studies from 1965 to 2010 (1965, 1974, 1977, 1980, 1990, 1997 and 2010). During this time period, pollution control legislation, both federal and state (e.g., FWCA 1972,

CWA 1977, WQA 1987 and others), was enacted in an attempt to improve stream and river water quality throughout the United States. To help understand the changes in water quality that have taken place over this 45-year time period on the Holston River in the vicinity of Kingsport, TN, biological oxygen demand (5-day), dissolved ammonium+ammonia, dissolved nitrate+nitrite, total phosphorus, dissolved chloride, and fecal coliforms data from the various Academy surveys were compiled, and the data are presented in Figures 5.1.8-5.1.13. These parameters were chosen to understand organic loadings (BOD5d) to the river and related nutrient (nitrate+nitrite and total phosphorus) impacts. Fecal coliform is also related to organic loadings from various sources, while dissolved chloride would be an indicator of major ions (e.g., sodium, potassium, and magnesium) and can be an indicator of the use of salt and discharges from wastewater treatment facilities.

The data were reviewed for unit and analytical comparability, and corrections were made, if necessary. Importantly, most methods used were from various editions of *Standard Methods for the Examination of Water and Wastewater*, which allows for data comparison due to method similarities and quality. However, there were some changes. For example, the data for nitrate (and nitrite) in 1965 and 1977 were originally presented as mg NO_3/L (i.e., as nitrate), and were converted to NO_3-N (i.e., as nitrogen) units for comparison to more recent data. Also, historic survey phosphorus units were converted (phosphate versus phosphorus). In addition, total phosphorus was not measured in the earlier surveys but was in the last three survey periods. Therefore, orthophosphate data were plotted for the first surveys, along with total phosphorus from the more recent surveys. In some surveys, samples were collected over a three-day period, while in the more recent surveys, samples were collected on the same day. The standard deviations of these values were used regardless of the time period sampled. In each plot, the mean and standard deviation are presented for the last three collections (1990, 1997 and 2010) to highlight temporal changes in water quality that have occurred after major pollution controls were enacted (early 1970s to mid-1980s). It should be noted that some of the reports use the term 'station' and some use the term 'zone.' In the material presented below, 'zone' is used for the figures. Finally, in some cases, the actual sampling location changed during the past 45 years.

Biological oxygen demand (BOD) generally decreased from about 5-15 mg O_2/L (Zones 3 to 6) in the older surveys to less than 1 mg O_2/L at all zones over the past 20 years (Fig. 5.1.8). The largest decrease occurred from 1965 to either 1977 or 1980. At Zone 2, upstream of Kingsport and the facility, BOD was slightly elevated in the past (up to 4.1 mg O_2/L in 1974) and decreased to < 1 mg O_2/L by 1997. The overall decrease in BOD is most likely related to advanced treatment of municipal and industrial effluents as a result of point source regulations.

Dissolved ammonium+ammonia and nitrate+nitrite concentrations showed slightly different trends over the time period (Figs. 5.1.9 and 5.1.10). Dissolved ammonium+ammonia concentrations upstream at Zone 2 averaged 0.03±0.02 mg N/L over the past 45 years, with no apparent temporal trend. At the downstream zones there was a substantial decrease from 1965 to the early 1970s, after which concentrations remained fairly constant. Presently, concentrations of ammonium+ammonia are generally similar among zones, with only slightly higher concentrations (and more variable) at Zone 5. Dissolved nitrate+nitrite concentrations at all zones decreased from 1965 to approximately 1980 after which concentrations increased slightly at most zones. The decrease may be due to better treatment of wastes, while the increase after

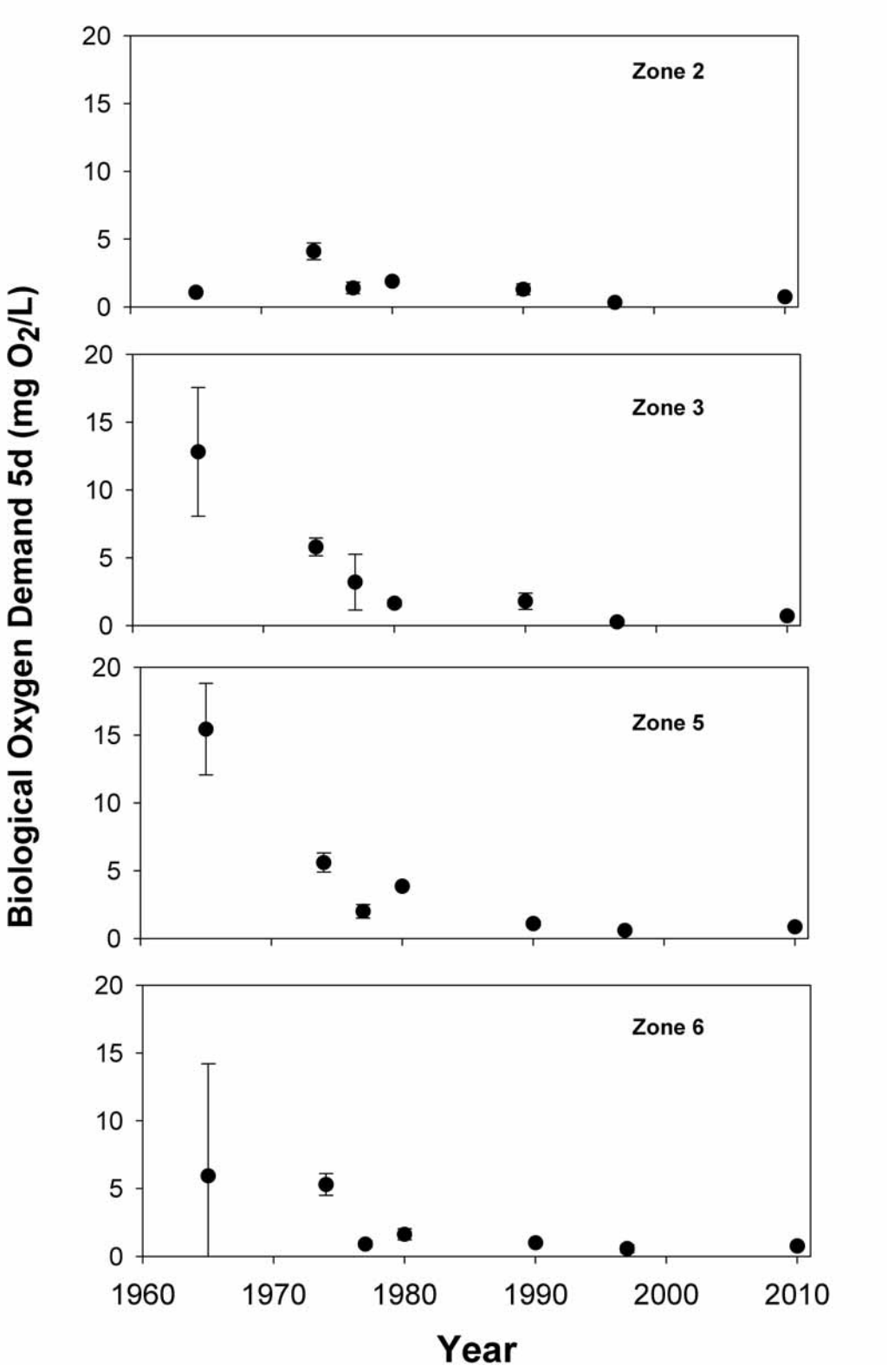
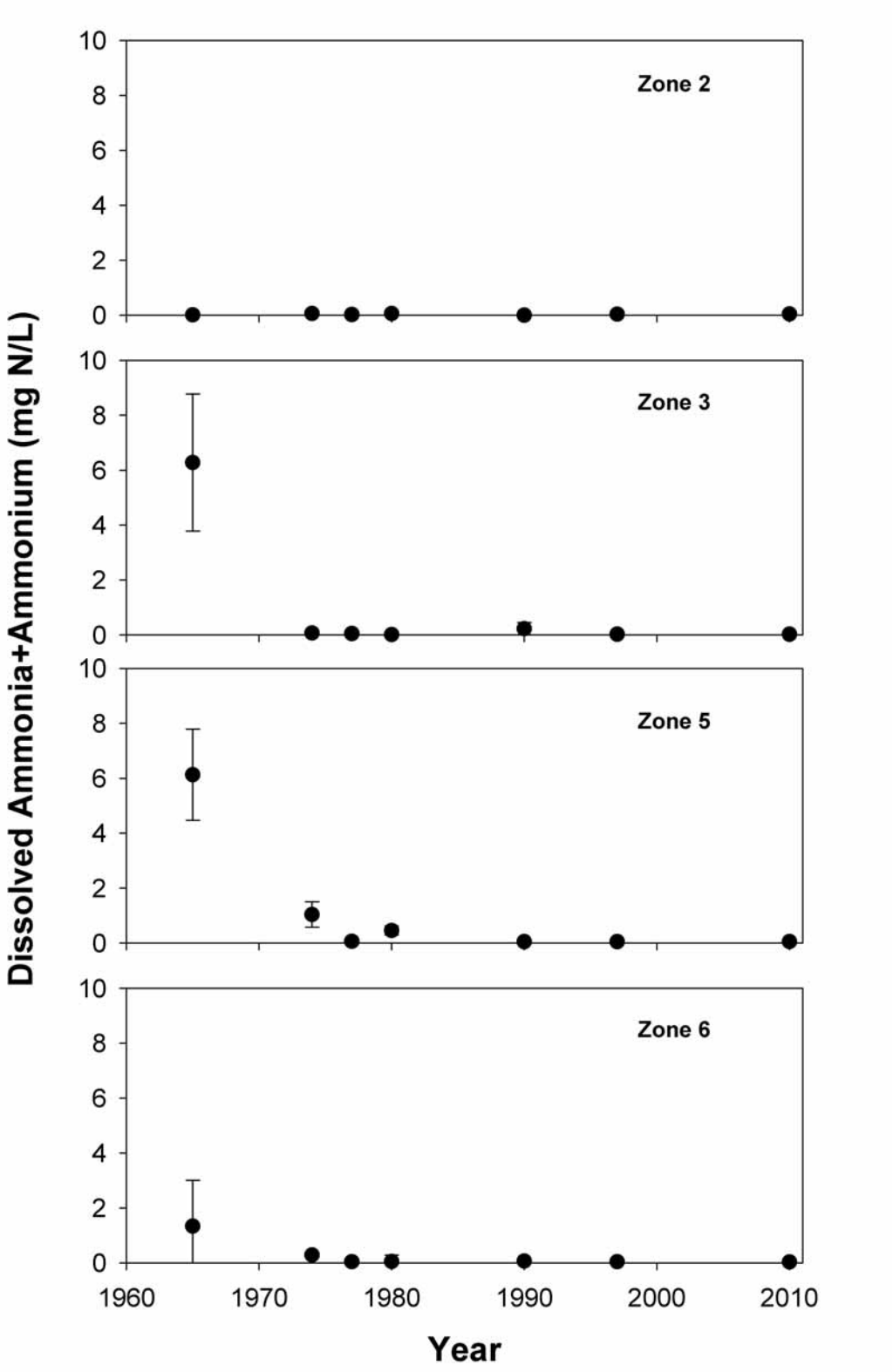


Figure 5.1.8. Biological oxygen demand (BOD_{5d} as mg O_2/L) at the four South Fork and mainstem Holston rivers zones from 1965 to 2010. The data are the averages (±1σ) for the sampling period regardless if the sampling was over three days or within one day.


Dissolved Ammonia+Ammonium (mg N/L)
Zone 2
Zone 3
Zone 5
Zone 6
0
2
4
6
8
10
1960
1970
1980
1990
2000
2010
Year

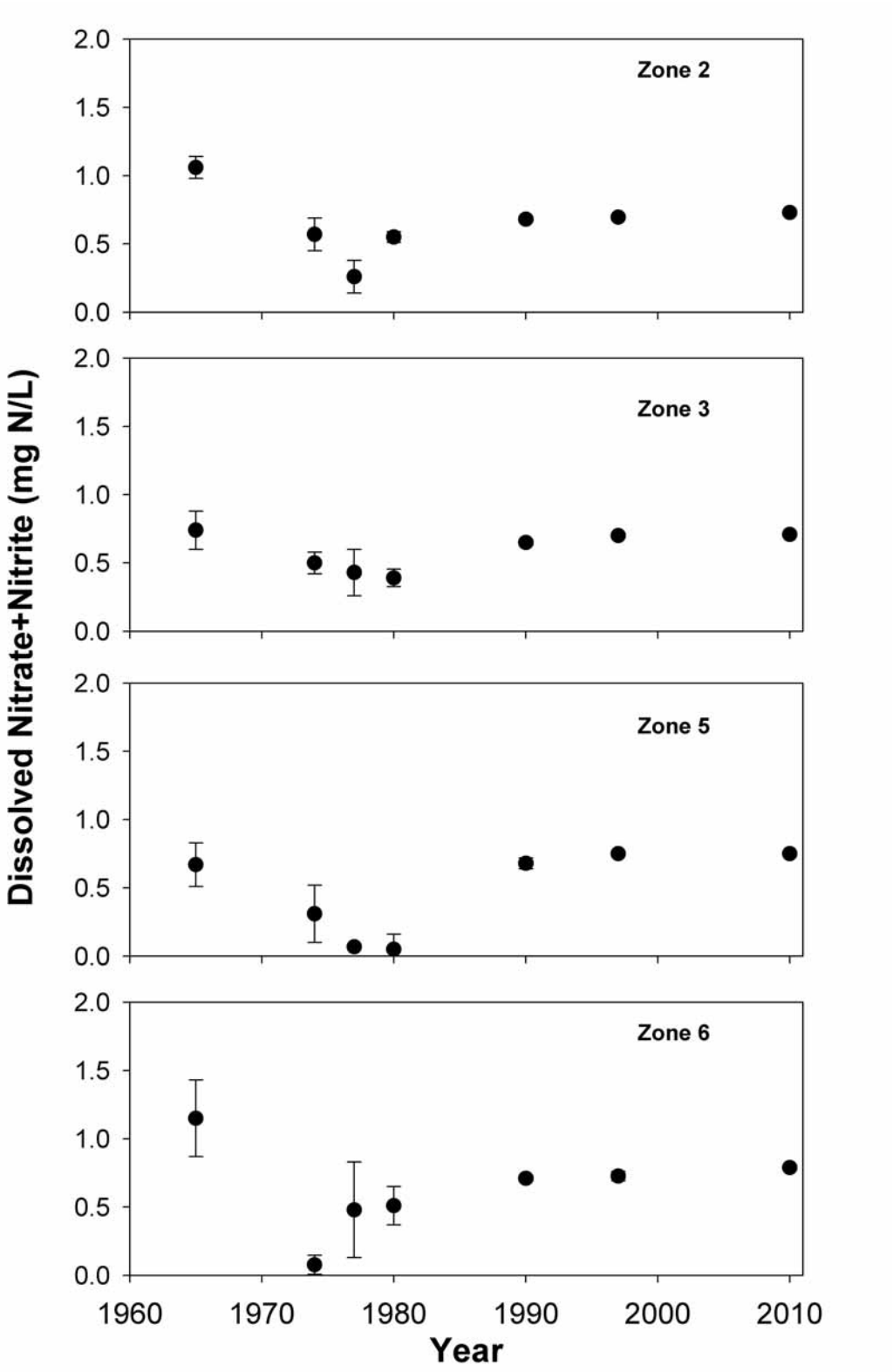


Figure 5.1.10. Dissolved nitrate+nitrite concentrations (in mg N/L) at the four South Fork and mainstem Holston rivers zones from 1965 to 2010. The data are the averages (±1σ) for the sampling period regardless if the sampling was over three days or within one day.

1980 could be due to changes in land use and population increases within the watershed. During the 2010 study, concentrations from Zone 2 to Zone 6 were similar and ranged from ~0.7 - 0.8 mg N/L.

Total phosphorus concentrations were only slightly higher before 1980, with a decrease in concentration over time (Fig. 5.1.11). Some of the variations may be due to changes in methods and the form of phosphorus analyzed. Prior to 1980, the form used was dissolved orthophosphate and not total phosphorus. The decrease with time is small (Zones 2 to 5), with no change observed at Zone 6, the most downstream zone. Over the past 20 years, TP concentrations were slightly higher downstream of Zone 2. Mean concentrations of TP were 0.02±0.001 mg P/L at Zone 2 (1990 to 2010), while they ranged from 0.05±0.02 to 0.08±0.05 mg P/L further downstream.

Dissolved chloride concentrations over the past 45 years showed similar trends at Zones 2, 3 and 5 and a slightly different trend at Zone 6 (Fig. 5.1.12). At Zone 2, concentrations were lowest overall and increased only slightly during the 1977 survey. A similar trend in chloride concentrations was observed at Zone 3, with generally similar concentrations. Concentrations at Zone 5 were elevated and more variable (1965 and 1977 surveys) and then decreased to the lowest observed values after 1980. At Zone 6, concentrations of dissolved chloride were extremely elevated in 1965 (395±240 mg/L). The elevated chloride levels were likely the result of the influence of the North Fork Holston River (Zone 5A), in which concentrations of dissolved chloride were elevated (2500 mg/L; ANSP 1965). Zone 6 is located downstream of the confluence of the north and south forks of the Holston River. Over the past 20 years, concentrations decreased to levels that are similar to the upstream zones.

Fecal coliform bacteria were measured in five of the seven surveys from 1965 to the present (Fig. 5.1.13). Overall, lower counts were observed at Zone 2 (85±141 colonies/100 mL) compared to the other zones (overall average ranged from 315±459 colonies/100 mL at Zone 6 to 883±764 colonies/100 mL at Zone 3). At all zones, there was a decrease in bacteria from the 1974-1977 surveys after which, the bacteria counts were fairly constant except at Zone 3 in 2010. Over the past 20 years (1990 to 2010), concentrations reflected a potential input between Zones 2 and 3. It should be noted that one of the highest values occurred during the 2010 survey at Zone 3, with a value of 2100±1184 colonies/100 mL.

5.1.4 Summary and Conclusions

The 2010 river survey included chemical and bacteriological analysis of water samples from various locations on the South Fork and mainstem Holston rivers in the vicinity of Eastman's Tennessee Operations facility. Trends of increasing concentrations were noted for several parameters along the South Fork and mainstem of the Holston River, with peak concentrations in Zone 5 (see ammonia and TP for example) or in some cases downstream in Zone 6 (see DOC and TN). For many parameters, BTS exhibited comparatively high concentrations. However, this spring is small and does not appear to impact the water quality downstream to any measurable extent. Other parameters showed no consistent spatial changes within the river system. A few parameters had higher concentrations in Horse Creek, including turbidity, TSS, conductivity,

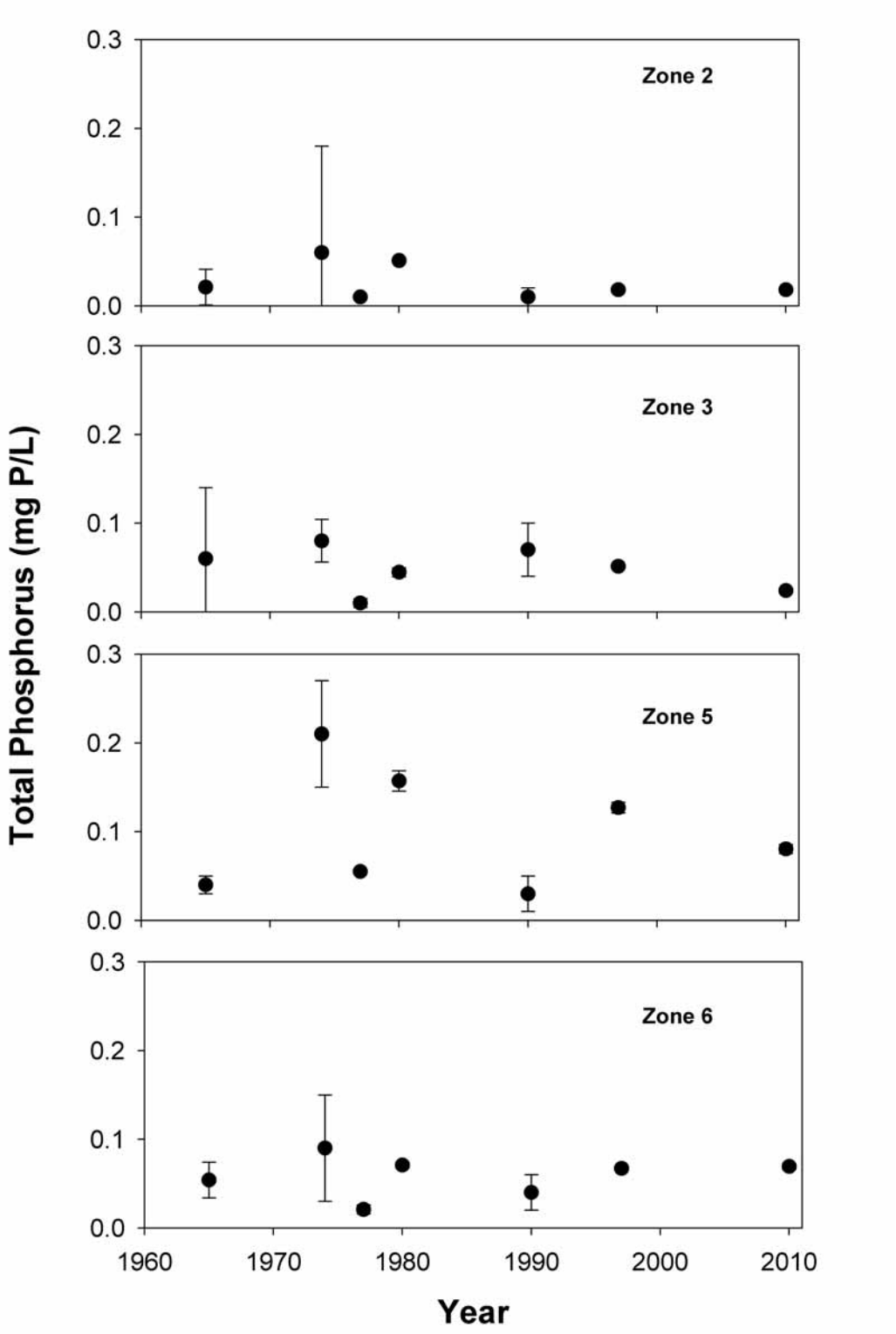


Figure 5.1.11. Total phosphorus concentrations (in mg P/L) at the four South Fork and mainstem Holston River zones from 1965 to 2010. The data are the averages (±1σ) for the sampling period regardless if the sampling was over three days or within one day.

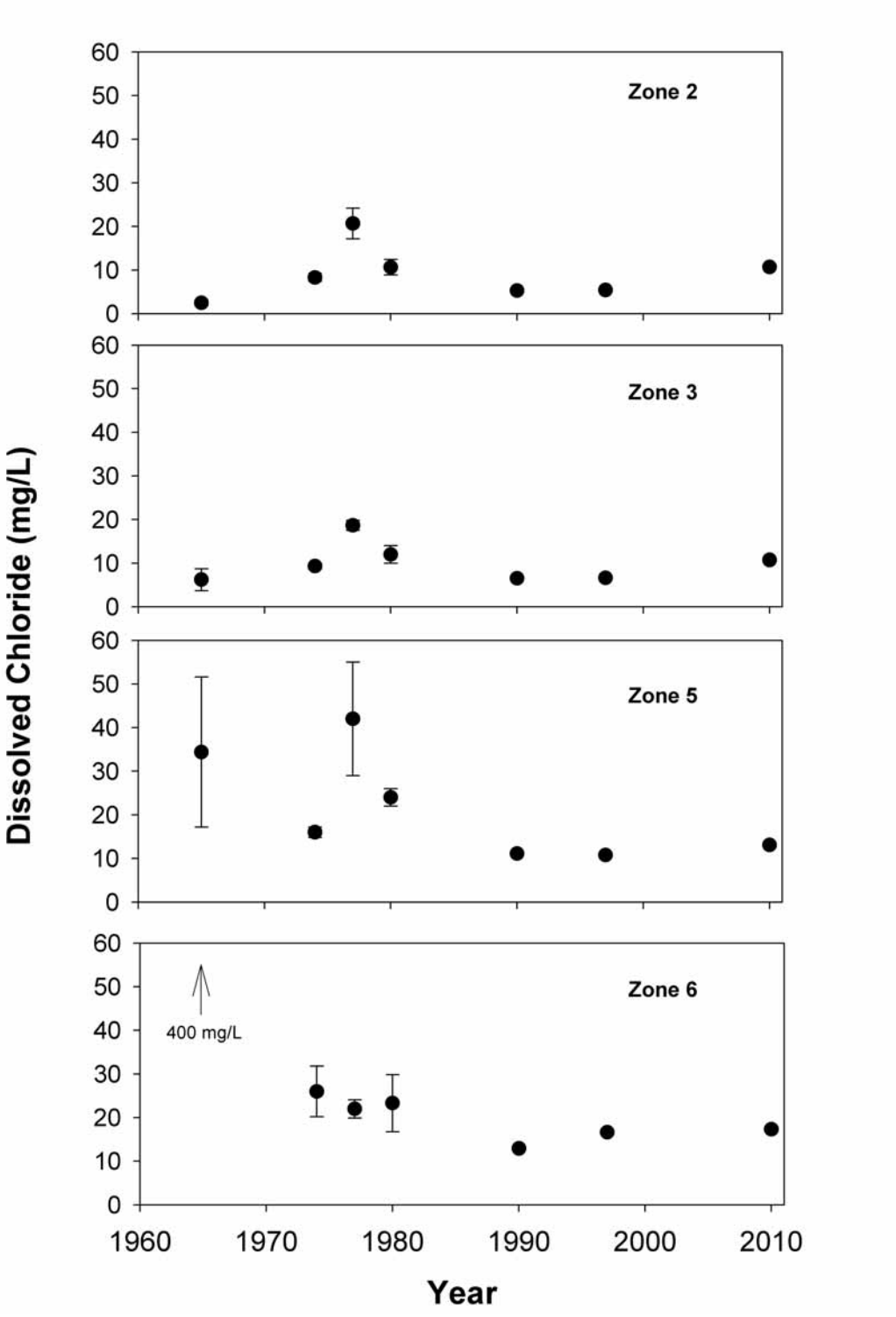


Figure 5.1.12. Dissolved chloride concentrations (in mg/L) at the four South Fork and mainstem Holston River zones from 1965 to 2010. The data are the averages (±1σ) for the sampling period regardless if the sampling was over three days or within one day.

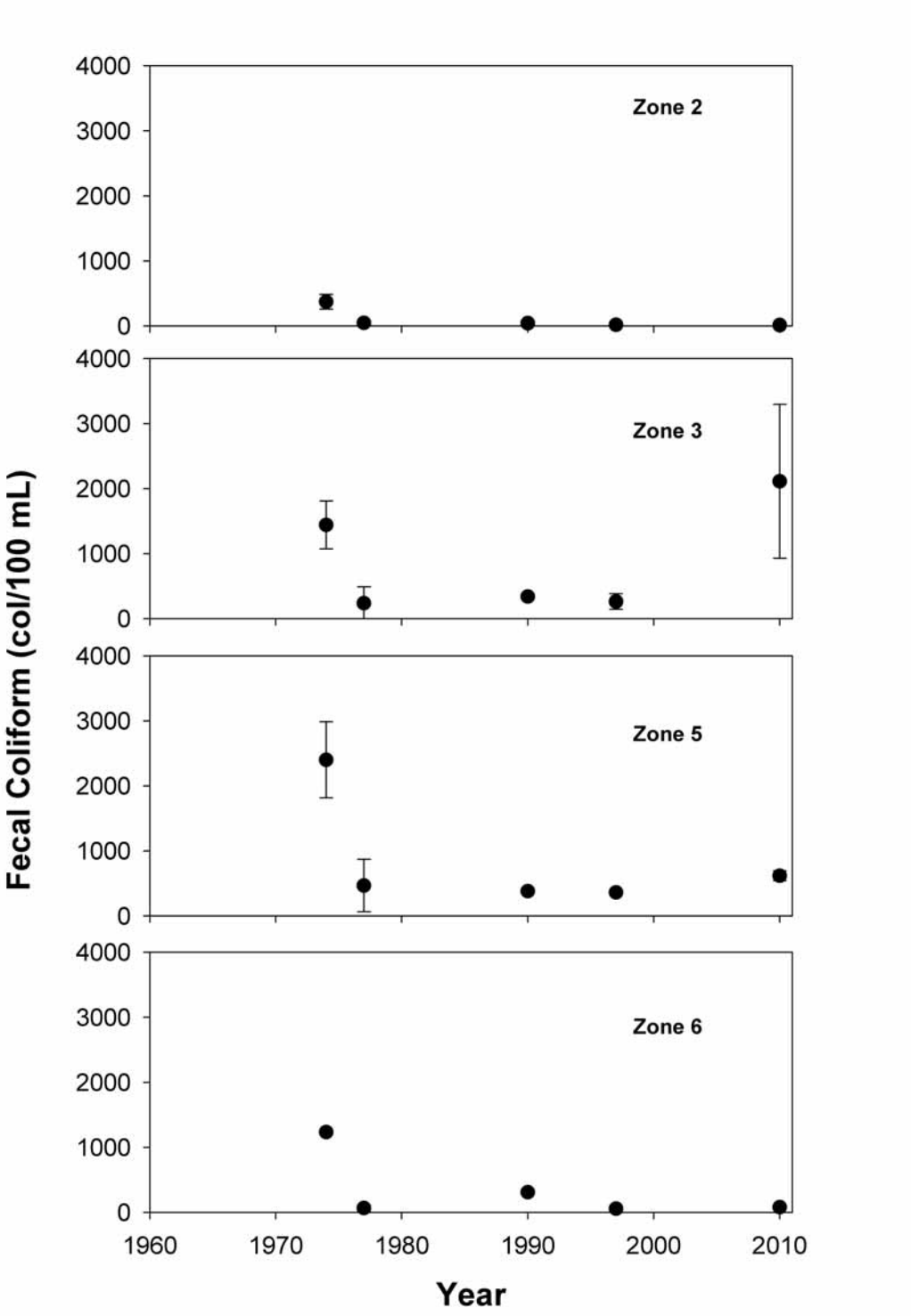


Figure 5.1.13. Fecal coliform concentrations (in colonies/100mL) at the four South Fork and mainstem Holston River zones from 1965 to 2010. The data are the averages (±1σ) for the sampling period regardless if the sampling was over three days or within one day.

TS, specific dissolved major ions, DOC and TOC, and SKN. Concentrations of selected parameters show an improvement over time. This is evident for BOD, fecal coliform and possibly dissolved ammonium+ammonia. Importantly, over the past 20 years the water quality appears to be stable with no substantial change in the parameters examined. Fecal coliform bacteria show an increase in concentration at Zone 3 in 2010, possibly due to a localized precipitation event during the survey. Generally, concentrations were higher downstream of Zone 2, reflecting the potential inputs of material from animal wastes, failed septic systems, and/or point and non-point source runoff between Zones 2 and 3 following the aforementioned rain event.

5.2 Algae and Aquatic Macrophytes

5.2.1 Overview

Species listings of algae and aquatic vascular plants are presented in Appendices 7.4.1 and 7.4.2. The diatom listings are comparable to previous studies for presence-absence, but because of a change in the counting method (during the 1977 survey), only general comparisons of the number of species are made.

With the use of modern taxonomic techniques, including electron microscopy, DNA sequencing and computer enhanced numeric analyses, there has been considerable change in algal taxonomy, especially for diatoms and blue-green algae (cyanobacteria). The number of taxonomic changes since the previous survey (1997) is probably greater than the changes observed since studies of Holston River algae started in 1965. Appendix 7.4.3 is a compilation of the taxonomic changes that were made to compare the current and previous survey (1997). Some of the changes noted were a result of combining (lumping) taxa that were not previously differentiated.

5.2.2 Holston River

5.2.2.1 Zone 2

The major algal substrate at Zone 2 was rocks and rock outcroppings covered with sediment. Because of dam operations, these substrates were dry for several periods of the day. Several sandy mudflats supported algae in pools and on the sediment surface. Along with a large stand of floating aquatic plants (*Elodea*), algal communities were growing on tree roots and rootlets protruding into the water. Much of this habitat was moderately to heavily sediment-covered.

Filamentous green algae, *Cladophora glomerata, Oedogonium* sp. and *Microspora* sp.*,* were found as “streamers” from silty-sand substrates (usually on top of rocks and cobble) in permanent flowing waters. In areas with greater current and less sediment, the green filamentous *Gongrosira debaryana* and *Stigeoclonium lubricum* were notable. In areas that were more pooled (and sometimes dry), blue-green algae and diatom communities were prominent on the heavy sediments. *Phormidium* sp. (*Microcoleus* in previous surveys) sheens were abundant in both wet and periodically dried areas.

Diatoms were widespread throughout the zone, especially on rocks and epiphytically (on plants) on other algae and aquatic macrophytes. There was very little relation between habitat and the larger diatom populations. *Achnanthidium minutissimum* (10-18%; *Achnanthes minutissima* in previous surveys), *Achnanthidium catenatum* (7-12%; previously a portion of *Achnanthes minutissima*), *Diatoma vulgaris* (2-17%; previously referred to as *D. vulgare*), *Cyclotella ocellata* (5-8%) and *Gomphonema minutum* (0-10%; previously *G. tenellum*) were the most abundant diatoms and were widespread throughout the zone. Several taxa, including *Cyclotella ocellata, C. michiganiana, Aulacoseira subartica, A. granulata* (previously *Melosira granulata*), *Encyonema reichardtii* (one population of 48%)*, E. subminuta* (previously *Cymbella minuta*), *Stephanodiscus hantzschii, S. minutulus, C. tholiformis, Staurosirella pinnata* (previously *Fragilaria pinnata*) and *Staurosira construens* var. *venter* (previously *Fragilaria construens* var. *venter*), had their highest populations in Zone 2, decreased in a downstream direction and were rarely found in Horse Creek zones. Of the notable populations, only *Amphora pediculus*, found epiphytically, and *Cocconeis pediculus,* found associated with much sedimentation, appeared to be habitat-related.

Floating just off the Cliffside Road landing, as in previous studies, was a large stand (30 x 50 ft) of *Elodea canadensis* with smaller populations of *Potamogeton crispus* and *P. nodosus* closer to shore. Upstream and closer to the Fort Patrick Henry Dam were several large floating growths (up to 5 ft in diameter) of *Heteranthera dubia* and, amongst the heavily sedimented rocks, *P. nodosus.*

Continued (starting with the 1990 survey) heavy sedimentation reduced the number of algal substrates found in comparison with previous surveys. The green and blue-green forms, however, were very abundant, and especially dominant as streamers from the heavily-sedimented rocks. As was observed in the past few studies (1980, 1990 and 1997), no red algae were found in 2010 (red algae had been found in 1965, 1967, 1974 and 1977).

5.2.2.2 Big Tree Spring

The algal community in the area of the Big Tree Spring was essentially a very large mat of filamentous green algae with epiphytic diatoms and several populations of blue-green algae near the muddy sand and water interface. The mat was *Cladophora glomerata* mixed with smaller portions of *Oedogonium* sp. There were notable populations of filamentous blue-green (*Phormidium autumnale, P.* sp. and *Lyngbya martensiana*) and green algae (*Oedogonium* sp. and *Ulothrix zonata*) on the mudflat area.

The epiphytic samples of diatoms were composed mostly of the taxa found throughout the study area – *Achnanthidium minutissima* (12-55%), *A. catenatum* (10-13%), *A. pyrenaicum* (2-8%), *Cyclotella ocellata* (2-11%), *Cocconeis pediculus* (2-10%) and *Diatoma vulgaris* (3-13%). *Achnanthes minutissima* var. *jackii* (8%) was the only diatom taxa greater than 1% that was notably more abundant in Big Tree Spring samples. The green *Characium* sp. and blue-green *Heteroleibleinii* sp. were other epiphytic forms found within the large mat.

There were no aquatic macrophytes in the general area of the spring. However, similar to the rest of Zone 2, upstream and downstream of the spring there were growths of *P. nodosus* amongst the heavily sedimented rocks.

5.2.2.3 Zone 3

During the 2010 survey, Zone 3 collections were made from areas that were defined as Zone (or Station) 3 for the 1965, 1967, 1974, 1977, 1990 and 1997 surveys and the area defined as Zone 3L in 1980. Areas on the left side of the island and near the left bank correspond to Zone 3L of the 1980 survey. Algal substrates included rocks with moderate to heavy sediment (even in a moderate to swift current), aquatic plants (rooted in slow and fast flow areas) and aquatic mosses. There were lesser areas of mudflat and a small amount of tree roots and rootlets.

Filamentous green algae, unlike the previous survey (1997), had several abundant forms in Zone 3. Streamers of the green algae *Cladophora glomerata* and *Microspora* sp. were found on the rocks with moderate to heavy sedimentation in both fast and moderate flow areas. Several populations of the brightly colored *Spirogyra* were observed on the sediment and sediment-laden objects in the slowest flow area. On a few rocks with reduced sedimentation were a couple of populations of *Schizomeris leibleinii*. A mat of *Hydrodictyon reticulatum* was floating/laying near the rocks in the slower flow area near the right bank of the left channel.

The abundance of blue-green algae was similar to the 1997 survey (less than earlier surveys), with only a few distinct populations on heavily sedimented objects. The filamentous blue-greens *Phormidium granulatum* (previously *Microcoleus lyngbyaceus*), *P.* sp. and *Pseudanabaena* sp. formed notable but small populations among diatom communities on sedimented rocks and logs. *Heteroleibleinii* sp. was a common epiphyte on several of the green algal filaments.

Diatom communities were found on most substrates, in varying flow (from slow to fast) and sedimentation (though somewhat less abundantly than in Zone 2). There were a few widespread forms, similar to Zone 2, although the abundances were lower, probably due to less sedimentation. *Achnanthidium minutissima, A. catenatum, Amphora pediculus, Cocconeis placentula* var. *lineata, Cyclotella ocellata, Diatoma vulgaris, Melosira varians and Navicula minima* were notable and widespread throughout Zone 3. However, populations were mostly below 10%. Two other small diatoms, *Achnanthidium deflexum* and *A. exiguum*, were found on most substrates and in higher proportions in Zone 3 than the other zones. *Tabularia fasciculata* and *T. tabulata* formed the largest populations as epiphytes of filamentous algae and aquatic mosses. On the heavily sedimented logs, species of the genus *Nitzschia* formed 40% of the diatom communities. *Pleurosira laevis,* a diatom that is large and filamentous, was not prevalent in the counts (probably because of the numerous small unicellular forms); however, it was observed in most algae counts similar to other algal "streamers."

Aquatic macrophytes in Zone 3 were abundant and very similar to what was observed in the last study (1997). Large stands of the broad-leafed *Potomogeton nodosus* grew all through the right channel area and because of shading, prevented other algal growth on the bottom sediments (there also were few epiphytic growths on these plants). In the swifter current in the left bank channel there were several stands of the grass-like *Potomogeton pectinatus* and a few clumps of

Heteranthera dubia. A large amount of aquatic moss and a few small stands of *Potomogeton crispus* were found on rocks in the swift current near the left bank. In this area, there was a notable amount of *Elodea canadensis*, most likely drifted in from just upstream.

Overall, there was a large amount of aquatic plant material in Zone 3, mostly as aquatic plants, mosses and filamentous green algae. Diatoms were widespread and notable, but the characteristic brownish sheens were less abundant than in prior years. Blue-green algae, similar to the previous survey (1997), were less abundant than in earlier surveys (1977 through 1990) with fewer species. The algal communities near the left side of the zone were dominated by diatoms and blue-green algae. However, there was a large amount of algal material (green algal mats and streamers) in the shallow area near the right bank of the left channel.

Although the right bank area was not extensively surveyed in 1980, the amount of aquatic plants found in 1997 and 2010 appeared to be greater than in 1977.

5.2.2.4 Zone 4

The layers of rock outcroppings that form fast-flow "falls" for algal "streamers" were the major feature and substrate of Zone 4. During the 2010 survey, there were very few, if any, large pooled areas in the rock outcroppings. Also, areas exposed during the lowest flow were rarer than in previous surveys. Algae grew in several different areas between the rock outcroppings, including as epiphytes of the abundant moss in fast-flow areas. The small backwater area near the left bank had tree roots and rootlets where algal communities were found.

The most abundant algae in Zone 4 were communities, mostly of filamentous algae, on and around the rocks in fast-flow areas. The green filamentous forms *Cladophora glomerata* and *Microspora* sp*.,* formed large growths as streamers on and around the rock outcroppings. In the fastest flow, several blue-green forms, including *Homoeothrix janthina, H. varians* and *Pleurocapsa minor* (previously *Entophysalis rivularis*), formed large communities tightly attached to the rocks and rock outcroppings (areas with reduced sedimentation). Amongst rocks and abundant mosses in moderate to fast-flow areas with more sediment, the prostrate green, *Gongrosira debaryana,* formed several notable populations. On the top of the rock outcroppings, with moderate sedimentation and mosses, were a few populations of the blue-green filamentous forms, *Plectonema* sp. and *Tolypothrix*. In the slower flow, more heavily-sedimented areas, there were many blue-green sheens composed of large populations of *Phormidium* sp. and *P. autumnale*. The green, bag-like algal form, *Tetraspora gelatinosa,* was only found once; in previous surveys this form was very common.

Diatom communities in Zone 4 were abundant, especially on the sedimented rocks. In addition to general and widespread species (*Achnanthidium minutissimum, Amphora pediculus, Diatoma vulgaris, Melosira varians* [18% as streamers from lightly sedimented rocks] and *Rhoicosphenia abbreviata* [formerly *R. curvata*]), the sedimented rocks had several notable diatom populations. These included *Cymbella turgidula* (22%) and several *Nitzschia* species (populations formed 16% of the diatom communities). *Cocconeis placentula* var. *lineata* was found to be widespread (along with *C. pediculus*), but formed large populations (23%) in the root materials. Several

diatom species, including *Achnanthidium eutrophilum, Gomphonema pumilum* var. *rigidum*, *G. kobayasii* (previously *G. cleveii*), *G. lagenula* (previously part of *G. parvulum*) and *Platessa conspicua* (previously *Achnanthes pinnata*), had notable populations in Zone 4 and the Horse Creek zones (the confluence is just upstream of Zone 4).

The major aquatic plants in Zone 4 were found in the pools behind or to the sides of the rock outcroppings. Small beds of *Potomogeton pectinatus* and *P. crispus* were found in the deepest pools just above the rock outcroppings. The water willow (*Justicia americana*) was rooted in shallow waters (stands up to 10 ft in diameter), mostly near the sides of the Sluice above the rock outcroppings. Aquatic moss covered most of the rocks in the fastest flow area. The bed of *Potomogeton nodosus* upstream of the rock outcroppings was smaller in 2010 than during several of the previous surveys (1980, 1990 and 1997).

The algal communities in Zone 4 were predominantly the filamentous forms around the rock outcroppings (blue-greens and greens). There were many widespread diatoms; however, when there was less sedimentation and less flow, there appeared to be differences in diatoms observed.

5.2.2.5 Zone 5

Collections at Zone 5 during the 1990, 1997 and 2010 surveys were made near the left bank, just off the Ridgefields Golf and Country Club, rather than from the stone breaker wall near the right bank as in previous studies (because North Fork Holston River water had penetrated through the breaker wall). Available substrate included logs and rocks with light sediment in moderate to fast flowing water, a mudflat, rocks with and without sediment in fast flow water and aquatic mosses.

Although diatoms were widespread and were at least as abundant as the filamentous green and blue-green algae, there were very few epiphytic diatoms. Most of the diatom taxa were found widespread in small populations (*Achnanthidium minutissima* 2-6%, *A. affine 2-8%*, *Cyclotella ocellata* 3%, *Diatoma vulgaris* 3%, *Navicula minima 2-5%, N.* aff. *subminuscula* 2-10% and *N. cryptotenella* 2-8%). There were high relative abundances of *Nitzschia,* ranging from 8% on tree roots, 29% on the mud/sandbar to 83% of the diatom communities on rocks.

The abundant rock material had filamentous forms on the sediment (mostly blue-greens) and small "streamers" from the rocks with more sediment. Distinct blue-green sheens on the rocks with mosses and moderate flow were composed of blue-green filaments *Homoeothrix janthina*, *H. varians* and the coccoid form *Pleurocapsa minor*. As sediment built up (slower flow), there were several populations of the green filaments *Cladophora glomerata* and *Microspora* sp. along with small mats (sheens) of the blue-greens, *Phormidium amoeneum*, *P*. sp. and *P. autumnale*.

Aquatic plants in Zone 5 were found most often in the fast current between the left bank and island. In the fastest areas, rooted pondweeds *Potamogeton nodosus* and *P. pectinatus* formed small stands; *Vallisneria americana* was found only rarely. Aquatic moss covered rocks in areas with the fastest current.

Because of changes in sampling location, it is difficult to compare algal and aquatic plant communities found in 1990, 1997 and 2010 with previous studies. A few more rooted aquatic

plants were observed with only a few beds of floating aquatic plants (e.g., no *Elodea canadensis* or *Heteranthera dubia* in 2010). In 2010, as in 1997, there were fewer aquatic plants and more diatoms. Aquatic mosses were still abundant in 2010, as in most previous studies, but appeared to have fewer diatom epiphytes. Diatoms were more generally distributed in most habitats, possibly due to the differing amounts of sedimentation, although this is hard to evaluate.

5.2.2.6 Zone 6

Algal substrates in Zone 6 were similar to those found at the other zones during 2010 studies. A small mudflat area extended onto the shore. Tree roots and rootlets extended into pooled areas near aquatic plant beds at the upper portion of the zone. Rock substrates were found in generally faster current and had algal communities along with epiphytized algal moss colonies.

Diatom communities were the most abundant algal forms in Zone 6 and were found abundantly on all substrates. *Cocconeis placentula* var. *lineata* was the most abundant form with populations of 26-86% (60 and 86% on tree roots and moss epiphytes, respectively). There were several other widespread forms including *Rhoicosphenia abbreviata* (2-16%), *Cocconeis pediculus* (3-11%) and *Navicula* aff. *subminuscula* (2-10%). On logs with heavy sedimentation several diatoms formed notable populations (*Diadesmis confervacea* [16%], *Melosira varians* [18%], *Nitzschia intermedia* [9%], *Navicula rostellata* [7%], *Navicula symmetrica* [7%] and *Nitzschia palea* var. *debilis* [6%]).

Filamentous algae were found on rocks and logs with and without much sediment, but were limited in the faster flow areas where there were mosses and diatom communities. The rocks in moderate flow and with light to moderate sedimentation supported the green *Cladophora glomerata* (with diatom and blue-green epiphytes) and the blue-greens *Homoeothrix janthina* and *Pleurocapsa minor*. With heavier sedimentation on rocks and logs, the filamentous forms *Rhizoclonium hieroglyphicum* (green algal), *Vaucheria* sp. (yellow-green) and *Phormidium* sp. (blue-green) formed notable populations. In the pooled area (slower flow) downstream of the bridge, a large mat of the green alga *Hydrodictyon reticulatum* had begun to die off.

Aquatic plants in Zone 6 were found in several large mats above the bridge, rooted in the fast current rocks and near the pooled area mudflat. Two *Potomogeton* species, *P. nodosus* and *P. pectinatus*, along with *Heteranthera dubia*, composed the large mats at the beginning of the zone. A few plants of *Vallisneria americana* were rooted around the rocks in the area of fastest current. A few strands of *Elodea canadensis* were found near the mudflat.

The major algal communities were found on rocks in riffle areas amongst the abundant aquatic moss and with varying amounts of sedimentation. Only a limited amount of algal material was found in areas of periodic desiccation. Aquatic plants were still abundant and occurred in several different forms.

5.2.3 Horse Creek

Only a few differences in algal substrates were found at the upper and lower Horse Creek zones. Algal communities were found on rocks and moss in moderately swift current with varying amounts of sediment. Algae were found on tree roots and rootlets that protruded into the creek. The upper portion of HC1 is more open above the road bridge and supports algal communities on sedimented substrates. In general, diatom communities were found more often on rocks in swift current at Zone HC1 than at Zone HC2; at Zone HC2 there were a few more diatom communities on sediment or mudflat areas than at Zone HC1.

Diatoms, the predominant algal form in Horse Creek periphyton communities during 2010 studies, were similar at both Horse Creek zones for most substrates with the possible exception of a few sedimented rocks and logs.

Other algal forms at the Horse Creek zones included several filamentous forms as small "streamers" from rocks in riffle areas and on sedimented objects in the slower-flow area. The greens *Cladophora glomerata* and *Oedogonium* sp. and blue-green *Phormidium* sp. were found on rocks with sediment in slow to moderate flow. Rocks with little sediment in riffle areas had notable populations of the blue-green *Homoeothrix juliana, H. janthina, H. varians, Phormidium* sp. and *P. autumnale.* Smaller populations of *Oedogonium* sp. and the red chantransia-stage were found in these riffle rocks. Of the filamentous forms, the only notable difference in the two Horse Creek zones was less of the *Tapinothrix*-like *Homoeothrix populations* (i.e., *H. janthina* and *H. varians*).

The only aquatic plants found at the Horse Creek zones were the water willow (*Justicia americana*), mosses and a small stand of *Polygonum* sp. (HC1) that may have been terrestrial in origin. The extensive area in Zone HC1 that supported plants that may or may not be considered aquatic had been filled in with completion of the Meadowview Golf Course construction.

5.2.4 Chlorophyll Analyses

Results of chlorophyll *a* analyses of rock and sediment substrates are presented in Tables 5.2.1 and 5.2.2. As observed in the previous study, results were highly variable. Mean chlorophyll *a* values from the rock substrates ranged from 0.21 to 9.45 $\mu g/cm^2$; mean values from the muddy sediment ranged from 2.47 to 36.18 $\mu g/cm^2$. Based on visual inspection of the data, it appears that values from rock and sediment substrates tended to be highest in Big Tree Spring and lowest in Zone 6. Disregarding the Big Tree Spring (as it was not sampled previously), the highest mean values for chlorophyll *a* were on Zone 3 rock substrates (6.82 $\mu g/cm^2$) and on Zone 4 muddy sediment substrates (22.19 $\mu g/cm^2$).

The proportion of organic material (as determined by ash-free dry weight; Table 5.2.3.) in the periphyton samples from rock substrates was highest in Zone 6 (61%). Zone 4 percentage of organic material from rock substrates was also high (42%). However, the two Horse Creek zones had low proportions of organic material (8 and 12% for zones HC1 and HC2, respectively) from these substrates.

Table 5.2.1. Chlorophyll *a* (in µg/cm^2) values from rock substrates in the South Fork and mainstem Holston rivers and Horse Creek, during July 2010 studies.

Zone	Replicate A	Replicate B	Replicate C	Average	s.d.
2	10.9	0.5	0.3	3.9	6.09
3	2.4	12.2	5.8	6.82	4.99
4	0.8	0.4	1	0.74	0.32
5	0.9	15.9	1.3	6.03	8.54
6	0.4	0.2	0.1	0.21	0.16
HC-1	17.4	0.1	1	6.19	9.75
HC-2	0.7	1.1	0.6	0.77	0.25
BTS	3.4	1.6	23.4	9.45	12.07

Table 5.2.2. Chlorophyll *a* (in µg/cm^2) values from sediment substrates in the South Fork and mainstem Holston rivers and Horse Creek, during July 2010 studies.

Zone	Replicate A	Replicate B	Replicate C	Average	s.d.
2	11.68	5.58	1.79	6.35	4.99
3	38.99	7.01	2.42	16.14	19.92
4	52.66	7.16	6.75	22.19	26.39
5	8.12	5.46	6.24	6.6	1.37
6	1.78	2.32	3.31	2.47	0.78
HC-1	1.06	1.46	8.29	3.6	4.06
HC-2	0.91	4.26	5.81	3.66	2.5
BTS	79.6	14.03	14.9	36.18	37.61

Table 5.2.3. Ash Free Dry Mass (AFDM) (% AFDM) values from rock substrates in the South Fork and mainstem Holston rivers and Horse Creek, during July 2010 studies.

Zone	Replicate A	Replicate B	Replicate C	Average	s.d.
2	31.3	28.6	23.9	27.9	3.7
3	14.9	10.2	5.7	10.3	4.6
4	44.1	38.6	42.8	41.8	2.9
5	28.2	20.6	17	21.9	5.7
6	62	47.1	73.7	60.9	13.3
HC-1	7	9.4	7.2	7.9	1.3
HC-2	8.9	10.7	15.3	11.6	3.3
BTS	22.6	6.3	27	18.6	10.9

For periphyton from sediment substrates, the proportion of organic material (AFDW; Table 5.2.4.) was highest in Zones 3 (30%) and HC1 (25%) and lowest in Zone HC2 (5%); values in downstream Holston River zones were also low (AFDW of 7% in Zones 5 and 6).

Table 5.2.4. Ash Free Dry Mass (AFDM) (% AFDM) values from sediment substrates in the South Fork and mainstem Holston rivers and Horse Creek, during July 2010 studies.

Zone	Replicate A	Replicate B	Replicate C	Average	s.d.
2	14.8	10.3	11.4	12.2	2.4
3	1.7	58.4	ND	30.1	40.1
4	12.4	12	12.7	12.4	0.4
5	4.6	14.4	3.3	7.4	6.1
6	10.4	8.6	1	6.7	5
HC1	61.7	2.4	10.7	24.9	32.1
HC2	3.5	3.7	6.8	4.7	1.9
BTS	31.9	5.9	2	13.3	16.3

5.2.5 Canonical Correspondence Analysis

To see how the diatom communities compared in the different zones, a species ordination was made for both data sets. On these ordinations, means of the species scores for each zone were "enveloped" (i.e., lines drawn around) to show the relationship between diatom communities in the different zones. The relative patterns for both data sets were similar (Figs. 5.2.1 and 5.2.2). Zone 2 diatom communities (and Big Tree Spring [BTS] for the qualitative samples) separated from the other zones on the Holston River and Horse Creek (BTS was near and partially overlapping Zone 2). Similarly, the Horse Creek zones overlapped and were separated from the Holston River zones. The other Holston River zones were near the center of the ordination with some separation, especially when similar substrates were compared (see Fig. 5.2.2). As would be expected by their position on the river, Zone 4 is positioned closer to the Horse Creek zones, and Zone 3 is positioned closer to Zone 2.

Relationships between the diatom communities and environmental factors are shown in a site (zone)/environment biplot (Fig. 5.2.3). There was only one set of environmental data for each zone, thus only one point rather than an area represented for each zone. In addition there were many more environmental factors than zones.

One of the biggest differences among samples is between the two Horse Creek zones and all others. The Horse Creek zones are on the right side of the graph, and all others are on the left side. The chemical characteristics that appear to account for most of the differences among the zones are alkalinity and turbidity. Alkalinity is higher, most likely because of geologic differences between the two watersheds. Turbidity may be influenced by higher agriculture-related soil erosion in the Horse Creek watershed and sedimentation in the impoundment above Fort Patrick Henry Dam.

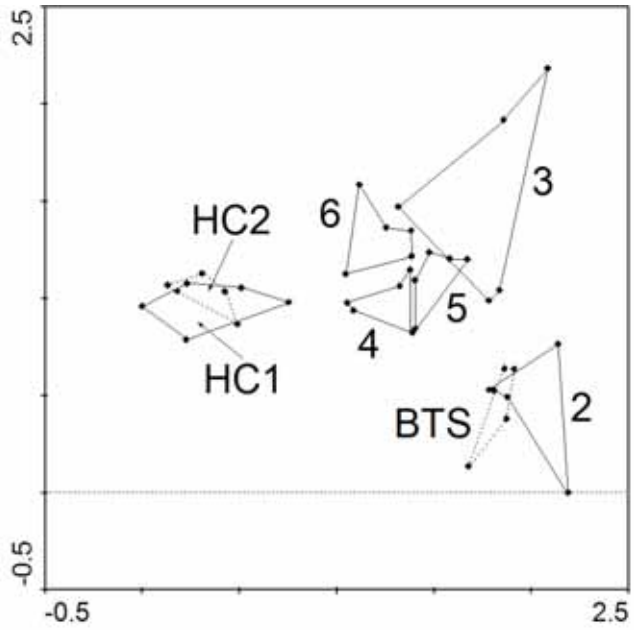


Figure 5.2.1. Relationship between diatom taxa and site location (zone) for the July 2010 samples. Samples are the qualitative hand collections (500 valve counts) from various substrates. The means of diatom taxa scores for all samples are plotted as diamonds. Samples are connected by the Zone in which they were collected.

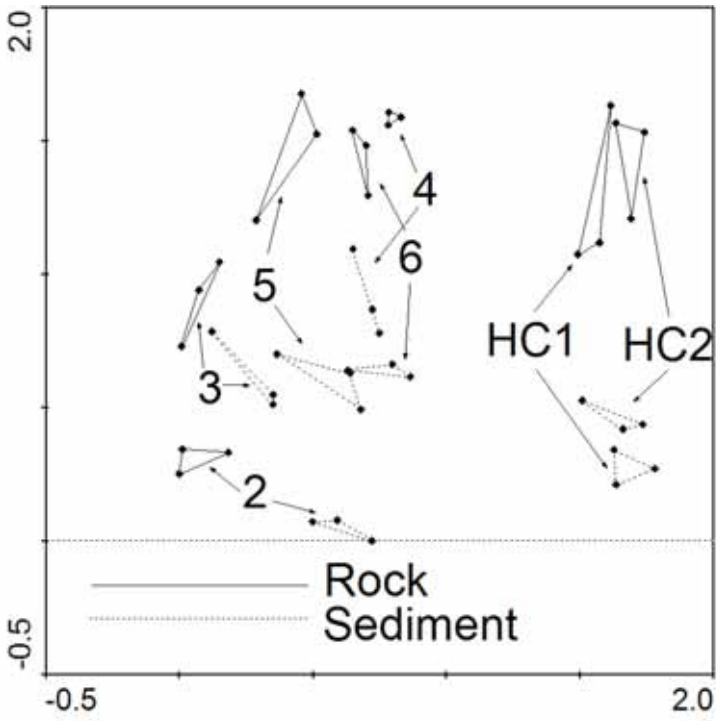


Figure 5.2.2. Relationship between diatom taxa, site location (zone) and substrate for the July 2010 samples. Samples are the quantitative samples from rock and sediment substrates (500 valve counts). The means of diatom taxa scores for all samples are plotted as diamonds. Samples are connected by the Zone/substrate in which they were collected.

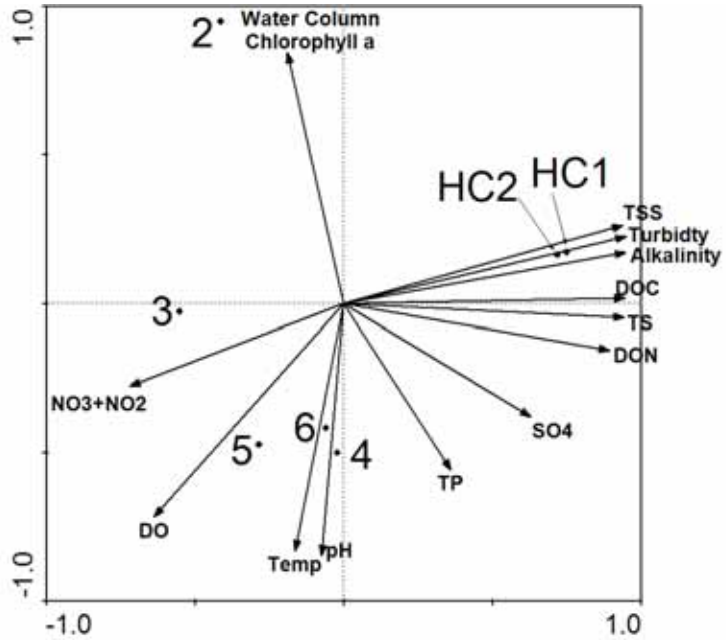


Figure 5.2.3. Relationship between diatom taxa and environmental variables for the July 2010 samples. Samples are represented by diamonds and measured environmental variables are represented by arrows and labeled as follows: alkalinity–total alkalinity, DOC–dissolved organic carbon, DON–dissolved organic nitrogen, SO4–sulfate, Temp–temperature, TSS–total suspended solids, Water Column Chlorophyll *a*–planktonic chlorophyll *a*, TS–total solids, TP–total phosphorus, DO–dissolved oxygen, Turbidity–total turbidity, NO3+NO2–nitrate plus nitrite nitrogen.

The Zone 2 diatom samples may differ from all others due to influence of the upstream dam. The amount of planktonic chlorophyll *a* (water column chlorophyll *a*) is an indication of how much planktonic algae there is that can settle and become part of the periphyton. Phosphorus values (TP) are lower than all other sites also, possibly suggesting that the reservoir behind the dam acts as a phosphorus sink causing outlet water to have below-average concentrations.

Zones 2 and 3 differ from Zones 5 and 6. This could be due to a variety of factors. There are clear differences in average values for the two sets of sites. Most are related to water quality characteristics that are influenced by industrial discharges, sewage treatment plants and other anthropogenic sources (e.g., phosphorus, nitrogen, BOD, major cations and anions and organic compounds).

Assemblages from Zones 4, 5 and 6 are relatively similar. The distances (i.e., differences) between samples from different zones are, in many cases, less than distances within a zone. Samples from similar habitats lie near each other. These relationships among samples indicate that for Zones 4, 5 and 6, physical habitat characteristics are more important than water chemistry.

5.2.6 Discussion

When using periphyton and aquatic macrophyte data to evaluate water quality, there are several things to consider. A large population of a species with a wide range of tolerances, a "generalist," might provide little information about water quality, whereas small- to moderate-sized populations of a species with very specific tolerances might be quite informative. Available substrates for algal colonization are also an important consideration. In addition, the physical environment must be factored in. During the 2010 study, the water fluctuations (from the operation of Fort Patrick Henry Dam) and sedimentation were very important considerations.

Filamentous algal forms (blue-greens, greens and yellow-greens), which can indicate enrichment when abundant (Hynes 1972), were found consistently throughout the study area, and probably more so in 2010 than in the previous study (1997) when the most pronounced forms were diatoms. The largest algal growths in the 2010 studies were small "streamers" of green algae from moderate to heavily-sedimented rocks in slow to moderate flow. *Cladophora glomerata* was the most common green filament, associated in these areas with *Oedogonium* sp. and *Microspora* sp. To a lesser extent in the faster water, there were several notable populations of the filamentous green *Gongrosira debaryana* and the blue-greens *Homoeothrix janthina, H. varians* and *Pleurocapsa minor.* Where there was heavier sedimentation, there were notable populations of blue-greens of the genus *Phormidium* (previously in the genus *Microcoleus*) and smaller amounts of the filamentous *Rhizoclonium hieroglyphicum* (green) and *Vaucheria* sp. were present. Small floating mats of *Hydrodictyon reticulatum*, a green filamentous form, were prominent in Zone 3 and especially Zone 6.

Most of the major diatom forms were widespread throughout the Holston River zones. However, there were a few abundant taxa that were found more often on a specific substrate or within a specific zone. *Achnanthidium minutissimum* (previously named *Achnanthes minutissimum*), the major diatom species in previous studies, was one of the most abundant species during 2010. This taxon was widespread on all types of substrates, with highest abundances in upstream zones and Horse Creek. Starting with the previous survey (1997), the abundances of *A. minutissimum* were lower; some of the lower abundances were due to taxonomic changes (several newly described species and subspecies were originally assigned to this taxon). However, population abundances have lowered from previous surveys. Other abundant diatoms were widespread on substrates but differed among zones; these taxa included *Amphora pediculus* (high in Horse Creek zones), *Diatoma vulgaris* and *Cocconeis pediculus* (both lower in Horse Creek), *Navicula* aff. *subminuscula* (not found in Zone 2, Big Tree Spring or on Horse Creek), *Rhoicosphenia abbreviata* (previously *R. curvata*; low in Zone 2 and Big Tree Spring) and *Gomphonema minutum* (highest in Zones 2 and 4). *Cocconeis placentula* var. *lineata* (the most abundant species in the 2010 survey) and *Navicula minima* were widespread, but more abundant in root and moss (epiphyte) material. Taxa of the genera *Nitzschia* were more common on sediment-laden substrates (*N. amphibia, N. palea* var. *debilis* and *N. dissipata* were commonly found).

The influence of the dam was noted in the diatom communities in Zone 2. The abundance of planktonic forms (species in the genera *Aulacoseira* [previously members of the genus *Melosira*]*, Cyclotella, Cyclostephanos, Stephanodiscus* and *Thalassiosira*), that originated in the reservoir was high in Zone 2 and reduced very quickly in a downstream direction. The tubed diatoms (*Encyonema* species), which can withstand periodic dessication (Werner 1977), were most abundant in Zone 2, where the fluctuating water levels encompass a larger portion of where algal communities were found.

Similar to the previous four surveys (1977, 1980, 1990 and 1997; ANSP 1978, 1981, 1992 and 1998), the abundant diatom species were indicative of waters with high nutrients, fitting into the categories defined as mesosaprobic (areas where biooxidations are proceeding) to oligosaprobic (areas where biooxidations are complete; Lowe 1974) and eutrophic (areas of high nutrients). *Achnanthidium minutissimum* (previously *Achnanthes minutissima*), the dominant diatom species, has a wide range of ecological tolerances, and is considered one of the "most ubiquitous species known" (Lowe 1974). Other abundant species, including *Melosira varians, Cocconeis placentula* v. *lineata, Navicula cryptotenella* (previously *N. radiosa* v. *tenella*) and *Cyclotella ocellata* have a wide range of tolerances and are indifferent to pH and salt concentrations. Of the information that is known about the 10-12 major species in Zones 2 through 6 for the 1977, 1980, 1990, 1997 and 2010 surveys, most are described as oligosaprobic with a range into mesosaprobic (many considered beta-mesosaprobic, or found in areas where bioxidation is almost complete). Although information is available for only about half of the abundant diatom species, the nutrient rating is, without exception, eutrophic.

Although there are many factors that explain the presence and absence of aquatic plants, the amount of plant material at several of the zones, especially Zones 2 and 3, was indicative of organic enrichment. The *Elodea canadensis* stand in Zone 2 was larger than in earlier surveys (prior to 1997). Also in Zone 2, in 2010 there were larger stands of rooted aquatics (*Potamogeton nodosus* and *Heteranthera dubia*). In Zone 3, the heavy *Potomogeton* growths that clogged the right channel and were abundant in the fast-flow areas of the left channel obliterated (by shading and crowding for root space) other algal and plant forms. Although there were small stands in pools and around the rock outcroppings, there was much less plant material in Zone 4 than in Zone 3; in Zone 4 the aquatic plant stands were more scattered (i.e., mixed with algal communities) and did not block light for other plants. The amount of aquatic plant material in Zone 5 was moderate, and highest in the channel at the left bank near the island. The abundance of aquatic plant communities in Zone 6 was increased from earlier surveys, but less than that found in Zones 2 and 3 and were composed of notable populations of several different species.

Overall comparisons of the algal and aquatic plant communities in 2010 with previous studies reveal few differences from the 1990 and 1997 studies; however, there was much improvement from the conditions observed in the '60s and early '70s. The algal communities observed in the general vicinity of Kingsport during the first studies in 1965 and 1967 were a result of conditions where pollutants had not been broken down into their inorganic constituents. Large growths of the sewage fungus (actually a bacterium) *Sphaerotilus* out-competed all but the most tolerant algal forms. Where algae grew, there were very few species, and only those considered tolerant of the most polluted condition. Improved conditions were noted in 1974 with little *Sphaerotilus*, but large aquatic plant and filamentous algal growth in Zones 3 and 5. In 1974 there was less

organic enrichment in Zones 4 and 6; the conditions in Zone 2 were, as previously observed, affected by its close proximity to the Fort Patrick Henry Dam. The 1977, 1980 and 1990 studies revealed organic enrichment in all areas above (although not as much) and below the Kingsport area; algal species considered tolerant of severe pollution conditions were not observed. Algal communities in Zone 4 and to an extent Zone 6, differed from Zones 3 and 5, especially in the amount of plant material observed.

Comparisons of epilithic (growing on rocks) algal biomass, as chlorophyll *a*, indicated lower average values in 2010 than in the previous study (1997). The highly variable values for chlorophyll *a*, collected from rock substrates, ranged from 0.21 µg/cm^2 to 9.45 µg/cm^2 and were highest in the samples from Big Tree Spring (BTS). For rock substrates, the highest average values in 2010 were lower than all of the 1997 average values (range of 15.40 µg/cm^2 to 61.22 µg/cm^2). Excluding BTS, values from Zones 3 and 5 were the highest (Zone 3 values were lowest in 1997) and lowest in Zone 6 (in 2010, average values were less than 1 µg/cm^2 in Zones 6, 4 and Horse Creek 2). In 1997, chlorophyll *a* values were highest at Zone 4.

Epipelic (growing on mud) algal biomass during 2010 studies was similar to 1997. Similar to the epilithic substrates, the highest values for the epipelic algal biomass were found at BTS. There was little difference in the range of values from 2010 and the previous studies (without BTS, average epipelic algal biomass ranged from 2.47 µg/cm^2 to 22.19 µg/cm^2 in 2010 and from 1.57 µg/cm^2 to 25.71 µg/cm^2 in 1997). As in 1997, Zone 3 epipelic algal biomass was among the highest (not including BTS) and for both studies, lowest values were found in Zone 6.

The types of algal species observed in 2010 were similar to those observed in 1980, 1990 and 1997. Diatom species with a wide range of ecological tolerances, but not indicative of severe pollution, were still the most abundant. *Achnanthidium minutissimum* was the dominant throughout Zones 2 and 3, but not as dominant, though widespread, as in 1980 and 1990. The flora in Zone 2 differed from the other zones (increased planktonic centric diatoms) probably due to dam effects. Ecologically, however, the flora was similar to the other zones (an enrichment effect was noted but not as much as in Zones 3 and possibly 5). Similar to previous studies, most flora differences in Zone 4 in 1997 were likely due to the unique substrate features (rock outcroppings and pools). In Zone 6, the species found were considered eutrophic (high nutrients), similar to the other zones, although there were differences in the actual species found.

The algal floras in the two Horse Creek zones were very similar, as in 1990 and 1997 studies. The differences in substrate (more open at the upper portion of Zone HC1 and more shaded mudflat area in Zone HC2) cannot be overlooked as the reason for most differences. Similar substrates like moss, tree roots and some rocks in fast-flow areas had similar diatom species; there were a few differences on heavily sedimented substrates. The overall diatom communities at Zone HCl had more species, probably due to an increase in substrates found in the open areas. Similar to algal communities in the river, the most abundant species found (*Cocconeis placentula* var. *lineata*, *Achnanthidium minutissimum*, *Amphora pediculus* and *Rhoicosphenia abbreviata*) were tolerant of a wide range of conditions. Overall conditions were characterized by high nutrients (eutrophic), as in the river zones, but were not indicative of severe pollution.

The major differences in diatom communities during 2010 Holston River studies were between those found in zones on Horse Creek and the zone just below the Fort Patrick Henry Dam, and the other Holston River zones. The diatom communities in Horse Creek zones were influenced by higher turbidity and alkalinity. Influences to the periphyton communities in Zone 2 were dam-related. There were more planktonic algae than in other downstream Holston River zones and, related to larger water fluctuations, there were greater amounts of diatoms that could withstand daily dessication.

No rare or endangered species were collected.

Didymosphenia geminata, an invasive diatom species, was reported to be found in the tailwaters of the Fort Patrick Henry Dam (above Zone 2; Spaulding and Elwell 2007) but was not observed during this survey.

In summary, the algal and aquatic macrophyte communities on the Holston River in the vicinity of Kingsport, TN were indicative of areas affected by organic enrichment. Severe pollution of organic material, observed in previous studies (ANSP 1966 and 1967), was not present.

5.3 Non-Insect Macroinvertebrates

5.3.1 Results

5.3.1.1 Sponges (Porifera)

Colonies of freshwater sponges (family Spongillidae) were common (Zones 2 and 3), uncommon (Zones 6, HC1 and HC2) and rare (Zone 5) on rocks or wood in moderate to fast currents. No sponges were collected in Zone 4. In 1997, colonies of sponges were rare (Zone 6), moderately common (Zone 2) and common (Zones 3 to 5) on rocks in slow to moderate currents. In 1990 sponges were common at Zones 2, 4 and 6 and present at Zones 3 and 5, although less common. Sponges were absent from the Horse Creek zones (HC1 and HC2) in 1997 and 1990. Sponges were present in the 1980 (Zones 2, 4, 5 and 6), 1977 (all zones) and 1974 (Zones 4 and 6) surveys.

The surveys reveal that sponges are a widespread and generally common component of the Holston River, although their presence varies among years. The appearance of sponges in both Horse Creek zones in 2010 was the first time they have been detected in this creek since survey work began in Horse Creek in 1990. However, in the 2010 survey sponges were less common in the Holston River than in most of the previous surveys. They were not found at Zone 4, which is the first time they were not collected at this zone since 1965. Sponges were also less common at Zone 5 in 2010 than they were in 1997.

5.3.1.2 Flatworms (Platyhelminthes)

In the 2010 survey, the planarian *Dugesia tigrina* was collected from a wide variety of habitats, including rocks (only on the undersides in swift currents), woody debris, tree leaves and in submerged aquatic vegetation (e.g., moss, *Elodea* and *Potamogeton*). Planarians were abundant

at all river zones including Horse Creek. Some specimens collected during the 1990, 1997 and 2010 surveys keyed to *Cura foremanii* (e.g., white proboscis), but additional work in 1997 indicated that these were likely atypical *D. tigrina* and morphologically fall within the range of variation for that species. In 1997 and 1990, *D. tigrina* (*C. foremanii*, in part during the 1990 survey) was abundant at all zones, but only moderately common at the Horse Creek zones. During the 1980 survey, *D. tigrina* was noted from Zones 3 through 6, all zones in 1977, Zones 3 through 6 in 1974, and only Zone 4 in 1965.

5.3.1.3 Moss Animals (Ectoprocta)

In the 2010 survey, the branching ectoproct *Plumatella repens* was observed at Zones 3 and 5 (common) and HC2 (uncommon) under large rocks in moderate to swift currents. In the previous three surveys (1980, 1990 and 1997), *P. repens* was found at all zones in the Holston River. In 1997, this freshwater bryozoan was rare to abundant on rocks in a range of habitats, from pools to rapidly flowing waters. This species was rare at Zones 3 and 6, moderately common at Zones 2 and 4, and abundant at Zone 5. It was also common at Zone HC1. In 1990, this same species was found on the undersides of rocks in a range of current velocities from pools to areas of fast running waters. At Zone 3, *P. repens* completely covered a piece of finished lumber approximately two-thirds of a meter long and resting against the shore. No bryozoans were observed in Horse Creek in 1990. In 1977 and 1974, colonies were noted at Zones 2 and 4 (in 1974 as *P. repens* in the text and *Plumatella* sp. in the table), while in 1965 this species was observed only at Zone 2. In the Holston River, *P. repens* appears to be an uncommon to common representative of the macroinvertebrate fauna. However, in 2010 it was less commonly observed than in the previous three surveys, when it was present at all five Holston River sites (vs. only at Zones 3 and 5 in 2010).

5.3.1.4 Segmented Worms (Annelida)

Annelids were represented by tubificids, naidids, earthworms and leeches. Tubificids are also known as sewer worms because of the ability of some species in the group to dominate the benthic fauna where oxygen levels are low. However, at the family level, Tubificidae are only good pollution indicators when they dominate faunal assemblages in which they previously had been uncommon or lacking. At these pollution levels, more sensitive species also begin to disappear from the ecosystem. Two species of tubificids, *Branchiura sowerbyi* and an undetermined taxon, were collected from the Holston River and Horse Creek in 2010. *Branchiura sowerbyi* was rare and collected with a moderately-common, undetermined tubificid in leaf litter-covered sediment at Zone 4 and by itself at Zone HC2. In 1997, *B. sowerbyi* was only collected at Zone 5 and had not been collected since the 1965 survey. It is possible that this taxon was collected previously and not identified beyond family level. However, this species has conspicuous gill filaments which makes it one of the more easily identified species in this family and therefore less likely to be left at the family level. In 2010 the undetermined tubificids were abundant (Zone 5), moderately common (Zones 2, 3 and 4) and rare (HC1) and were usually collected from soft sediments and detritus in backwaters and less commonly from riffles. In Zone 2, this taxon was also collected from detritus and algae associated with smartweed (*Polygonum* sp.) above the permanently wetted zone. Undetermined tubificids were not collected from Zones 6 or HC2 in 2010. In 1997, Tubificidae was moderately common at Zone

2, uncommon at Zone 3, and rare at Zone 6. Undetermined tubificids obtained in 1990 were found at Zones 3 and 4, while in 1980 specimens were collected at all zones except Zone 5. Undetermined tubificids were not collected during the faunal survey in 1977, while in 1974 they were found at Zone 5. The first Holston River field effort in 1965 collected individuals at all zones. It was during this study that the tubificid assemblage was found to be abundant at Zone 3 and was identified to the species level (8 species).

In 2010, the naidid *Stylaria lacustris* was uncommon at Zones 2 and 6. It was collected from the same habitats as the tubificids. Naidid oligochaetes were noted for the first time in the Holston surveys in 1997 and were also collected at Zones 2 and 6. Members of this taxon are probably typical of the South Fork and mainstem Holston rivers fauna but are not typically recorded because of their small size.

The earthworm *Eiseniella* cf. *tetraedra* was abundant at Zones 2, 3 and 4, moderately common at Zone 5 and uncommon at Zones 6 and HC2. It was not collected from Zone HC1. This species was collected from soft sediments, root mats, detritus, algae and sometimes in riffles. Examination of material from the 1997 and 1990 surveys indicated that *E.* cf. *tetraedra* was previously identified as *Lumbriculus variegatus* in these surveys. Earthworms were also collected in the 1980, 1977, 1974 and 1965 surveys; and it is also possible that in these earlier surveys specimens identified as *L. variegatus* were *E.* cf. *tetraedra*. However, without an examination of vouchers from these earlier surveys, the taxonomy of these worms cannot be confirmed. As a result, the determination of *L. variegatus* for the 1980, 1977, 1974 and 1965 surveys is retained in this report. In 1997, *E.* cf. *tetraedra* was found at all Holston River zones, but was not collected from Horse Creek. In 1990, *E.* cf. *tetraedra* was collected from all zones except HC2. A species of segmented worm identified as *L. variegatus* was taken at Zones 4 and 6 in 1980, Zones 2 through 5 in 1977, Zones 2 through 6 in 1974 and only at Zone 5 in 1965.

Eight species of leeches (*Erpobdella punctata*, *Mooreobdella microstoma*, *Gloiobdella elongata*, *Helobdella stagnalis*, *Helobdella triserialis*, *Placobdella papillifera*, *Placobdella parasitica* and *Piscicolaria reducta*) were collected in the 2010 survey. One species or both species of the erpobdellids, *E. punctata* and *M. microstoma*, were collected from soft sediments and detritus in backwaters in Zones 2, 4 and 5. *Erpobdella punctata* was abundant under rocks along the river bank at Zone 3 and a single specimen was collect from a riffle at Zone 6. *Gloiobdella elongata* was collected from Zones 2, 3 and 5 from detritus in backwaters and less commonly in macrophytes (Zone 2). The taxonomy of this species is somewhat unclear and molecular studies have placed it back into the genus *Helobdella* (Siddall and Borda 2003). *Helobdella stagnalis* was collected from Zones 3 and 5 in detritus and soft sediments. *Helobdella triserialis* was collected from Zones 2, 3, 5 and 6 from soft sediments and detritus in backwaters. In Zone 2, *H. triserialis* was also collected from macrophytes and in Zone 3 it was found under rocks along the river banks. *Placobdella papillifera*, a largely free-living species (Klemm 1991), was collected from Zones 3, 4 and 5 from soft sediments in backwaters. A single specimen of *P. parasitica* was collected during the 2010 survey at Zone 6 from detritus and algae in a backwater on the left bank. This species is free-living or a parasite of turtles (Klemm 1991). *Piscicolaria reducta* was collected from the darter *Etheostoma zonale* at Zones 5 and 6. This fish parasite was the only leech species collected from Horse Creek (HC2) and it was collected by the fish crew on a darter. This is the first record of this leech from the Academy surveys.

Leeches were widespread but usually only moderately common to rare in the Holston River study areas except at Zones 3 and 5. At Zone 3, *E. punctata* was abundant under rocks along the banks. In Zones 3 and 5, the leech *H. triserialis* (Zone 3) and *G. elongata* (Zone 5) were common. In addition to the high numbers of *E. punctata* and *H. triserialis* at Zone 3, five of the eight leech species were found at this zone. Zone 5 had the highest richness of leeches with seven of the eight species collected in 2010. Leeches were rare in Horse Creek as only a single specimen parasitizing a darter was collected from HC2 during the 2010 survey.

A similar community of leeches was collected in 1997 and included six species (*E. punctata*, *M. microstoma*, *H. stagnalis*, *H. triserialis*, *P. papillifera* and an undetermined species of glossiphoniid). All but one of these species (*M. microstoma*) was found at Zone 3. *Helobdella triserialis* was found at Zones 4 and 5 while *E. punctata* was present at Zones 3, 5, 6 and HC2. *Mooreobdella microstoma* occurred at Zones 4, 5 and 6. It is possible that the undetermined glossiphoniid leech in 1997 was *G. elongata* as this is a small, nondescript leech, but material was not examined to confirm this species' identity. Five species of leeches (*E. punctata, Desserobdella phalera*, *H. triserialis*, *P. papillifera* and an undetermined species of erpobdellid) were found in 1990. *Desserobdella phalera* was found at both zones in Horse Creek. This leech and an undetermined species of erpobdellid from Zone HC2 were the only species taken from this small stream and not at all from the river. *Placobdella papillifera* was collected from Zones 2 and 3, *H. triserialis* at Zones 3 and 4 and *E. punctata* at Zone 3, 4 and 5. The 1980 survey produced five taxa of leeches consisting of *H. triserialis* (as *H. lineata)* at Zones 4 through 6*, G. elongata* at Zones 5 and 6, the fish leech *Myzobdella lugubris* at Zone 4, *E. punctata* at Zones 3, 5 and 6, and two undetermined species of fish leeches (Piscicolidae) at Zone 4. In the 1977 study, three taxa of leeches were observed. *Helobdella triserialis* (as *H. lineata)* was collected at Zones 3 and 4*, E. punctata* at Zones 3 through 6, and *P. parasitica* at Zone 6. The 1974 field effort produced six species of leeches consisting of *H. triserialis* (as *H. lineata)* at Zones 3 and 5*, G. elongata* at Zones 3, 5 and 6*, H. stagnalis* at Zone 6*, E. punctata* at Zones 3 through 6, and *P. parasitica, Nephelopsis obscura* and *Helobdella* species at Zone 6. The 1965 survey resulted in three taxa of leeches consisting of *H. triserialis* (as *H. lineata* and *H. punctata-lineata)* at Zones 4 and 6, *E. punctata* [(as *M. microstoma, E. punctata,* an undetermined piscicolid species and an undetermined *Dina* (identification uncertain) species] at Zones 3, 5 and 6, and *H. stagnalis* [as *Illinobdella alba* and undetermined species of *Illinobdella* (identification uncertain) and *Helobdella* (identification uncertain)] at Zone 6.

5.3.1.5 Molluscs (Mollusca)

Ten species of gastropods were collected during the 2010 survey. The snails were *Campeloma decisum* (pointed campeloma), *Pleurocera uncialis* (pogoda hornsnail), *Leptoxis praerosa* (onyx rocksnail), *Fossaria obrussa* (golden fossaria), *Gyraulus parvus* (ash gyro), *Micromenetus dilatatus* (bugle sprite), *Helisoma anceps* (two-ridge rams-horn)*, Physella heterostropha* (pewter physa), *Ferrissia rivularis* (creeping ancylid) and *Laevapex diaphanus* (cymbal ancylid). The snail *Novisuccinea ovalis* (oval ambersnail) was also collected on terrestrial vegetation along the banks. However, this species is considered terrestrial (Clarke 1981, Turgeon et al. 1998) and is not discussed further.

The pointed campeloma was abundant in backwaters with soft, muddy or sandy sediments at Zones 4, 5, 6 and HC1. The pogoda hornsnail was abundant at all Holston River and Horse Creek zones and was collected primarily from hard substrates (e.g., rocks, bedrock and woody debris), although it was also found on macrophytes and detritus. The waters in which the pogoda hornsnail was observed ranged from slow to swift currents. The onyx rocksnail was abundant at Zones 5 and 6, where it was found on hard substrates in slow to swiftly flowing currents. The golden fossaria was abundant at Zones 2 and 3, where it was collected from rocks along the banks (Zone 2) and macrophytes and backwaters (Zones 2 and 3). This species was also uncommon to rare at Zones 5, 6, HC1 and HC2 and was generally collected from backwater areas in association with detritus. The ash gyro was abundant at Zones 2 and 3 and rare at Zone 6, where it was collected from submerged aquatic vegetation. The bugle sprite was rare during the 2010 survey and a single specimen was taken from macrophytes at Zone 3. The two-ridge rams-horn was abundant at Zone 2 and moderately common at Zones 3, 4 and 5, where it was collected from macrophytes or emergent vegetation. Pewter physas were generally the most common and widespread species and were collected from a variety of habitats including on rocks (Zones 2, 5), macrophytes (Zones 2, 3), emergent and hanging vegetation (Zones 2, 4, 5 and HC1) and soft sediments and leaf litter (Zones 2, 3, 6, HC1, HC2). It was found primarily in slow waters, but also occasionally in runs and areas of moderately swift current. It was abundant at all Holston River zones (except Zone 4) and the two Horse Creek zones. Two species of limpets were found in the survey zones. The cymbal ancylid was abundant on rocks and woody debris with slow to moderate currents at Zone 6. It was abundant, but less common at this zone than the creeping ancylid. The creeping ancylid was found at Zones 3, 4, 5, 6, HC1 and HC2 on rocks (in each of these zones), on trash (e.g., bottles, plastic buckets, boards and plastic siding; Zones 4 and 6), on woody debris (Zone 6) and on macrophytes (Zone 3). The creeping ancylid was abundant at Zones 4 through 6 and Zones HCl and HC2 and moderately common at Zone 3. It was not collected at Zone 2. The cymbal ancylid was most common in quiet water habitats it shared with the creeping ancylid, while the creeping ancylid ranged from moderately flowing to quiet waters.

Snails were one of the most common components of the non-insect macroinvertebrate fauna in the study area. The pewter physa and pogoda hornsnail were the most abundant and widespread species. The onyx rocksnail was abundant where suitable conditions prevail in the Holston River at Zones 5 and 6. The highest snail richness was found at Zone 6 where 8 of the 10 species were collected. The pagoda hornsnail and pewter physa were conspicuous faunal elements in Horse Creek.

Ten species of gastropods were also obtained during the 1997 survey. The same taxa were collected with the exception of the presence of the two-ridge rams-horn in 2010 and the presence of the slender walker in 1997. The pointed campeloma was rare at Zone 4 and moderately common at Zone 6. The pogoda hornsnail was taken from Zones 4, 5, 6, HCl and HC2. The onyx rocksnail was found at Zones 4, 5 and 6. The slender walker, which was not collected in the 2010 survey, was moderately common at Zone 2 and rare at Zone 6 in 1997. The golden fossaria was abundant at Zone 2, moderately common at Zone 3, common at Zones 4 and HC2, uncommon at Zone HC1 and rare at Zone 6. The ash gyro was moderately common at Zone 2 and abundant at Zone 6. The bugle sprite was taken from all zones with the exception of HC1. Pewter physas were abundant at Zones 2, 3, HC1 and HC2, common at Zone 6, and moderately

common at Zones 4 and 5. As in 2010, the cymbal ancylid was abundant at Zone 6, the only zone at which it was collected. The creeping ancylid was abundant at Zones 4, 5, 6 and, HCl, common at Zones 3 and HC2, and moderately common at Zone 2.

Nine species of gastropods were collected during the 1990 survey. The snails were the pointed campeloma, pogoda hornsnail, onyx rocksnail, slender walker, golden fossaria, bugle sprite, pewter physa, creeping ancylid and cymbal ancylid. In 1990, the pointed campeloma was collected from Zones 4, 5 and 6. The pogoda hornsnail was taken at Zones 4, 5, 6, HC1 and HC2. The onyx rocksnail was found only at Zone 6. The slender walker was found at Zone 2. The golden fossaria was collected at Zones 2, 3 and 4. The bugle sprite was collected at Zones 3, 4 and 5. Pewter physas were present at all Holston River and Horse Creek zones. The cymbal ancylid was collected at Zone 6 and the creeping ancylid was found at all zones.

The 1980 study produced seven species of snails at Zones 2 through 6. These taxa included the pagoda hornsnail (as *P.* cf. *unciale*) at Zones 4 through 6, onyx rocksnail (as *Anculosa subglobosa*) at Zones 5 and 6, golden fossaria (as *Lymnaea* sp.) at Zones 3 and 4, bugle sprite (as *Menetus dilatatus*) at Zone 6, ash gyro at Zone 6, pewter physa (as *Physa* sp.) at Zones 2 through 6 and creeping ancylid (as undetermined species of ancylid) at Zones 2 and 4 through 6.

The 1977 study also resulted in the collection of seven species of snails consisting of the pointed campeloma (as *Campeloma* sp.) at Zone 4, pagoda hornsnail (as *P. canaliculata*) at Zones 4 through 6, onyx rocksnail (as *Anculosa subglobosa*) at Zone 5, golden fossaria (as *Lymnaea* cf. *humilis* at Zones 2, 3 and 6), pewter physa (as *Physa pomilia*) at Zones 2, 3, 5 and 6, marsh rams-horn (as *Helisoma* cf. *trivolvis*) at Zones 4 and 6, and creeping ancylid (as *F. rivularis* and *F. tarda*) at Zones 2, 3 and 6.

The six taxa of snails obtained in 1974 were the pointed campeloma (as *Campeloma* sp.) at Zone 4, pagoda hornsnail (as *P.*cf. *unciale*) at Zone 5, slender walker (as *Pomatiopsis* cf. *lapidaria*) at Zone 2, golden fossaria (as *Lymnaea* sp.) at Zones 2, 5 and 6, pewter physa (as *Physa pomilia*) at Zones 2 through 6 and marsh rams-horn (as *Helisoma* cf. *trivolvis*) at Zone 6.

The first survey in 1965 collected only three species of snails in Zones 2 through 6 and consisted of the pointed campeloma (as *Campeloma subsolida*) at Zone 4, golden fossaria (as *Lymnaea obrussa*) at Zones 4 and 6 and the pewter physa (as *Physa microstoma*) at all zones.

Bivalve molluscs in the 2010 survey consisted of five species including the introduced Asian clam, native fingernail clams and pea clams. The Asian clam, *Corbicula fluminea*, was abundant at all zones in a variety of substrates consisting of sand (Zones 2, 5, 6, HC2), gravel (Zones 2, 3, 5, 6, HC1, HC2), and soft sediments (Zones 2, 3, 4, 5, 6, HC1). The first record of this non-native species during Academy surveys was at Zones 5 and 6 in 1977 (ANSP 1978). In the 1980 survey, this introduced species was still found at the same two zones, but by 1990 had become a common and widespread component of the river. The pea clam and fingernail clams (sphaeriids) were represented by four taxa in 2010. These were an undetermined species of *Pisidium* (undetermined pea clam), *Sphaerium fabale* (river fingernail clam), *S. striatinum* (striated fingernail clam) and *Musculium securis* (pond fingernail clam). Pea clams were abundant at Zones 2, 3, 4 and 5 and uncommon at Zones 6 and HC1 but were not collected at

Zone HC2. This taxon was collected from macrophytes (Zone 2) and mud covered with leaf litter (Zones 2, 3, 4, 5, 6 and HC1). The pond fingernail clam was only collected at Zone 5, where it was abundant and associated with soft sediment and detritus. The river fingernail clam was abundant at Zone 5 and uncommon at HC1. At both zones, the river fingernail clam was collected from soft sediment and detritus. The striated fingernail clam was abundant at both Horse Creek zones and Zone 5, where it was collected in soft sediment, leaf litter and gravel.

Bivalve molluscs in 1997 consisted of the same five species collected in 2010. The Asian clam, *Corbicula fluminea*, was found at all zones. Pea clams were abundant at Zones 2, 4 and 5, common at Zones 6, HC1 and HC2, and rare at Zone 3. At Zone 5 the river fingernail clam was uncommon and the pond fingernail clam abundant. The striated fingernail clam was collected at both Horse Creek zones. During the 1990 study, the Asian clam was found at all zones. The pea clam and fingernail clams in 1990 were represented by four species. Pea clams were taken from Zones 3 and 4. The river and pond fingernail clams were collected from Zones 4 and 5, respectively. The striated fingernail clam was collected at both Horse Creek zones and Zone 4.

In 1980 only two taxa of these sphaeriid clams were noted: an unidentified *Musculium* species at Zones 3 and 6 and an unidentified *Pisidium* species from Zones 3, 5 and 6. The 1977 survey listed four taxa of sphaeriid clams consisting of *P. compressum* (ridged-beak pea clam) (Zone 4), *P.* cf. *fallax* (Zone 4), the river fingernail clam (Zone 4) and the pond fingernail clam (as *S. securis*) (Zones 4 and 6). Four species of sphaeriid were also identified in the 1974 survey and consisted of *P.* cf. *fallax* at Zones 5 and 6, *P.* cf. *variable* at Zones 4 and 5, river fingernail clam at Zone 4 and pond fingernail clam (as *S. securis*) at Zone 6. The first (1965) survey collected two species of these small clams consisting of the striated and pond fingernail clams. The former was collected at Zone 4 (listed as *S. fabale* in the species list and *S. striatinum* in the text) and the latter from Zone 6 (as *S. lacustre* and considered the probable taxonomic equivalent of *M. securis* [as *S. securis*] [ANSP 1975: 100] by the same macroinvertebrate zoologist who conducted both surveys).

Among the mussel fauna, only the yellow sandshell, *Lampsilis teres* (as *Lampsilis anadontoides*), has ever been collected (1965) live from the study area. Mussels have undoubtedly been a part of the native fauna of this portion of the South Fork and mainstem Holston rivers in the past, and their absence is a measure of inadequate water quality and habitat loss from the construction of dams which create lentic environs or subject these mollusks to cold, irregular water levels. An examination of the lower portion of Horse Creek (not part of historic Zones HC1 or HC2) for mussels in 1976 (ANSP 1976) discovered four species of live mussels and the valves of an additional two taxa. The living mussels included an undetermined pigtoe of the *Fusconaia flava* complex (op. cit., "probably the nominate one"), mountain creekshell (*Villosa vanuxemensis*), rainbow (*Villosa iris*) and pocketbook (*Lampsilis ovata*). The shell material was from the rough rabbitsfoot (*Quadrula cylindrica strigillata*) and wavy-rayed lampmussel (*L. fasciola*). These five species probably represent faunal relics of species that once occurred in the Holston River. Of note is the status (Special Concern) of the rough rabbitsfoot on the Tennessee Heritage Program's list of endangered and threatened species in the state (Bogan and Parmalee 1983).

Shell material was collected during the 2010 survey, but no live mussels were found. Some material was recent, but most shells were older and did not provide information on possible extant populations. No shell material was found at Zones 2 and 3. Shells of *Amblema plicata* (threeridge) were found on the Big Sluice at two locations (Zone 4 and Kit Bottom). *Pleurobema sintoxia* (round pigtoe) shells were found at Zones 5, 6, HC1 and HC2. Recent shells of *Villosa iris* (rainbow) were found at Zone 6. Recent shells of *Villosa vanuxemensis* (mountain creekshell) were also found at Zones HC1 and HC2. Shells of *Fusconaia flava* (Wabash pigtoe) and *Lasmigonia costata* (flutedshell) was found at HC2. Recent material from the rainbow, mountain creekshell, and Wabash pigtoe were found at Zones 6, HC1 and HC2. This pattern of only finding recent material far downstream from the dam or in tributaries suggests that hydromodifcation of the Holston River resulting from the Fort Patrick Henry Dam is part of the cause of the loss and lack of recovery of the mussel fauna in this river. Material collected from Zones 4 and 5 suggest that extant populations of the threeridge or round pigtoe may still be present in these areas, but the limited amount of shell material suggests that if they are extant these populations are small.

5.3.1.6 Crustaceans (Crustacea)

Seven species of crustaceans were collected in 2010: the crayfishes *Cambarus bartonii cavatus*, *C. girardianus*, *C. striatus* and the introduced *Orconectes rusticus* (rusty crayfish); the isopod or water slater, an undetermined *Caecidotea* species; the gammarid amphipod, an undetermined *Crangonyx* species; and the hyalellid amphipod, *Hyalella azteca*. *Cambarus b. cavatus* was moderately common at Zone 2, uncommon at HC2, and rare at Zone 3. This species was found under rocks along the river banks (Zones 2, 3 and HC2) and in root mats of riparian trees (HC2). *Cambarus b. cavatus* is stenothermic in the Ridge and Valley province and therefore more common in springs and small, first order streams. It was the dominant crayfish at Zone 2, where the cold tailwaters from Fort Patrick Henry Dam provide a cooler, more stable water temperature. *Cambarus girardianus* was moderately common at Zones 2, 5, 6, HC1 and HC2 and rare at Zones 3 and 4. This crayfish species was most commonly collected from rocky riffles (Zones 2, 3, 4, 5, 6, HC1 and HC2) and less commonly from macrophytes (Zones 2 and 6). The *C. girardianus* specimens were collected by the fish crew in Zone HC2. The taxonomic status of members of the subgenus *Hiaticambarus* (*C. girardianus* and *C. longirostris*) in the Tennessee River drainage of the Ridge and Valley province is unclear. However, inasmuch as *C. girardianus* bears a bold color pattern and *C. longirostris* a plain color pattern and populations of *Hiaticambarus* in the study area exhibit the bold pattern, they are herein referred to as *C. girardianus*. A single specimen of *C. striatus* was collected at Zone 4 from under trash in a backwater. The introduced rusty crayfish was abundant at Zones 3, 4, 5, 6, HC1 and HC2 and rare at Zone 2. The rusty crayfish was found in a wide variety of habitats including rocky riffles, macrophytes, emergent vegetation and root mats. This species was unknown from any of the pre-1990 surveys, although the crayfishes collected in 1980 were not identified to species. It seems likely that the close proximity of Fort Patrick Henry Lake and its tailwater fisheries is the source of a bait bucket introduction to the area. There exists an unpublished record for this species from Fort Patrick Henry Lake. The single female bearing eggs was found entangled in a gill net on 20 April 1972. The Academy survey collections (1980, 1990, 1997 and 2010) were conspicuous by the absence of *O. forceps*. This species would be expected to occur in large

riverine habitats in the Ridge and Valley province and the rusty crayfish appears to have replaced *O. forceps* in this portion of the Holston River.

The undetermined species of isopod (*Caecidotea* sp.) was found at Zones 2, 5 and 6 from soft sediments and detritus in backwaters and from beds of macrophytes. This taxon was common at Zone 2 and uncommon at Zones 5 and 6. The amphipod fauna was dominated by an undetermined species of *Crangonyx*. The *Crangonyx* species was abundant at Zone 5, common at Zone 2, moderately common at Zone 4, and rare at Zones 3 and 6. This species was taken from leaf litter, root mats and aquatic macrophytes. A second species of amphipod, *Hyalella azteca*, was only collected at Zone 6 from aquatic macrophytes, where it was uncommon. Amphipods are widespread in the Holston River system, but optimal conditions for large population sizes vary from zone to zone. Amphipods and isopods were not collected in Horse Creek.

There appears to be a common macrocrustacean fauna in the Holston River consisting of one species of water slater, two amphipods, and a spotty, but widespread crayfish fauna which appears to have undergone a transition with the introduction of the rusty crayfish. The rusty crayfish and *C. girardianus* were widespread while *C. b. cavatus* is moderately common only at Zone 2. The native crayfish species *O. forceps* seems to have been replaced in the study area by the rusty crayfish.

Six species of crustaceans were collected in 1997 and included *C. b. cavatus*, *C. girardianus*, *C. striatus*, rusty crayfish, an undetermined *Caecidotea* species and an undetermined *Crangonyx* species. *Cambarus girardianus* was collected from all Holston River and Horse Creek zones. *Cambarus b. cavatus* was moderately common at Zones 2, 4 and HC1. As in 2010, a single specimen of *C. striatus* was found at Zone 4. The rusty crayfish was collected from all zones with the exception of Zone 2. The undetermined species of isopod was common at Zones 2 and 6 and uncommon at Zone 4. The *Crangonyx* species was abundant at Zones 2, 4 and 5 and common at Zone 6.

Six species of crustaceans were also collected in 1990 and consisted of *C. b. cavatus*, *C. girardianus* (as *C. longirostris*), rusty crayfish, an undetermined *Caecidotea* species, *H. azteca* and an undetermined *Crangonyx* species. *Cambarus b. cavatus* was found at Zones 2, 5, HC1 and HC2. *Cambarus girardianus* was collected at all zones except Zone 3. The rusty crayfish was collected from all seven zones. *Caecidotea* was collected from Zones 2, 4, 5 and 6. A single *H. azteca* specimen was collected at Zone 6. The *Crangonyx* species was common at Zones 2, 4 and 5 and rare at Zones 3 and 6.

Only four species of crustaceans were found in 1980 and consisted of an undetermined species of crayfish, *Caecidotea* species (as *Asellus* sp.) at Zones 2, 3, 5 and 6, *Crangonyx* species (as *C.* cf. *floridanus*) at all zones and *H. azteca* at Zone 4. The 1977, 1974 and 1965 surveys each produced five species of crustaceans. The taxa collected in 1977 included *C. b. cavatus* at Zones 2 and 5, *O. forceps* at Zones 5 and 6, *Caecidotea* sp. (as *A. communis*) at Zones 2 and 4 through 6, the gammarid amphipod (as *Crangonyx* cf. *floridanus*) at Zones 2, 4, 5 and 6 and *H. azteca* at Zone 4. In 1974 the crustacean fauna at Zones 2 through 6 included *C. b. cavatus* (as *C. bartonii* and *C. robustus*) at Zones 2, 4 and 6; *Caecidotea* sp. (as *A. communis*) at Zones 2, 4, 5 and 6; and

the talitrid (Zone 4) and gammarid (as *C.* cf. *floridanus* at Zones 2 through 6) amphipods. A *Lirceus* species recorded from Zone 2 was a terrestrial taxon. The 1965 crustacean fauna included *C. b. cavatus* (as *C. bartonii* and *Cambarus* species) and *C. striatus*; two species of *Caecidotea* consisting of one epigean (as *A. militaris* at Zones 2 and 4) and one troglobitic (as *A.* (identification uncertain) *stygius* at Zone 2) taxon; and the amphipod *H. azteca*. The collection of a blind troglobitic species that was washed to the surface waters from a spring source is a fortuitous addition to the list that has not been duplicated. A terrestrial isopod listed as *Lirceus* species was also noted from Zone 4.

5.3.1.7 Water Mites (Acari)

In 2010 two taxa of water mite were collected: *Lebertia* sp. and *Hydrachna* sp. *Lebertia* was uncommon at Zones 3 and 6 and rare at Zone 2. A single specimen of an undetermined species of the genus *Hydrachna* was collected from Zone 6. Water mites were collected from macrophytes (Zones 2 and 6) and riffles (Zone 3). In 1997 an undetermined species of *Lebertia* was rare at Zones 2 and 5. In 1980, undetermined species of *Lebertia* (Zones 3 through 6) and *Hygrobates* (Zone 6) were encountered in backwater beach pools or in submerged vegetation. During the first survey (1965), mites belonging to the genera *Tyrellia* and *Hygrobates* were recorded from unidentified habitats at Zone 3.

5.3.2 Conclusions

5.3.2.1 Species Richness Among Zones (2010)

A total of 39 non-insect invertebrate taxa were collected during the 2010 survey. Most taxa collected during the survey were classified as tolerant to pollution (59%), and only two taxa (5%) were classified as sensitive. The remaining taxa were classified as having moderate tolerance to pollution (26%) or were not classified (10%) due to a lack of information on those taxa. The large proportion of tolerant taxa is not unexpected as non-insect invertebrates in general tend to be more pollution tolerant than insects. However, there were still tolerant or sensitive non-insect invertebrates that were informative of the conditions in the Holston River.

A comparison of Zones 2-6 reveals a range of 17 to 28 species, with the lowest number at Zone 4 and the greatest at Zone 5. Zone 2 supported 21 species of non-insect macroinvertebrates. This zone supported a larger than expected population of the stenothermic, small stream crayfish *C. b. cavatus* for a large river in the Ridge and Valley province, likely due to the cold tailwaters of Fort Patrick Henry Dam. These cold tailwaters were probably also the cause of the rarity of the rusty crayfish. Nine taxa were abundant at Zone 2, including *D. tigrina, E.* cf. *tetraedra*, pagoda hornsnail, golden fossaria, ash gyro, pewter physa, two-ridge rams-horn, pea clam and the Asian clam. Zone 2 differed from many other zones in that amphipods and isopods were common. At Zone 3, a diverse leech (five species) and snail (seven species) fauna was present. Crustaceans consisted of three crayfishes and one amphipod and all were rare with the exception of the rusty crayfish. In addition to the rusty crayfish, nine other taxa were abundant at Zone 3 (*D. tigrina, E.* cf. *tetraedra*, *E. punctata*, pagoda hornsnail, golden fossaria, ash gyro, pewter physa, pea clam, Asian clam). The leech abundance was greater than that found at any other zone. The least taxa-rich zone (4) was the only zone where sponges were not observed, and this zone also

supported a low leech richness compared to the other zones. The abundant taxa at Zone 4 included *D. tigrina, E.* cf. *tetraedra*, pointed campeloma, pagoda hornsnail, creeping ancylid, pea clam, Asian clam and rusty crayfish. Although fewer species were found at Zone 4 than the other zones, the pollution-tolerant *E. punctata* was not collected, whereas pollution-sensitive pogoda hornsnail were abundant. Zone 5 had the greatest species richness (28) among the zones, and the abundant taxa at this zone included *D. tigrina,* undetermined tubificid, pointed campeloma, pagoda hornsnail, onyx rocksnail, pewter physa, creeping ancylid, pea clam, fingernail clams (3 species), Asian clam, gammarid amphipod and rusty crayfish. There were also several common or moderately common leeches. Six abundant taxa at Zone 5 were pollution tolerant (i.e., *D. tigrina*, undetermined tubificid, pewter physa, river fingernail clam, striated fingernail clam and gammarid amphipod). Zones 5 and 3 were the only zones that supported at least a moderately-sized population of the leech *E. punctata*. Zone 6 supported 25 species, the second greatest richness. Among these 25 species, nine were abundant and included *D. tigrina,* pointed campeloma, pagoda hornsnail, onyx rocksnail, pewter physa, cymbal ancylid, creeping ancylid, Asian clam and rusty crayfish. The cymbal ancylid was only collected at this zone. The fauna at Zone 6 was similar to Zone 5 in that it supported abundant pollution-sensitive species, including the pogoda hornsnail and onyx rocksnail. In addition, there were only three abundant taxa considered pollution tolerant (i.e., *D. tigrina*, pewter physa, cymbal ancylid).

Differences among the five Holston River zones were not significant (Cochran's Q value = 9.46, which is less than the Chi-square value [9.49] at the 95.0% confidence limit). This analysis was also run using the Horse Creek data, and a significant difference between the sites was identified (Cochran's Q value = 23.54, which is greater than the Chi-square value [12.59] at the 95.0% confidence limit), which suggests that there was a difference between the Holston River and Horse Creek zones. Non-metric multidimensional scaling (NMDS) did not identify strong patterns or clusters (Figure 5.3.1). The Horse Creek zones and Zone 4 were separated from the other Holston River zones on Dimension 2 largely by the absence of a number of taxa that were present at Zones 2, 3, 5 and 6 on the Holston River. Among the Holston River zones, Zones 2 and 3 were most similar to each other which was partly driven by the presence of *C. b. cavatus* and the absence of the pointed campeloma. Zones 4 and 6 were least similar to the other Holston River zones. Zone 4 had lower richness and differed from the other zones by the absence of several taxa and the presence of *C. striatus* and *B. sowerbyi,* which were not found at other Holston River zones. Zone 6 differed from the other zones due to the presence of four taxa not found elsewhere and the absence of several taxa (e.g., several leeches and two-ridge rams-horn).

5.3.2.2 Species Richness Among Years

When comparing totals among surveys and zones, it was necessary to adjust the numbers of taxa in some groups. Tubificid worms were determined to species level in 1965 and in subsequent years were identified as morphospecies, as Tubificidae, or a combination of species-level and Tubificidae. For comparative purposes, those species easily identified (i.e., *B. sowerbyi*) were left at species level and all other taxa were lumped into Tubificidae. The pea clam genus *Pisidium* was identified to species in the 1965 and 1974 surveys, but left at the genus level in subsequent years. For consistency all *Pisidium* were left at the genus level. Microinvertebrates including nematodes (1990) and rotifers (1965) were both omitted from the species totals. The

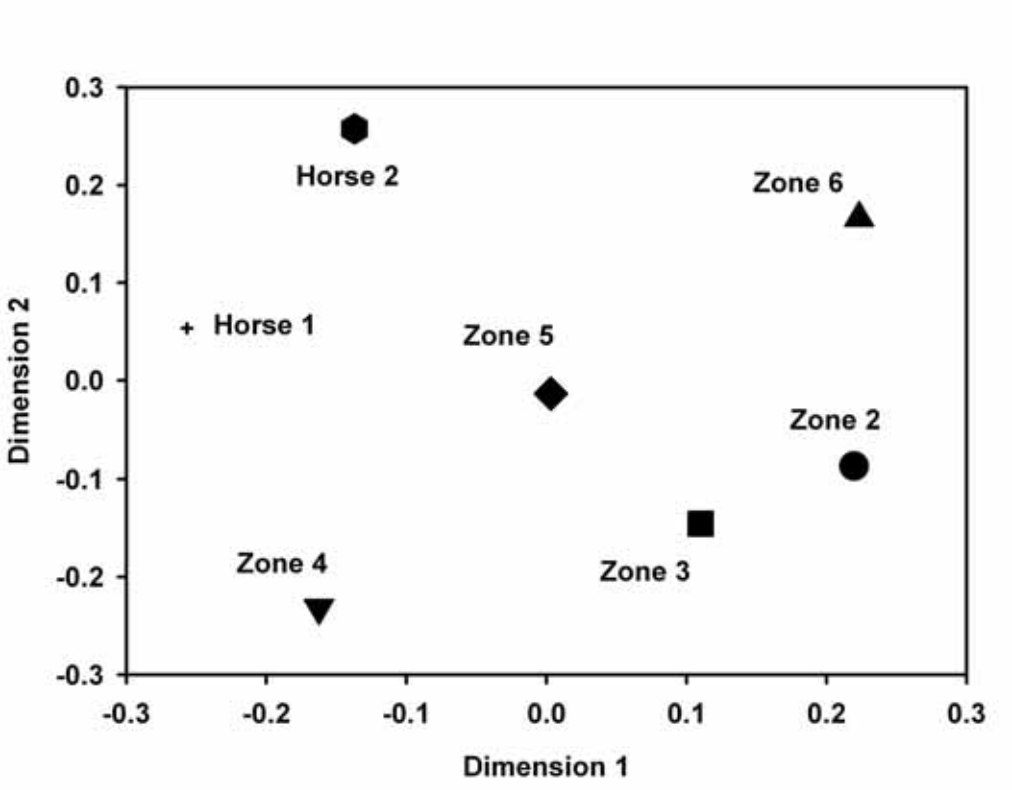


Figure 5.3.1. Non-metric multidimensional scaling using Bray-Curtis dissimilarity for non-insect macroinvertebrate taxa collected in July 2010 at South Fork and mainstem Holston rivers Zones 2, 3, 4, 5 and 6 and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, Tennessee.

oval ambersnail was not included in totals because this taxon is considered terrestrial. The numbers of species collected in any one year, adjusted for comparative consistency among years, are presented in Table 5.3.1 and displayed graphically in Figure 5.3.2.

A total of 39 species of non-insect macroinvertebrates were collected from the study area during July 2010, which was greater than in any previous survey. Despite an increase in the number of taxa collected in 2010, there were only three taxa (*Piscicolaria reducta*, *Helisoma anceps* and *Hydrachna* sp.) new to the Holston River surveys. The increases in taxa richness in the 1990, 1997 and 2010 surveys have been largely a result of collecting more of the taxa historically found in the Holston River (Appendices 7.5.1 and 7.5.2). The only pattern observed for total richness was a general increase in richness from 1965 through 2010 (Table 5.3.2). There was an increase in species richness between the 1997 and 2010 surveys at all the zones except Zone 4. The number of taxa at Zone 4 dropped from 21 to 17 taxa between 1997 and 2010. One more species was collected at Zone 6 (25), two more at Zone 2 and six more at Zones 5 and 3 (Table 5.3.1). The Zone 2 fauna was increased by the addition of three leech taxa (*M. microstoma*, *G. elongata* and *H. triserialis*) and the collection of the pollution-sensitive pagoda hornsnail. The Zone 3 fauna was increased by the addition of three species of snail (pagoda hornsnail [sensitive], ash gyro [moderate] and two-ridge rams-horn [moderate]), the crayfish *C. b. cavatus* (tolerant), the gammarid amphipod *Crangonyx* sp. [tolerant] and the water mite *Lebertia* sp. (tolerant). Zone 5 had increases in the number of leech (+4 species), snail (+2 species), clam (+1 species) and crustacean (+1 species) taxa. The population of sensitive onyx rocksnail increased

Table 5.3.1. Non-insect macroinvertebrate taxa richness between 1965 and 2010 from South Fork and mainstem Holston rivers on Zones 2, 3, 4, 5 and 6 and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, TN. For comparative purposes, some taxonomic groups were aggregated to higher taxonomic levels (e.g., Tubificidae with the exception of easily identified species *Branchiura sowerbyi*) and taxa not typically collected as part of these surveys were eliminated (e.g., Nemata and Rotifera). Note: These totals differ somewhat from Table 5.3.1 in the 1997 survey report due to differences in how some taxa were treated between surveys (i.e., *Novisuccinea* and Nematoda not included in 2010, *Pisidium* and *Caecidotea* aggregated to the genus level, and morphospecies aggregated to genus or family level). *Zone 6 in 1965 was approximately 4.8 km (3 mi) upriver of the location of this zone in subsequent survey years.

	Holston						Horse	
	2	**3**	**4**	**5**	**6***	**Total**	**1**	**2**
2010	21	24	17	28	25	39	14	15
1997	19	18	21	22	24	34	12	12
1990	15	16	21	17	16	28	11	11
1980	9	11	19	15	22	27	-	-
1977	9	8	15	12	15	23	-	-
1974	9	8	13	12	15	25	-	-
1965	7	6	11	4	8	20	-	-

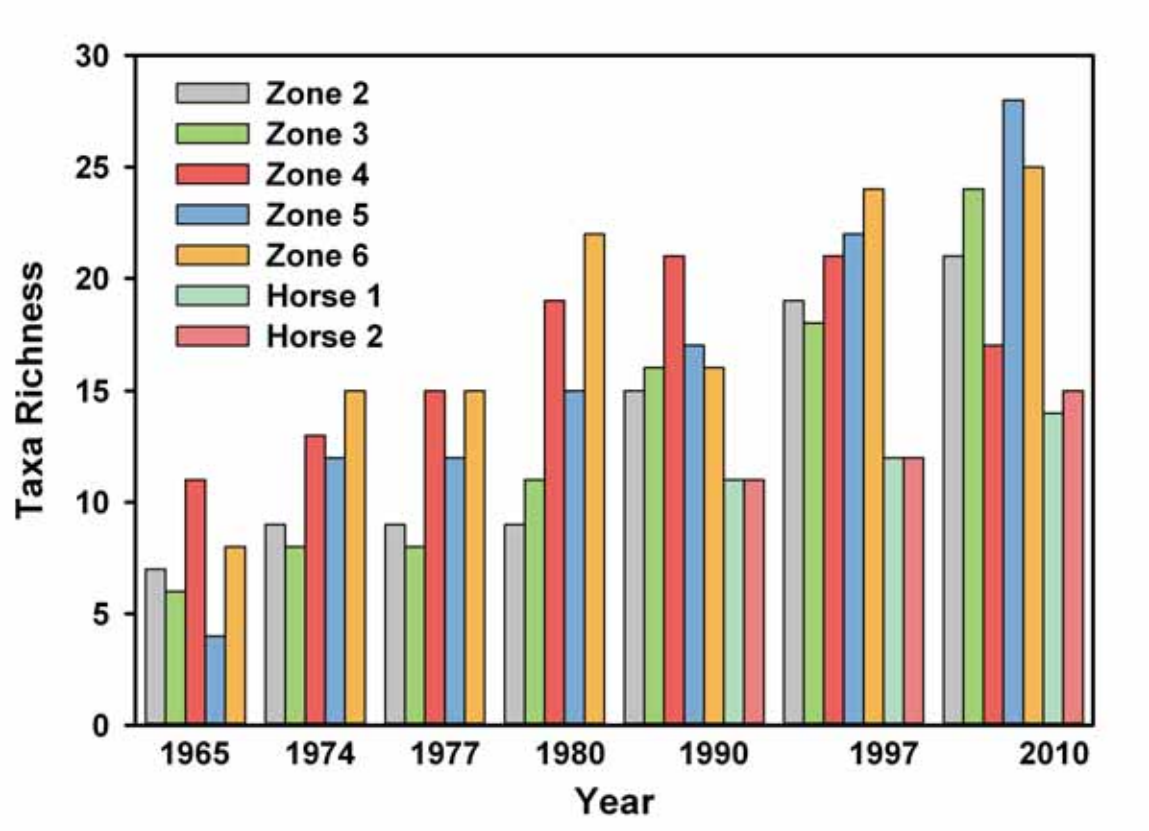


Figure 5.3.2. Taxa richness for non-insect macroinvertebrate taxa collected 1965-2010 at South Fork and mainstem Zones 2, 3, 4, 5 and 6 and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, TN.

Table 5.3.2. Richness of dominant taxonomic non-insect macroinvertebrate groups collected between 1965 and 2010 from South Fork and mainstem Holston rivers Zones 2, 3, 4, 5 and 6, Hawkins and Sullivan counties, TN. Note: These totals differ somewhat from Table 5.3.2 in the 1997 survey report due to differences in how some taxa were treated between surveys (i.e., *Novisuccinea* and Nematoda not included in 2010, *Pisidium* and *Caecidotea* aggregated to the genus level, and morphospecies aggregated to genus or family level).

	Snails	Clams	Crustaceans	Leeches	Worms	Other	Total
2010	10	5	7	8	4	5	39
1997	10	4	6	6	4	4	34
1990	9	5	6	3	2	3	28
1980	7	3	4	5	2	6	27
1977	7	4	5	3	1*	3	23
1974	6	3	5	6	2*	3	25
1965	3	2	4	3	3*	5	20

* Additional worm taxa were identified in the 1965, 1974 and 1980 surveys, but these taxa were lumped for comparability among subsequent years.

from moderately common to abundant. This pollution-sensitive species was not collected in 1990, although unknown numbers were taken from Zone 5 in 1977 and 1980. Sponges were less abundant in the 2010 survey compared to the 1997 survey and were not observed at Zone 4.

A NMDS analysis using data from all seven surveys indicated several patterns (Fig. 5.3.3). Some of the largest differences between samples were among years with many of the samples from the 1965, 1974, 1977 and 1980 surveys more similar to each other than zones across years. An exception to this was Zone 2 samples which clustered together across survey years 1965-1980. The 2010, 1997 and 1990 surveys grouped together and indicated that the non-insect macroinvertebrate communities have been similar during this period. During the three most recent surveys, it is also apparent that zones are more similar to each other across years, which suggests that the differences among the zones have been maintained during these years. In the years when the Horse Creek sites were sampled (i.e., 1990, 1997 and 2010), these sites clustered apart from the Holston River zones, indicating that the Horse Creek and Holston River zones were less similar. Examination of the Bray-Curtis dissimilarity values can also provide additional insight into changes at these zones over time. The Bray-Curtis values are on a scale of 0 to 1, with a score of 1 indicating complete dissimilarity and 0 indicating complete similarity between samples. Dissimilarity among Holston River zones was lower during the years 1990, 1997 and 2010 and ranged from 0.28 to 0.33 (Appendix 7.5.3). In contrast these values ranged from 0.41 to 0.57 during the 1965-1980 surveys. This indicates that since the 1990 surveys the non-insect macroinvertebrate communities across zones were more similar to each other than in previous surveys. Dissimilarity between Zones 2 and 3 also changed from 1965 to 2010 (Appendix 7.5.3). Dissimilarity between Zones 2 and 3 ranged from 0.20 to 0.35 during the 1990, 1997 and 2010 surveys with the lowest dissimilarity identified in 2010 (0.20). In contrast these values ranged from 0.41 to 0.65 during the 1965, 1974, 1977 and 1980 surveys.

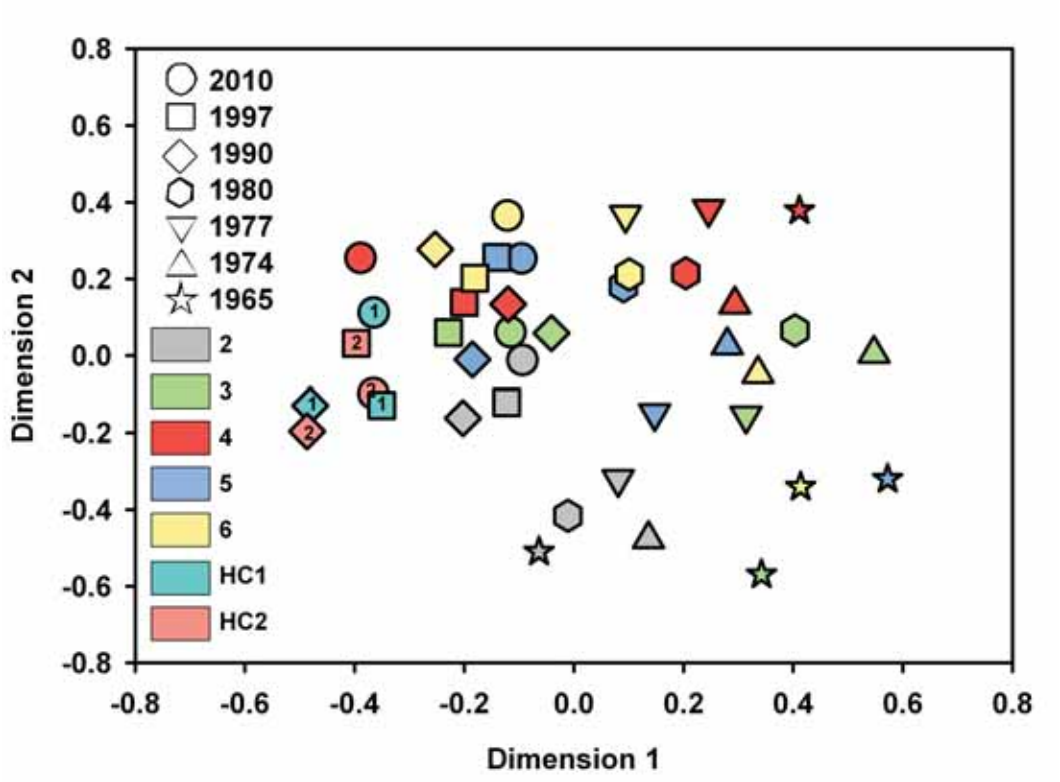


Figure 5.3.3. Non-metric multidimensional scaling using Bray-Curtis dissimilarity for non-insect macroinvertebrate taxa collected in the 1965, 1974, 1977, 1980, 1990, 1997 and 2010 surveys at South Fork and mainstem Holston rivers Zones 2, 3, 4, 5 and 6 and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, Tennessee. Zones are indicated by symbol color.

The 1965 (ANSP 1966) survey produced the fewest taxa (20), although seven species of tubificid were not included in this count for comparability among the different survey years. In 1965 this tolerant tubificid element was conspicuous with eight species. The low number of taxa probably reflected the initial sampling effort, as well as ambient river water quality ("polluted" to "very polluted" at Zones 3 and 5, respectively). The survey was also conspicuous in the smaller number of snail species collected compared to later studies. Only 3 species were found in 1965 compared to 6 to 10 species in the 1974 to 2010 investigations (Table 5.3.2). The snail *Physella heterostropha* (as *Physa microstoma*), tolerant of a wide range of environmental variables, was one of the few snail species found at Zones 3 and 5 in 1965. A small population of this species was noted from Zone 3 and vast numbers were present at Zone 5. None of the pollution-sensitive pleurocerid snails was found at any of the zones downriver from Fort Patrick Henry Dam.

The 1974 (ANSP 1975) survey marked an overall improvement in water quality as measured by the non-insect macroinvertebrate fauna. There was a conspicuous reduction in tubificid species (eight species in 1965 and two in 1974) and biomass, especially at Zone 3. The greatest increase in richness across all zones can be seen in two of the dominant groups, the leeches and snails. The number of snail and leech species was twice that observed in 1965 (Table 5.3.2). At the individual zones, the greatest increase in richness occurred at Zones 5 and 6, with the largest

gain at Zone 5 (8 species, Table 5.3.1). Especially noteworthy was the presence of the pollution-sensitive pogoda hornsnail at Zone 5. At Zone 6, the increase was across the taxonomic spectrum and included new snail, clam, crustacean and leech taxa. It should be noted that Zone 6, the downriver-most zone in 1974, was 4.8 km (3 mi) downriver from the sampling site in 1965. The new Zone 6 (designated Zone 6A in the report) was considered to differ somewhat in physical features, including certain habitats that made it easier to survey (ANSP 1975). However, the increase in species at Zone 6 was attributed to an improvement in water quality (ANSP 1975), although not as dramatic as at Zone 5. Little change was apparent at Zone 3 with an increase of only two species (an actual decrease of three taxa, if you include the tubificids at species level taken at Zone 3 in 1965—treated as a single taxon for comparative purposes, see Tables 5.3.1 and 5.3.2). The decrease in the numbers of species and relative abundance of tubificids at Zone 3 was as dramatic an improvement in water quality at Zone 3 as the greater increase in numbers of species found at Zones 5 and 6. However, Zones 2 and 3 support fewer species of non-insect macroinvertebrates than Zones 4 through 6, reflecting the effects of the Fort Patrick Henry Lake tailwater at Zone 2 and lowered water quality at Zone 3.

The 1977 (ANSP 1978) study revealed no overall increase in total numbers of species from Zones 2 through 6 nor significant additions at any of the zones except Zone 4. At this site, compared to 1974, two additional taxa (from 13 to 15 species) were found (3 additional taxa if *Pisidium* is taken to species level). The increase was in two of the dominant groups (leeches and clams) that occur in the Holston River. In 1977, both species of pleurocerids (pogoda hornsnail and onyx rocksnail) were collected at Zone 5 and the pogoda hornsnail was also recorded from Zones 4 and 6. The introduced Asian clam was first observed in the Holston River study region at Zones 5 and 6 in 1977. As in 1974, Zones 2 and 3 exhibited reduced faunas compared to Zones 4 through 6.

The 1980 (ANSP 1981) investigation found a consistent increase in overall species richness (23 in 1977 and 27 in 1980) as well as at each zone (three species at Zones 3 and 5, four species at Zone 4 and 7 species at Zone 6) except at Zone 2 below Fort Patrick Henry Dam which had no increase. The increase in total richness was due in part to increased leech richness from three in 1977 to five in 1980. Among individual zones, the large increase at Zone 6 is also attributable to no specific group and reflects an overall increase in species across the diverse range of non-insect macroinvertebrates. One snail new to Zone 6 was the pollution-sensitive onyx rocksnail (also present at Zone 5). The other pleurocerid snail, the pogoda hornsnail, was present at Zones 4, 5 and 6. In 1980, with overall improvement, Zones 2 and 3 still exhibited comparatively lower species richness.

The 1990 investigation (ANSP 1992) was conducted under high water conditions at Zones 2, 5 and 6. At Zone 6 the only decrease in species richness (16 taxa in 1990) compared to 1980 (22 species) was recorded. There was a slight increase in species richness across all zones from 27 in 1980 to 28 in 1990. The overall increase in species between the two surveys is seen in the snails and clams (two additional species for each group). Among the snail fauna in 1990, the cymbal ancylid was collected for the first time and a third taxon not collected in 1980 was the slender walker (taken from Zone 2 in 1974). The pogoda hornsnail was found at Zones 4, 5 and 6, while the onyx rocksnail was abundant at Zone 6. Zones 2 and 3 showed more improvement in species richness than Zones 4 and 5, and Zone 6 was mostly impacted by high water levels.

The lack of pleurocerid snails and isopods, reduced numbers of amphipods, and an abundant population of the leech *E. punctata* indicated that Zone 3 was still less diverse than the downriver zones.

In 1997 (ANSP 1998) a total of 34 taxa were collected, which represented a 6 species increase from 1990. Much of this increase was the result of three additional leech taxa collected in 1997. Two new taxa collected in 1997 not present in the previous surveys were the worm *S. lacustris* and the leech *M. microstoma*. All zones with the exception of Zone 4 had an increase of two to eight species. The largest increase was observed at Zone 6 (8 species). Both Zones 2 (4 species) and 3 (2 species) had increases in taxa richness but these were less than the increases at Zones 5 and 6. Zone 4 had the same number species (21 species) recorded in 1990.

Two zones on Horse Creek were sampled in 1990, 1997 and 2010. Two more species were collected at Zone HC1 and three more at HC2 in 2010 compared to the 1997 survey (Table 5.3.1). In 2010, the Horse Creek zones were similar. Different taxa included an undetermined Tubificidae, pointed campeloma, pea clam and river fingernail clam at Zone HC1 and a bryozoan, *B. sowerbyi*, an earthworm, a leech and *C. b. cavatus* at Zone HC2. In 1997, both Horse Creek zones produced 12 species constituting only a slightly different fauna (a bryozoan and crayfish at Zone HC1 and a leech and bugle sprite at Zone HC2). A comprehensive Horse Creek survey was conducted for the first time in 1990, when 11 species were collected at each zone. As in 1997, the faunas between zones were similar with the only differences an earthworm at Zone HC1 and an undetermined species of leech at Zone HC2. An early survey of an undefined zone on lower Horse Creek discovered several species of mussels (ANSP 1976) and no Asian clams. No living mussels were observed at either Horse Creek sampling zone in 1990 or 1997. Again in 2010 no living mussels were observed in Horse Creek, although relic material was found at Zones HC1 (2 species) and HC2 (4 species) indicating possible extant populations.

5.3.2.3 Dominant Taxa

Five groups of non-insect macroinvertebrates have dominated the faunal surveys in the study area (Table 5.3.2). In 2010, the 39 taxa collected included 10 snail, 7 crustacean, 8 leech, 4 worm and 5 clam taxa. These five groups constituted 87% of the non-insect macroinvertebrate fauna from the Holston River, and it is in these larger groups that changes in fauna among the years can often be observed. In 2010, the remaining groups were either widely collected (e.g., planarian) or spotty in distribution (e.g., sponges, ectoprocts and water mites). Of the 34 (adjusted number) taxa collected in 1997, the dominant groups were snails (10 species), crustaceans (6 taxa), leeches (6 taxa), worms (4 taxa) and clams (4 taxa). For 1990, 89% of the fauna consisted of snails (9 taxa), clams (5 taxa), crustaceans (6 taxa), worms (2 taxa) and leeches (3 taxa). In 1980, these 5 groups out of a total of 27 taxa (78%) consisted of 7 kinds of snails, 4 kinds of crustaceans and 5 kinds of leeches, 2 kinds of worm and 3 kinds of clams. A lower number of taxa (23) was collected in 1977 when the numbers in the 5 dominant groups consisted of 7 taxa of snails, 5 of Crustacea, 4 of clams, 1 of worms and 3 taxa of leeches. The 1974 total of 25 species comprised 6 kinds each of snails and leeches, 3 kinds of clams, 5 kinds of Crustacea and 2 kinds of worm. The first survey in 1965 produced only 20 taxa consisting of 4 taxa of Crustacea, 2 taxa of clams and 3 taxa each of snails, worms and leeches.

5.3.3 Summary

The results of the 2010 survey indicate that the non-insect macroinvertebrate fauna of the South Fork and mainstem Holston rivers is broadly similar to recent surveys (1997 and 1990; ANSP 1998 and 1992). Five groups of non-insect macroinvertebrates have dominated the faunal surveys in the study area (Table 5.3.2). Of the 39 species collected in 2010, the 5 dominant groups, as in the past, include the snails (10 species), crustaceans (7 species), leeches (8 species), worms (4 species) and clams (5 species). The remaining species were scattered among four diverse classes of non-insect macroinvertebrates. The non-insect macroinvertebrates from Zones 2 through 6 indicate impacts at Zones 2, 3 and 4 in relation to Zones 5 and 6. No rare or endangered species were collected.

Zone 2 had larger than expected numbers of the normally stenothermic crayfish *C. b. cavatus* and no pollution-sensitive onyx rocksnails were present. However, the pollution-sensitive pagoda hornsnail was collected in this zone for the first time. Numbers of species were greater than in 1997 (21 versus 19 species) and may reflect sampling of an additional area in the zone. Impacts on Zone 2 from the Fort Patrick Henry dam made this area a poor control or reference zone for comparisons with downstream potentially impacted, impacted, or recovery zones, which were much less impacted by the dam's effects. However, it remains a more appropriate control for evaluating point source and other impacts than areas above the impounded reservoir.

Zone 3 produced large numbers of a pollution-tolerant flatworm, earthworm, golden fossaria, pewter physa and the leech *E. punctata* as well as a diverse leech fauna (five species). As in Zone 2, the pollution-sensitive pagoda hornsnail was collected in Zone 3 for the first time. Compared to 1997, Zone 3 exhibited a large increase in species richness (24 versus 18), which included 3 new snail species, an amphipod, *C. b. cavatus* and a water mite. These mixed results indicate a small improvement between 1997 and 2010 at Zone 3. Based on NMDS and Bray-Curtis dissimilarity, Zones 2 and 3 were more similar to each other than to any of the other zones sampled in 2010. This similarity and the presence of the stenothermic *C. b. cavatus* indicate that the cool, fluctuating water levels from the dam may also be impacting Zone 3.

Zone 4 exhibited a lower richness than any of the other Holston River zones. Compared to 1997 the species richness was reduced (17 versus 21 species), and Zone 4 was the only zone sampled in 2010 to have a lower richness compared to 1997. The loss of species included a few notable taxa such as the pollution-sensitive onyx rocksnail (collected for the first time 1997), sponges, moss animals and isopods (moderately common in 1997). Tubificids were rare in 1997 and were not collected in 1990, but in 2010 this pollution-tolerant taxon was present in all zones. Water quality, as measured by the non-insect macroinvertebrates, at Zone 4 indicates a decline in conditions between 1997 and 2010. However, differences between Zone 4 and the other Holston River zones may also be partly driven by differences in habitat with Zone 4 having less habitat diversity.

Zone 5 supported the greatest taxa richness of any of the zones sampled in 2010. Zone 5 indicated an improvement in water quality over Zone 3, although pollution-tolerant tubificids were abundant (moderately common at Zones 2-4 and not collected at Zone 6) and the tolerant

leech *E. punctata* was moderately common (absent at Zone 2, rare at Zone 6 and abundant at Zone 3). The pogoda hornsnail and onyx rocksnail were abundant (the pogoda hornsnail was abundant at all zones and the onyx rocksnail was abundant at Zone 6). Compared to 1997, Zone 5 had a large increase in the numbers of species (28 versus 22) and an increase in the pollution-sensitive pogoda hornsnail (abundant in 2010 and common in 1997) and onyx rocksnail (abundant in 2010 and moderately common in 1997). In addition, pollution-tolerant taxa which increased in numbers from 1990 to 1997 did not increase further in 2010 (*E. punctata* and tubificids), and isopods were collected in 2010 despite being absent in 1997. Comparisons between 1997 and 2010 at Zone 5 also indicate some improvement in water quality because there was an increase in abundances of the sensitive onyx rocksnail. The downriver-most Zone 6 supported 25 species in 2010 compared to 24 in 1997. Large numbers of the pollution-sensitive pleurocerid snails (pagoda hornsnail and onyx rocksnail) carpeted substrates. Amphipods and isopods were rare and uncommon in 2010, but they were both common in 1997. The pollution-tolerant tubificids were absent and the leech *E. punctata* was rare in 2010 which was similar to 1997, when these taxa were both rare. Compared to 1997, the total richness and numbers of taxa in 2010 at Zone 6 was similar indicating that conditions have not changed.

In Horse Creek, two (HC1) and three (HC2) additional species were collected in 2010 compared to 1997. As in 1997, the two Horse Creek zones support similar faunas that did not differ greatly in species composition. However, differences between taxa in these two zones were greater than in previous years. These differences may reflect habitat differences between the two zones.

A summary of 2010 results compared to the 1997 survey is as follows:

Zone 2: Small improvement (possibly reflecting sampling of an additional area)

Zone 3: Small improvement

Zone 4: Moderate decline

Zone 5: Moderate improvement

Zone 6: Similar conditions

Zone HC1: Similar conditions

Zone HC2: Similar conditions.

5.4 Aquatic Insects

5.4.1 Qualitative Collections

The comprehensive taxa list, development of which was discussed in Section 4.5.3.1, provides a more robust comparison among communities and among years than use of the qualitative collections alone (ANSP 1997). This list combines the taxa from qualitative and quantitative collections and is appropriate because earlier surveys focused exclusively on the qualitative collection of species. Before 1997, investigators spent an entire day sampling each zone and searched qualitatively-collected detritus under magnification. When a quantitative sampling plan was implemented in 1997, qualitative efforts had to be reduced. Using the comprehensive species list makes the data more comparable with earlier field efforts by including small, cryptic specimens found through microscopic examination of detritus (quantitative samples), as well as accounting for species collected in the qualitative sampling, which samples habitats which are not covered by the quantitative samples (the PIBS samples). The comprehensive taxa list for all zones is presented in Table 5.4.1 (tables appear at the end of Section 5.4).

The qualitative collections were the primary method whereby the comprehensive taxa list was augmented with species from the orders Odonata, Hemiptera and Coleoptera. However, other orders were also abundant among qualitative collections (e.g., Ephemeroptera, Trichoptera, Megaloptera and Diptera). Most of the Chironomidae specimens examined were small, immature specimens collected through quantitative collections using the PIBS.

5.4.1.1 Holston River

Zone 2 clearly had the lowest number of taxa in 2010. There was a total of 30 aquatic insect species, of which 11 were chironomid midges. Richness was low because habitat diversity was low. The stream bed was armored, with interstices filled with sand. Most exposed cobble surfaces were covered with filamentous algae. The water level at Zone 2 fluctuated widely, and the intermittently inundated zone was extensive. Thus, much of the benthic substrata were colonized by species that can dwell among filamentous algae strands, live among sand grains and tolerate frequent short periods of desiccation (or rapidly recolonize re-wetted habitat). Many of the larger species collected were found among the sheltered substrata of a single small backwater on the river-left bank. Especially noteworthy was the lack of hellgrammite larvae (*Corydalus cornutus*) in Zone 2. Hellgrammite larvae are ubiquitous in Appalachian rivers and are large predators that hunt among the interstices of benthic substrata.

In 2010, Zone 3 had more taxa than Zones 2 and 5. Zone 3 supported 59 total taxa, of which 13 were Chironomidae. The change in community structure from 1997 (previously the second-most diverse year in the survey's history) to 2010 was easily observed qualitatively in the field. In 1997, three dominant taxa occurred among the riffle substrata at Zone 3: midges, flatworms and blackflies. Hours of searching among the macrophyte beds along the river-right bank at Zone 3 in 1997 produced only two damselflies in poor condition. In 2010, flatworms were tiny and

uncommon, and midges and blackflies made up a comparatively small portion of the community. In 2010, mayflies and caddisflies were abundant in riffles, and damselflies were ubiquitous among macrophytes. The aquatic insect communities of Zone 3 represented a significant improvement relative to Zone 2 in 2010 and relative to all previous collections within the zone.

Zone 4 is the historical site on the Big Sluice. Although the zone was augmented with sites upstream and adjacent to Kit Bottom, Zone 4 was the only zone near Kit Bottom from which qualitative samples were collected. The physical condition of the zone appeared similar to the 1997 survey, when 29 taxa were collected. In 2010, 62 taxa were collected, with 16 taxa of Chironomidae. The only mayfly taxon collected in 1997 was *Tricorythodes*. Although this taxon was also abundant in 2010, there were 10 other mayfly species. Similarly, the species list from 1997 included only one caddisfly taxon (*Oecetis* spp.) but included nine additional caddisfly taxa in 2010. Thus, the mayflies and caddisflies, generally considered to be pollution-sensitive species, increased by 19 species from 1997 to 2010. The location was less diverse than Zone 3 but much more diverse than Zone 2.

In 2010, Zone 5 supported 48 taxa, of which 13 were chironomid midges. This was less than the zones immediately upstream and downstream, but much more than the 33 taxa collected in 1997. In 1997, *Tricorythodes* was the only mayfly taxon collected from Zone 5; 10 taxa were collected in 2010 (however, after correction for changes in taxonomic standards between the surveys the equivalent mayfly richness was 7 taxa in 2010). Three caddisfly taxa were collected in 2010 that were not collected in 1997: *Psychomyiia, Leucotrichia* sp. and *Oecetis persimilis*. However, the rocks were covered with empty *Leucotrichia* cases in both 1997 and 2010. Thus, Zone 5 appears to have improved since 1997. The zone lacked some of the marginal habitats that were common at Zones 3 and 6.

The greatest number of taxa occurred at Zone 6. Zone 6 offered a complex mix of habitats and microhabitats that supported very high diversity. A total of 72 taxa, 13 of which were chironomid midges, were collected from the zone, which is more than ever collected previously. 1997 was previously the richest year observed at Zone 6 with 55 taxa (of which 18 were midges). Although the order Plecoptera was sparse at this zone, it was the only zone to support extensive populations of the semivoltine[1] stonefly *Pteronarcys*. In the southeastern United States, *Pteronarcys* is not quite as sensitive to disturbance as it is thought to be in other regions (such as western mountains and the northern Appalachians), but it could be called moderately sensitive to disturbance. As a large, prominent semivoltine insect, the presence of two apparent age classes indicates that Zone 6 has supported populations of this taxon for more than 2 years. Members of this genus feed under large cobbles and boulders on accumulations of coarse detritus in the form of leaf packs. Long-term alteration of the Holston River's flow regime has eliminated these habitats from much of Zone 2, where interstices are filled with fine sediments. The impoundment of Fort Patrick Henry Dam may also act as a sink for coarse organic material, reducing their amounts sufficiently to hinder the establishment of *Pteronarcys* populations at upstream study sites.

1 Semivoltine insects are insects which complete only part of their life cycle in one calendar year. Thus they live for two or three years as aquatic larvae before reproducing as terrestrial adult insects. Most of the insects collected in this survey were univoltine, completing their entire life cycle over a 1-year period. Midges are an example of a multivoltine insect that can complete several life cycles in a 1-year period.

There was a shift in the relative abundance of the two dragonfly species *Boyeria vinosa* and *Basiaeschna janata* between 1997 and 2010. Both of these aeshnid species inhabit similar habitats among root wads and branches of undercut banks near flowing water. In 1997, *Boyeria vinosa* was nearly ubiquitous in this habitat, while *Basiaeschna janata* was absent or uncommon. In 2010, *Basiaeschna janata* was ubiquitous, and *Boyeria vinosa* was uncommon. This difference occurred throughout all zones and is probably due to factors other than the operation of the Eastman facility.

More taxa were collected from all zones except Zone 2 in 2010 than during any of the earlier surveys. Most importantly, many of the additional taxa were mayflies and caddisflies, which are somewhat more sensitive to pollution than many other orders of aquatic insects. The increase in the richness of these groups existed even after correcting for some changes in taxonomic standards occurring between 1997 and 2010—indicating that these are real ecological improvements, not artifacts of different laboratory procedures. The qualitative findings indicate a marked improvement in water quality of the Holston River over the last 45 years.

5.4.1.2 Horse Creek

Qualitative collections at the Horse Creek sites ran the entire range of Zone HC1 (to include riffles at HC1U and HC1L) and Zone HC2. Habitats included rock ledges, undercut banks, fast and slow riffles, leaf packs, sand and gravel deposits, boulders and woody debris. Identical (Zone HC2) or somewhat fewer (Zone HC1) numbers of taxa were collected at Horse Creek zones in 2010 (51 and 51 taxa, respectively, Table 5.4.1) during the quantitative and qualitative sampling when compared with 1997. 1997 was the most diverse year on record for Horse Creek when 60 and 51 taxa were collected from Zones HC1 and HC2, respectively. In 2010, there were missing taxa from the Ephemeroptera, Plecoptera and Trichoptera compared to 1997. These changes occurred across both Zones HC1 and HC2 and were possibly due to earlier emergences of some species (caused by warm weather, e.g., *Sweltsa* which was abundant in 1997, but not collected in 2010) or urbanization of the Horse Creek watershed, which is known to depress the richness of these groups. More frequent surveys would help determine the causes with less ambiguity.

One rare species of stonefly was collected in 2010 that was not collected in previous surveys. A PIBS sample from Zone HC1 (upper) contained *Hansonoperla appalachia*. The species is not currently listed as federally threatened or endangered. The species is known to occur only rarely through its range, which extends through the Appalachian Mountains from New Hampshire to northern Georgia, although the USFWS (2011) data base only reports it from Tennessee, South Carolina, and Kentucky. The *Encyclopedia of Life* (NatureServe 2010; http://www.natureserve.org/explorer/servlet/NatureServe?searchSciOrCommonName=hansonoperla&x=10&y=4) cites the species' conservation status as Globally Vulnerable (G3) to extirpation, and indicates the state conservation status in West Virginia is "Imperiled" (S2). They list the status in Tennessee as "vulnerable" (S3), but the Tennessee Department of Environment and Conservation does not list *Hansonoperla* among their list of rare species (Withers 2009). Only a single *Hanosperla* specimen was collected, and there is no evidence of its survival being affected by operation of the Eastman facility.

5.4.1.3 Kit Bottom

Qualitative collections and assessments were not made at Kit Bottom, which was only surveyed by quantitative methods. It is likely that qualitative collections would have been of little use given the extremely localized nature of the assessment and of the potential impacts. Quantitative assessments provided a comprehensive assessment of the fine-scale distributions of aquatic insects dwelling near Kit Bottom, including cryptic and small species only observed through microscopic examination of detritus.

5.4.2 Quantitative Collections

Most of the effort to describe and compare aquatic insect assemblages was drawn from the collection of quantitative samples collected by means of a Portable Invertebrate Box Sampler. From all zones, just under 106,000 macroinvertebrates were collected, comprised primarily of aquatic insects and a few non-insect taxa (mostly mites and worms). There were 135 distinct taxa, of which 121 taxa were aquatic insects. The average density was greatest at Zone 2 (about 82,000 organisms per square meter); the other zones were less dense but more diverse (average of 8,000-37,000 organisms per square meter). Invertebrate abundance data were used in descriptive multivariate analyses of broad structural differences among zones, as well as to calculate several ecological summary measures (commonly called "metrics") for each sample. The metrics were used to statistically compare and contrast the structure of benthic assemblages among zones on the Holston River and Horse Creek, and also to contrast changes along a gradient near Kit Bottom on the Big Sluice. Sample-specific covariates were used to ensure that the influence of habitat was accounted for in statistical comparisons when necessary.

5.4.2.1 Covariate Measures

Near-substrate velocity measures recorded for each PIBS sample (Fig. 5.4.1) were used to stratify sample collection within the riffles of each zone measures; no significant differences were observed among zones ($P<0.503$). Lack of suitable high-flow riffles at Zone 2 required that a lower range of velocities be sampled at the other zones to prevent confounding zone differences with differences related to water velocity.

Analysis of the field-estimated particle size distribution for each PIBS (Fig. 5.4.2) indicated that Zone 4 had a significantly greater proportion of sand in the sample, which significantly ($P<0.017$) reduced the particle size index for the zone, but no other significant differences were observed among the particle size index scores of the other zones (Tukey's HSD $P>0.50$).

Analysis of field-measured water depth associated with each PIBS was tenuous at best. The water level fluctuated widely at Zones 2 and 3 (although much less at Zone 3), and samples were collected near the maximum effective depth of the PIBS. However, by the time the water level began to decrease, the locations from which the first PIBS samples were collected were very shallow. Thus, the data from the sample location of the first Zone 2 PIBS samples were recorded at about 0.4 m depth, but the sampling varied over a range of depths daily from about 0.1-1.0 m. Thus, the data probably mean very little for Zone 2, but might be more significant for other

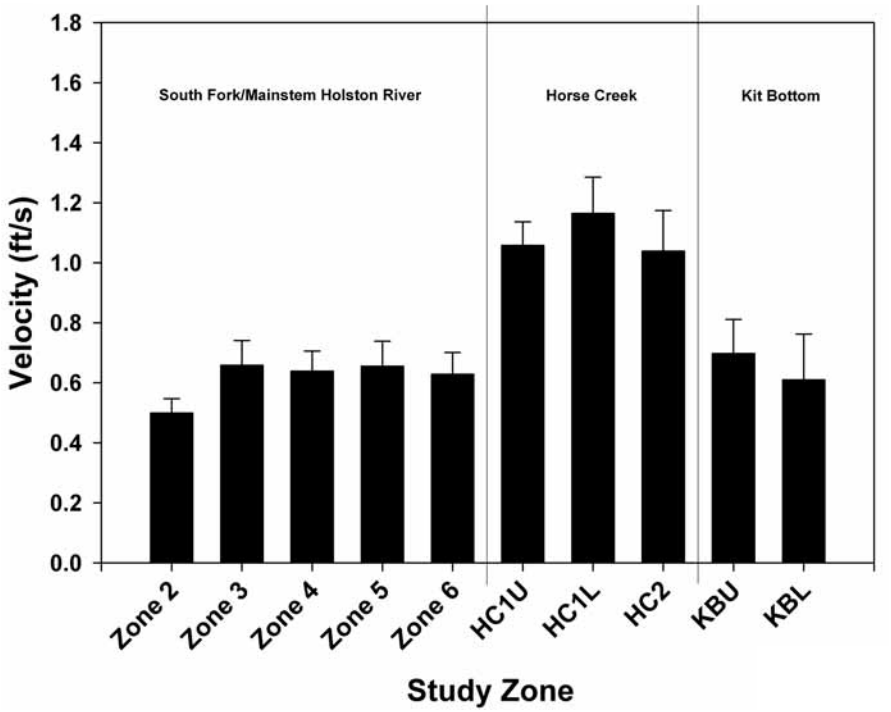


Figure 5.4.1. Mean velocity (ft/s) (±1 standard error) at zones on the Holston River, Horse Creek and Kit Bottom, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

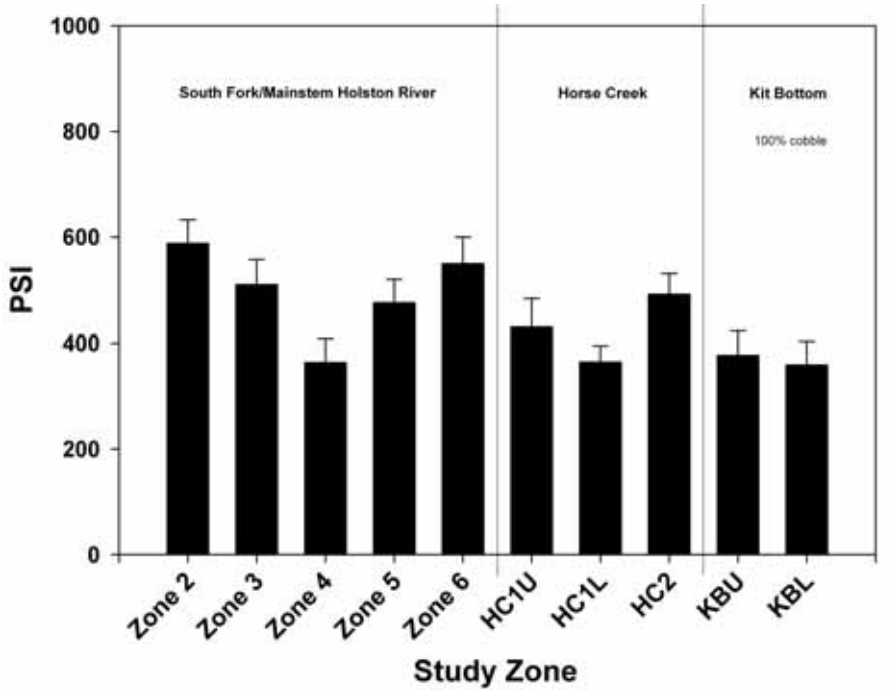


Figure 5.4.2. Mean particle index (±1 standard error) at zones on the Holston River, Horse Creek and Kit Bottom, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed

zones, where the fluctuations in depth were much less extreme. The analysis of this covariate (DEPTH) reflected efforts to ensure samples were collected from perennially wetted stream channels. Significant differences among the sample sites ($P<0.001$) were observed. Tukey's HSD test resulted in two homogeneous groups with Zones 2 and 3 being significantly deeper than Zones 4, 5 and 6. Deeper riffle areas were sampled when depth fluctuations were observed that could inadvertently result in samples being collected from intermittently inundated areas. Therefore the differences observed probably reflect the degree to which the water depth fluctuated at the zones, more than depth *per se.*

There was also a significant difference in the amount of photosynthetic pigments in the samples ($P<0.001$; Fig. 5.4.3). The greatest concentrations were observed in samples collected from Zone 2, but the zone was only marginally significantly different from observations at Zones 3 and 5. Samples from Zone 4 were significantly lower in pigment concentration than Zones 2, 3 and 5, but not significantly different from Zone 6. However, samples from Zone 5 contained marginally significantly more pigment than observed in Zone 6. Moss and algae contributed greatly to pigment at a number of sites. At Zone 2 all exposed benthic substrate was coated with moss or filamentous algae (or both). At Zones 3 and 5, the finer substrate was not covered with plant material, but tightly appressed patches of filamentous algae were abundant on larger substrata. At Zone 6 the pigment in samples was more likely due to vascular hydrophytes, such as *Elodea* sp., which was much more abundant in the flow regime sampled. Pigment concentrations were low to intermediate at the Horse Creek and Kit Bottom zones, respectively.

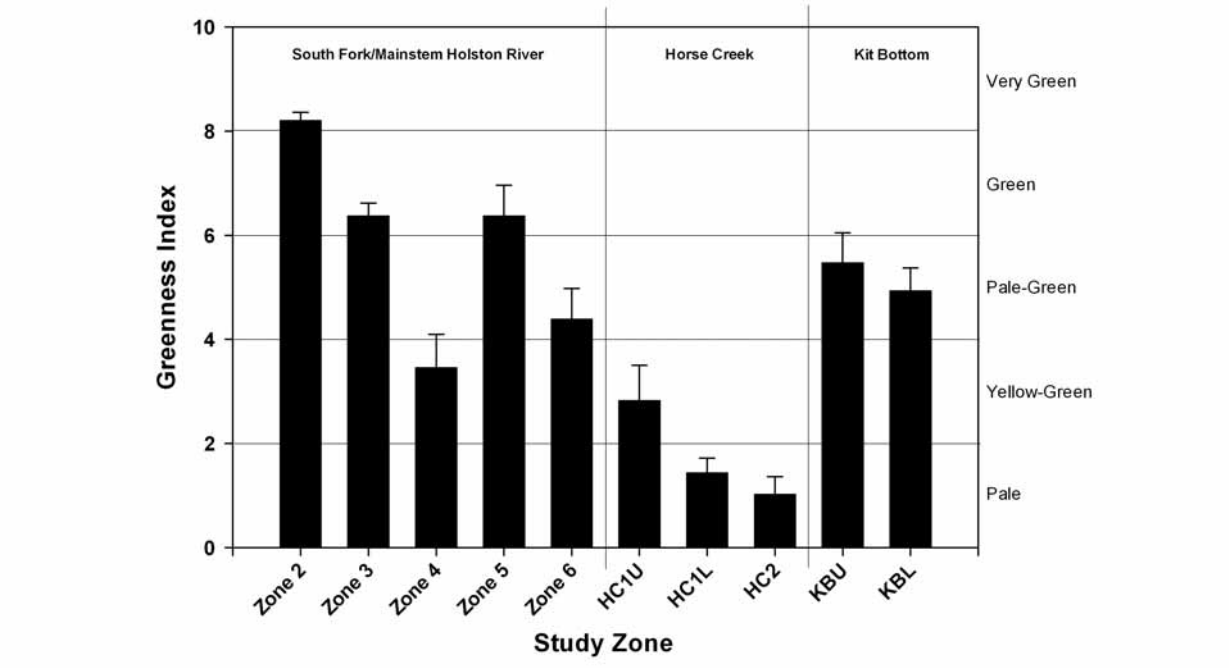


Figure 5.4.3. Mean pigment index (±1 standard error) at zones on the Holston River, Horse Creek and Kit Bottom, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

5.4.2.2 Multivariate Summaries

Two multivariate methods were used to describe the differences in the overall community structure among zones. Cluster analysis used the Bray Curtis (1957) index of dissimilarity to form linkages using the unweighted pair group, arithmetic means algorithm (UPGAMA; Wilkinson 2009) method to generate a single-linkage cluster dendrogram to illustrate the underlying similarities in community structure (Fig. 5.4.4). The figure summarizes several trends in the overall community composition very well. First, at the top of the figure, all the samples collected from Zone 2 cluster together indicating that the zone was characterized by both a unique and fairly consistent community structure. Although Zone 3 was more diverse than Zone 2, both sites shared many dominant taxa. Similarly, Zones 3 and 5 supported very comparable assemblages. Samples from Zones 3 and 5 usually formed linkages with samples from the same zone, but then formed linkages between the two zones before forming a linkage with Zone 2.

Similarly, the benthic assemblages of some of the Lower Kit Bottom samples were most similar to the Zone 4 macroinvertebrates. Samples from Horse Creek zones routinely formed linkages with other Horse Creek zones, indicating that samples from all Horse Creek zones supported structurally similar benthic assemblages. Furthermore, some upper Kit Bottom zone samples were most similar to Horse Creek samples while others were closer to some lower Kit Bottom zone samples. This was due to proximity of the confluence of the two water bodies a short distance upstream from the upper Kit Bottom zone and the tendency of invertebrates to drift downstream. Most samples from Zone 6 formed a single branch before linking with the Horse Creek and Big Sluice zones and then ultimately forming the last link with Zones 2, 3 and 5, most likely reflecting the greater influence of widely fluctuating water levels at the upstream zones.

The other multivariate statistical method used to examine differences in aquatic insect community composition was Detrended Correspondence Analysis (DCA)[2]. The analysis was run only on Holston River Zones 2-6 (Fig. 5.4.5). When the other zones were included in the analysis[3], Horse Creek samples forced the other sites together, making it difficult to draw meaningful conclusions about actual trends in the Holston River. Horse Creek clearly supports an insect fauna that is quite different from the Holston River, and it influenced community structure of the Kit Bottom sites as well. For example, “water pennies” (*Phephenus herricki*) were abundant in all Horse Creek samples and occurred in the Kit Bottom sites in decreasing abundance below the confluence of Horse Creek and the Big Sluice. When the Horse Creek sites were included in the analysis, they occurred in the upper right of the plot, with the differences among other zones appearing small in comparison. Since the purpose of this survey is to detect anthropogenic influences, not to describe differences in fundamentally different ecosystems, the fundamentally different ecosystems were excluded from the analysis.

2 A Nonmetric Multidimensional Scaling (NMS) ordination was also performed, but the results were nearly identical to the DCA. The DCA was preferable for this report because there is a long history of using the technique for the Holston River and other surveys the Academy has conducted for Eastman.

3 Note that the Horse Creek samples were actually excluded from the DCA as described in the text and figure. That is, rather than simply omitting the ordination results for certain zones and re-scaling the graph, the whole ordination was re-run without the influence of HC or KB sites on the multivariate data space.

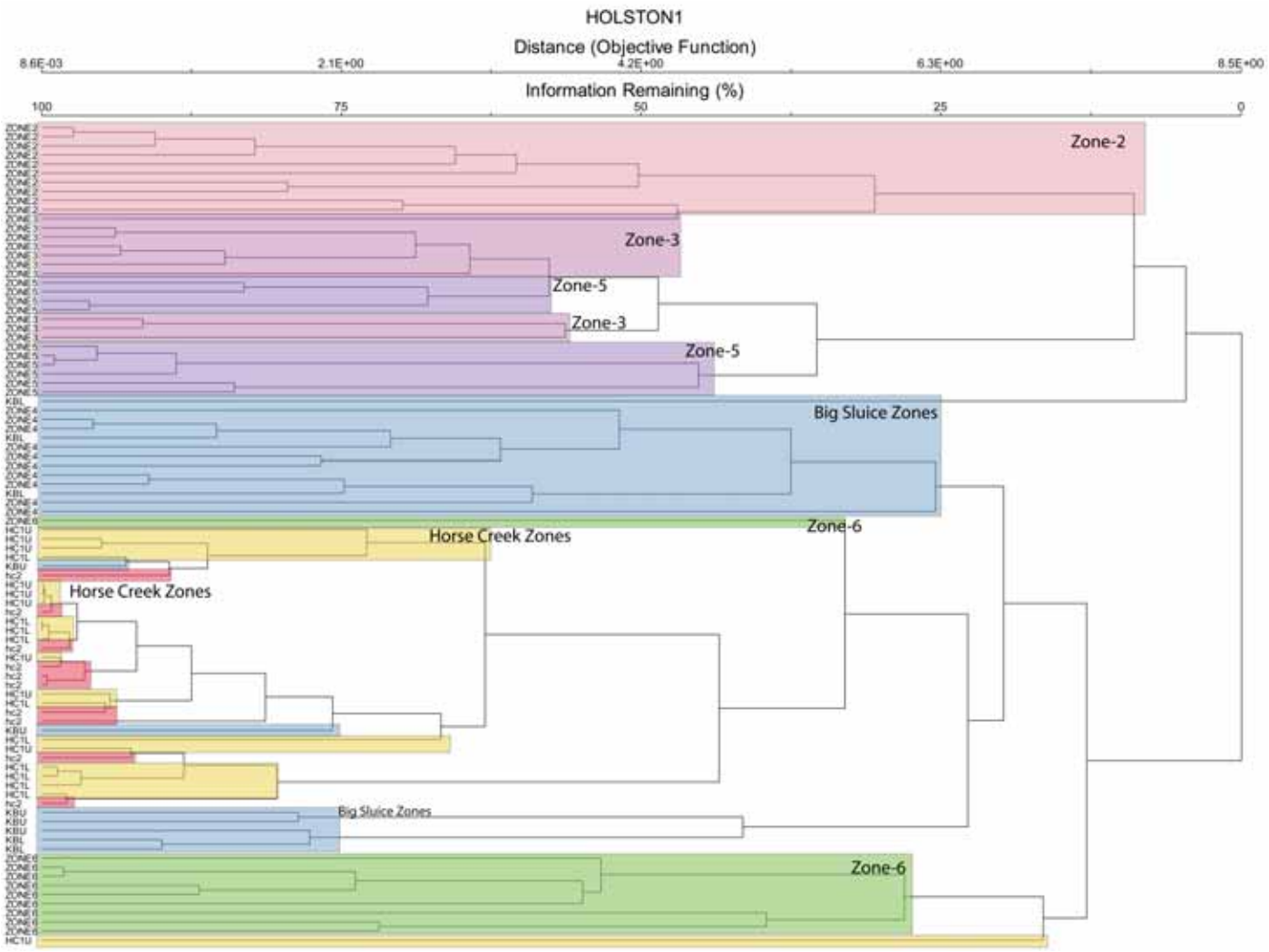


Figure 5.4.4. Cluster dendrogram depicting the degree of similarity among samples of the insect taxa collected from the Holston River in July 2010. This analysis included all the zones studied, including samples from the Horse Creek and Kit Bottom zones in addition to the Holston River zones. The left side of the diagram shows the zone from which the sample was collected. Samples linked together to the far left represent very similar community composition, whereas samples or sample groups that are not grouped up until the extreme right are very dissimilar.

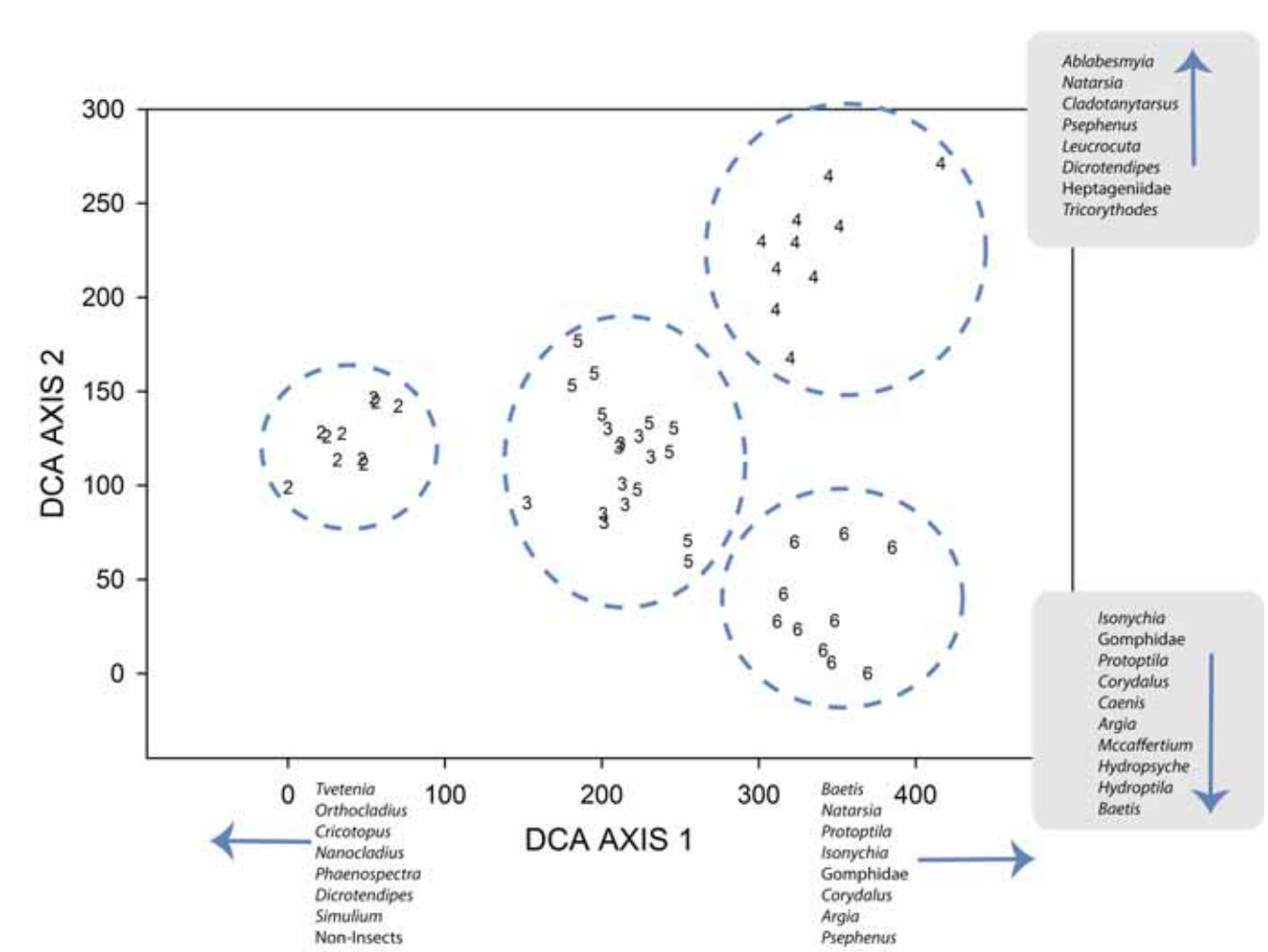


Figure 5.4.5. Detrended Correspondence Analysis (DCA) derived from quantitative samples of aquatic insect assemblages (non-insects represented as a single taxon) collected from Zones 2-6 on the Holston River, July 2010. Taxa tolerant to stress tended to pull samples to the right along the X-axis (DCA Axis-1), whereas species common in Horse Creek tended to pull samples upward along the Y-axis (DCA Axis 2). Most zones formed clusters of homologous groups highlighted by circles, which serve to identify the region occupied by each zone and have no statistical significance. Zone 3 and Zone 5 overlapped greatly in DCA-space and were intermediate on the disturbance gradient between Zones 2 and 6.

Generally, organisms indicating disturbance tended to pull samples to the left side of the DCA Axis-1[4]. Specific taxa with a high influence to pull the taxa to the left included midges (*Tvetenia*, *Orthocladius, Cricotopus, Nanocladius, Phaenospectra, Dicrotendipes*), black flies (*Simulium* sp.) and non-insects. The species with a right-ward pull on DCA Axis-1 included mayflies (*Baetis*, *Isonychia*), caddisflies (*Protoptila* sp.), damselflies/dragonflies (*Argia*, Gomphidae), midges (*Natarsia*), helgramites (*Corydalus*), and water pennies (*Psephenus*).

With regard to the axis explaining the second-most variance, DCA Axis-2, samples were pulled upwards by midges (*Ablabesmyia*, *Natarsia*, *Dicrotendipes*, *Cladotanytarsus*) water pennies (*Psephenus*) and mayflies (Heptageniidae, *Leucrocuta*, *Tricorythodes*). Samples were pulled downward by mayflies (*Isonychia*, *Caenis*, *Mccaffertium*, *Baetis*), dragonflies/damselflies (Gomphidae, *Argia*), caddisflies (*Hydropsyche*, *Protoptila*, *Hydroptila*) and hellgrammites (*Corydalus*).

Overall, the taxa with a strong left-ward influence are often associated with filamentous algae, sediment, disturbance, or a similar combination of factors. Many of the species with a pull to the lower right are more sensitive. The species that pulled samples to the upper-right were species abundant in Horse Creek. All samples from Zone 2 occurred in the far left of the plot, whereas zones minimally influenced by the dam (Zones 4, 6) occurred on the far right. The occurrence of samples from Zones 3 and 5 overlapping centrally underscores both the similarity of those two zones to each other and less influence of species tolerant to disturbance than at Zone 2.

5.4.2.3 Biological Metrics

Biological metrics synthesize community structure data in a form that can test hypotheses about changes in community function. When examining the figures associated with the biological metrics from each zone, it is important to keep in mind that the figures display the unadjusted mean metric scores (±1 standard error of the mean), but that the actual hypothesis tests were often performed on means corrected for one or more covariates. Thus, some figures may suggest a significant trend in the data that is actually not statistically significant after correction for environmental factors, and vice-versa. The interpretation of the pattern exhibited in each graph is based on the appropriate statistical test, and should be considered with each figure. It is also important to remember that statistical analyses of Horse Creek were performed separately from those for the other zones. Although the results are presented on the same graph, no comparisons of Horse Creek and the other zones were performed.

5.4.2.3.1 Total Abundance

Holston River: Abundance data were analyzed using natural logarithms. The transformed data passed both Levene's test of variance homogeneity and the Kolmogorov-Smirnov test of normality (Table 5.4.2, Fig. 5.4.6). The total abundance metric, the number of organisms per sample, was significantly greater at Zone 2 than at all other zones because midges were very

4 The first DCA Axis is a multidimensional axis that explains the greatest variation among all samples. It is expressed as a series of loading factors that are applied to each taxon in the n-dimensional hypervolume, where n=the number of taxa. Thus, species with a high negative loading factor move samples to the left, whereas species with a high positive loading factor move samples to the right along the DCA axis. Similarly, species with a high negative loading factor on the second DCA axis tended to pull samples downwards, and high positive loadings pulled samples upwards in the plot.

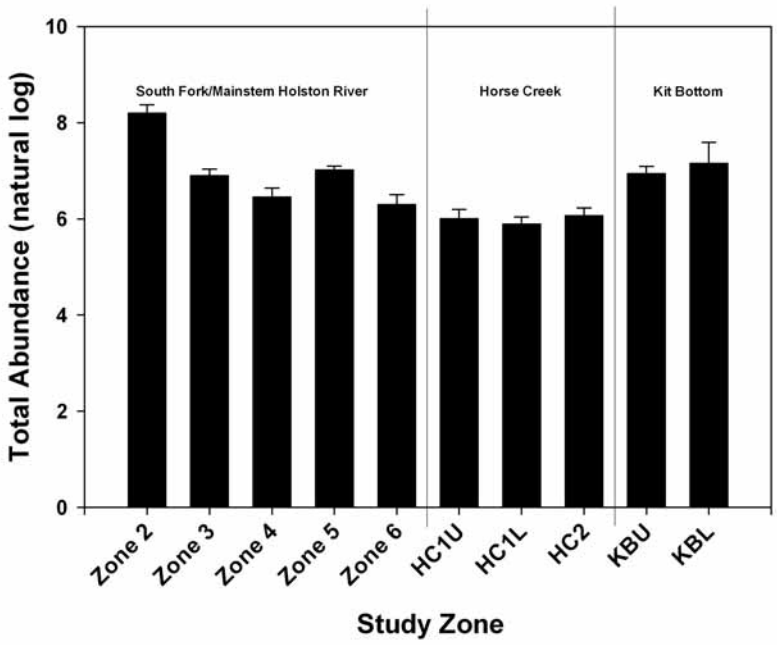


Figure 5.4.6. Natural log of the total abundance of aquatic insects per sample (±1 standard error) of benthic communities collected quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

abundant among the dense, closely appressed tufts of filamentous algae and moss that covered immobile (armored) substrata. Frequent and intense flow pulses and loss of upstream sediment have caused the rocky substrata to become tightly interlocked (armored) and relatively immobile, reducing the effects of scour and allowing fine particles to accumulate in the interstitial spaces among larger, interlocked spaces. This favored certain species adapted for life in tight spaces and excluded many of their predators.

Total benthic abundance is a notoriously difficult metric to interpret because some disturbances cause it to increase, whereas others may cause it to decrease. However, it is the foundation upon which all other metrics are calculated. The total density at Zone 2 ranged from about 32,000-149,000 organisms/m^2 (approximate average 82,000 org/m^2), whereas the aquatic invertebrate density Zones 3-6 ranged from about 3,000-36,000 individuals/m^2 (averages for Zones 3-6: 22,000, 15,000, 23,000, 13,000 organisms/m^2).

This pattern in abundance is common below impoundments, even without stream bed armoring and the accumulation of fine materials in interstitial spaces. For example, reservoirs act as nutrient sinks and establish planktonic production, exporting a steady crop of fine particulate organic material (FPOM) to lotic consumers downstream. The result is a predictable increase in the density of FPOM-consuming organisms below dams (collector-filterers, collector-gatherers). Note that other organisms will inhabit the tailwaters, but their relative abundance is lower because the total abundance has increased with the addition of gatherers.

The response signature of this metric on the South Fork and mainstem Holston rivers is the typical response signature observed below dams in eastern North America. The metric does not demonstrate an apparent response to Eastman's ongoing activities in the area.

In 1997, all zones had about the same average total abundance of invertebrates; 5,000/sample. In 2010, Zone 2 averaged about 4,000 individuals per sample, but all other sites had about 400-1,900 organisms per sample, on average. Total abundance is a highly variable measure. Possible reasons for the difference are numerous and include temperature, flexible life histories and life cycles of the insects, and impacts of the hydrological regime imposed by Fort Patrick Henry Dam. It would be irresponsible to speculate on the actual cause of this reduction without more data on emergence patterns of the aquatic insect populations of the Holston River.

Horse Creek: The natural log-transformed total abundance of invertebrates passed both Levene's test of homogeneity of variances and the Kolmogorov-Smirnov normality test. There were no significant differences in total abundance among the Horse Creek study zones (P= 0.905; Fig. 5.4.6; Table 5.4.3).

Kit Bottom: The transformed abundance data passed the tests for variance homogeneity and normality. There were no statistically significant differences among the zones on the Big Sluice (P=0.147; Fig. 5.4.6; Table 5.4.4), with none of the three covariates contributing significantly to the analysis of covariance model.

5.4.2.3.2 Taxa Richness

Holston River: The biological metric taxa richness failed Levine's test for homogeneity of variance because Zone 2 had significantly less variance in the richness of benthic invertebrates than the other sites—especially Zone 3 and Zone 6, both of which were represented by samples with a wide range of richness. Although richness rarely needs transformation, sometimes, when low values are compared to high values, transformation is required. Therefore, the comparison of the richness of benthic assemblages used log-transformed values. The metric passed the Kolmogorov-Smirnov test for deviation from normality.

After the transformation, the richness values passed Levine's test of variance homogeneity. Significant differences among the zones persisted after the variance due to covariates FLOW and PART were accounted for (Table 5.4.2, Fig. 5.4.7). Taxa richness exhibited a monotonic increasing gradient below the dam, with Zone 2 having significantly fewer taxa per sample than all other sites, and Zone 3 having fewer taxa per sample than subsequent zones.

In 1997, the metric was low on average at Zones 2-4 (~ 10 taxa per sample), moderate at Zone 5 (~15 taxa per sample) and high at Zone 6 (~ 22 taxa per sample). In 2010, Zone 2 averaged about 10 taxa, Zone 3 had ~14, Zone 4 had ~17, Zone 5 had ~20, and Zone 6 had ~22 taxa per sample. Thus, Zones 2 and Zone 6 exhibited very similar richness measures in 1997 and 2010, whereas the intervening sites all supported richer aquatic insect assemblages in 2010, indicating that conditions between Zones 2 and 6 have improved since 1997.

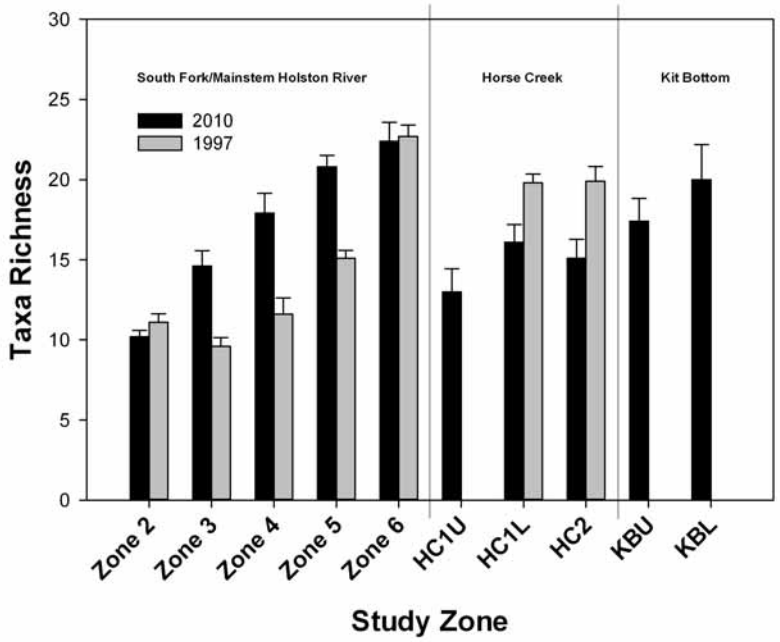


Figure 5.4.7. Mean number of insect taxa (taxa richness) (±1 standard error) of benthic communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010 and July/August 1997. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

Horse Creek: The taxa richness metric passed the tests for variance homogeneity and normality. There were no statistically significant differences in the richness of benthic insect assemblages of the three Horse Creek sites (P=0.215; Table 5.4.3).

Kit Bottom: The taxa richness metric passed the tests for variance homogeneity and normality. There were no statistically significant differences among the zones on the Big Sluice (P=0.553; Table 5.4.4).

5.4.2.3.3 Diversity (H')

Holston River: Shannon-Wiener Diversity passed the tests for normality and homogeneity of variances and was analyzed without transformation. The average diversity (H') ranged from about 1.7 at Zone 2 to about 2.6 at Zone 6. This gradient is similar to that observed for taxa richness; a monotonic gradient increasing downstream. Diversity was especially low below the Fort Patrick Henry Dam at Zone 2, and generally increased downstream (Fig. 5.4.8). Differences among zones persisted after the variance due to significant covariates (FLOW and PART) was accounted for. After correction for particle-size and near-substrata velocity, Zone 2 had the lowest diversity and Zones 3 and 4 were not significantly different from each other, but were significantly less diverse than Zones 5 and 6—which also were not different from each other (Table 5.4.2, Fig. 5.4.8). The metric did not demonstrate an apparent response to Eastman's ongoing activities in the area.

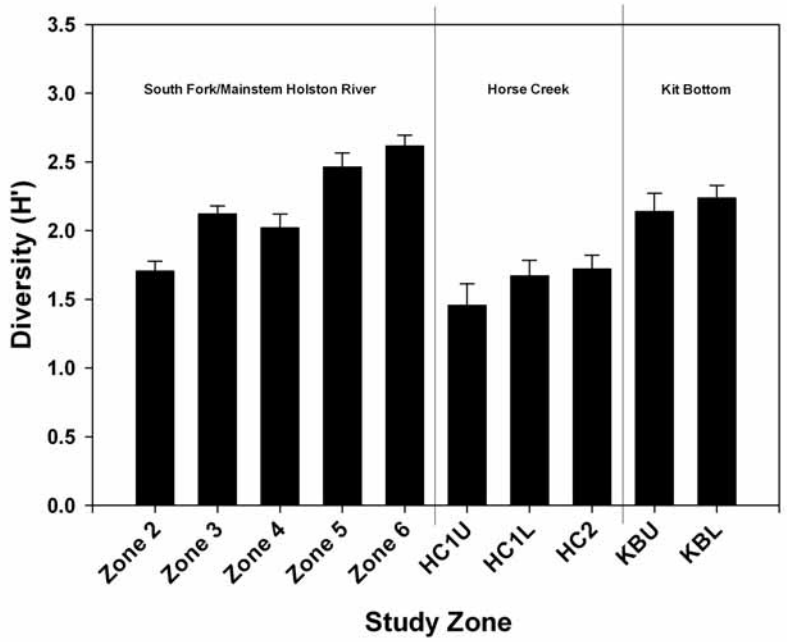


Figure 5.4.8. Mean Shannon-Wiener diversity (±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

Horse Creek: Shannon-Wiener Diversity passed the tests for normality and homogeneity of variances. There were no statistically significant differences among the three Horse Creek sites in terms of diversity (P=0.309; Table 5.4.3).

Kit Bottom: The Shannon-Wiener Diversity Index (H') data passed the tests for homogeneity and normality. There were no statistically significant differences among the zones on the Big Sluice, including Zone 4 (P=0.400; Table 5.4.4).

5.4.2.3.4 Evenness (J')

Holston River: The index of community evenness, Pielou's J', passed the tests for normality and homogeneity of variance and was analyzed without transformation. After correction for influence of FLOW and PART, statistically significant differences among sites persisted. Zone 2 and Zone 4 were not significantly different from each other but exhibited significantly lower evenness than the other three Holston River zones, which were not significantly different from each other. Zone 3 exhibited an intermediate level of evenness and was not significantly different from Zone 4, nor from Zones 5 and 6 (Table 5.4.2, Fig. 5.4.9).

The hypothesized response of this metric to detrimental effects of discharge at Zone 3 would have this metric significantly lower than the upstream reference and downstream recovery sites. However, this metric was significantly greater than the upstream reference and not significantly different from the downstream sites. The metric did not demonstrate an apparent response to Eastman's ongoing activities in the area.

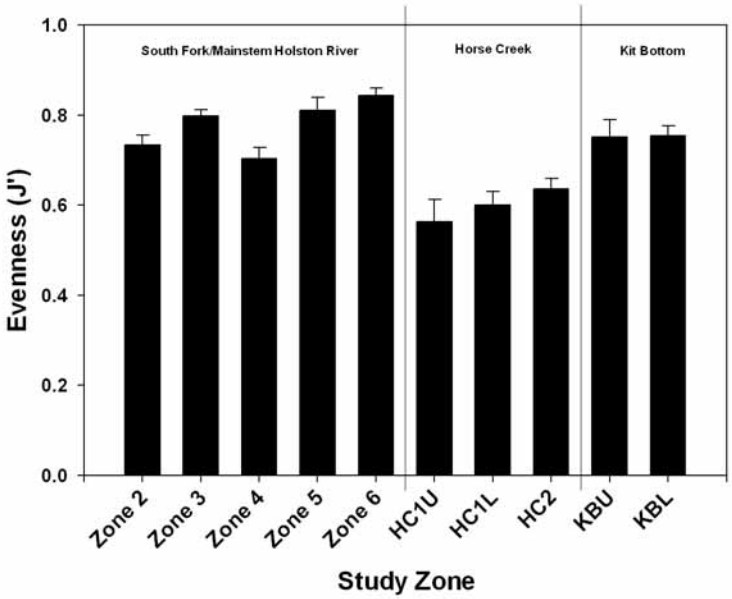


Figure 5.4.9. Mean community evenness (±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

Horse Creek: Community Evenness (J') passed the tests for normality and homogeneity of variances. There were no statistically significant differences in the amount of evenness expressed among the three Horse Creek sites (P=0.373; Table 5.4.3).

Kit Bottom: Community Evenness (Pielou's J') data passed the tests for variance homogeneity and normality. There were no statistically significant differences among the zones on the Big Sluice (P=0.347; Table 5.4.4).

5.4.2.3.5 EPT Index

Holston River: The EPT Index (richness) metric failed Levine's test for homogeneity of variance because Zone 2 had significantly lower richness than the other sites and therefore lower variation richness metrics than the other sites. Thus, although richness metrics do not usually require transformation, the EPT index was transformed using natural logarithms for the same rationale discussed for the metric Taxa Richness (above). When transformed, the EPT Index data passed Levene's test of homogeneity of variances, but failed the Kolmogorov-Smirnov test for normality. The problem with these data is that they appear to compare a binary variable with a continuous one, because samples from Zone 2 usually had zero EPT taxa and occasionally, one. When Zone 2 was excluded, the other zones passed the Kolmogorov-Smirnov normality test. This emphasizes how different the communities from Zone 2 are from the other zones.

The only covariate to explain a significant amount of variation in the richness of EPT orders was PART. After the variance related to PART was accounted for, significant differences among sites

persisted. Specifically, Zone 2 had significantly fewer kinds of EPT organisms than all others sites, and Zone 6 exhibited significantly more kinds of EPT organisms than all other zones, with samples averaging about 10 EPT taxa per sample. Zones 3, 4 and 5 did not differ significantly from each other in the richness of EPT orders (Table 5.4.2, Fig. 5.4.10). Note that although the p-values should be interpreted conservatively, Zones 3, 4, 5 and 6 were not likely to consist of congruent populations with those observed at Zone 2 (Tukey's HSD P<0.001).

This response signature is typical of the longitudinal gradient occurring below impoundments. Some species become so abundant downstream from dams that other species comprise a smaller portion of the overall community. Sorting protocols that use subsampling will always find lower richness when the abundance of a few species increases dramatically. The metric did not demonstrate an apparent response to Eastman's ongoing activities in the area. In 1997, the richness of EPT insects was very low at the farthest upstream zones; 0-1 at Zones 2 and 4, 0 at Zone 3, and 2 at Zone 5. Zone 6 was the only zone to have typical EPT richness in 1997 (~8 EPT taxa/sample). In 2010, all the zones except Zone 2 exceeded the results of 1997. This is especially true of Zone 3, which had no EPT taxa in 1997 and six taxa in 2010. Thus, this metric describes a substantial improvement of conditions on the Holston River in 2010, relative to 1997.

Horse Creek: The EPT Index passed the tests for normality and homogeneity of variances. There were no statistically significant differences in the amount of evenness expressed among the three Horse Creek sites (P=0.812; Table 5.4.3).

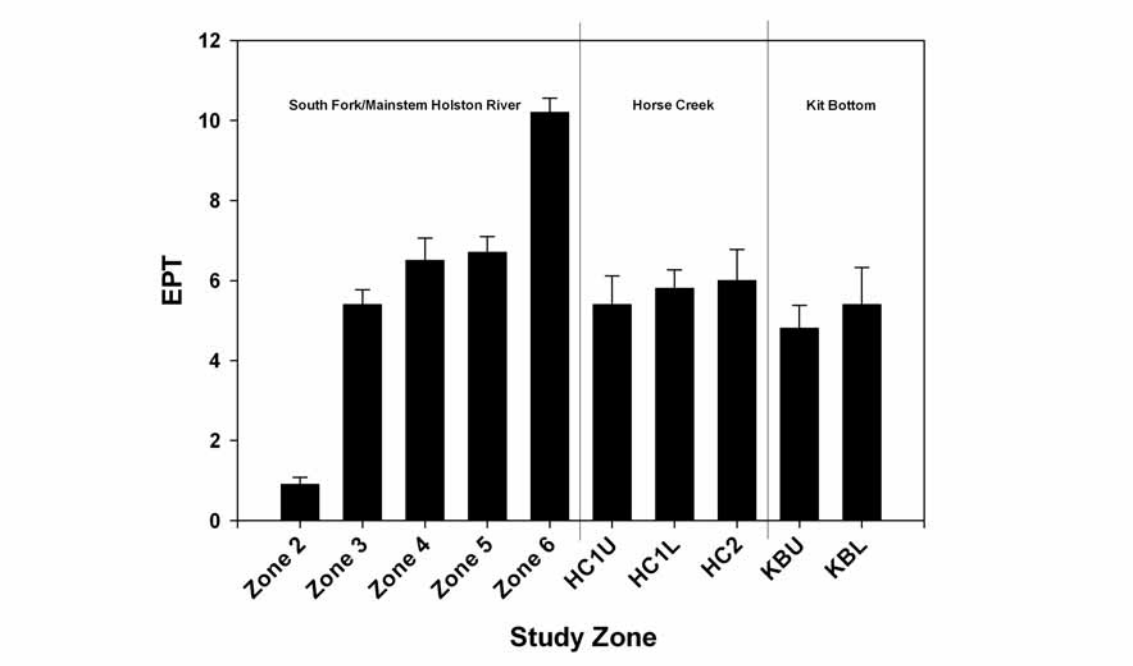


Figure 5.4.10. Mean EPT Richness Index (±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

Kit Bottom: EPT Index (richness) data passed the tests for variance homogeneity and normality. There were no statistically significant differences among the zones on the Big Sluice (P=0.201; Table 5.4.4).

5.4.2.3.6 Percent EPT

Holston River: The percent of the community represented by EPT insects failed both the test for homogeneity of variance and the test for normality. This often happens when proportional measures (such as percent EPT) have values below 30% and above 70%. A frequently-used transformation for these measures is the arc-sine transformation (e.g., Krebs 2009). After the transformation, the metric passed the test for variance homogeneity and the test for normality.

After transformation, none of the covariates explained a significant portion of the variance in the relative abundance of EPT insects. Thus the model was run without covariates (Table 5.4.2, Fig. 5.4.11). Zone 2 had significantly lower relative abundance of EPT insects than all other sites. Zone 4 had a significantly greater relative abundance of EPT insects than Zone 3 and Zone 5 because of one taxon in particular. The mayfly *Tricorythodes* sp. comprised a large portion of the samples collected from the Big Sluice. *Tricorythodes* mayflies have protective hairs, which help them resist abrasion from sand and sediment. Furthermore, they are often abundant among mossy substrata. Thus, the comparisons of other zones with EPT abundance at Zone 4 is influenced by the very high abundance of *Tricorythodes*, which is likely greatest at Zone 4 because of physical habitat availability. Zone 6 had a high EPT relative abundance represented by a more-moderate increase in the abundance of several taxa.

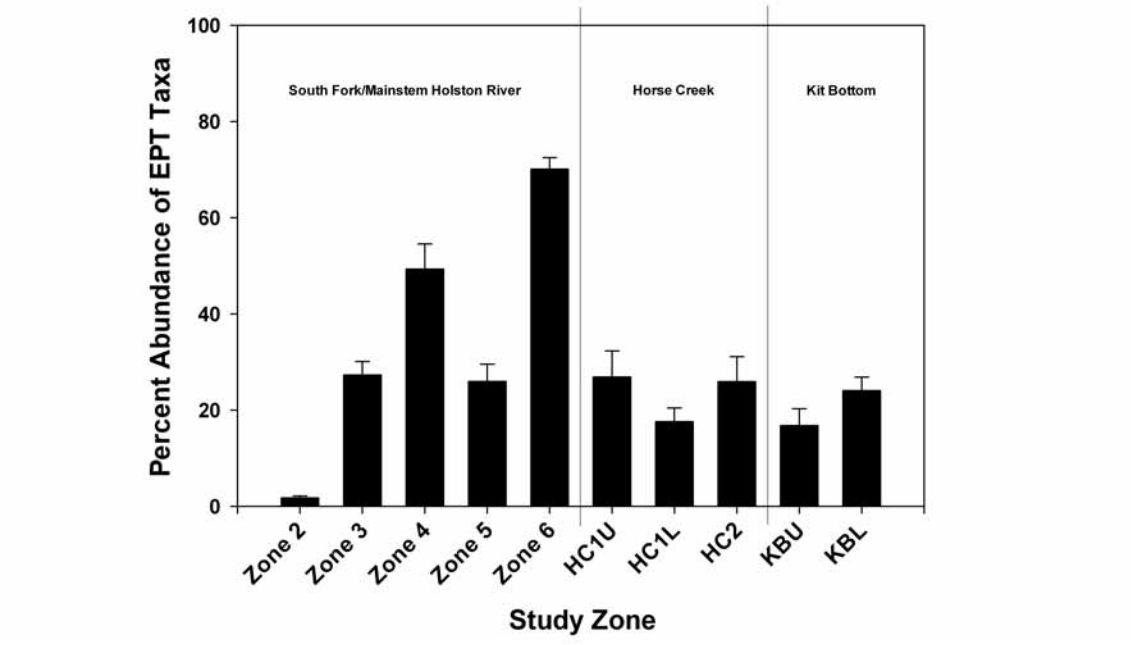


Figure 5.4.11. Mean percent relative abundance of EPT taxa (±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

Although the relative abundance of EPT insects at Zone 3 is lower than optimal for mid-reach rivers in the Appalachian Mountains (e.g., Zone 6, Fig. 5.4.11), it is a marked improvement from Zone 2 and, perhaps more importantly, from the results of the 1997 Holston insect survey, when no EPT organisms were in the quantitative samples at Zone 3 (note that the 1997 survey did not use the Percent EPT Abundance metric).

Horse Creek: The relative abundance of EPT insects passed the tests for normality and homogeneity of variances. There were no statistically significant differences in the amount of evenness expressed among the three Horse Creek sites (P=0.312; Table 5.4.3).

Kit Bottom: The relative abundance of EPT insects passed the tests for normality and homogeneity of variances. Significant differences persisted after the variance related to the only significant covariate (GREEN) was accounted for (Table 5.4.4, Fig. 5.4.11). Specifically, Zone 4 (below Kit Bottom) had significantly more EPT insects than either KBU or KBL. The zone above Kit Bottom (KBU) was not significantly different from the zone more potentially influenced by Kit Bottom (KBL). The pattern of EPT abundance in the Big Sluice zones presented a subtle gradient of increasing EPT relative abundance downstream. KBU had the lowest abundance of EPT organisms, KBL had an intermediate abundance of EPT organisms, and Zone 4 had many more EPT organisms in samples than KBU or KBL. The aquatic insect assemblages of Kit Bottom are expected to provide one of two response signatures if Kit Bottom were having a detrimental effect on the abundance of EPT species. Either the EPT abundance at KBL would be significantly lower than that observed for KBU and Zone 4, or KBU would have significantly greater EPT abundance than KBL and Zone 4. The former would occur if the insects found the Kit Bottom area locally inhospitable, but Zone 4 was suitable for colonization. The latter would occur if the zone above Kit Bottom was suitable for colonization, but the Kit Bottom area was inhospitable to EPT insects and degradation continued downstream through Zone 4. Neither alternative was observed, and the response appears to be a simple gradient of improvement, with the zone above the influence of Kit Bottom having the lowest contribution of EPT insects and the farthest downstream site having the greatest abundance of EPT insects.

5.4.2.3.7 Percent Chironomidae

Holston River: Although the non-transformed data passed the Kolmogorov-Smirnov normality test and Levene's test of homogeneity of variances, the test was run using arc-sine transformed data because some sites averaged less than 30% (Zones 4 and 6) or greater than 70% (Zone 2)—these extremities of the percent measures may be distorted by being forced between 1 and 100, and numerous authors caution against using untransformed percentages under these circumstances (e.g., Krebs 2009, Zar 1999).

The relative abundance of midges was greatest at Zone 2, where they comprised about 76% of the community (Table 5.4.2, Fig. 5.4.12). For a cobble-pebble bottomed Appalachian river, Zone 3 exhibited higher midge abundance than expected (53% of the community). This could easily be part of the recovery from the midge-dominated ecosystem below Fort Patrick Henry Dam, though it is noteworthy that the benthic substrata were cleaner at Zone 3 and not as armored as at Zone 2.

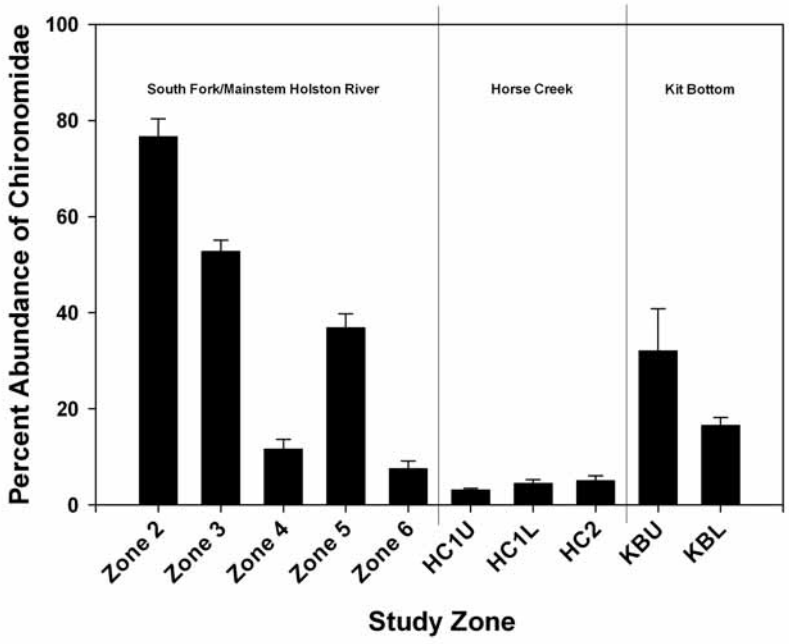


Figure 5.4.12. Mean percent abundance of chironomid midges (±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

In 1997, Zones 2, 3 and 5 all exhibited about 45-50% dominance by chironomid midges. Zone 4 had the highest percent of the community represented by Chironomidae (~68%) and Zone 6 had the lowest dominance by midges (~15%). Thus, in 2010, improvements in the abundance of midges were observed at Zones 4, 5 and 6. Zone 2, influenced by the frequent and extreme changes of flow, has had midges become a much larger constituent of the community. Zone 3 remains essentially unchanged compared to 1997.

Horse Creek: The arcsine-transformed percent abundance of Chironomidae passed the tests of homogeneity of variances and normality. There were no statistically significant differences among the three Horse Creek zones in terms of the percent abundance of chironomid midges (P=0.214; Table 5.4.3). Moreover, the abundance of midges was relatively low for all three of the sites; average midge abundance was less than 10% of the community for all three study zones. This is typical for small Appalachian streams, but atypical of streams in lightly to moderately urbanized streams. Thus this metric underscores the similarity among the three Horse Creek zones, but also suggests that the abundance of some tolerant organisms is surprisingly low for all three zones (Table 5.4.3).

Kit Bottom: The arcsine-transformed percent abundance of Chironomidae passed the tests of homogeneity of variances and normality. The particle size index and pigment index (PART, GREEN) explained a significant amount of the variation in midge abundance among these sites, but significant differences among the sites persisted (P=0.044). Specifically, KBU had significantly greater chironomid abundance than Zone 4, but was not significantly different from

KBL (Table 5.4.4, Fig. 5.4.12). Functionally, this response appears to represent a gradual downstream decline in the relative abundance of midges in the community.

If the community were impaired by leaching near KBL, one of two response signatures would be expected. Either the relative abundance of chironomids at KBL would be significantly greater than that observed for KBU and Zone 4, or KBU would have significantly fewer midges than KBL and Zone 4. The first scenario would occur if most insects found the Kit Bottom area locally inhospitable. The second scenario would occur if the impacts of leaching were sufficient to cause extensive reaches below Kit Bottom to be unsuitable for most aquatic insect species. Neither of these two response signatures was observed. The abundance of midges does not indicate any ecological impairment of the Big Sluice near Kit Bottom.

5.4.2.3.8 Non-insect Percent Abundance

Holston River: The percent abundance of non-insect taxa (Fig. 5.4.13) failed both Levene's test of equality of variances and the test of normality. Arcsine transformations also resulted in failure of these tests, but the combined transformation of ln-arcsine allowed the dataset to pass both the tests of normality and variance homogeneity. Zero values in the data set were responsible for the problems with both tests because non-insects (mostly tiny aquatic worms and mites) were especially low in percent abundance at Zone 6.

The covariate FLOW was the only one to explain a significant portion of the variance in the percent abundance of non-insects among the Holston River benthic assemblages in 2010. After correction for this covariate, a significant difference persisted among zones (P=0.002). Tukey's HSD test indicated that Zone 5 had significantly greater non-insect percent abundance than

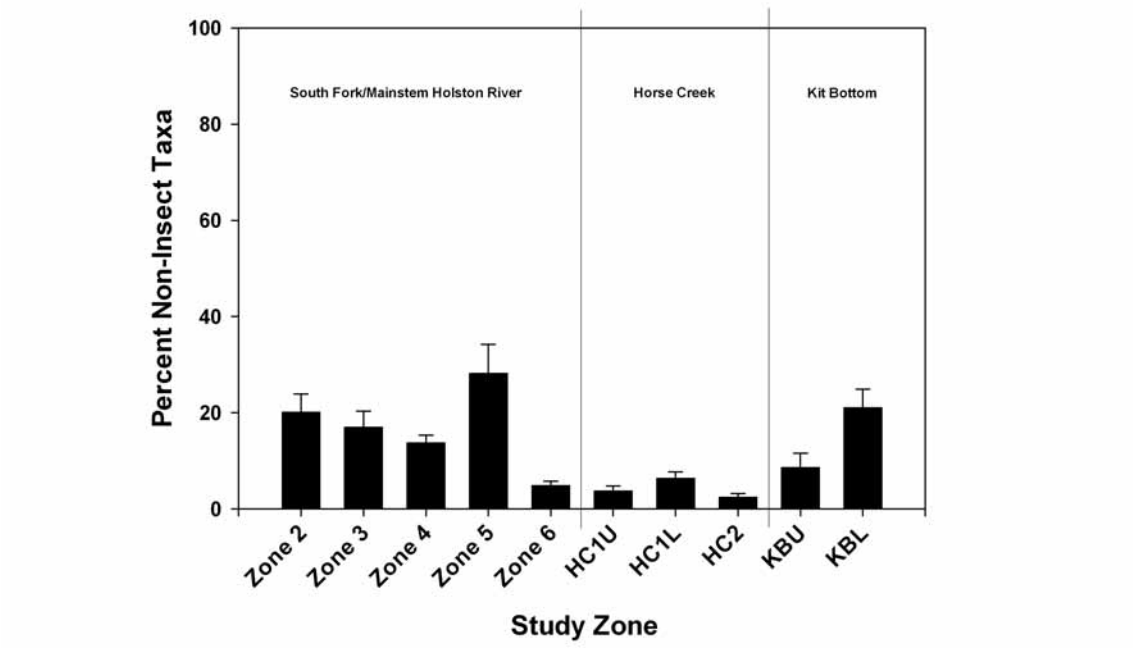


Figure 5.4.13. Mean percent abundance of non-insects (±1 standard error) of benthic communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

Zones 2 and 6, whereas Zone 3 was not significantly different from Zones 2, 4 or 5 (Table 5.4.2; Fig. 5.4.13). The non-insects of Zone 5 were primarily aquatic mites (often associated with the refugia provided by filamentous algae), but also included aquatic worms. Whereas, at Zone 2, they were primarily aquatic worms, but also included some aquatic mites. The locations with the greatest non-insect abundance appeared to occur in samples with filamentous algae. The pigment index was not significant because both moss and vascular hydrophytes contributed to the pigmentation of sample preservative. Thus, the macroinvertebrates of quantitative samples from Zone 5 may be reflective of localized anthropogenic influences.

Horse Creek: The percent abundance of non-insects passed the tests for normality and variance homogeneity after arcsine transformation. There were significant differences among the three Horse Creek zones (P=0.003; Table 5.4.3, Fig. 5.4.13). Zone HC1L had significantly more non-insects than HC2. Zone HC1U was not significantly different from Zone HC1L or HC2.

The expected response signature for impairment at Zone HC2 is that non-insects would have been most abundant at Zone HC2. However, Zone HC2 had significantly fewer non-insects than one upstream site and was not significantly different from the other one. Thus these results do not indicate any impairment of Zone HC2.

Kit Bottom: The percent abundance of non-insects passed the tests for variance homogeneity after arcsine transformation, but failed the test for normality. Therefore the Kruskal-Wallis non-parametric ANOVA was used to test for differences among zones to assess the communities near Kit Bottom on the Big Sluice (Table 5.4.4, Figure 5.4.13). The Dwass-Steel-Critchlow-Fligner test indicated that KBU and KBL had significantly more non-insects than Zone 4, but that KBU and KBL were not significantly different from each other.

Efforts to make Zone 4 samples comparable with other Holston River samples with respect to depth and velocity required sampling around and below mid-channel bars. The only significant difference that suggested an impact was the relative abundance of non-insect taxa at KBL. This metric often increases in places where conditions become inhospitable to aquatic insects. A re-evaluation of these data indicated that the higher average relative abundance of non-insects was due to one sample from KBL which exhibited elevated oligochaete worm density of about 1,440 worms/m^2; whereas most other samples contained less than 50 worms/m^2. These are small aquatic worms hardly visible to the naked eye. One of the reasons 10-16 samples are collected from a zone is to minimize the influence of a single aberrant sample. We collected fewer samples from the Kit Bottom sites, which allowed a single outlier to have a greater influence on the site average for this metric. When this single sample was removed, the difference among zones was no longer statistically significant. Kit Bottom samples were collected very close to the bank, where physical characteristics can cause localized high concentrations of some small worm species. Therefore, we do not believe this metric provides evidence that Kit Bottom significantly altered the benthic community structure of the Big Sluice.

The expected response signature for this metric is that impaired sites should have had more non-insects than others. Thus, if the success of aquatic insects in the Sluice were influenced by leachate from Kit Bottom, the non-insect macroinvertebrates (mostly small mites and worms) would be expected to be greatest at KBL, with latent effects possibly expressed as far

downstream as Zone 4. However, Zone 4 had significantly fewer non-insect invertebrates than the two other zones on the Sluice, which were not significantly different from each other. Thus these results do not indicate that Kit Bottom has impaired communities of aquatic insects.

5.4.2.3.9 The North Carolina Biotic Index (NCBI)

Holston River: The biotic index score was calculated using the tolerance values provided primarily by the North Carolina Biotic Index (NCBI; Lenat 1993) and augmented by Hilsenhoff (1987) and Barbour et al. (1999) when necessary. The actual tolerance values used are presented in the methods section of this report. The NCBI failed Levene's test of variance homogeneity and most reasonable transformations similarly failed the test. Ultimately, the Kruskal-Wallis non-paramentric test was used, which is less sensitive to assumptions of normality and variance homogeneity. The NCBI values were significantly different ($P<0.001$) among the zones and the Conver-Inman Test for Pairwise Comparisons indicated that each zone was significantly different ($P<0.05$) from each other, with the exception of Zones 3 and 5, which were not significantly different from each other.

The general trend was a decline in NCBI from upstream to downstream (Fig 5.4.14). High values of NCBI indicate communities dominated by species that are tolerant to organic enrichment. Generally, these are species with tolerance to low dissolved oxygen concentrations, tolerance to sedimentation, or association with filamentous algae. Zone 2 had the greatest NCBI scores, followed by Zones 3 and 5, then Zone 4. Zone 6 had the lowest NCBI scores according to the Conver-Inman Test. Other than Zone 6, the zones had a somewhat higher NCBI score than anticipated for central Appalachian rivers. The apparent NCBI suggests a downstream gradient below the dam. The influence of the non-insects at Zone 5 (on average 30% of the assemblage) caused the NCBI to increase significantly.

The relationship of the NCBI was not tested in the context of covariates in the GLM model, because it failed the test of assumptions even after transformation. However, the metric was correlated with the amount of photosynthetic pigments in the sample (Fig. 5.4.15).

Horse Creek: The NCBI for Horse Creek zones passed the tests of homogeneity of variances and normality without transformations. The GLM model did not detect any significant differences among the three zones on Horse Creek. Moreover, the average scores were relatively low indicating the presence of sensitive organisms. Thus, this metric does not suggest any impairments of the HC2 zone (Table 5.4.3).

Kit Bottom: The NCBI metric passed the tests for variance homogeneity, but failed the test for normality even after several transformations. Therefore the Kruskal-Wallis non-parametric ANOVA was used to test for differences among the zones to assess the communities near Kit Bottom on the Big Sluice (Table 5.4.4, Fig. 5.4.14). The Dwass-Steel- Critchlow-Fligner test indicated that KBU and KBL had significantly greater NCBI scores than Zone 4, but that KBU and KBL were not significantly different from each other. Associations with aquatic plants appeared to have a moderately strong influence on this metric, most likely due to the non-insects associated with algae and moss (most of these taxa have above average tolerance values in the NCBI and HBI).

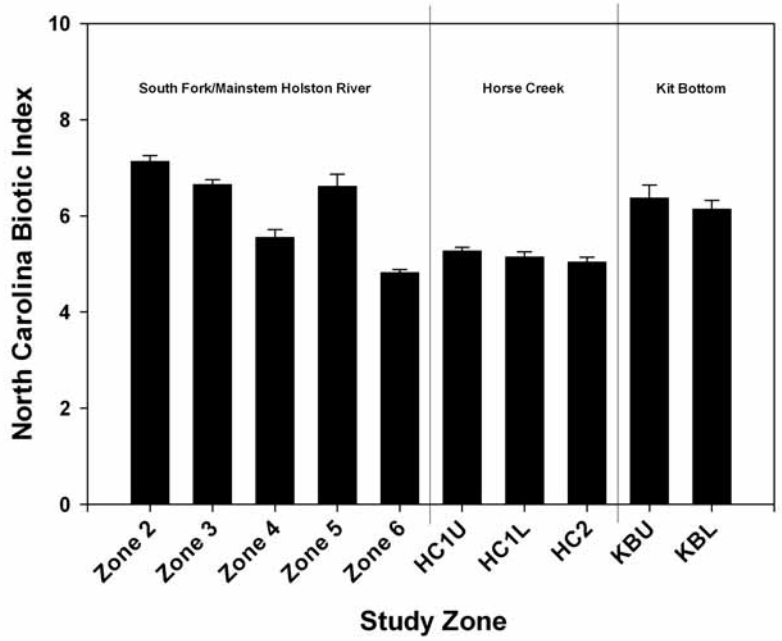


Figure 5.4.14. Mean North Carolina Biotic Index (±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

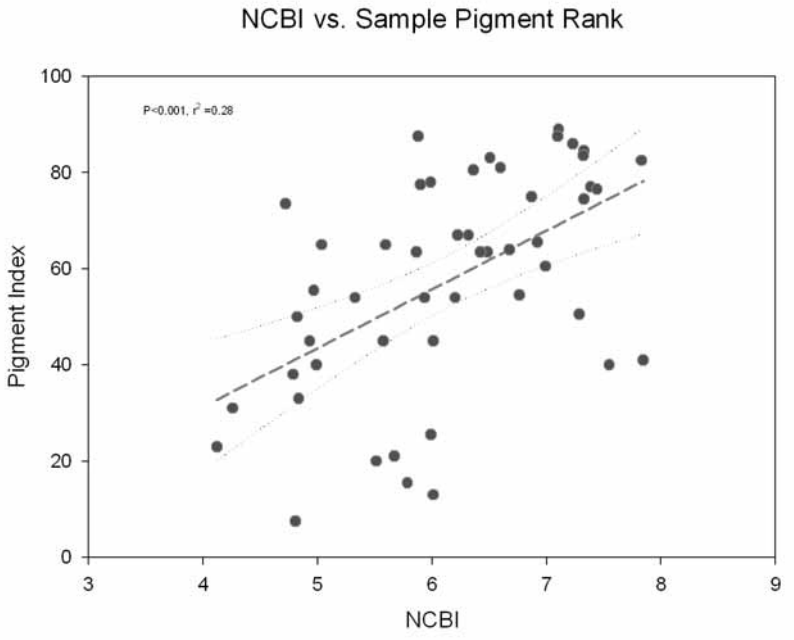


Figure 5.4.15. Relationship between NCBI and the Pigment Index of samples collected from the Holston River Zones (Zones 2-6). The line denotes the linear least-squares regression plot with 95% confidence interval of the regression noted by dotted curves to either side of the regression ($n=50$, $p<0.001$, $r^2=0.285$).

The expected response signature for this metric is that impaired sites should have had greater NCBI metric scores than others. Thus, if the success of aquatic insects in the Sluice was influenced by leachate from Kit Bottom, the NCBI score would be expected to be greatest at KBL, with latent effects possibly expressed as far downstream as Zone 4. However, Zone 4 had significantly lower NCBI scores than the two other zones on the Sluice, which were not significantly different from each other (Fig. 5.4.14). Thus these results do not indicate that anthropogenic stressors related to Kit Bottom have impaired the success of aquatic insects.

5.4.2.3.10 Functional Feeding Group Analysis

When the effects of ecological perturbations are sufficiently pervasive, they may cause changes in the abundance of whole functional groups of organisms. Thus the relative abundance of the five major functional feeding groups is often used as a surrogate to assessing changes in benthic food web structure. Relying on these measures alone to assess changes in community function has been criticized because large changes in the trophic structure are required before these metrics respond (e.g., Karr and Chu 1999). Functional feeding group metrics have been found to respond to urbanization (ANSP 2001) and ground water contamination.

The relative abundances of different functional feeding groups are not independent variables; when the percent abundance of collector-gatherers increases, the relative abundance of one or more groups must decrease. It makes more sense to consider the changes simultaneously rather than as individual metrics.

Holston River: The percent abundance of collectors (collector-gatherers + collector-filterers) was arcsine transformed as usually required for proportional measures to meet the assumptions of normality. However, the abundance of collectors failed Levene's test for variance homogeneity because many of the samples were 100% collectors and most samples were very close to that value (Table 5.4.2; Fig. 5.4.16). None of the covariates were correlated significantly with the abundance of collectors (step-wise GLM $P>0.15$). The test was re-run with the more conservative Kruskal-Wallis test followed by the Dwass-Steel-Critchlow-Fligner test for determining the significance ($\alpha=0.05$) of inter-zone differences. The abundance of collectors was significantly different among zones (Kruskal-Wallis $P<0.001$) and Zone 2 had significantly more collectors than all other zones, which were not significantly different from each other (Table 5.4.2).

Collectors are generalists, which often become dominant at disturbed locations in streams. There are two noteworthy observations regarding the relative abundance of collectors. First, the metric suggested that Zone 2 was more disturbed than any other zone. Second, the abundance of collectors at Zone 3 was not significantly different from Zones 5 and 6.

Sometimes, more intermediate disturbances result in an increase in primary production, without dramatic increases in collectors. In these cases, scrapers or herbivore-piercers increase in dominance. These data also failed Levene's test for variance homogeneity because of the near-complete dominance of collectors at Zone 2. Therefore, the same procedure used for collectors was used to assess the percent abundance of scrapers and herbivore-piercers.

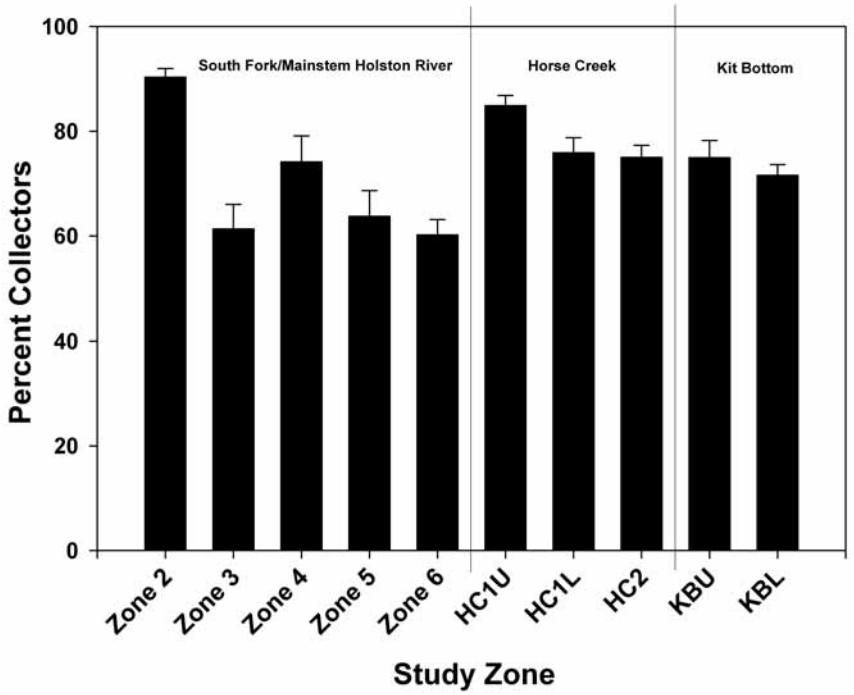


Figure 5.4.16. Mean percent collectors (gatherers + filterers) dominance (percent ±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

The percent abundances of scrapers were significantly different among the Holston River zones. As expected, Zone 2 had significantly lower scraper abundance than all other sites (Fig. 5.4.17; Table 5.4.2). Zone 6 had significantly greater scraper percent abundance than Zones 3, 4 and 5, which were not significantly different from each other. These results do not indicate any impairment of the zones. About 24% of the community at Zone 6 was comprised of scrapers, which is not unduly dominant for a mid-reach river. The herbivore-piercers (mostly *Hydroptila*) were most abundant at Zone 6 (Fig. 5.4.18), with Zones 2 and 4 having very few herbivore-piercers. The abundance of these organisms probably reflects the greater abundance of macrophytes in the sampling area at Zone 6.

Shredders are one of the most important groups of aquatic insects in central Appalachian streams because their feeding affects the quality and quantity of food available to all other functional feeding groups. Their role is an especially important linkage between riparian and stream communities of deciduous forests. However, in mid-reach rivers, like the Holston around Kingsport, their role is not quite as prominent as it is in headwaters because there is less deciduous cover, greater solar radiation input, increase in the role of autochthonous production, a corresponding decrease in the role of allochthonous production, and an increase in the role of scrapers. Thus, although shredders are an important constituent, they were not expected to dominate in any particular zone of the Holston River.

The abundances of shredders were low at all sites, and zero for most Zone 2 samples. Thus, the metric failed the test of homogeneity of variances and the Kruskal-Wallis test was used to assess

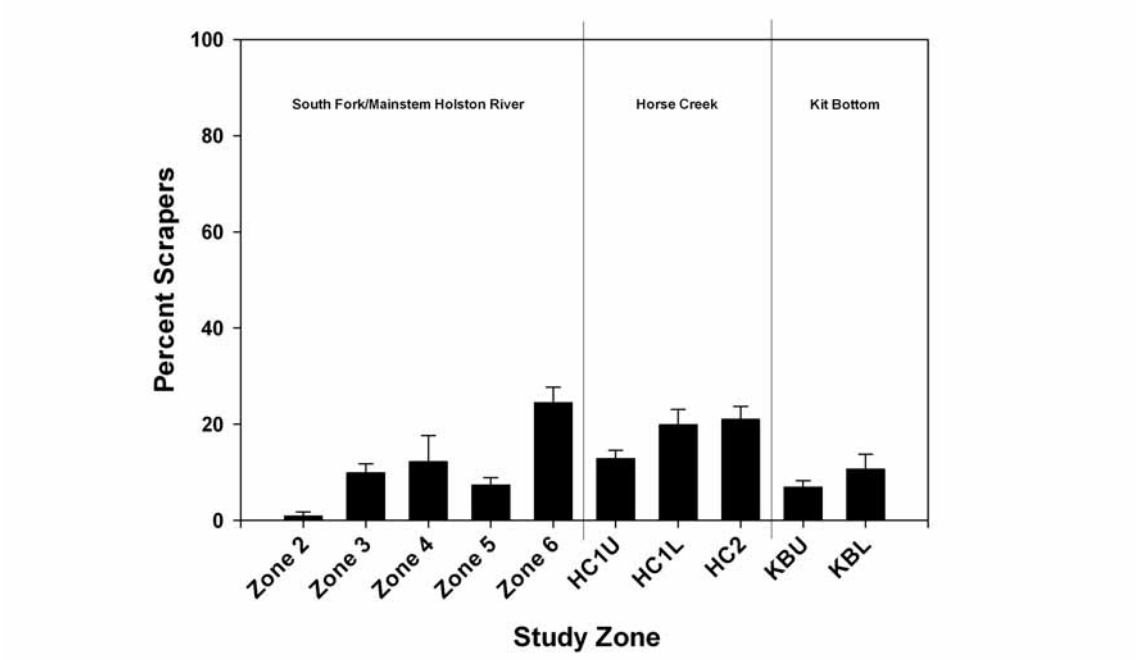


Figure 5.4.17. Mean percent scraper dominance (percent ±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

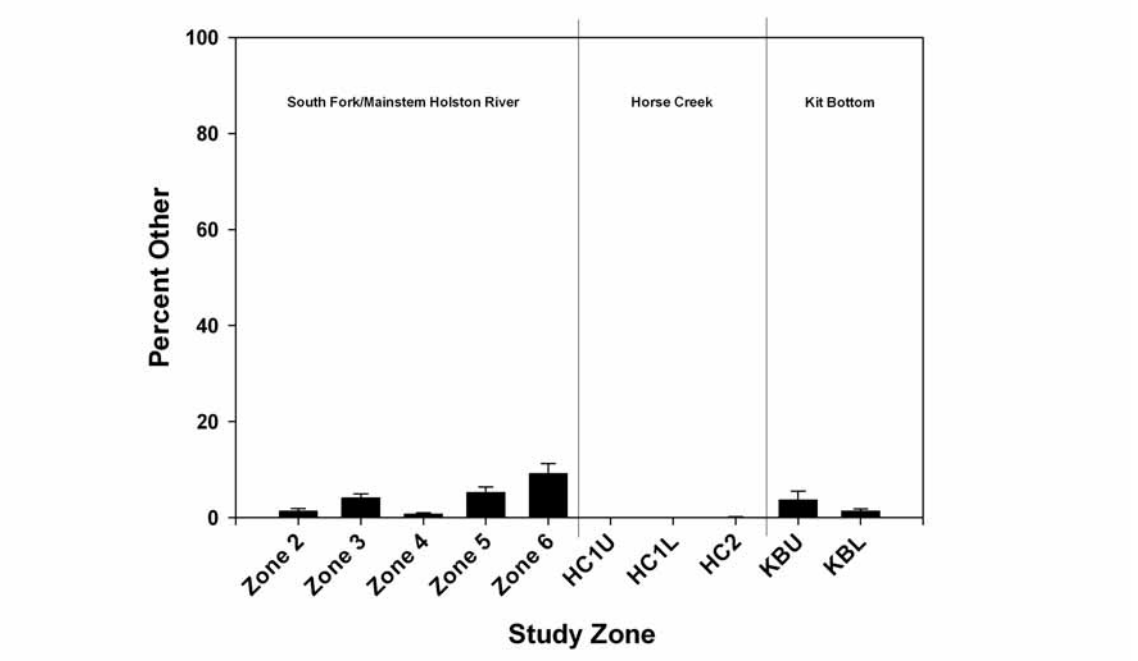


Figure 5.4.18. Mean percent herbivore-piercer (“other”) dominance (percent ±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

differences among zones. Although there were significant differences among the zones ($P<0.001$), the Dwass-Steel-Critchlow-Fligner test indicated that shredder abundance was greatest at Zones 3 and 5. Lowest relative abundance of shredders occurred at Zone 2, where the community below the dam was dominated by collectors. The shredder metric can vary dramatically because of the clumped distribution of the resources upon which they feed. Thus, values from 5-30% of the community are not uncommon. The only location with an unusual shredder abundance was Zone 2, from which many samples contained no shredders, due to influence of the Fort Patrick Henry Dam.

Horse Creek: The percent abundances of most functional feeding groups among the Horse Creek zones passed the tests for normality and homogeneity of variances. A non-parametric ANOVA was used for shredders, because they had zero abundance in several samples. Significant differences were observed among the shredders, scrapers and collectors, but not herbivore-piercers, which had zero abundance in most samples and could not be tested (Table 5.4.3, Figs. 5.4.16-5.4.19).

All covariates influenced the abundance of collectors, whereas scrapers were only influenced by the flow velocity and particle size. They were most likely not affected by the pigment index (GREEN) because there were very few green plants in the riffles of Horse Creek. Scrapers were mostly water pennies (*Psephenus herricki*) which were very abundant and feed almost exclusively on diatoms and the related biofilms. No covariates contributed significantly to the variation in the abundance of shredders.

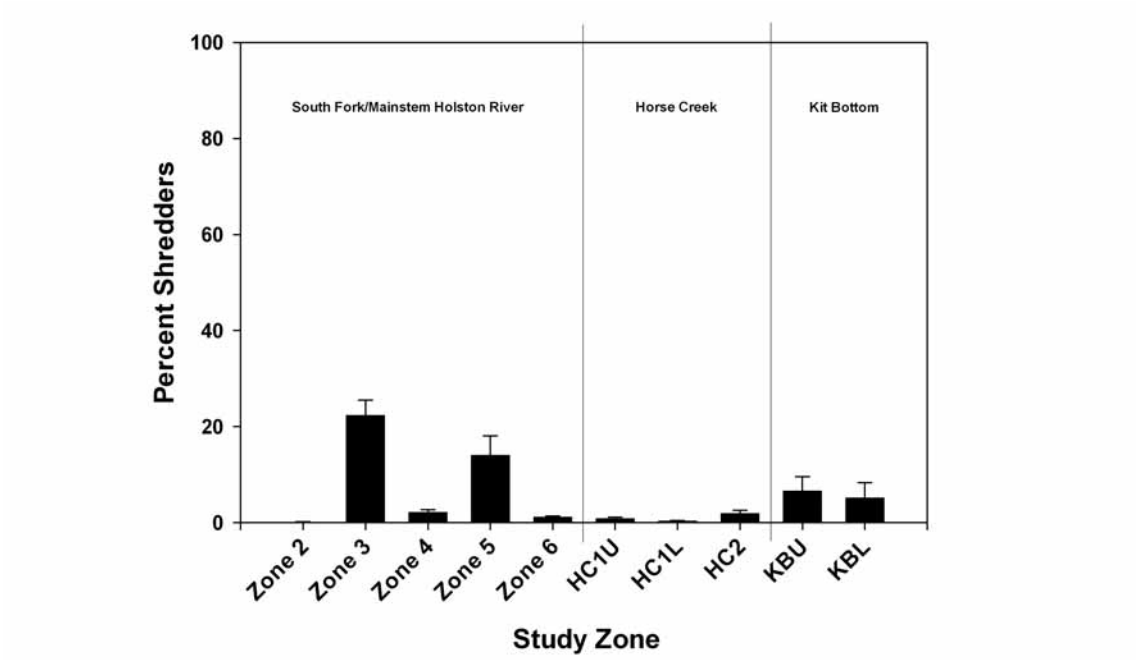


Figure 5.4.19. Mean shredder dominance (percent ±1 standard error) of aquatic insect communities sampled quantitatively at zones on the Holston, Horse Creek, Kit Bottom and the Big Sluice, July 2010. Data are means and standard error of untransformed data. Statistical analyses typically used data transformations, whose means will differ from those displayed here.

Collectors were significantly more abundant at Zone HC1U than at Zones HC1L and HC2, which were not significantly different from each other (Table 5.4.3). The response signature that would indicate impairment at Zone HC2 would include an elevated contribution of collector-gatherers at Zone HC2 relative to the other zones. However, Zone HC1U had about 10% more collectors than both Zones HC1L and HC2. Thus these results do not indicate any disturbance of Zone HC2 relative to the other sites, but do suggest that there may be some fundamental differences between the two upstream sampling locations.

If a disturbance at Zone HC2 propagated a change in trophic state in the river by providing nutrients for plant growth, an increase in scrapers or herbivores-piercers at Zone HC2 relative to the other zones would be expected. The results indicate (Table 5.4.3, Fig. 5.4.18) that Zone HC1L had significantly more scrapers than at Zone HC1U (about 8%). This is likely because part of Zone HC1U is partially covered by a four-lane bridge which shades the stream. This reduction in scrapers could be responsible for the corresponding increase in the percent abundance of collectors at HC1U.

Shredders are a keystone element of shaded small streams in eastern North America. All three of the Horse Creek zones had an average relative abundance of shredders of about 0.5-2.0% of the community, which is relatively low for Appalachian forest streams. The low values are likely related to seasonality and life-histories of shredders, which are adapted to follow an autumn pulse of detritus with rapid growth and spring emergence. Many shredders are sensitive to pollution, which can foul their gills or contaminate their food supply. Therefore, the response signature of an impact at Zone HC2 would be a marked decrease in shredders at Zone HC2 relative to the other sites. However, most zero-values for the abundance of shredders occurred at Zone HC1L, which had significantly fewer shredders than Zone HC2. Zone HC2 was not significantly different from Zone HC1U. Thus, the survey did not indicate any reduction in the success of shredders at Zone HC2.

Kit Bottom: The relative abundances of the functional feeding groups among the Kit Bottom zones failed either the tests of normality or variance homogeneity, so all of the comparisons were made using the Kruskal-Wallis non-parametric ANOVA followed by the Dwass-Steel-Critchlow-Fligner test when appropriate. Since all the tests used the Kruskal-Wallis test, it was not possible to account for the influence of covariates on the relative abundance of different functional feeding groups near Kit Bottom. However, there were no statistically significant differences detected in any of the functional feeding groups among the zones collected from Kit Bottom (Table 5.4.4).

5.4.2.4 Dominant Taxa

This section briefly presents the 10 dominant taxa at each zone. These data are from quantitative samples, but observations are qualitative and did not involve any statistics.

5.4.2.4.1 Holston River

Zone 2 was dominated by the midges *Orthocladius, Cricotopus, Tvetenia,* and *Dicrotendipes*. Small oligochaete worms and flatworms were also dominant. Together, these tolerant taxa comprised well over three quarters of the macroinvertebrates collected (Fig. 5.4.20).

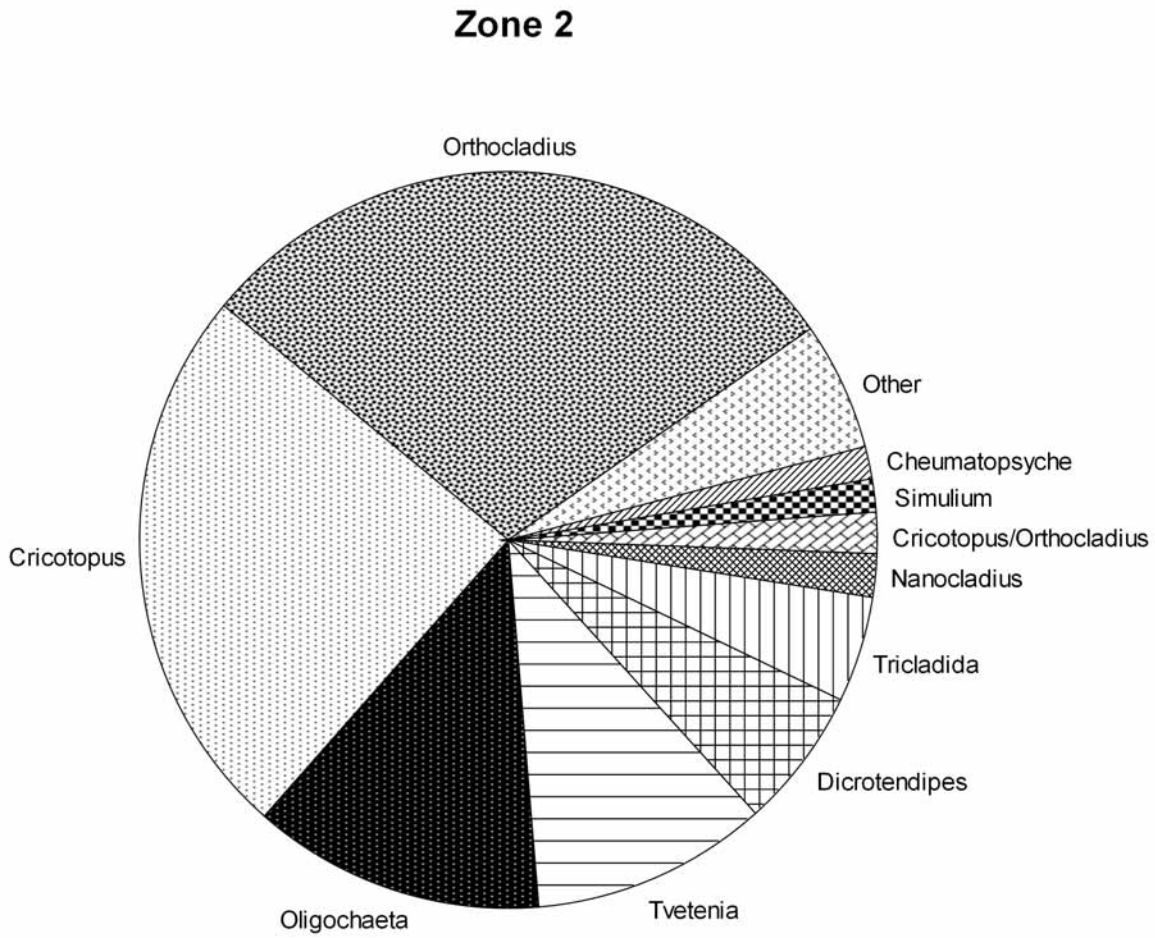


Figure 5.4.20. Relative abundance of the 10 most abundant taxa at Zone 2. These figures are based on the average (n=10) abundance of all taxa from each zone.

Zone 3 was also dominated by some midges (*Polypedilium*, *Cricotopus*, *Micropsectra* and *Dicrotendipes*) and oligochaete worms, but these taxa together only comprised about half of the sample (Fig. 5.4.21). It was noted in the qualitative section of this report that mayflies and caddisflies occurred at Zone 3 in 2010, but not in 1997. Some caddisflies (*Cheumatopsyche, Psychomyia* and *Hydroptila*) were among the dominant taxa in 2010.

The sediment-tolerant mayfly *Tricorythodes* was by far the most abundant taxon at Zone 4 in 2010 and comprised about one third the community (Fig. 5.4.22). Other mayflies were found (*Baetis* and *Maccaffertium*), but these only accounted for a small portion of the community.

Zone 5 was dominated by tiny oligochaete worms that were often associated with algae or moss on the substrata. Two midges (*Polypedilium* and *Dicrotendipes*) were also among the 10 dominant taxa (Fig. 5.4.23). Together these three taxa comprised about one third of the community. Although dominance by midges is often an indicator of stress, it is noteworthy that there were actually fewer midges among the dominant taxa at this zone than at the upstream zones.

Mayflies (*Maccaffertium*, *Isonychia*, *Baetis*, *Tricorythodes* and *Caenis*) were the most abundant organisms at Zone 6 and collectively comprised almost one half of the community. Caddisflies (*Hydropsyche*, *Protoptila*, *Hydroptila* and *Cheumatopsyche*) comprised about a quarter of the community (Fig. 5.4.24). Thus, the EPT taxa (mayflies, caddisflies and stoneflies), comprised nearly three quarters of the community.

5.4.2.4.2 Horse Creek

The similarity of all Horse Creek zones is further underscored by the list of dominant taxa. The riffle beetle *Stenelmis* comprised most of the communities at all three zones (Figs. 5.4.25, 5.4.26, 5.4.27). Water pennies (*Psephenus herricki*) were also abundant in Horse Creek and among the dominant taxa at all three zones. They were less abundant at Zone HC1U than at the two lower sites. This may be due to decreased food quantity or quality from the bridge that shades much of Zone HC1U. The finer particle size distribution at Zone HC1U compared to Zone HC2 may also contribute to the reduced abundance of *Psephenus*.

5.4.2.4.3 Kit Bottom

Both Kit Bottom (Figs. 5.4.28, 5.4.29) sites were dominated by *Stenelmis*, which was also abundant farther downstream at Zone 4 (Fig. 5.4.22). Both sites also supported abundant *Tricorythodes* mayflies—though they did not comprise as much of the community as observed downstream at Zone 4 (Fig. 5.4.22). The two Kit Bottom sites supported similar proportions of oligochaete worms, water mites (*Acari*), *Polypedilium* midges, and other taxa. There were very few differences between these two zones in terms of dominant taxa.

5.4.3 Quality Assurance

Sorting efficiency checks indicated that 93.5% of all individuals quantitatively collected were separated from associated debris and enumerated. A voucher collection has been made representing the species collected in this survey.

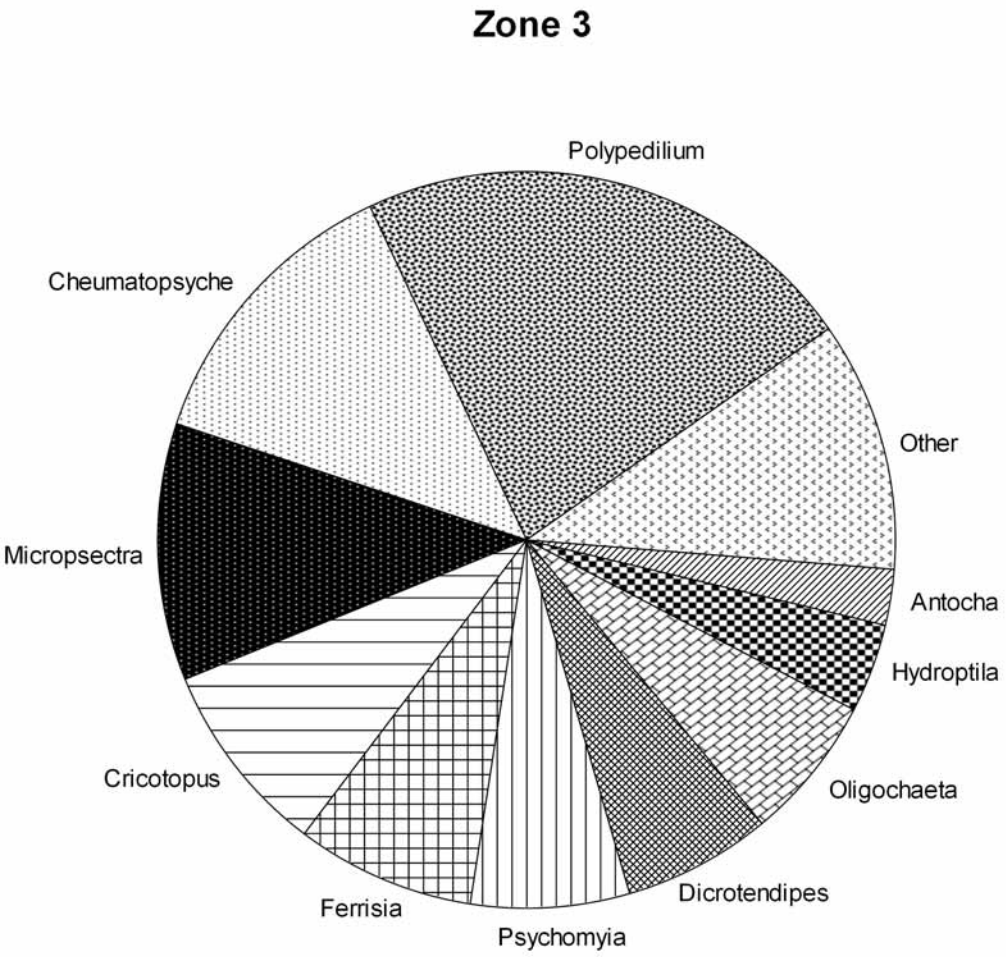


Figure 5.4.21. Relative abundance of the 10 most abundant taxa at Zone 3. These figures are based on the average (n=10) abundance of all taxa from each zone.

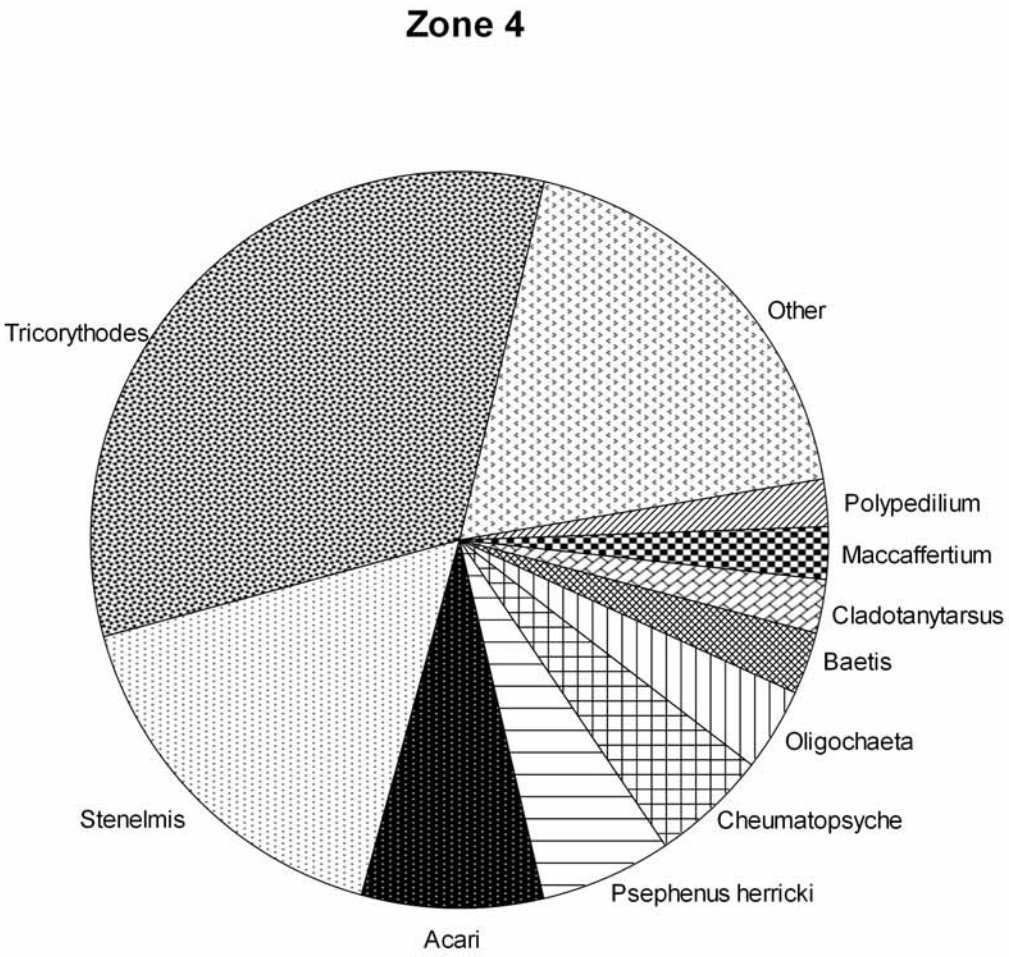


Figure 5.4.22. Relative abundance of the 10 most abundant taxa at Zone 4. These figures are based on the average (n=10) abundance of all taxa from each zone.

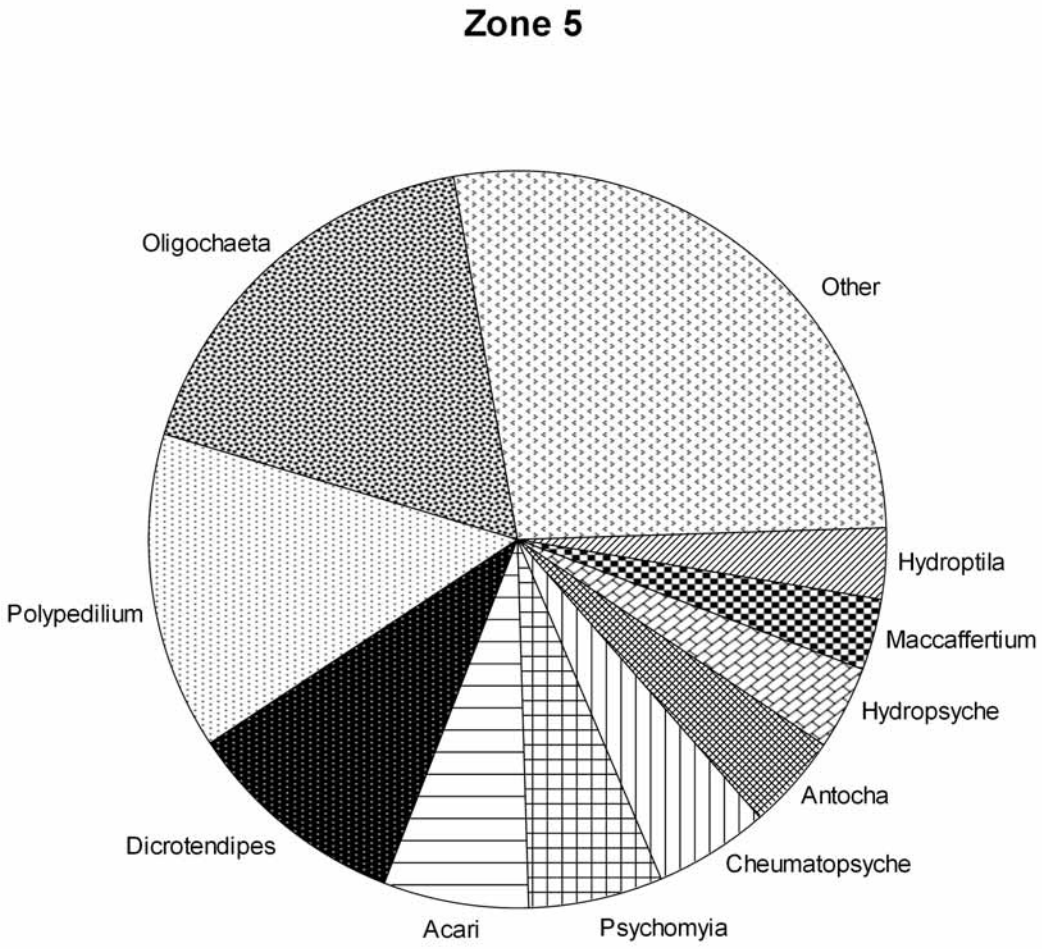


Figure 5.4.23. Relative abundance of the 10 most abundant taxa at Zone 5. These figures are based on the average (n=10) abundance of all taxa from each zone.

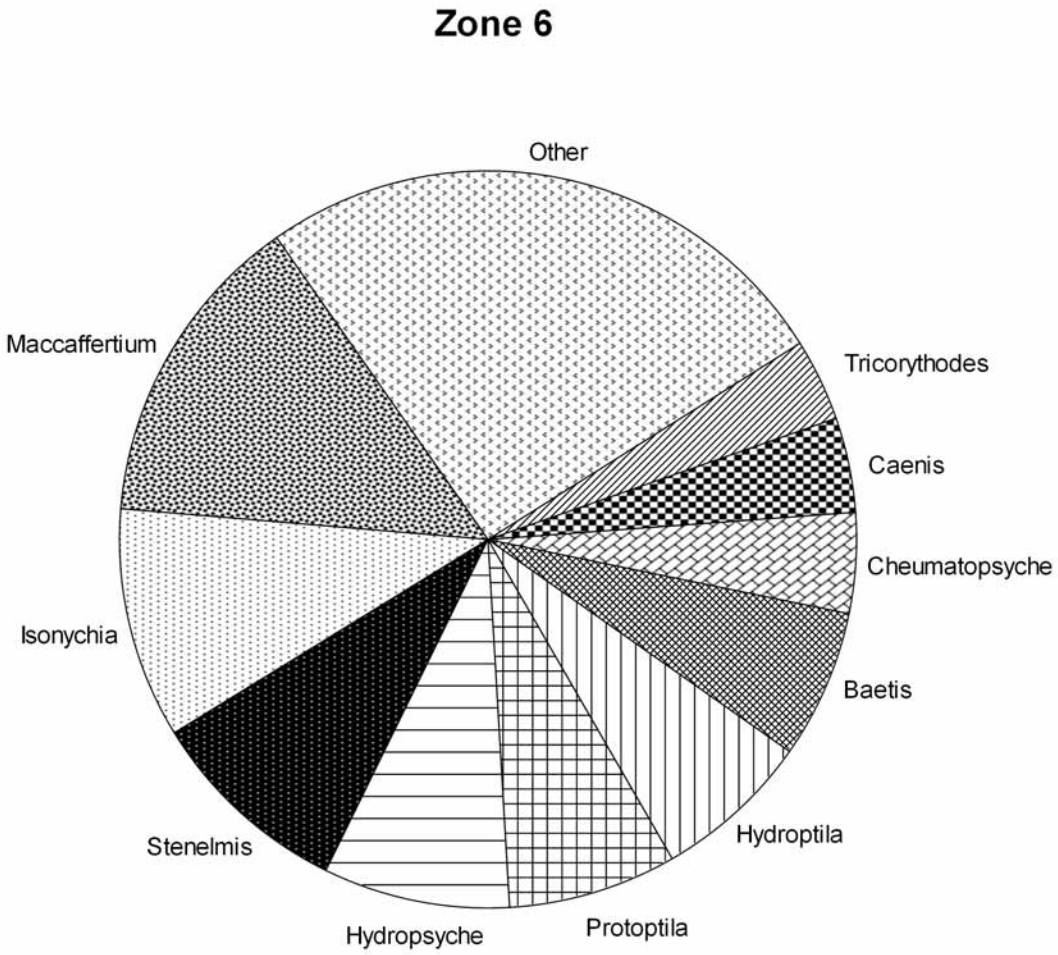


Figure 5.4.24. Relative abundance of the 10 most abundant taxa at Zone 6. These figures are based on the average (n=10) abundance of all taxa from each zone.

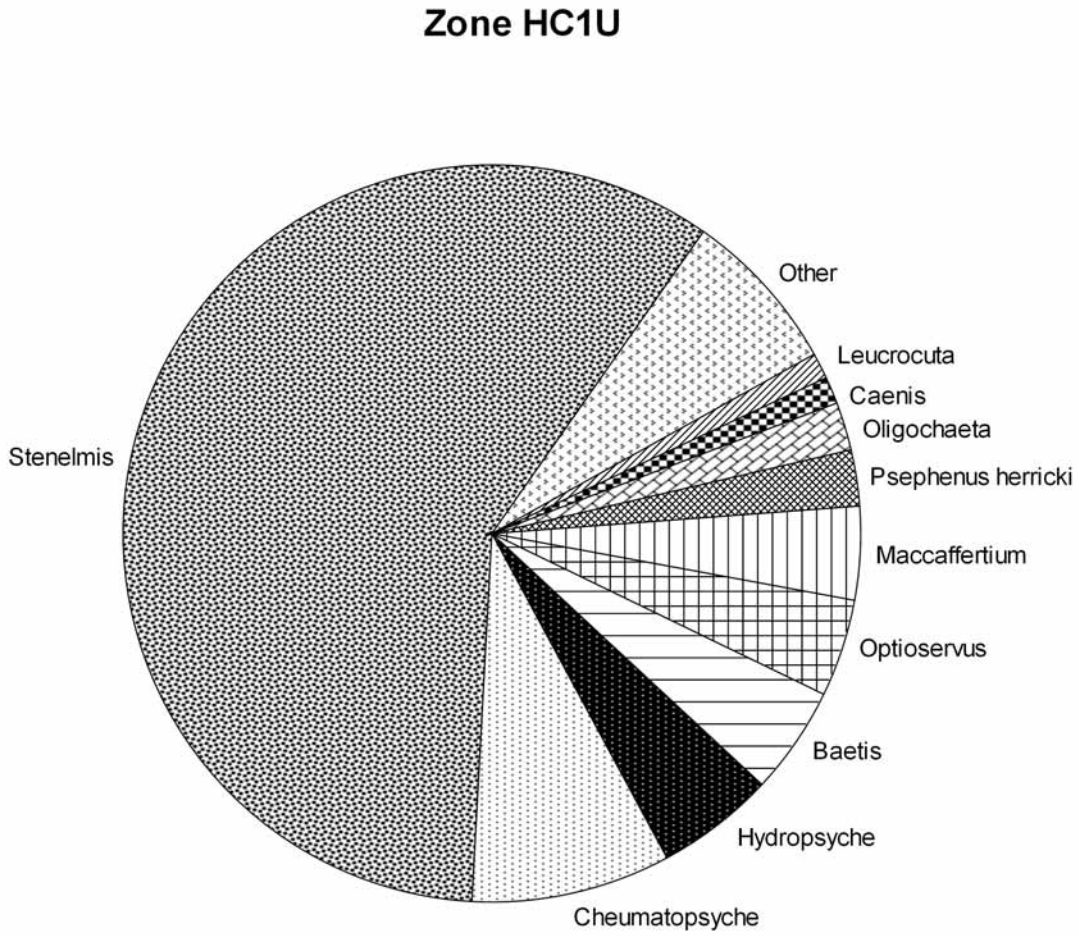


Figure 5.4.25. Relative abundance of the 10 most abundant taxa at Zone HC1U. These figures are based on the average (n=10) abundance of all taxa from each zone.

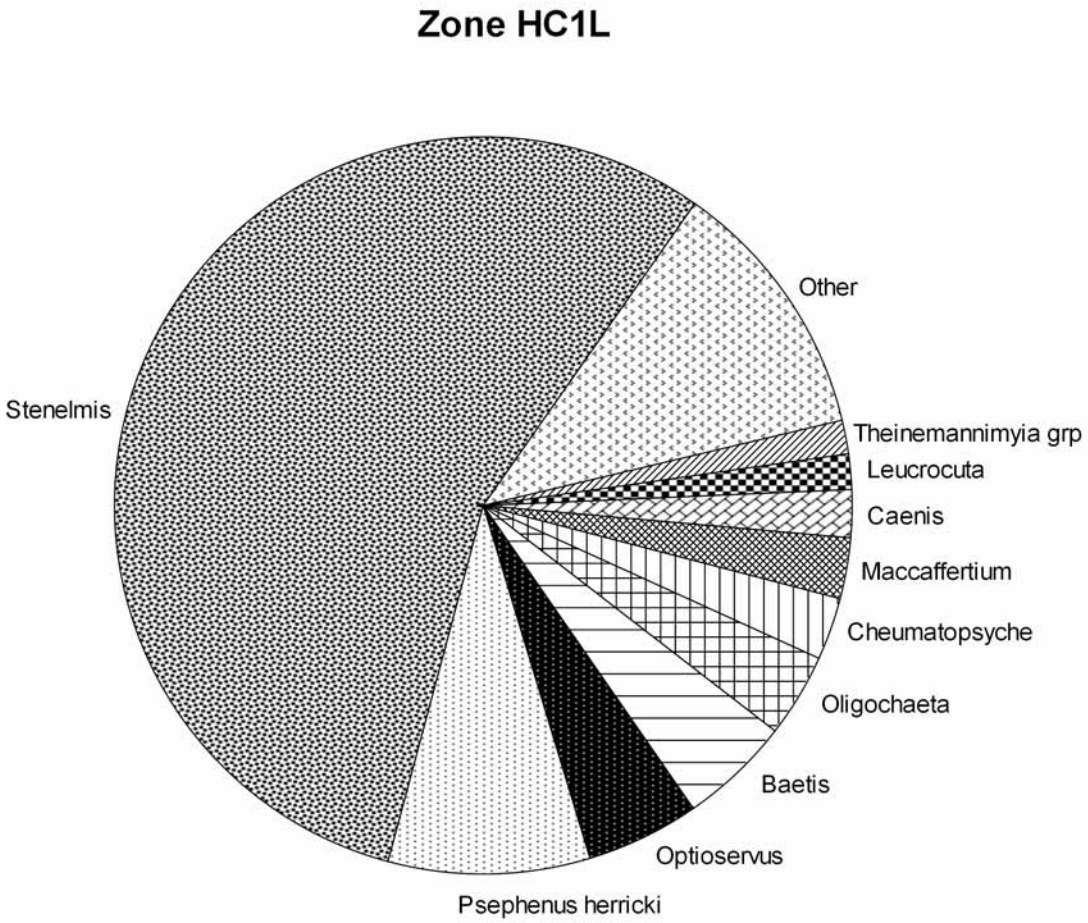


Figure 5.4.26. Relative abundance of the 10 most abundant taxa at Zone HC1L. These figures are based on the average (n=10) abundance of all taxa from each zone.

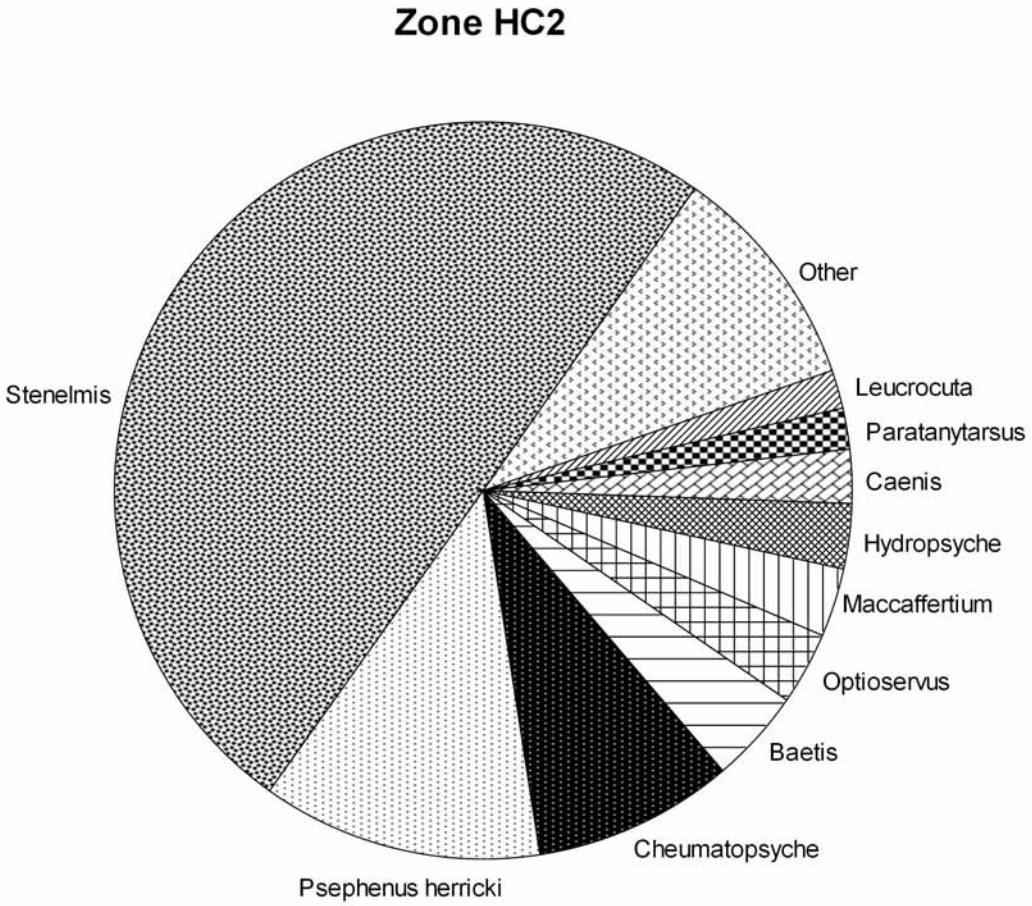


Figure 5.4.27. Relative abundance of the 10 most abundant taxa at Zone HC2. These figures are based on the average (n=10) abundance of all taxa from each zone.

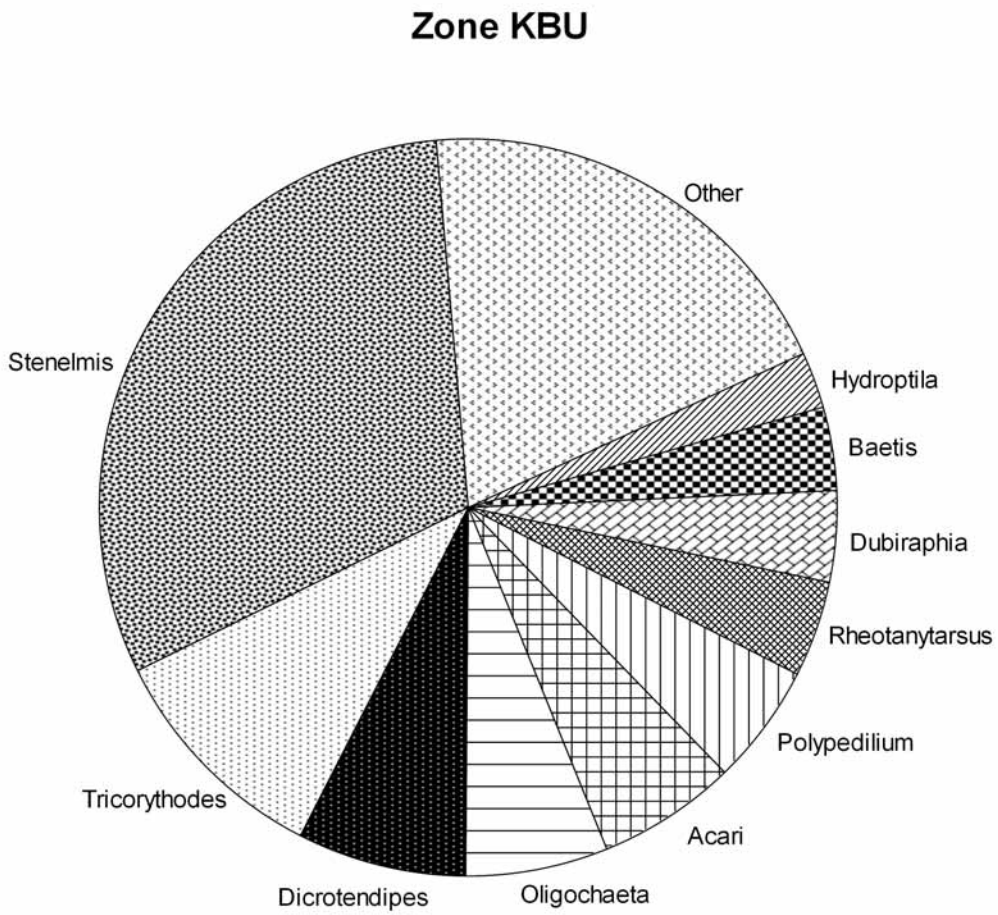


Figure 5.4.28. Relative abundance of the 10 most abundant taxa at Zone KBU. These figures are based on the average (n=10) abundance of all taxa from each zone.

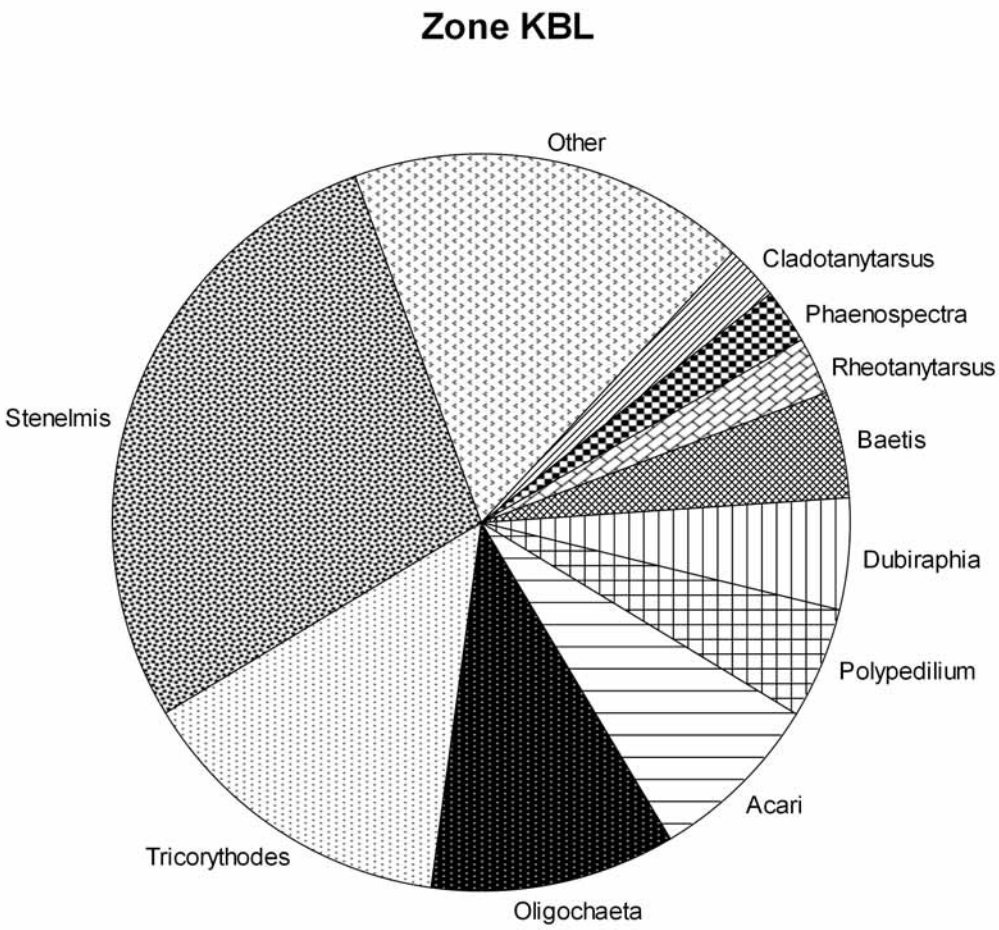


Figure 5.4.29. Relative abundance of the 10 most abundant taxa at Zone KBL. These figures are based on the average (n=10) abundance of all taxa from each zone.

5.4.4 Summary

5.4.4.1 South Fork of the Holston River and Holston River Mainstem

The aquatic insect surveys found multiple lines of evidence for two major conclusions regarding the aquatic insect assemblages of the Holston River. First, the operation of the Fort Patrick Henry Dam has had extensive effects on the biota of the river, more so in 2010 than in 1997. Second, generally the conditions of the river have improved markedly at most zones, not withstanding the effects of the dam on Zone 2.

5.4.4.2 Horse Creek

Horse Creek aquatic insect communities were generally very similar to the previous years' findings, especially to 1997, which supported the greatest number of aquatic insect species on record.

One rare species of stonefly, *Hansonoperla appalachia*, was collected in 2010 that had not been collected in previous surveys. The species is not currently listed as federally threatened or endangered. Probably the greatest threat to *H. appalachia* is continued urban development within the Horse Creek watershed. No evidence was found that Eastman's activities have imperiled the species locally or otherwise.

Part of the survey compared the suitableness of the previous upstream reference site. The HC1 zone of 1997 and previous years was near the downstream margin of HC1 in an area potentially affected by golf course operations. In 2010, the old zone was designated HC1L (Horse Creek-1 Lower) because of its proximity to the lower margin of Zone HC1 and a new sampling area near the upstream margin of Zone HC1 (HC1U) was added, to compare with the other zones. This zone was instrumental in assessing Zone HC2. When Zone HC1L metrics alone would have produced ambiguous results regarding the condition of Zone HC2, Zone HC1U helped clarify that Eastman-related activities did not impair the structure and function of Horse Creek aquatic insect communities. Continued sampling of both locations is recommended.

5.4.4.3 Kit Bottom

The survey found no statistically significant impairments related to potential leaching from Kit Bottom. When an assessment makes such a broad statement, it is useful to re-examine the suitability of the assessment to determine the limits of the finding. A statistical power analysis on the Kit Bottom Assessment was performed because it used fewer samples than the rest of the aquatic insect survey. The metric used for the power analysis was taxa richness. The effect sizes of 20% and 30% were selected based on the greatest taxa richness observed among the zones. The results indicate that replication levels used were insufficient to detect a 20% change in the richness of aquatic insect samples, but were adequate to assess a 30% change in richness. Thus, it is clear that moderate or strong effects (Cohen 1988) related to Kit Bottom were not overlooked by the survey. However, the survey may have overlooked changes less than 25%.

5.4.4.4 Overall Summary

- The most pervasive impairment to development of natural aquatic insect communities in the Holston River is the hydrological regime imposed by Fort Patrick Henry Dam.
- The aquatic insect communities at Zone 3 were more diverse than in any of the previous Holston River surveys and now include relatively sensitive orders of aquatic insects (mayflies and caddisflies).
- No relevant changes in the community structure of Horse Creek or in the Big Sluice near Kit Bottom were observed.
- A species of conservation concern was collected, but it was not federally listed as rare, threatened or endangered. Only a single specimen was collected, and there is no evidence of its survival being affected by operation of the Eastman facility.
- The comprehensive taxa list for each Holston River zone was equal to or greater than in previous years, while the quantitative assessment indicated that abundance and diversity in riffles were similar to previous surveys.

Table 5.4.1. Insect taxa in hand and quantitative collections from Zones 2-6 on the Holston, and Zones 1U, 1L and 2 on Horse Creek, July 2010 (Page 1 of 4). (*not counted in zone total.)

	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	HC1	HC2
Class Hexapoda							
Group Insecta							
Order Ephemeroptera							
Family Baetidae	X						
Acentrella		X			X		
Acerpenna			X		X	X	X
Baetis		X	X	X	X	X	X
Callibaetis sp.		X			X		X
Centroptilum		X	X		X		X
Plauditus		X		X	X		X
Pseudocleon				X	X		
Heterocleon				X		X	
Family Isonychidae							
Isonychia	X	X	X	X	X		X
Family Heptageniidae							
Leucrocuta		X	X			X	X
Maccaffertium		X	X	X	X	X	X
Stenacron		X	X	X	X	X	X
Stenonema femoratum	X	X	X			X	X
Family Ephemerellidae							
Serratella			X	X	X		
Family Caenidae							
Caenis		X	X	X	X	X	X
Family Leptohyphidae							
Tricorythodes		X	X	X	X	X	
Family Ephemeridae							
Hexagenia						X	
Order Plecoptera							
Family Pteronarcydae							
Pteronarcys sp.					X		
Family Leuctidae							
Leuctra							X
Family Capniidae						X	X
Family Perlidae							
Hansonoperla appalachia						X	
Order Odonata							
Suborder Anisoptera							
Family Gomphidae					X*		
Dromogomphus spinosus			X	X			X
Gomphus lividus	X	X					
Hagenius brevistylus			X		X	X	X
Stylogomphus albistylus	X		X			X	X
Family Aeshnidae							
Anax sp.					X		
Basiaeschna janata		X	X	X	X	X	X
Boyeria vinosa		X	X	X	X	X	
Family Corduliidae							
Epicordula princeps		X					

Table 5.4.1 (continued). Insect taxa in hand and quantitative collections from Zones 2-6 on the Holston, and Zones 1U, 1L and 2 on Horse Creek, July 2010 (Page 2 of 4). (*not counted in zone total.)

	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	HC1	HC2
Suborder Zygoptera							
Family Caloptyerigidae							
Hetaerina americana		X	X		X	X	X
Family Coenagrionidae							
Argia		X	X	X	X	X	X
Enallagma		X	X		X		X
Ischneura		X			X		
Order Hemiptera							
Family Hydrometridae							
Hydrometra sp.							X
Family Mesoveliidae							
Mesovelia mulsanti		X				X	
Family Veliidae							
Rhagoveliia obesa		X	X		X	X	X
Microvellia spp.	X						
Family Gerridae							
Aquarious							X
Gerris comutus	X				X		
Rheumatobates riley						X	
Family Belostomatidae							
Belostoma spp.		X	X		X	X	X
Family Nepidae							
Rantara spp.		X			X		
Family Corixidae							
Sigara spp.	X				X		
Trichocorixia spp.		X					
Family Saldidae					X		
Order Megaloptera							
Family Sialidae							
Sialis sp.		X	X				
Family Corydalidae							
Corydalus cornutus			X	X	X	X	
Order Trichoptera							
Philopotamidae							
Chimmara						X	
Family Psychomyiidae							
Psychomyiia		X	X	X	X	X	X
Family Hydropsycidae							
Hydropsyche	X	X	X	X	X	X	X
Cheumatopsyche	X	X	X	X	X	X	X
Family Glossosomatidae							
Protoptila sp.			X		X		
Family Helicopsychidae							
Helicopsyche borealis			X		X		
Family Hydroptilidae							
Hydroptila		X	X	X	X		
Leucotrichia		X		X			

Table 5.4.1 (continued). Insect taxa in hand and quantitative collections from Zones 2-6 on the Holston, and Zones 1U, 1L and 2 on Horse Creek, July 2010 (Page 3 of 4). (*not counted in zone total.)

	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	HC1	HC2
Family Brachycentridae							
Brachycentrus lateralis			X		X		
Micrasema			X		X		
Family Uenoidae							
Neophylax sp.			X				
Family Limnephilidae							
Pycnopsyche sp.		X					
Family Leptoceridae							
Nectopsyche					X		
Oecetis persimlis			X	X	X		
Order Lepidoptera							
Petrophila		X	X	X	X		
Order Coleoptera							
Family Gyrinidae							
Dinetus sp.					X		
Family Haliplidae							
Peltodytes sp.	X	X	X		X		
Family Dytiscidae							
Laccophilus spp.					X		
Laccophilus maculosus	X						
Neoporus venustus		X			X		X
Thermonectus basilaris	X			X			
Family Hydrainidae				X			
Family Hydrophilidae							
Berosus sp.				X	X	X	
Enochrus sp.					X		
Troposternus spp.	X	X	X	X	X		
Troposternus lateralis	X	X					
Family Dryopidae							
Helichus sp.		X	X		X		
Family Scirtidae		X				X	X
Family Elmidae							
Dubiraphia		X	X	X	X	X	X
Macronychus glabratus		X	X	X		X	X
Optioservus					X	X	X
Oulimnius						X	X
Stenelmis	X	X	X	X	X	X	X
Heterelmis					X		X
Family Psephenidae							
Psephenus herricki			X		X	X	X
Ectopria			X				
Order Diptera							
Suborder Nematocera							
Family Ceratopogonidae							
Atrichopogon sp.							X
Palpomyia/Bezzia Complex		X		X	X	X	X

Table 5.4.1 (continued). Insect taxa in hand and quantitative collections from Zones 2-6 on the Holston, and Zones 1U, 1L and 2 on Horse Creek, July 2010 (Page 4 of 4). (*not counted in zone total.)

	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	HC1	HC2
Family Culicidae					X		
Anopheles sp.					X		
Family Simulidae							
Simulium	X		X	X	X		
Family Tipulidae							
Antocha spp.		X	X	X	X	X	X
Hexatoma spp.		X				X	X
Tipula spp.	X		X	X		X	X
Family Empididae							
Hemerodromia		X	X	X	X	X	X
Family Tabanidae				X			
Family Stratiomyidae							
Stratiomys sp.	X						
Family Chironomidae							
Subfamily Tanypodinae							
Ablabesmyia			X				
Natarsia			X			X	
Theinemannimyia grp			X	X	X	X	X
Subfamily Orthocladinae							
Theinemanniella			X		X	X	
Cricotopus/Orthocladius	X*	X*		X*	X*		
Cricotopus	X	X	X	X	X		X
Orthocladius	X	X	X	X	X		
Eukeifferella	X	X		X	X		X
Lopescladius						X	
Nanocladius	X				X		
Tvetenia	X	X	X				
Synorthocladius		X					
Parametriocnemus						X	
Subfamily Chrironominae							
Tribe Chironomoni							
Chironomus spp.		X					
Cryptochrionomus			X	X	X		
Dicrotendipes	X	X	X	X	X		
Microtendipes	X	X	X	X		X	X
Phaenospectra	X	X	X	X			
Polypedilium	X	X	X	X	X	X	X
Pseudochironomus				X	X		
Tribe Tanytarsini							
Cladotanytarsus			X				
Micropsectra				X	X	X	
Paratanytarsus		X					X
Rheotanytarsus	X	X	X	X	X	X	X
Stempellinella						X	X
Sublettea	X	X	X	X			
Tanytarsus			X		X	X	X

Table 5.4.2. Statistical results of comparisons among Holston River zones.

Metric	Zone P-value	Covariates				Tukey HSD grouping					Covariates	
		FLOW	PART	DEPTH	GREEN	Zone 2	Zone 3	Zone 4	Zone 5	Zone 6	Changed Tukeys Groups	Changed Trend?
Ecological Community Metrics												
Abundance	<0.001	**	NS	NS	NS	A	C	BC	BC	B	Yes[1]	No
Taxa Richness	<0.001	*	**	NS	NS	A	B	C	C	C	Yes[1]	No
Diversity (H')	<0.001	***	**	NS	NS	A	B	B	C	C	Yes[1]	No
Evenness (J')	<0.001	*	***	NS	NS	A	BC	AB	C	C	Yes[1]	No
Disturbance Metrics												
EPT Richness	<0.001	NS	*	NS	NS	A	B	B	B	C	Yes[1]	No
% EPT	<0.001	NS	NS	NS	NS	A	B	C	B	D	n/a	n/a
% Chironomidae	<0.001	**	*	NS	NS	A	B	C	D	C	No	No
% Non-Insect	0.002	***	NS	NS	NS	BC	AB	AB	A	C	Yes[1]	No
NCBI [n.p.]	<0.001	--	--	--	--	A	B	C	B	D	n/a	n/a
Functional Groups												
% Collectors [n.p.]	<0.001	--	--	--	--	A	B	B	B	B	n/a	n/a
% Scrapers [n.p.]	<0.001	--	--	--	--	A	B	B	B	C	n/a	n/a
% Shredders [n.p.]	<0.001	--	--	--	--	A	C	B	C	B	n/a	n/a
% Herbivore-piercers [n.p.]	<0.001	--	--	--	--	AB	BC	A	C	C	n/a	n/a

n.p. denotes a non-parametric test was used to describe significant differences among zones because transformations did not resolve violations in the assumptions of variance homogeneity and/or normality.

* indicates the covariate explained a marginally significant portion of the variance in the model (0.15 > P >0.05).

** indicates the covariate explained a significant portion of the variance in the final GLM model (0.05 > P >0.01).

*** indicates the covariate explained a very high portion of variance in the final GLM model (P < 0.01)

— indicates the relationship was not tested because the difference among sites was not statistically significant or a non-parametric test precluded determining the interrelations among the variables.
n/a means non-applicable (no significant covariates)
Yes[1] Without covariates, there were fewer significant pairwise differences among stations (i.e., broader groups)

Table 5.4.3. Statistical results of comparisons among Horse Creek zones.

Metric	Zone P-value	Covariates				Tukey HSD grouping			Covariates Changed Tukeys Groups	Covariates Changed Trend?
		FLOW	PART	DEPTH	GREEN	HC1U	HC1L	HC2		
Ecological Community Metrics										
Abundance	0.905	--	--	--	--	--	--	--	n/a	n/a
Taxa Richness	0.215	--	--	--	--	--	--	--	n/a	n/a
Diversity (H')	0.309	--	--	--	--	--	--	--	n/a	n/a
Evenness (J')	0.373	--	--	--	--	--	--	--	n/a	n/a
Disturbance Metrics										
EPT Richness	0.812	--	--	--	--	--	--	--	n/a	n/a
% EPT	0.312	--	--	--	--	--	--	--	n/a	n/a
% Chironomidae	0.214	--	--	--	--	--	--	--	n/a	n/a
% Non-Insect	0.003	***	--	--	--	AB	B	A	n/a	
NCBI	0.293	--	--	--	--	--	--	--	n/a	n/a
Functional Groups										
% Collectors	<0.001	**	**	*	*	A	B	B	No	No
% Scrapers	0.017	**	*	NS	NS	A	B	AB	Yes[1]	No
% Shredders	0.019	NS	NS	NS	NS	AB	A	B	n/a	n/a
% Herbivore-piercers	n/a	n/a	n/a	n/a	n/a	n/a	n/a	n/a	--	n/a

n.p. denotes a non-parametric test was used to describe significant differences among zones because transformations did not resolve violations in the assumptions of variance homogeneity and/or normality.

* indicates the covariate explained a marginally significant portion of the variance in the model (0.15 > P >0.05).

** indicates the covariate explained a significant portion of the variance in the final GLM model (0.05 > P >0.01).

*** indicates the covariate explained a very high portion of variance in the final GLM model (P < 0.01)

— indicates the relationship was not tested because the difference among sites was not statistically significant or a non-parametric test precluded determining the interrelations among the variables.
n/a means non-applicable (no significant covariates)
Yes[1] Without covariates, there were fewer significant pairwise differences among stations (i.e., broader groups)

Table 5.4.4. Statistical results of comparisons among Kit Bottom zones (including Zone 4).

Metric	Zone P-value	Covariates				Tukey HSD grouping			Covariates	
		FLOW	PART	DEPTH	GREEN	KBU	KBL	Zone-4	Changed Tukeys Groups	Changed Trend?
Ecological Community Metrics										
Abundance	0.147	--	--	--	--	--	--	--	n/a	n/a
Taxa Richness	0.553	--	--	--	--	--	--	--	n/a	n/a
Diversity (H')	0.400	--	--	--	--	--	--	--	n/a	n/a
Evenness (J')	0.347	--	--	--	--	--	--	--	n/a	n/a
Disturbance Metrics										
EPT Richness	0.201	--	--	--	--	--	--	--	No	n/a
% EPT	>0.001	NS	NS	NS	*	A	A	B	No	No
% Chironomidae	0.044	NS	**	NS	*	A	AB	B	No	No
% Non-Insect [n.p.]	0.036	--	--	--	--	A	A	B	No	n/a
NCBI [n.p.]	0.016	--	--	--	--	A	A	B	--	n/a
Functional Groups										
% Collectors [n.p.]	0.576	--	--	--	--	--	--	--	--	n/a
% Scrapers [n.p.]	0.804	--	--	--	--	--	--	--	--	n/a
% Shredders [n.p.]	0.446	--	--	--	--	--	--	--	--	n/a
% Herbivore-piercers [n.p.]	0.095	--	--	--	--	--	--	--	--	n/a

n.p. denotes a non-parametric test was used to describe significant differences among zones because transformations did not resolve violations in the assumptions of variance homogeneity and/or normality.

* indicates the covariate explained a marginally significant portion of the variance in the model (0.15 > P >0.05).

** indicates the covariate explained a significant portion of the variance in the final GLM model (0.05 > P >0.01).

*** indicates the covariate explained a very high portion of variance in the final GLM model (P < 0.01)

— indicates the relationship was not tested because the difference among sites was not statistically significant or a non-parametric test precluded determining the interrelations among the variables.
n/a means non-applicable (no significant covariates)
Yes[1] Without covariates, there were fewer significant pairwise differences among stations (i.e., broader groups)

Table 5.4.5. Summary of insect taxa richness for the Holston River and Horse Creek, 1965-2010.

	Zone							2010 Total		
Collection Method	2	3	4	5	6	HC1	HC2	Holston	Horse Cr	All
Quantitative	15	29	41	34	43	44	36	59	53	79
Comprehensive	30	59	62	48	72	51	51	108	66	121

Year	Historical Taxa Richness				
1997	30	16	29	33	55
1990	35	17	40	23	38
1980	6	5	23	10	16
1977	10	6	21	10	33
1974	14	8	24	10	21
1965	8	0	45	8	16

5.5 Fish

5.5.1 Overview

A total of 3948 individuals of 47 species was collected in the 2010 survey (Table 5.5.1; all tables appear at the end of Section 5.5), including 17 species of carp and minnow, 5 species of sucker, 8 species of centrarchid (bass and sunfish) and 8 species of darter. Mimic shiner, mountain madtom, speckled darter, striped bass and shorthead redhorse, have not been reported in previous ANSP Holston River surveys. Overall, the most widespread species (Table 5.5.2; Appendix 7.6.1) were the Tennessee snubnose darter, telescope shiner (all zones), central stoneroller (all zones except 2), greenside darter, smallmouth bass (all zones except 2 and T2), and rock bass, banded sculpin, northern hog sucker, redline darter and redbreast sunfish (collected at 8 of the 11 zones). The Tennessee snubnose darter was the most abundant species overall. The banded sculpin was the second-most common, but it was found in greatest abundance at Zones HC1 and HC2. The Tennessee snubnose darter, telescope shiner and banded sculpin composed 53% of the total catch. This partly reflects high catches of these at Zones HC1 and HC2, although the snubnose darter and stoneroller were also the most abundant species excluding the Horse Creek zones.

As in previous surveys, relatively few species were collected at Zones 2 and 3, with an increase downstream at Zones 5 and 6. The comparisons need to account for the use of boat electrofishing only at Zones 2, 5 and 6. Comparing backpack and hand samples only, the fewest species (7) were collected at Zone 2 and the most at Zones 6 (29) and HC2 (23) (Appendix 7.6.1). Zone 6 was notable for the collection of a large variety of minnows (14 species) and darters (6 species).

5.5.2 Block Backpack Samples

Block backpack sampling provided a consistent technique across the main river zones. Samples were taken in a similar habitat (shallow riffles with predominance of cobble substrates), although there were some differences in habitat among and within zones. Examination of directions of flows and conductivity indicated that water on the left side of the island at Zone 3 derived from the right side, so this area (called 3LR) is considered part of Zone 3R. In past years, this area was considered part of Zone 3L. The area on the left side of the island provided riffle habitat similar to that at the other zones, and two block backpack samples were taken in this area. Block backpack electrofishing was not done below the dam at Zone 3R (as was done in 1997), because accessible habitat in this area was not comparable to that in other block backpack samples. Zone 4 contains a series of bedrock ledges. Block samples were taken at the middle part of the zone, where these ledges had a relatively shallow slope and were partly covered by cobble and gravel. The presence of a shallow layer of loose rocks over bedrock was unique among the sampling sites. Within zones, there were differences in current velocity and depth, with slower, shallower areas usually close to shore.

Twenty-two species were collected in the block samples (Table 5.5.3), although only seven species (central stoneroller, telescope shiner, northern hog sucker, greenside darter, redline

darter, Tennessee snubnose darter and banded darter) were collected at three or more of the six zones. There was a trend to increasing species diversity (average number of species per sample and total species per zone) downstream. The banded sculpin, Tennessee snubnose darter and rainbow trout were the only species caught in block samples at Zone 2, while 17 species, including 6 darter species, were caught at Zone 6 (Table 5.5.3). Total density was much greater at the Zone 6 sites and at the Zone 4 sites than at the other sites (Table 5.5.3; Fig. 5.5.1).

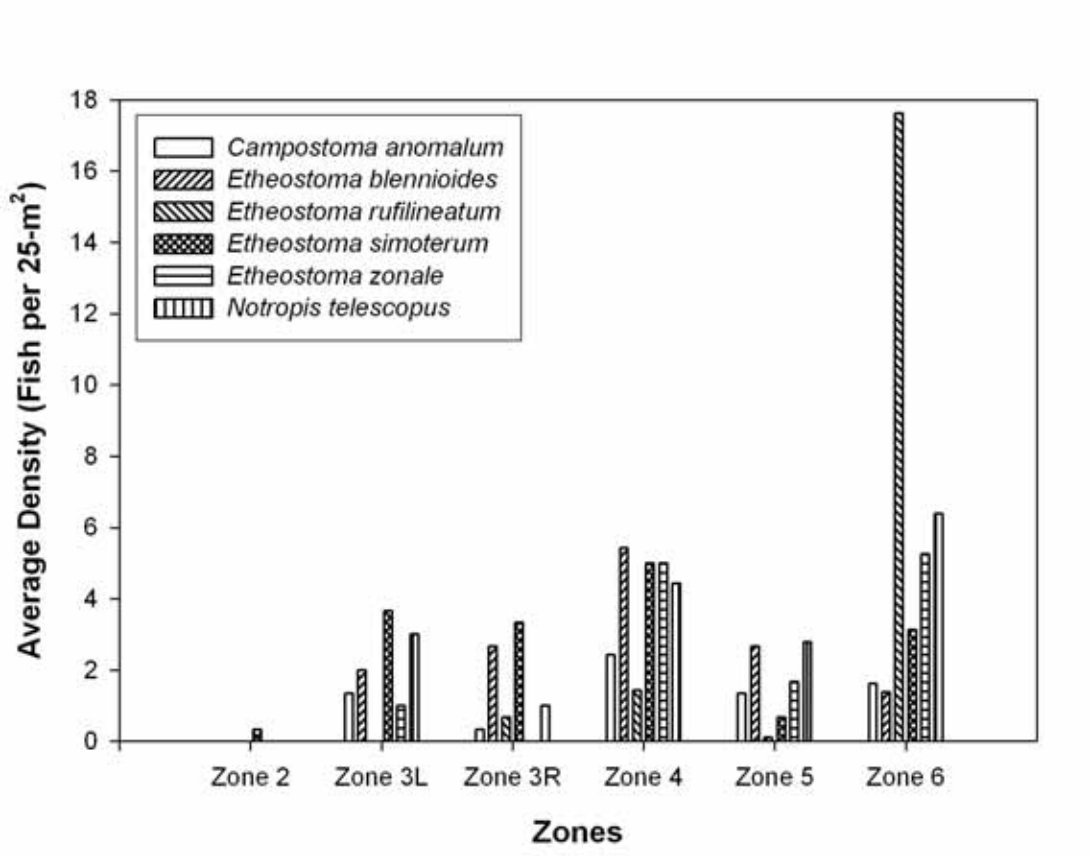


Figure 5.5.1. Average density (number of fish/25 m^2) of common species in block net electrofishing samples, in the July 2010 South Fork and mainstem Holston rivers survey.

The density and diversity of fish among zones were compared using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). ANCOVA was used to compare zones after adjusting for relationships between density and habitat factors (average and maximum velocity, depth and substrate within each sampling site). Densities of the six most common species (central stoneroller, telescope shiner, redline darter, Tennessee snubnose darter, banded and greenside darters), species richness and Shannon-Wiener diversity were analyzed. Densities were ln-transformed (i.e., ln(density per 25 m^2 + 0.05)) and used as the dependent variable.

There were significant differences among zones in species richness and in density for all species except central stoneroller and telescope shiner (Table 5.5.4). There was a marginally significant difference among zones in species diversity (Table 5.5.4). The inclusion of microhabitat differences (depth, velocity and substrate) did affect the conclusions concerning zone differences for

greenside and Tennessee snubnose darters, resulting in no differences among zones. There were no significant relationships between substrate type or depth and the biological variables. A significant relationship was found between average velocity and density of redline darter, but the inferences about zone differences were the same for models with and without velocity.

Three planned pairwise comparisons were used to compare differences among zones when a significant zone effect existed (Table 5.5.4). Zones 3L and 3R, Zones 5 and 6, and Zone 2 and all other zones were compared. Post hoc tests were also used to determine differences among zones, even though these were not very powerful in determining significant differences. The comparisons typically showed significant differences among the most extreme zones. For example, the planned pairwise comparison showed that densities were significantly higher for redline darter at Zone 6 than at Zone 5, and the post hoc showed higher densities at Zone 6 than at all other zones. The banded darter densities were significantly lower at Zone 2 than at all other zones combined. Post hoc analysis showed Zones 4 and 6 having higher densities than Zone 2. Species richness was significantly higher at Zones 4 and 6 than at Zone 2 and was significantly higher at Zone 6 than at Zone 5 in the post hoc analysis.

Principal Components Analysis (PCA) was used to test for correlations between the five microhabitat covariates used in the ANCOVA tests. The Eigenvalues showed that 98.1% of the total variance was explained by the first three PCA Factors (Table 5.5.5). Factor 1 was correlated with the velocity and depth variables (i.e., high values of Factor 1 represent shallower and lower velocity samples) but was not correlated with substrate. Factor 2 was positively correlated with velocity and substrate and negatively correlated with depth. Thus, high values of Factor 2 indicate samples with shallower water, higher velocities and coarser substrates. Shallow cobble-boulder riffles with broken surface flows would show high values of Factor 2. Factor 3 is positively correlated with substrate, and reflects variations in substrate only weakly correlated with depth and velocity. ANCOVAs using the PCA factors were similar to those using raw microhabitat variables. The abundance of redline darter was significantly related to Factor 2. This ANCOVA showed similar zone relationships as the ANCOVA using velocity.

Riffle habitats at all the main river zones (2, 3, 5 and 6) and Big Sluice (Zone 4) were affected by water level fluctuations associated with dam releases. The fluctuations were most extreme at Zone 2, where shallow riffles were partly exposed at low release levels, and deeper riffles were "washed out" (few waves or surface roughness) at high dam releases. Although evident, the fluctuations were much smaller at the other zones.

5.5.3 Shore Backpack Electrofishing Samples

While the block electrofishing samples concentrated on small areas of one habitat type, the shore electrofishing samples covered a variety of shallow, nearshore habitats. Shore electrofishing was done at each zone (Table 5.5.6), with one sample taken at Zones 2, 3L, 3R and 5, three samples at Zone 4 and two samples at Zone 6. Two samples were taken at Zone KL, three samples at Zone KU and one sample at Zone T2. The shore electrofishing at the two Horse Creek zones (Table 5.5.7) was somewhat different, since the sampling areas could be blocked at these sites, and two passes were done in one site in each of the two zones.

Overall, densities were low at Zones 2 and 3R (Table 5.5.6), averaging from 2.0 to 4.2 fish per 25 m^2. Densities were much higher at the three Big Sluice sites, Zones KU, KL and 4 (19.6-24.0 fish per 25 m^2) and high at Zones 3L and 5 (11.8-12.4 fish per 25 m^2) as well. The relatively high density at Zones KU, KL and 4 reflect high catches of Tennessee snubnose darter. Similarly, high catch rates at Zones 5 and 3L reflected dominance by a few species (snubnose darter and stoneroller [both zones] and smallmouth bass [Zone 5]), as well as lower catch rates of a number of other species. Densities at Zone HC1 were much higher than those at Zone HC2, with high densities of stoneroller, banded sculpin, snubnose, greenside and redline darters and telescope shiner. Densities at the Horse Creek zones (Table 5.5.7) are not directly comparable with those in the river samples, since collecting efficiency should be much higher at the Horse Creek sites, because of the narrower sites, blocks and use of two passes.

Among the main river zones, species richness increased greatly downstream, with the exception of the nine species found at Zone 3L (Table 5.5.6). Species richness was lowest (three species) at Zone 2, where most fish were collected near the mouth of Rock Springs Branch. Species richness was highest (18 species) at Zone 6. Species richness was high at the Horse Creek zones as well (20 and 23 species at Zones HC1 and HC2, respectively).

At Zone 2, few fish were caught in the backwater at the mouth of Rock Spring Branch. However, high densities of fish were found in the pools in the creek just upstream of the spring (the T2 sample). Fish may have used these pools as refuge during high flow portions of the dam release cycle. Although the catch rate at Zone 3R was relatively low, young-of-year of smallmouth and largemouth bass were caught in the pool above the dam at the zone.

The use of two passes in blocked reaches at the Horse Creek zones allows estimation of total density based on depletion estimates. (Table 5.5.7; Appendices 7.6.2 and 7.6.3). These estimates use the difference in catch among sequential passes, assuming a constant probability of capture of each individual on each pass. For Zone HC1, catch rates on the second pass were near that (bluegill and redbreast sunfish) or greater than that on the first pass for several species (telescope shiner, mimic shiner and northern hog sucker). This indicates either low collecting efficiency or violation of the assumptions, so that total densities are imprecise or cannot be calculated. Catch rates on the first pass were clearly greater than second pass rates for stoneroller, banded sculpin and snubnose darter. Since the density of captured fishes was high at the zone, this suggests high total densities at the zone. The depletion estimates are more reliable for Zone HC2. Larger fish were caught at the zone, which are more efficiently captured by electrofishing. Overall, calculations suggest that about half to three-quarters of fish in the site were collected.

5.5.4 Boat Electrofishing Samples

A total of 33 species was collected by boat electrofishing (Tables 5.5.8, 5.5.9). Eight species (channel catfish, warmouth, redear sunfish, streamline chub, common carp, gizzard shad, striped bass and brown trout) were collected only by boat electrofishing in this survey (Appendix 7.6.1). Large adults of some other species (e.g., golden and black redhorse, and smallmouth bass) were caught by this technique. The number and diversity of species was low at Zone 2, despite sampling twice as long. One (18.0 cm) brown trout and twenty-one rainbow trout were caught. These species are stocked in the cool tailwaters of Fort Patrick Henry Dam. The rela-

tively high catch rate at Zone 5 reflects catches of a number of small sunfishes and rock bass along riprap on the right bank of the pool of the zone. The high catch rate at Zone 6 reflects high catches of redhorses and a number of species of minnows. Redhorse, including both large adults and young-of-year, were caught in the center of the channel as well as along either shoreline. Most of the minnows were collected in eddies along and behind the bridge abutments at the right bank of the river. Several large smallmouth bass were also collected at this zone.

5.5.5 Other Techniques

A few specimens were collected by dip netting. These were useful in documenting presence of some species at several zones.

5.5.6 Condition and Anomalies

Condition was assessed by analyzing the weight-length relationships among individual fish of stonerollers and Tennessee snubnose darters. ANCOVA of the ln-transformed weight of fish was used to compare condition. In these analyses, zone differences in ln(weight) are modeled after adjustment for length (by using ln(total length) as a covariate in the statistical model). There were highly significant zone differences in the weight-length relationships for both species analyzed (Table 5.5.10). For stoneroller, there were no differences in the slopes of the weight-length relationships among zones, i.e., the differences among zones were consistent across different sizes of fish. For stoneroller, the highest condition (i.e., greatest weight at any given length) was at Zones KL, 3L and KU. Planned pairwise comparisons of the least squares means (KU vs KL, 3L vs 3R, 5 vs 6 and KU vs 4) indicated no significant difference in condition. A comparison of KU, KL and 4 (Big Sluice zones) vs all other zones was significant with higher condition for the Big Sluice zones.

The comparisons for the Tennessee snubnose darter (*Etheostoma simoterum*) were complicated by a weakly significant difference in slopes among zones ($p<0.03$). However, when the slopes were graphed, the length-weight regression curves did not intersect over the range of fish size, allowing for interpretations to be made. The same planned comparisons used for the stoneroller were used. However, only Zone 6 showed significantly greater condition than Zone 5. Comparison of the Least Square Means indicates that condition was highest at Zones HC1 and KU and lowest at Zone 3R.

External examination of fish for anomalies and parasites was done as part of routine handling. These examinations looked for several types of anomalies, as well as presence of parasites (Tables 5.5.11 and 5.5.12):

1) Structural (presumably skeletal) deformities. These included malformation of head bones, deformed spiny dorsal fins, disproportionally small heads or deformed mouths and

malformation of the vertebral column. No fish in this survey were observed to have any skeletal deformities.

2) Lesions, growths and other skin abnormalities. Lesions (open sores on the body) were seen on several specimens. Four largemouth bass, one smallmouth bass and one rock bass from Zone 5 had lesions around their mouths; these are considered to be hook wounds. Another rock bass from Zone 4 and a yellow bullhead from Zone HC1 had lesions. At Zone HC2 a stoneroller had a small dorsal lesion and a smallmouth bass had a lesion on the caudal peduncle and missing caudal rays.

3) Fin erosion. A few specimens had broken or eroded portions of fins. This was seen on one rainbow trout from Zone 2, one white sucker from Zone 2, two greenside darters from Zone 4, one banded darter from Zone 5, one redbreast sunfish from Zone 2 and one smallmouth bass from Zone HC2.

4) Emaciation. One bigeye chub (Zone HC2) was extremely emaciated.

5) Leeches (Table 5.5.12). Parasitic leeches are known to attack fish; these are usually different taxa than free-living leeches. One or more leeches were found on a number of fish. Leeches were usually attached to the fins. Leeches were found mainly on darters and sunfishes. Among common groups, leeches were not found on minnows or sculpins. Most leeches were found on Tennessee snubnose darters, for which 0.4-38.1% of the specimens per zone had leeches (overall, 4.5% of all snubnose darters had leeches). About 3% of all redbreast sunfish and 1% of rock bass had leeches. Leeches were most common at Zones 3R and 5. No leeches were found on fish from Zones 2 or HC2. While many leeches remained attached through the preservation and curation process, some leeches fell off (some were found in the sample bottles), so that the frequency of leeches may be underestimated.

6) Other parasites. Black spot was noted on several stonerollers at Zones HC1 (12 specimens) and HC2 (27 specimens) (Table 5.5.11).

The frequency of observed anomalies is summarized in Table 5.5.11. These frequencies are approximate, since minor anomalies may have been missed, especially on released fish. Anomalies were found on specimens from all zones except 3L, 6, KL and KU, but were most frequent on specimens from Zones HC2, 2 and 5. It is not possible to assign causes to many of the anomalies observed. Some lesions may result from parasites.

5.5.7 Size Distributions and Growth Rates

The size distributions of stonerollers differed among zones (Fig. 5.5.2), although relatively small sample sizes impedes comparisons. Two types of differences were noted: size of the smallest mode of fish (young-of-year, YOY) and occurrence of larger fish. Most or all YOY fish were less than 6 or 7 cm, based on the presence of a single mode of smaller fish. Within this mode, fish tended to be smallest at Zones HC1 and HC2 and largest at Zones 3L and 5. Too few YOY were caught at Zones 3R, 4, 6 and KU to determine their size distribution. Larger fish (presumably yearling and 2+ or older fish) were caught in most zones. However, fish greater than

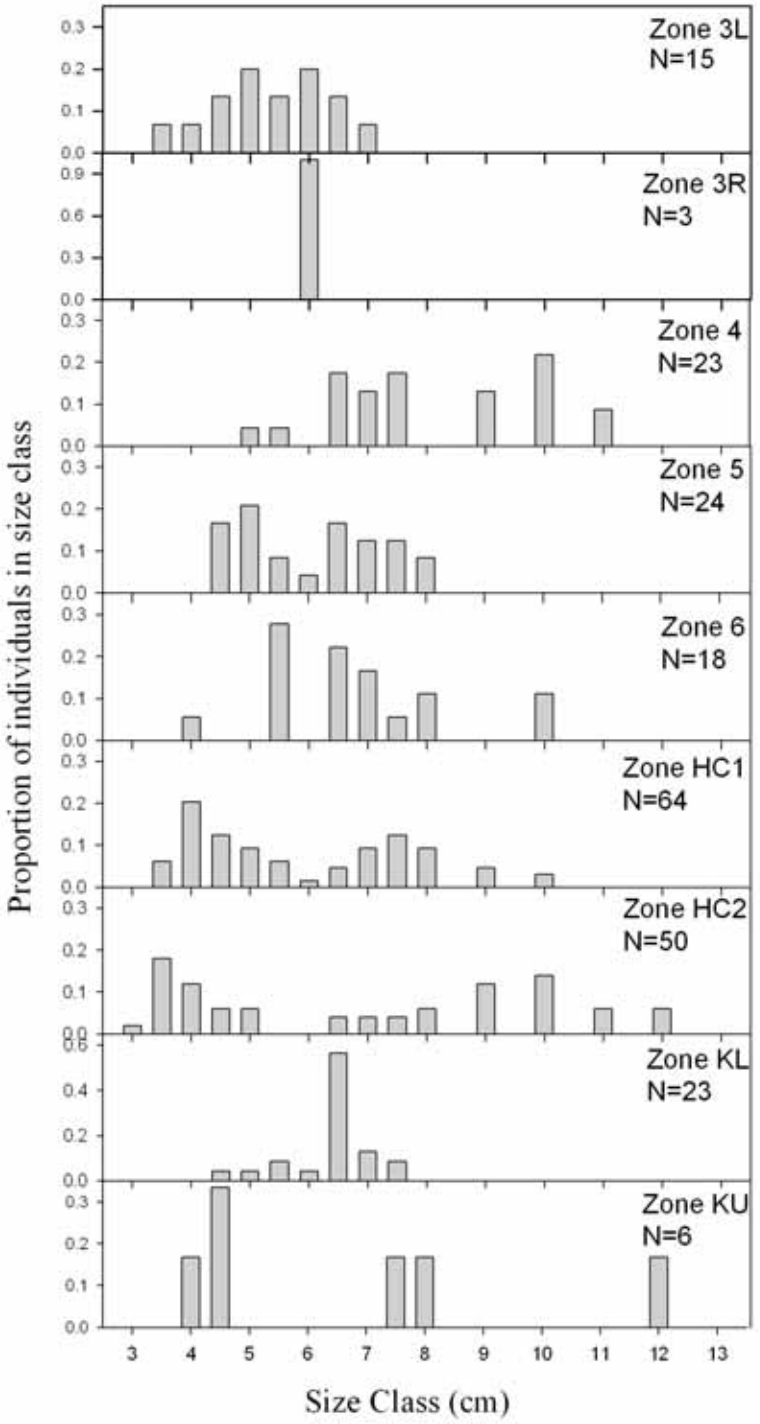


Figure 5.5.2. Frequency of lengths (total length, cm) of stonerollers collected at different zones as part of the July 2010 ANSP survey (0.1 = 10% of total, etc.).

8 cm were caught only at Zones 4, 6, HC1, HC2 and KU. Very few large stonerollers (greater than 11 cm) were caught: one at Zone HC2 and one at Zone KU.

The size distribution of Tennessee snubnose darters (Figure 5.5.3) showed several modes. A major mode of smaller fish, presumably YOY, was seen at all zones. This mode was at 3 cm at Zone HC1; 3.5 cm at Zones 4, 6, HC2 and KL; and at 4 cm at Zones 3L, 3R and KU. The mode was at 3.5-4 cm at Zone 5. No clear mode was seen in field-measured fish from Zone 2. However, small darters, 13.0-21.7 mm were found in hand collections from Zone 2. A second mode of larger fish was seen at most zones, but often with few individuals. This mode was around 4.5 cm at Zone HC1; and 5 cm at Zones 5, 6 and HC2. Too few larger fish were caught to locate the larger mode at the other zones.

5.5.8 Otolith Analyses

Ageing was done on 43 specimens of the Tennessee snubnose darter. In addition to examining otoliths, scales were examined from some specimens to determine whether all were young-of-year fish. Ages were estimated from presumed daily rings on the otoliths, and hatching date estimated as the date of the first ring. It is possible that a few rings are formed prior to hatching, so the date of the first ring may predate the actual hatching date by a few days. Without information on ring formation in embryos, the date of the first ring will be referred to as the hatching date. Based on the age estimates and collection dates for all specimens, estimated hatching dates ranged from 14 March through 27 May. However, there were uncertainties about the accuracy of age estimates for several specimens. One specimen from Zone 2 showed what appeared to be an annulus on scales. There was no indication of an annulus on the otolith, although there was a mark near the edge of the otolith. This specimen had the earliest estimated hatch date among all specimens and was the largest specimen caught at Zone 2. This specimen was excluded from further analysis. For several other otoliths, counts of the number of rings differed greatly on different sections of the otolith. This difference probably results from differences in the ability to distinguish (and not count) subdaily rings. Where multiple counts differed by more than 10%, the specimens were excluded from further analysis, because of uncertainty in age. Where multiple counts differed by less than 10%, the average of the two counts was used.

After exclusion of specimens as noted above, estimated hatch dates ranged from 30 March to 27 May. Total length increased approximately linearly with age (Fig 5.5.4) to about 95 days, when growth appeared to slow. Except for Zone 2, there were no clear patterns in estimated hatch dates among the zones (Fig. 5.5.5). At Zone 2, all but one young-of-year were small and had late estimated hatching dates (21 April to 27 May, compared to 30 March to 10 May for other zones). The difference in hatching dates among zones was highly significant (one-way ANOVA, $p<0.000001$). The significance was due to the difference between Zone 2 and the other zones (planned comparison, $p < 0.0000001$). Hatch dates at Zone 3 were not different from those at the other zones (Fig. 5.5.5).

Potential differences in growth rates were tested in two ways. First, an average daily growth rate was estimated as (total growth)/(days since hatch) (Fig. 5.5.6), where total daily growth is the total length minus 0.5 cm (i.e., assuming a size at hatch of 0.5 cm). Zone differences in the ln of

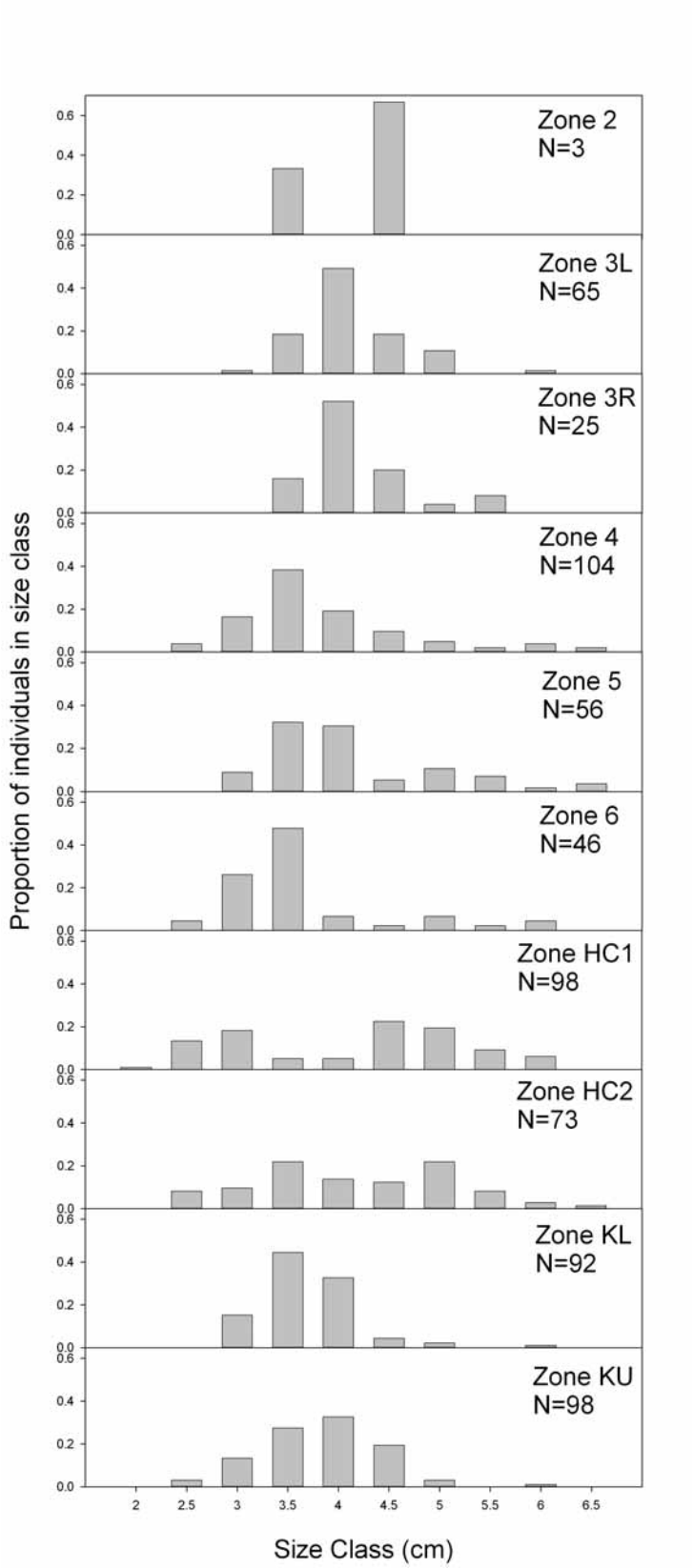


Figure 5.5.3. Frequency of lengths (total length, cm) of Tennessee snubnose darter collected at different zones as part of the July 2010 ANSP survey. Data are based on field measurements and do not include some specimens collected as part of macroinvertebrate sampling (0.2 = 20% of total, etc.).

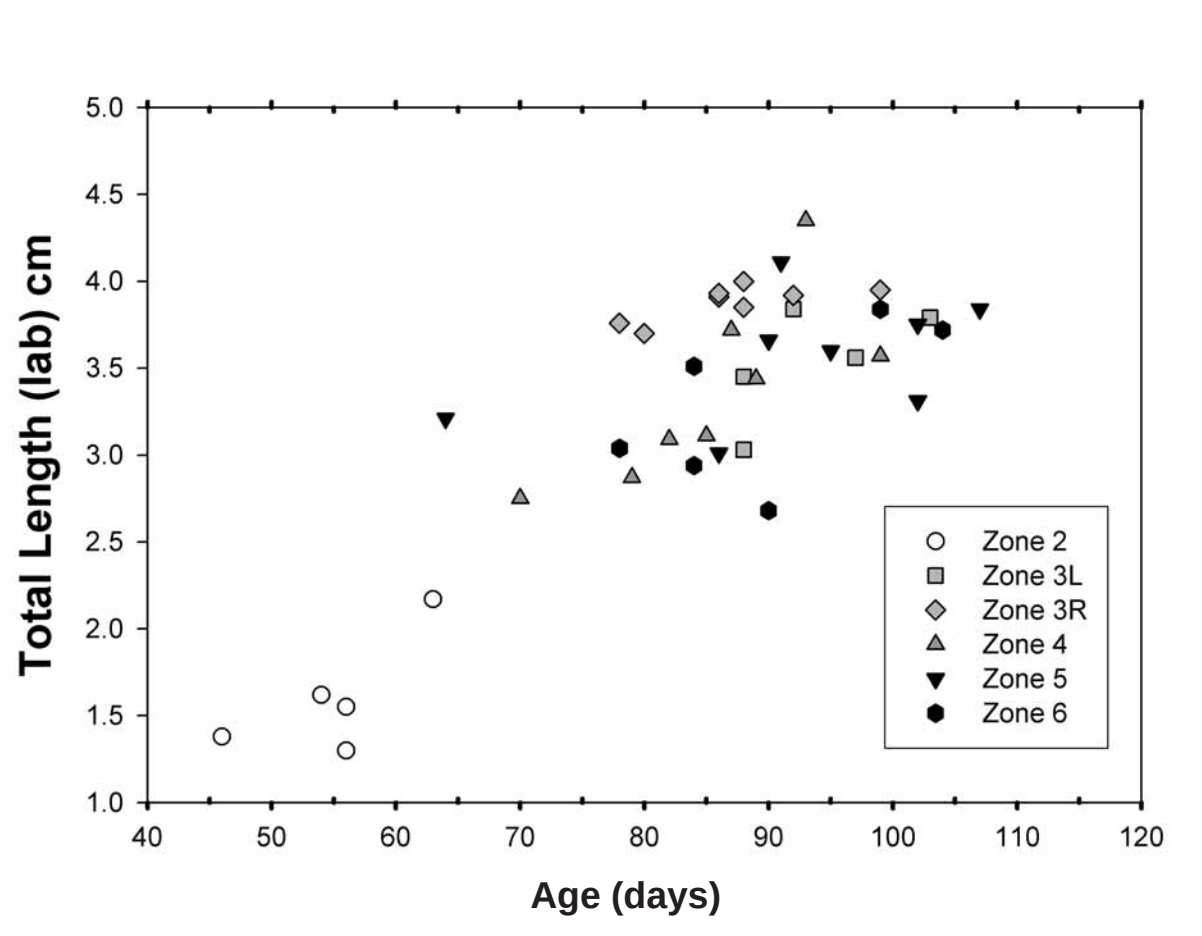


Figure 5.5.4. Relationship between total length (measured in lab) and age (estimated from the number of otolith rings) in Tennessee snubnose darters from the July 2010 ANSP survey.

these daily growth rates (Fig. 5.5.7) were tested using one-way ANOVA. The difference was highly significant ($p < 0.0000001$). As with hatching dates, the difference largely reflects low growth rates at Zone 2 (planned comparison, $p < 0.0000001$). Growth rates were not significantly higher at Zone 3 than at Zones 4-6 (planned comparison, $p < 0.07$). However, growth rates at Zone 3R were significantly higher than those at other zones ($p<0.0018$, planned comparison excluding Zone 2). Secondly, zone differences in regression of the total growth on age were assessed using ANCOVA. Results were similar to those using ANOVA of growth rates: there was a highly significant zone difference due to low growth rates at Zone 2. Growth rates at Zone 3 were weakly significantly different than those at Zones 4-6 (planned comparison, $p < 0.02$). As in the ANOVA, this difference reflects higher growth rate at Zone 3R compared to other zones ($p<0.0000001$, planned comparison excluding Zone 2).

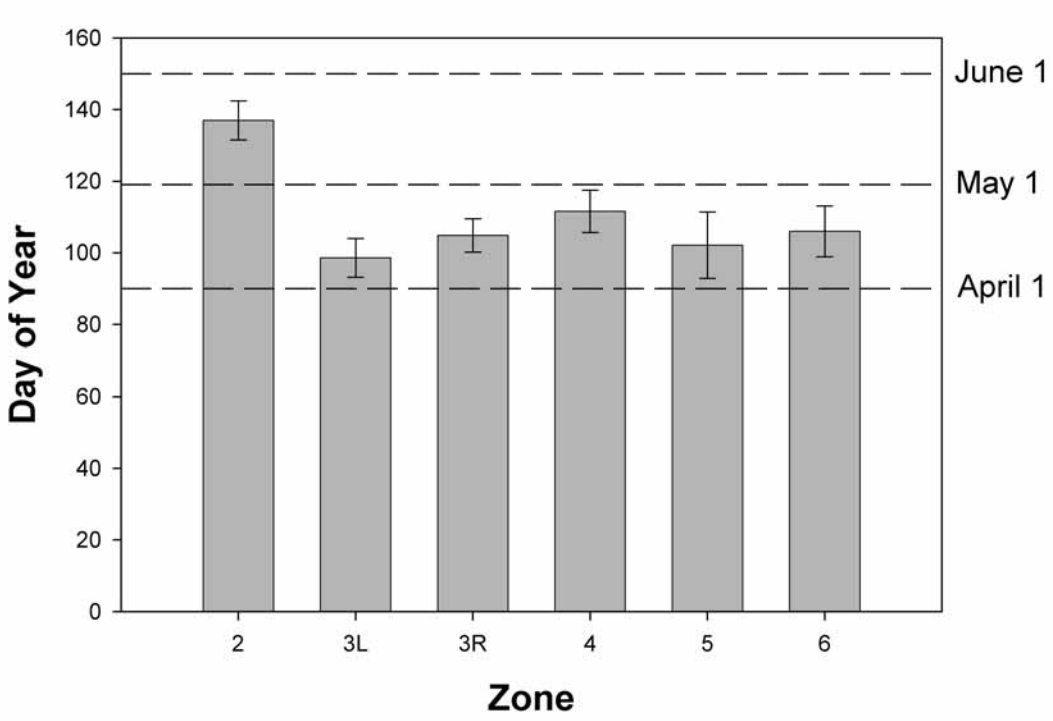


Figure 5.5.5. Mean and standard errors of estimated hatch dates (i.e., the date of the first otolith ring) in Tennessee snubnose darters from the July 2010 ANSP survey.

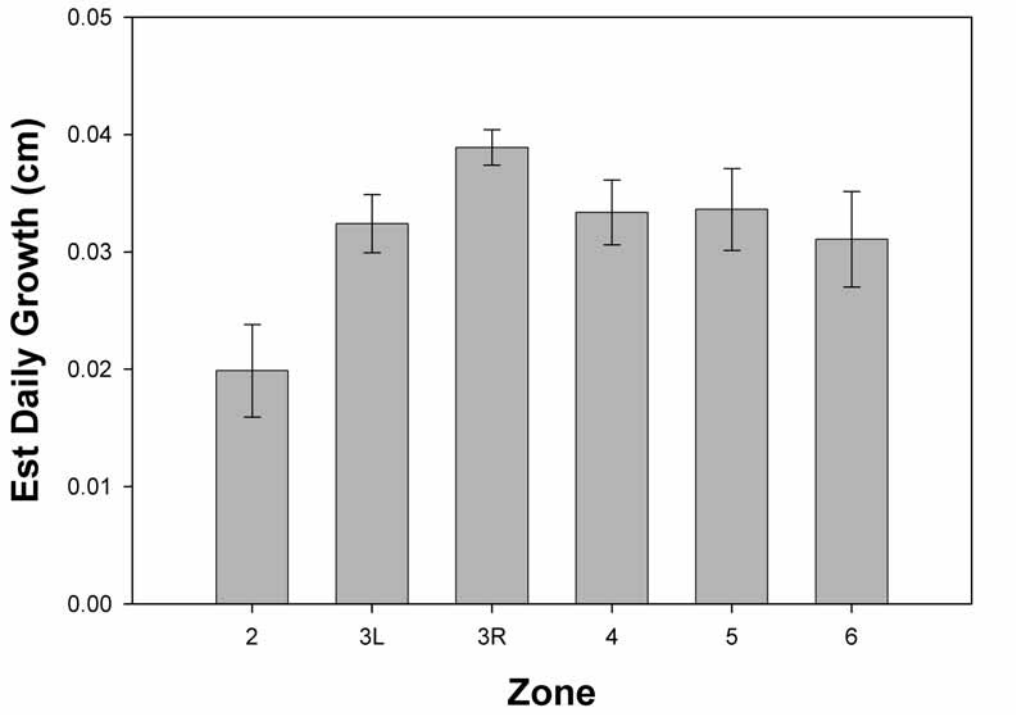


Figure 5.5.6. Mean and standard errors of estimated daily growth rate of Tennessee snubnose darters from the July 2010 ANSP survey. Growth rates are estimated assuming a hatching total length of 0.5 cm.

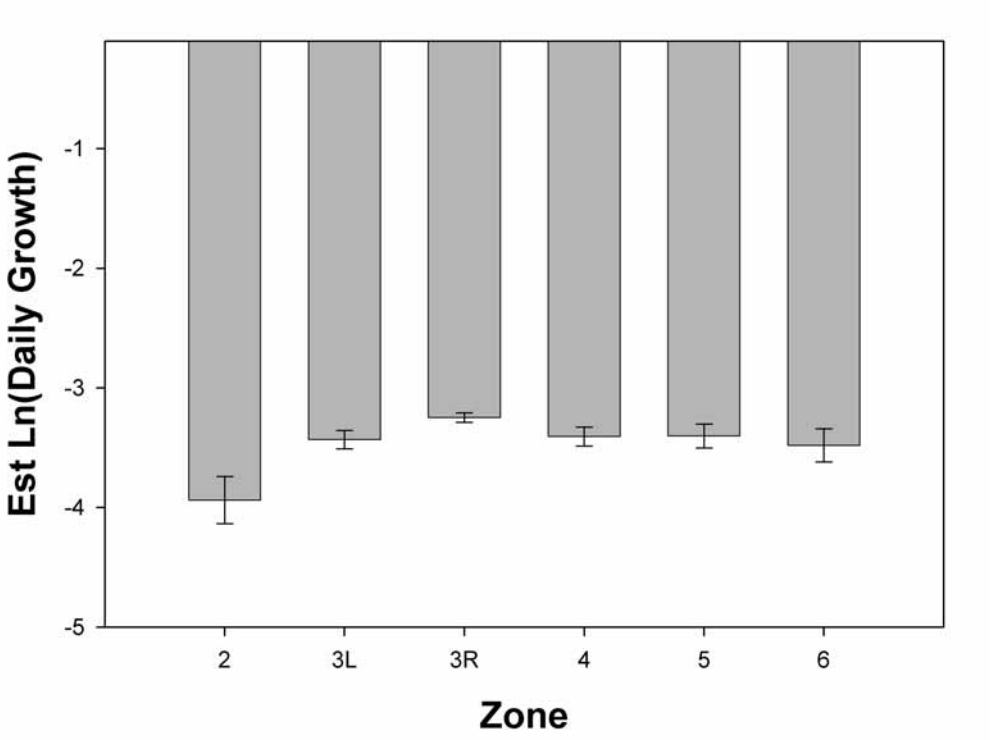


Figure 5.5.7. Mean and two standard errors of the ln(estimated daily growth rate) of Tennessee snubnose darters from the July 2010 ANSP survey.

5.5.9 Discussion

5.5.9.1 Differences Among Zones

Differences among zones could reflect impacts of human disturbance as well as differences in habitat occurrence. Potential anthropogenic stressors differ among the sites, with effects of Fort Patrick Henry Dam most important at Zone 2 and decreasing in importance from Zones 3 to 6. Point-source and urban impacts of Kingsport and local industries would be evident at Zones 3 and 5, as well as the Big Sluice Zones KL and 4, and Zone HC2 on Horse Creek. Zone 6 could show recovery from these various upstream effects but could also show effects of disturbance on the North Fork Holston River. No sampling was done immediately downstream of the North Fork Holston River to address this possibility.

Several comparisons highlight differences between Zone 2 and the other zones. Zone 2 had very low species richness and abundance in the block electrofishing samples; these differences were statistically significant in the ANOVA and ANCOVA analyses. A similar pattern was seen in the one shore electrofishing sample. The high abundance of fish in the lower part of Rock Spring Branch (T2) suggests that this site serves as a refuge for minnows during high flow portions of the dam release cycle. Boat electrofishing showed the presence of rainbow and brown trout in Zone 2. These coldwater fish are maintained by stocking. White sucker was caught primarily at Zone 2. A large striped bass was observed in Zone 2. The white sucker and striped bass are tolerant (or prefer) cool water. Boat electrofishing also found adult black and golden redhorse and several species of sunfish, although catch rates of these were generally lower than at the

other zones. Sunfish were caught mainly in the lower part of Zone 2. The presence of very small postlarval white sucker, telescope shiner and Tennessee snubnose darter suggests later spawning and/or slower growth rate of these species in the cooler water at Zone 2. Temperature measurements during sampling showed cooler temperatures (17.2-18.6^{o}C) compared to other zones (22.0-27.9^{o}C).

Zone 6 had higher species richness and abundance of some species in several types of sampling. Several species, such as streamline chub, logperch and bluebreast darter, were found only at Zone 6. Species richness and abundance of redline darters were statistically higher in the block electrofishing samples from Zone 6 than other zones. The boat electrofishing showed similar patterns. The shore electrofishing samples from Zone 6 had relatively high species richness, but rather low abundances. This partly reflects differences in habitat coverage. In several zones (e.g., Zones 3L, 4 and 5), shore electrofishing covered a mix of riffle and run habitats. At Zone 6, some riffle habitat was covered, but much of the sampling was in runs and coves alongshore. There was relatively low collecting efficiency in these habitats, as evidenced by observations of fish escaping capture.

Patterns among the other riverine zones (Zones 3L, 3R and 5) were not as clear. Overall species richness was higher at Zone 5 than at upstream river zones in both block and shore electrofishing, but there was no clear pattern in average species richness.

The Big Sluice zones (KU, KL and 4) were sampled by shore electrofishing to examine effects of potential landfill leachate. The assemblages were similar among the three zones. All three zones showed high densities of the Tennessee snubnose shiner, moderately high abundance of the greenside darter, and lower abundances of central stoneroller, Northern hog sucker, banded sculpin, smallmouth bass and banded darter. There was no pattern in abundance or species occurrence suggesting negative impacts of the discharge.

The otolith analyses, excluding the questionable samples, indicated a range in hatching dates from 30 March to 10 May for fish from Zones 3-6 and 21 April to 27 May for fish from Zone 2. This is earlier than found in the 1997 study (13 April to 10 June); no specimens from Zone 2 were available in 1997. These dates are consistent with literature values of peak spawning in April to early May (Etnier and Starnes 1993). The most striking aspect of the otolith analyses was the late spawning of darters at Zone 2. Combined with lower growth rates at Zone 2, young-of-year fish were markedly smaller at Zone 2 than at the other zones. The late spawning mode probably reflects cooler spring temperatures at Zone 2 because of the bottom releases from Fort Patrick Henry Dam. Zone 3R showed higher growth rates than the other zones; this could reflect warmer temperatures resulting from cooling-water discharges upstream.

There were relatively few anomalies in 2010 and no clear spatial patterns, except for the occurrence of presumed hook wounds in fish from Zone 5, which may reflect higher fishing pressure related to more public access at that zone than others. Two types of parasites were seen. Black spot was seen only in stonerollers from the two Horse Creek zones. The prevalence of leeches was highest at Zones 5, 3L and 3R and intermediate at Zones KL and KU.

Given the gradient in conditions from Zone 2 downstream, it is difficult to separate possible effects of the multiple stressors in Kingsport on the lower zones. Fish diversity and abundance were relatively low at Zones 3L and 3R, but were generally higher than at Zone 2. There was no clear step change in fish communities from Zone 2 down through Zone 6 suggestive of Kingsport impacts.

5.5.9.2 Temporal Trends in Fish Communities

The Academy has conducted fish surveys on the Holston River since 1965, allowing analysis of changes in the fish fauna. Over the survey period, 60 species have been recorded (Table 5.5.13). One of these (fatlips minnow *Phenacobius crassilabrum*) was recorded only once and at Zone 1, which was not sampled in the 1990-2010 surveys. Twelve other species collected in earlier surveys were not collected in 2010 (goldfish, river chub, popeye shiner, sand shiner, stargazing minnow, fathead minnow, river carpsucker, quillback carpsucker, flathead catfish, longear sunfish, spotted bass and white crappie). Most of these have been rare in the surveys. For example, the only records of popeye shiner, stargazing minnow and quillback carpsucker are single fish caught by boat electrofishing in 1997. Several (goldfish, flathead catfish and white crappie) were only caught in earlier surveys (before 1974). However, river chub, longear sunfish and spotted bass have occurred fairly frequently in recent samples. Five species were recorded for the first time in the 2010 survey: mimic shiner, striped bass, mountain madtom, shorthead redhorse, and speckled darter. Several other species have been caught at current Zones 2-6 in only one other survey: brown trout, creek chub, channel catfish, green sunfish, warmouth, and gilt darter (also in 1997), and the redear sunfish (also in 1990). The streamline chub was recorded for the first time since 1977. Several of these species, such as mountain madtom, streamline chub, and gilt darter, are generally considered sensitive to water quality or habitat degradation.

The sand shiner was caught in Horse Creek in 1990 and 1997. No sand shiners were caught in 2010, but mimic shiners, not previously reported, were found in several sites, including Horse Creek. The two species are similar in appearance, and specimens from 1997 were re-examined to confirm identification. Causes for the loss of one and addition of the other are not known. Similarly, one speckled darter was caught in Horse Creek in 2010. It is not known whether the species is a recent colonist or has occurred in small numbers or in different parts of Horse Creek.

Three species of redhorse suckers were caught in the 2010 survey, one of which (shorthead redhorse) had not been previously recorded and one (golden redhorse) which was found only in Horse Creek prior to 1997. The number of adult redhorse collected in 2010 partly reflects sampling efficiency of the boat electrofishing equipment used in 2010, which was able to capture redhorse in midchannel habitats. However, adult redhorse are vulnerable to gill nets as well as electrofishing and have been sampled on earlier surveys. Furthermore, young-of-year redhorse are often found in shallow water where they are vulnerable to rotenone, backpack electrofishing and even dip netting (several young-of-year black redhorse were captured by dip netting at Zone 6 in 2010). Redhorses are often considered relatively sensitive to habitat and water quality degradation, related to direct toxicity, increased turbidity, or loss of spawning habitat due to sedimentation. The shorthead and black redhorses are more sensitive to these impacts, while the golden redhorse is more tolerant.

Although not previously collected, in 1997 the green sunfish was the most common species of sunfish collected overall, and was the dominant sunfish at several zones. The presence of a number of hybrid sunfish in 1997 (mainly of green sunfish with other species) suggested recent introduction to the area with disruption of behavior of other species. Although present in 2010, the green sunfish was less common than several other species, and only one hybrid sunfish was observed. The green sunfish is a widespread species whose range has expanded greatly historically (Lee et al. 1980). It is very tolerant of poor water quality and environmental variation and is often considered an indicator of degradation (Karr et al. 1986, Miller et al. 1988). In 1997, the redbreast and longear sunfish appeared less widespread and common in the river than in past surveys, suggesting replacement by green sunfish. Redbreast sunfish were relatively common in the 2010 survey, but no longear sunfish were caught. The source of the green sunfish is not known. It is common in small ponds and could have escaped from ponds; it may also have been introduced intentionally or accidentally with other species. In 1997, a number of fathead minnows were caught at Zone HC1, and the species was recorded at Zone 2 as well. The fathead minnow is frequently used as a bait species, and its range has spread due to "bait-bucket" release. It is considered tolerant of poor water and environmental quality. It was not found in 2010.

In general, there appeared to be more aquatic macrophytes in the Holston River than in most previous surveys, especially at Zones 2, 3R and 6. Many fishes use aquatic macrophytes as cover, and many Tennessee snubnose darters and one of the three mountain madtoms caught were captured in cover. The high abundance of the snubnose darter and occurrence of the mountain madtom in 2010 may reflect habitat provided by macrophytes. In contrast, relatively few stonerollers, especially large individuals, were caught in 2010 relative to other surveys. The stoneroller feeds on attached algae, and its abundance may be linked to changes in algal abundance.

The block electrofishing samples provide a consistent basis for comparison among years, although they reflect occurrence in only a single habitat. The 2010 and 1997 block electrofishing results differed among zones. Few fish were caught in Zone 2 in both 2010 and 1997. In 1997, banded sculpins were the only species caught and were caught in three of five samples. In 2010, fish were caught in two of six samples (two snubnose darters and one rainbow trout in one and one banded sculpin in another). Many more species and individuals were caught at Zone 3L in 2010 (average of 19 fish per 25 m^2) than in 1997, with more minnows and darters in 2010. More species were caught in total in 2010, although the average number per sample was similar in both surveys. Only one block sample was taken at Zone 3R in 1997, preventing a good comparison. At Zone 4, 2010 samples had higher abundances, higher average species richness and more species over all samples. A similar pattern was seen at Zone 5, although the difference is not as great. At Zone 6, average densities and species richness were similar, although more species were caught overall in 2010 (17 species) than in 1997 (8 species).

The information on presence-absence among all surveys provides a longer temporal view of zone trends. These comparisons are affected by differences in the amount of sampling by different techniques and protocols and by differences in collecting conditions. The pattern of dam releases in 2010 led to higher minimum flows than in 1997, when daily cycles led to relatively low water levels in the morning. The difference was especially notable at Zones 2, 3R and 5, where

accessible habitats were more limited in 2010. Even more extreme fluctuations were noted in some of the early surveys, when the river channel was reduced to isolated pools at the lowest release levels.

More species have been recorded at Zone 2 in the three most recent surveys (Table 5.5.14; Appendices 7.6.4-7.6.12), but this is mainly due to the use of additional techniques (boat electrofishing, trapping and gill nets), and sampling over larger areas. Among similar techniques, there has been little trend in species occurrence. There is some tendency to fewer species at Cliffside in recent surveys, but this probably reflects lower effort at this site as other parts of the zone were sampled. Three sport species, the rainbow trout, brown trout and striped bass, were caught in Zone 2 in 2010, all by electrofishing (with the exception of a single rainbow trout caught in a block electrofishing sample). The two trout species are stocked in the area to form a tailwater trout fishery, and striped bass are stocked in downstream reservoirs. The banded sculpin is the only species which has been caught in all surveys at the zone, suggesting low or transient populations of most species, particularly in the upper part of the zone. In 2010, adult and young-of-year white suckers and adult carp, black and golden redhorse and several species of sunfish were also caught in Zone 2.

Zone 3L was sampled only in the 1980, 1990, 1997 and 2010 surveys (Table 5.5.14 and Appendix 7.6.8). There has been an increase in the number of species caught at the zone in both 1997 and 2010. Two minnow species, two species of bass and two darter species were first caught at Zone 3L in 2010. Together these suggest some improvement in the zone, although the species richness is still lower than in downstream zones. White sucker was not caught at Zone 3L for the first time.

Zone 3R (Appendix 7.6.9) has shown a dramatic increase in species richness and abundance over the survey period. No fish were caught in 1965. Comparisons at Zone 3R are complicated by the location of capture. Reports indicate that the area below the dam was sampled prior to 1980, but the location was changed to above the dam because of changes in discharges in the zone. In 1997 and 2010, both areas were sampled. The earliest collections were of tolerant species (yellow bullhead, mosquitofish and white sucker), and these species were common in 1990, as well. These species were less common or absent in 1997 and 2010, and several species of minnows, sunfish, bass and darters were caught. The redline and gilt darters, both relatively intolerant species, were caught at Zone 3LR (treated as part of Zone 3R) in 2010; these had not been recorded in earlier samples.

Fewer species were caught at Zone 4 in 2010 than in 1997 (Appendix 7.6.10). Several species typical of cover and backwaters, such as mosquitofish, yellow bullhead and green sunfish, were caught in 1997 but not in 2010. Some species indicative of good habitat and water quality, such as river chub, bigeye chub and bluebreast darter, were caught in 1997 at Zone 4 but not in 2010. Recent surveys (from 1990 on) have found smallmouth bass and more species of darters than previous surveys.

Comparisons of Zone 5 (Table 5.5.14; Appendix 7.6.11) are complicated by changes in the location of sampling. Up to 1977, sampling was done on the right bank and both banks were sampled in 1980. Sampling in 1990 was done on the left bank because of possible leakage of

water from the North Fork through the dividing levee. Sampling in 1997 and 2010 was done mainly along the left bank, with some right bank collections upstream of the North Fork Holston levee. Collections at Zone 5R have been highly variable over time, possibly related to differences in sampling conditions. The high richness recorded at the right bank in 1980 occurred during low flows when fish were concentrated into small pools. More species were caught at Zone 5 in 2010 than in any other survey (Appendix 7.6.11), but this partly reflects boat electrofishing catches in 2010. Excluding boat electrofishing, fewer species were caught in 2010 than in 1997. This could be partly due to greater effort and collecting efficiency in shore electrofishing in 1997. Species richness in the lower riffle and run areas on the left bank (Zone 5L) was similar in 1980 (13 species) and 1990 (12 species), and lower than the 1997 and 2010 results. These results suggest improvements in the fish communities at the zone after 1990. It is difficult to discern any changes after 1997, given differences in collecting conditions and effort between the two most recent surveys.

Species richness at Zone 6 (Table 5.5.14; Appendix 7.6.12) has been relatively constant over the 1965-1990 period. Excluding 1977 (which was sampled only by seines, which are less effective than rotenone or electrofishing), 15-18 species were collected each year (excluding trapping and gill netting in 1990). In 1997, 23 species were collected (plus 10 additional species collected by boat electrofishing). In 2010, 28 species were collected (plus 7 additional species collected by boat electrofishing). Several species were caught in 2010 which have not been previously caught at the zone, including gizzard shad, streamline chub, shorthead redhorse, mountain madtom, mimic shiner, green sunfish, banded darter and logperch. However, several species caught in 1997, such as river chub, popeye shiner, stargazing minnow and gilt darter, were not caught in 2010. It is likely that sampling effort, particularly by boat electrofishing, was not large enough to document all uncommon and rare species at species-rich zones like 5 and 6, so that many of the apparent differences between 1997 and 2010 collections reflect collecting completeness.

In 1997, two specific types of fin anomalies were noted. One involved fin erosion without a healed edge, sometimes including the flesh of the caudal peduncle as well. The second involved shortened or asymmetric caudal fins, often with thickened skin and with a healed edge. The former was hypothesized to represent bacterial or fungal infection resulting from fish stress and/or high abundance of bacteria or fungi. The second may have represented healed response to the first type or may have been due to another cause. In 2010, eight individual fish were noted with some kind of caudal injury, but none showed either of the two types noted in 1997. Caudal injuries in the 2010 fish might have been due to predation by other fish or handling by fishermen (five of the eight fish were species vulnerable to angling), rather than reflecting disease.

In summary, the fish assemblage of the South Fork and mainstem Holston rivers appears similar to that of 1997, with some differences in species recorded likely reflecting incomplete sampling and/or year-to-year variation in abundance. Several of the species documented in 2010 which have not been found in recent or in all previous surveys are species indicative of good habitat and water quality, such as shorthead redhorse, streamline chub and mountain madtom. However, some relatively intolerant species recorded in 1997 were not recorded in 2010, such as spotted bass and river chub. Several species which appeared to be less common in 2010 than in 1997, such as green sunfish and fathead minnow, are very tolerant species. The gradient in impairment of the fish assemblages from Zone 2 downstream to Zone 6 was seen in 2010, as in previous

surveys. This gradient can be explained by decreasing effects of flow regulation by the Fort Patrick Henry dam, but effects of various other anthropogenic impacts of the Kingsport area are probably also important. There seem to be greater changes in assemblages in some parts of the study area. The assemblages at Zones 3L and 3R were richer, with more intolerant species, and some anomalies observed in 1997 were not seen in 2010. The cold tailwaters at Zone 2 continue to support sport species, including rainbow trout, brown trout and striped bass. However, the density and species richness of other species is low. The size structure of some species at Zone 2 indicates either delayed spawning or slow growth due to water temperature or other factors. The different sampling techniques provided somewhat conflicting information on conditions at Zone 4. However, in general, Zone 4 appeared intermediate in metrics relative to the other zones. In 2010, a new comparison of assemblages along Big Sluice was made; this showed no consistent differences among the three zones, KU, KL and 4.

Table 5.5.1. Common and scientific names of fish caught in the 2010 ANSP survey of the South Fork and mainstem Holston rivers and Horse Creek near Kingsport, TN (Page 1 of 2).

Scientific Name	Common Name	Total Caught
	Clupeidae - herrings	
Dorosoma cepedianum	gizzard shad	5
	Cyprinidae - carps and minnows	
Campostoma anomalum	central stoneroller	234
Cyprinella galactura	whitetail shiner	2
Cyprinella spiloptera	spotfin shiner	6
Cyprinus carpio	common carp	14
Erimystax dissimilis	streamline chub	2
Luxilus chrysocephalus	striped shiner	36
Luxilus coccogenis	warpaint shiner	14
Notropis amblops	bigeye chub	67
Notropis leuciodus	Tennessee shiner	1
Notropis photogenis	silver shiner	9
Notropis rubellus	rosyface shiner	24
Notropis sp. (sawfin)	sawfin shiner	39
Notropis telescopus	telescope shiner	340
Notropis volucellus	mimic shiner	12
Pimephales notatus	bluntnose minnow	5
Rhinichthys atratulus	blacknose dace	14
Semotilus atromaculatus	creek chub	1
	Catostomidae - suckers	
Catostomus commersoni	white sucker	58
Hypentelium nigricans	northern hog sucker	26
Moxostoma duquesnei	black redhorse	35
Moxostoma erythrurum	golden redhorse	32
Moxostoma macrolepidotum	shorthead redhorse	14
Moxostoma species*	redhorse species	3

Table 5.5.1 (continued). Common and scientific names of fish caught in the 2010 ANSP survey of the South Fork and mainstem Holston rivers and Horse Creek near Kingsport, TN (Page 2 of 2).

Scientific Name	Common Name	Total Caught
	Ictaluridae - bullhead catfishes	
Ameiurus natalis	yellow bullhead	8
Ictalurus punctatus	channel catfish	1
Noturus eleutherus	Mountain madtom	3
	Salmonidae - trout	
Oncorhynchus mykiss	rainbow trout	22
Salmo trutta	brown trout	1
	Poeciliidae - livebearers	
Gambusia affinis	western mosquitofish	40
	Cottidae - sculpins	
Cottus carolinae	banded sculpin	479
	Percichthyidae - temperate perches	
Morone species	striped bass or hybrid striper	1
	Centrarchidae - sunfishes	
Ambloplites rupestris	rock bass	111
Lepomis auritus	redbreast sunfish	67
Lepomis cyanellus	green sunfish	13
Lepomis gulosus	warmouth	2
Lepomis hybrid*	sunfish hybrid	1
Lepomis macrochirus	bluegill	35
Lepomis microlophus	redear sunfish	7
Micropterus dolomieu	smallmouth bass	159
Micropterus salmoides	largemouth bass	22
	Percidae - perches	
Etheostoma blennioides	greenside darter	297
Etheostoma camurum	bluebreast darter	18
Etheostoma rufilineatum	redline darter	252
Etheostoma simoterum	Tennessee snubnose darter	1278
Etheostoma stigmaeum	speckled darter	1
Etheostoma zonale	banded darter	135
Percina caprodes	logperch	1
Percina evides	gilt darter	1

*not counted in total.

Table 5.5.2. Occurrence of different fish species caught on the South Fork and mainstem Holston rivers and Horse Creek during ANSP sampling in July 2010. (X: collected, -: not collected) (Page 1 of 2)

	Zone											
Species	**2**	**3L**	**3R**	**3L+3R**	**4**	**5**	**6**	**HC1**	**HC2**	**KL**	**KU**	**T2**
Dorosoma cepedianum	-	-	-	-	-	X	X	-	-	-	-	-
Campostoma anomalum	-	X	X	X	X	X	X	X	X	X	X	X
Cyprinella galactura	-	-	-	-	-	X	X	-	-	-	-	-
Cyprinella spiloptera	X	-	-	-	-	X	X	-	-	-	-	-
Cyprinus carpio	X	-	-	-	-	X	X	-	-	-	-	-
Erimystax dissimilis	-	-	-	-	-	-	X	-	-	-	-	-
Luxilus chrysocephalus	-	-	X	X	X	X	X	X	X	-	X	-
Luxilus coccogenis	-	-	-	-	X	X	X	X	-	-	-	-
Notropis amblops	-	-	-	-	-	X	X	X	X	-	-	X
Notropis leuciodus	-	-	-	-	-	-	X	-	-	-	-	-
Notropis photogenis	-	-	-	-	-	-	X	-	X	-	-	-
Notropis rubellus	-	X	-	X	-	-	-	-	X	-	-	-
Notropis sp (sawfin)	-	-	X	X	-	X	X	-	-	-	-	-
Notropis telescopus	X	X	X	X	X	X	X	X	X	X	X	X
Notropis volucellus	-	-	-	-	-	X	X	X	X	X	-	-
Pimephales notatus	-	-	-	-	-	-	X	-	X	-	-	-
Rhinichthys atratulus	X	-	-	-	-	-	-	X	X	-	-	X
Semotilus atromaculatus	-	-	-	-	-	-	-	-	X	-	-	-
Catostomus commersoni	X	-	-	-	-	-	-	-	X	-	-	-
Hypentelium nigricans	-	X	-	X	X	X	X	X	X	X	X	-
Moxostoma duquesnei	X	-	-	-	-	X	X	-	-	-	-	-
Moxostoma erythrurum	X	-	X	X	-	X	X	-	-	-	-	-
Moxostoma macrolepidotum	-	-	-	-	X	-	X	-	-	-	-	-
Moxostoma species	-	-	-	-	-	X	-	-	-	-	-	-
Ameiurus natalis	-	-	-	-	-	-	X	X	-	X	-	-
Ictalurus punctatus	-	-	-	-	-	X	-	-	-	-	-	-
Noturus eleutherus	-	-	-	-	-	X	X	-	-	-	-	-
Oncorhynchus mykiss	X	-	-	-	-	-	-	-	-	-	-	-
Salmo trutta	X	-	-	-	-	-	-	-	-	-	-	-
Gambusia affinis	-	X	-	X	-	-	X	X	X	-	-	-
Cottus carolinae	X	-	-	-	X	X	X	X	X	X	X	-
Morone species	X	-	-	-	-	-	-	-	-	-	-	-

Table 5.5.2 (continued). Occurrence of different fish species caught on the South Fork and mainstem Holston rivers and Horse Creek during ANSP sampling in July 2010. (X: collected, -: not collected) (Page 2 of 2).

	Zone											
Species	**2**	**3L**	**3R**	**3L+3R**	**4**	**5**	**6**	**HC1**	**HC2**	**KL**	**KU**	**T2**
Ambloplites rupestris	X	X	-	X	X	X	X	X	X	X	-	-
Lepomis auritus	X	X	X	X	X	X	X	X	X	-	-	-
Lepomis cyanellus	-	-	-	-	-	X	X	-	X	-	-	-
Lepomis gulosus	X	-	-	-	-	X	-	-	-	-	-	-
Lepomis hybrid	-	-	-	-	-	-	X	-	-	-	-	-
Lepomis macrochirus	X	-	-	-	-	X	X	X	X	-	-	-
Lepomis microlophus	-	-	-	-	-	X	-	-	-	-	-	-
Micropterus dolomieu	-	X	X	X	X	X	X	X	X	X	X	-
Micropterus salmoides	-	X	X	X	-	X	X	-	X	-	-	-
Etheostoma blennioides	-	X	X	X	X	X	X	X	X	X	X	-
Etheostoma camurum	-	-	-	-	-	-	X	-	-	-	-	-
Etheostoma rufilineatum	-	X	X	X	X	X	X	X	X	X	-	-
Etheostoma simoterum	X	X	X	X	X	X	X	X	X	X	X	X
Etheostoma stigmaeum	-	-	-	-	-	-	-	X	-	-	-	-
Etheostoma zonale	-	X	-	X	X	X	X	X	-	X	X	-
Percina caprodes	-	-	-	-	-	-	X	-	-	-	-	-
Percina evides	-	-	X	X	-	-	-	-	-	-	-	-

Table 5.5.3. Average fish density (fish per 25 m^2) in block net electrofishing samples at each zone on the South Fork and mainstem Holston rivers during ANSP sampling in July 2010.

Scientific	Zone					
	2	3L	3R	4	5	6
Campostoma anomalum	-	1.33	0.33	2.43	1.33	1.63
Cottus carolinae	0.17	-	-	-	-	0.25
Cyprinella galactura	-	-	-	-	-	0.13
Etheostoma blennioides	-	2.00	2.67	5.43	2.67	1.38
Etheostoma camurum	-	-	-	-	-	2.13
Etheostoma rufilineatum	-	-	0.67	1.42	0.11	17.75
Etheostoma simoterum	0.33	3.67	3.33	5.00	0.67	3.13
Etheostoma zonale	-	1.00	-	5.00	1.67	5.25
Hypentelium nigricans	-	-	-	0.29	0.22	0.13
Luxilus chrysocephalus	-	-	-	0.14	-	0.25
Luxilus coccogenis	-	-	-	0.14	-	0.13
Micropterus dolomieu	-	0.33	-	-	0.22	-
Moxostoma macrolepidotum	-	-	-	0.29	-	-
Notropis leuciodus	-	-	-	-	-	0.13
Notropis photogenis	-	-	-	-	-	0.25
Notropis rubellus	-	7.67	-	-	-	-
Notropis sp. (sawfin)	-	-	-	-	0.11	0.38
Notropis telescopus	-	3.00	1.00	4.43	2.78	6.38
Noturus eleutherus	-	-	-	-	0.11	0.13
Oncorhynchus mykiss	0.17	-	-	-	-	-
Percina caprodes	-	-	-	-	-	0.13
Percina evides	-	-	0.33	-	-	-
Total	0.67	19.00	8.33	24.56	9.89	39.51
Total No. of Fish	4	57	25	172	89	316
Avg. No. of Species	0.5	3.33	2.67	4.57	3.44	6.25
Total No. of Species	3	7	6	10	10	17
No. of Samples	6	3	3	7	9	8
Total Area Sampled (m^2)	150	75	75	175	225	200

Table 5.5.4. Results of ANOVA and ANCOVA tests comparing density of major species, species richness and diversity of fishes among zones in the 2010 ANSP survey. Rows for the same species indicate tests using different variables. Rows with no entry for velocity or PCA indicate ANOVA tests of zone differences with no covariate tests. Rows with an entry for velocity indicate ANCOVA tests of zone differences with velocity as a covariate. Rows with an entry for PCA indicate ANCOVA tests of zone differences with the second PCA axis as a covariate (the first PCA axis was not significant for any test). "p" indicates p-values of tests, "ns" indicates non-significant, and "-" indicates that the variable was not included in the test.

Dependent Variable	r^2	p value			Significant Comparisons	
		Zone	Velocity	PCA	Planned Comparisons	Post Hoc (Pairwise) Comparisons
Stoneroller (*Campostomas anomalum*)	0.17	ns	-	-	-	-
Greenside darter (*Etheostoma blennoiodes*)	0.32	0.0343	-	-	3L,3R,4,5,6>2	4>2
	-	ns	ns	-	-	-
Redline darter (*E. rufilineatum*)	0.63	<0.0001	-	-	6>5	6>2,3L,3R,4,5
	0.78	<0.0001	0.0055	-	6>5	6>2,3L,3R,4,5
	0.71	0.0002	-	0.0143	6>5	6>2,3L,3R,4,5
Tennessee snubnose darter (*E. simoterum*)	0.33	0.0296	-	-	none	-
	-	ns	ns	-	-	-
Banded darter (*E. zonale*)	0.45	0.0024	-	-	3L,3R,4,5,6>2	4,6>2
	0.64	0.0074	ns	-	3L,3R,4,5,6>2	4,6>2
Telescope shiner (*Notropis telescopus*)	0.15	ns	-	-	-	-
Number of species	0.57	0.0001	-	-	6>5 and 3L,3R,4,5,6>2	4,6>2 and 6>5
Shannon-Wiener diversity	0.44	0.051	-	-	3L,3R,4,5,6>2	none

Table 5.5.5. Summary of the percent variance explained by and the correlation of environmental covariates to the Principal Components Analysis Factors.

	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5
Total % Variance	47.43	37.37	13.30	1.25	0.66
Cumulative % Variance	47.43	84.80	98.10	99.34	100.00
Avr Velocity	-0.78	0.57	-0.21	-0.13	0.08
Max Velocity	-0.76	0.59	-0.22	0.15	-0.07
Avr Depth	-0.80	-0.56	0.17	-0.11	-0.10
Max Depth	-0.74	-0.60	0.26	0.11	0.10
Substrate	0.00	0.72	0.69	-0.01	-0.01

Table 5.5.6. Average fish density (fish per 25 m^2) in shore electrofishing samples at each zone on the South Fork and mainstem Holston rivers and Horse Creek during ANSP sampling in July 2010.

	Zone										
Species	2	3L	3R	4	5	6	HC1	HC2	KL	KU	T2
Campostoma anomalum	-	1.37	0.19	0.70	1.22	0.22	1.73	1.36	1.92	0.59	0.50
Cyprinella galactura	-	-	-	-	0.10	-	-	-	-	-	-
Cyprinella spiloptera	0.12	-	-	-	-	-	-	-	-	-	-
Luxilus chrysocephalus	-	-	-	-	0.41	0.26	0.32	0.16	-	0.06	-
Luxilus coccogenis	-	-	-	-	0.71	-	0.13	-	-	-	-
Notropis amblops	-	-	-	-	-	0.04	0.53	0.73	-	-	0.33
Notropis photogenis	-	-	-	-	-	-	-	0.03	-	-	-
Notropis rubellus	-	-	-	-	-	-	-	0.03	-	-	-
Notropis telescopus	1.79	-	-	-	0.20	0.09	1.09	0.08	0.17	0.06	19.50
Notropis volucellus	-	-	-	-	-	-	0.03	0.08	0.17	-	-
Pimephales notatus	-	-	-	-	-	0.04	-	0.03	-	-	-
Rhinichthys atratulus	-	-	-	-	-	-	0.11	0.03	-	-	1.00
Semotilus atromaculatus	-	-	-	-	-	-	-	0.03	-	-	-
Catostomus commersoni	-	-	-	-	-	-	-	0.03	-	-	-
Hypentelium nigricans	-	0.25	-	0.04	0.20	0.18	0.05	0.08	0.08	0.37	-
Moxostoma duquesnei	-	-	-		-	0.09	-	-	-	-	-
Moxostoma erythrurum	-	-	0.09	-	-	0.13	-	-	-	-	-
Moxostoma macrolepidotum	-	-	-	0.04	-	0.22	-	-	-	-	-
Ameiurus natalis	-	-	-	-	-	-	0.03	-	0.17	-	-
Gambusia affinis	-	0.12	-	-	-	0.04	0.29	0.31	-	-	-
Cottus carolinae	-	-	-	0.05	0.10	-	8.61	3.53	0.25	0.28	-
Ambloplites rupestris	-	-	-	0.42	0.51	0.04	0.37	0.52	0.08	-	-
Lepomis auritus	-	-	0.84	0.04	-	-	0.08	0.24	-	-	-
Lepomis cyanellus	-	-	-	-	-	0.39	-	0.05	-	-	-
Lepomis hybrid	-	-	-	-	-	0.04	-	-	-	-	-
Lepomis macrochirus	-	-	-	-	-	-	0.03	0.31	-	-	-
Micropterus dolomieu	-	0.25	1.59	0.78	1.63	-	0.58	0.60	0.33	0.89	-
Micropterus salmoides	-	0.25	0.19	-	-	0.04	-	0.03	-	-	-
Etheostoma blennioides	-	1.12	-	2.65	0.71	0.35	1.14	0.86	1.83	2.78	-
Etheostoma rufilineatum	-	0.12	-	0.78	-	0.04	1.20	0.81	0.17	-	-
Etheostoma simoterum	0.12	7.71	1.31	13.61	5.79	1.32	3.06	2.59	18.67	14.94	1.00
Etheostoma stigmaeum	-	-	-	-	-	-	0.03	-	-	-	-
Etheostoma zonale	-	0.62	-	0.46	0.81	0.31	0.16	-	0.17	0.11	-
Total	2.02	11.82	4.20	19.58	12.40	3.86	19.56	12.49	24.00	20.07	22.33
Total No. of Fish	17	95	45	343	122	88	736	478	288	345	134
Avg. No. of Species	3.0	9.0	6.0	7.7	12.0	11.0	17.0	18.0	9.0	5.3	5.0
Total No. of Species	3	9	6	11	12	18	20	23	12	9	5
No. of Samples	1	1	1	3	1	2	2	2	2	3	1
Average width (m)	3.0	3.0	4.0	3.0	3.0	3.0	5.13	7.65	3.0	3.0	-
Total Area Sampled (m^2)	210	201	268	420	246	570	940.5	956.5	300	345	150

Table 5.5.7. Raw density (fish/25 m^2) of fish caught using shore electrofishing at Horse Creek Zones HC1 and HC2 during ANSP sampling in July 2010.

Species	Raw Density Zone HC1	Raw Density Zone HC2
Cyprinidae		
Campostoma anomalum	1.73	1.36
Luxilus chrysocephalus	0.32	0.16
Luxilus coccogenis	0.13	-
Notropis amblops	0.53	0.73
Notropis photogenis	-	0.03
Notropis rubellus	-	0.03
Notropis telescopus	1.09	0.08
Notropis volucellus	0.03	0.08
Pimephales notatus	-	0.03
Rhinichthys atratulus	0.11	0.03
Semotilus atromaculatus	-	0.03
Catostomidae		
Catostomus commersoni	-	0.03
Hypentelium nigricans	0.05	0.08
Ictaluridae		
Ameiurus natalis	0.03	-
Poeciliidae		
Gambusia affinis	0.29	0.31
Cottidae		
Cottus carolinae	8.61	3.53
Centrarchidae		
Ambloplites rupestris	0.37	0.52
Lepomis auritus	0.08	0.24
Lepomis cyanellus	-	0.05
Lepomis macrochirus	0.03	0.31
Micropterus dolomieu	0.58	0.60
Micropterus salmoides	-	0.03
Percidae		
Etheostoma blennioides	1.14	0.86
Etheostoma rufilineatum	1.20	0.81
Etheostoma simoterum	3.06	2.59
Etheostoma stigmaeum	0.03	-
Etheostoma zonale	0.16	-
Total	19.56	12.49
Area (m^2)	940.50	956.5

Table 5.5.8. Numbers of fish collected in boat electrofishing samples with a second chase boat also netting on the South Fork and mainstem Holston rivers during ANSP sampling in July 2010.

	Total Number of Fish			Average Fish per 100 m		
Species	Zone 2	Zone 5	Zone 6	Zone 2	Zone 5	Zone 6
Dorosoma cepedianum	-	3	2	-	0.26	0.27
Cyprinella spiloptera	-	1	4	-	0.08	0.68
Cyprinus carpio	1	12	1	0.05	1.97	0.14
Erimystax dissimilis	-	-	2	-	-	0.27
Luxilus chrysocephalus	-	-	2	-	-	0.25
Luxilus coccogenis	-	-	-	-	-	-
Notropis amblops	-	9	7	-	0.74	0.92
Notropis photogenis	-	-	6	-	-	1.29
Notropis sp. (sawfin)	-	-	3	-	-	0.76
Notropis telescopus	1	2	27	0.04	0.16	7.60
Notropis volucellus	-	2	4	-	0.16	0.51
Pimephales notatus	-	-	3	-	-	0.37
Catostomus commersoni	56	-	-	3.22	-	0.00
Hypentelium nigricans	-	1	3	-	0.09	0.76
Moxostoma duquesnei	11	6	13	0.62	0.60	2.39
Moxostoma erythrurum	1	8	19	0.05	0.77	4.11
Moxostoma macrolepidotum	-	-	6	-	-	1.51
Moxostoma species	-	3	-	-	0.26	-
Ameiurus natalis	-	-	1	-	-	0.12
Ictalurus punctatus	-	1	-	-	0.17	-
Oncorhynchus mykiss	21	-	-	1.08	-	-
Salmo trutta	1	-	-	0.04	-	-
Morone species	1	-	-	0.05	-	-
Ambloplites rupestris	4	46	5	0.25	6.56	0.61
Lepomis auritus	3	28	12	0.12	3.49	1.68
Lepomis cyanellus	-	2	-	-	0.34	-
Lepomis gulosus	1	1	-	0.04	0.17	-
Lepomis macrochirus	2	17	3	0.08	2.57	0.37
Lepomis microlophus	-	7	-	-	0.85	-
Micropterus dolomieu	-	16	28	-	1.87	5.73
Micropterus salmoides	-	16	-	-	2.05	-
Etheostoma blennioides	-	1	-	-	0.09	-
Etheostoma camurum	-	-	1	-	-	0.31
Etheostoma simoterum	2	-	1	0.09	-	0.12
Total	105	182	153	5.68	23.25	30.76
Number of Species	13	19	22			
Number of Samples	7	3	3			
Total Duration Shocked (min)	95	52	45			
Total Distance Shocked (m)	1823.4	986.8	613.6			

Table 5.5.9. Numbers of fish collected in boat electrofishing samples by the primary boat only on the South Fork and mainstem Holston rivers during ANSP sampling in July 2010.

Species	Total Number of Fish			Average Fish per 100 m		
	Zone 2	Zone 5	Zone 6	Zone 2	Zone 5	Zone 6
Dorosoma cepedianum	-	3	2	-	0.26	0.27
Cyprinella spiloptera	-	1	4	-	0.08	0.68
Cyprinus carpio	1	12	1	0.05	1.97	0.14
Erimystax dissimilis	-	-	1	-	-	0.12
Luxilus chrysocephalus	-	-	2	-	-	0.25
Luxilus coccogenis	-	-	-	-	-	-
Notropis amblops	-	9	4	-	0.74	0.49
Notropis photogenis	-	-	6	-	-	1.29
Notropis sp. (sawfin)	-	-	2	-	-	0.61
Notropis telescopus	1	2	25	0.04	0.16	7.31
Notropis volucellus	-	2	3	-	0.16	0.37
Pimephales notatus	-	-	3	-	-	0.37
Catostomus commersoni	53	-	-	3.06	-	-
Hypentelium nigricans	-	1	2	-	0.09	0.45
Moxostoma duquesnei	11	5	10	0.62	0.51	1.78
Moxostoma erythrurum	1	8	17	0.05	0.77	3.68
Moxostoma macrolepidotum	-	-	4	-	-	1.23
Moxostoma species	-	3	-	-	0.26	-
Ameiurus natalis	-	-	1	-	-	0.12
Ictalurus punctatus	-	1	-	-	0.17	-
Oncorhynchus mykiss	20	-	-	1.04	-	-
Salmo trutta	1	-	-	0.04	-	-
Morone species	1	-	-	0.05	-	-
Ambloplites rupestris	4	39	5	0.25	5.71	0.61
Lepomis auritus	3	24	12	0.12	3.06	1.68
Lepomis cyanellus	-	2	-	-	0.34	-
Lepomis gulosus	1	1	-	0.04	0.17	-
Lepomis macrochirus	2	16	3	0.08	2.39	0.37
Lepomis microlophus	-	4	-	-	0.43	-
Micropterus dolomieu	-	9	23	-	1.11	4.38
Micropterus salmoides	-	13	-	-	1.79	-
Etheostoma blennioides	-	1	-	-	0.09	-
Etheostoma camurum	-	-	1	-	-	0.31
Etheostoma simoterum	2	-	1	0.09	-	0.12
Total	101	156	132	5.54	20.29	26.63
Number of Species	13	19	22			
Number of Samples	7	3	3			
Total Duration Shocked (min)	95	52	45			
Total Distance Shocked (m)	1823.4	986.8	613.6			

Table 5.5.10. Summary of analyses of condition. Weight-length regressions are analyzed by ANCOVA on ln-transformed total weight (g) and total length (cm). Columns report p-values of zone effects and slope differences (zone-length interactions), model correlation coefficients, numbers of specimens analyzed, and least squares mean weights (i.e., weights adjusted for weight-length regressions).

						Zone					
	p zone diff/slope differences	r^2	3L	3R	4	5	6	HC1	HC2	KL	KU
		Etheostoma simoterum **(Tennessee snubnose darter)**									
Model and # of specimens	0.0018/0.0312	0.93	59	25	75	48	41	90	51	86	75
LSMEANS			-0.43	-0.55	-0.46	-0.44	-0.43	-0.33	-0.42	-0.43	-0.38
Significant pairwise differences	6>5										
		Campostoma anomalum **(Stoneroller)**									
Model and # of specimens	0.0020/ns	0.97	15	3	23	23	17	57	47	23	4
LSMEANS			1.00	0.83	0.94	0.88	0.90	0.88	0.87	1.03	0.99
Significant pairwise differences	none										

Table 5.5.11. Summary of information on observed external anomalies on fishes from the July 2010 ANSP study. Anomalies are keyed as: C (caudal erosion), L (lesions), E (emaciation), Oh (Open wound or hook wound), B (black spot). Caudal erosion is modified by: I (large), s (small).

Zone	Species	Total No.	Number With Anomalies	% Observed With
2	*Oncorhynchus mykiss*	22	1 C	4.5
	Lepomis auritus	3	1 Cl	33.3
	Catostomus commersoni	57	1 C	1.8
	All species	136	3	2.2
3L	All species	183	0	0.0
3R	*Lepomis auritus*	9	1 Cl	11.1
	All species	114	1	0.9
4	*Etheostoma blennioides*	75	2 C	1.3
	Ambloplites rupestris	9	1 L	11.1
	All species	515	3	0.6
5	*Micropterus salmoides*	16	4 Oh	25.0
	Micropterus dolomieu	34	1 Oh	2.9
	Etheostoma zonale	23	1 Cs	4.3
	Ambloplites rupestris	51	1 Oh	2.0
	All species	393	7	1.8
6	All species	601	0	0.0
HC1	*Campostoma anomalum*	65	12 B	18.5
	Ameiurus natalis	1	1 L	100.0
	All species	743	13	1.7
HC2	*Notropis amblops*	28	1 E	3.6
	Micropterus dolomieu	23	1 LCl	4.3
	Campostoma anomalum	52	27 LB	51.9
	All species	493	29	5.9
KL	All species	288	0	0.0
KU	All species	346	0	0.0

Table 5.5.12. Number (and percentages) of specimens collected with leeches during the July 2010 survey.

Species	Zone											All
	2	3L	3R	4	5	6	HC1	HC2	KL	KU	T2	
Ambloplites rupestris Rock bass	-	-	-	-	1 (2.0)	-	-	-	-	-	-	1 (1.0)
Etheostoma blennioides Greenside darter	-	-	2 (13.3)	1 (1.3)	6 (18.75)	-	-	-	1 (4.5)	-	-	10 (3.4)
Etheostoma simoterum Tennessee snubnose darter	-	1 (10.0)	15 (20.3)	1 (0.4)	24 (38.1)	1 (1.4)	1 (0.87)	-	5 (4.1)	9 (3.3)	-	57 (4.5)
Etheostoma zonale Banded darter	-	6 (75.0)	-	1 (2.4)	5 (21.7)	-	-	-	-	-	-	12 (8.9)
Lepomis auritus Redbreast sunfish	-	-	2(22.2)	-	-	-	-	-	-	-	-	2 (3.0)
All species	0 (0)	7 (3.8)	19 (16.7)	3 (0.6)	36 (9.2)	1 (0.2)	1 (0.1)	0 (0)	6 (2.1)	9 (2.6)	0 (0)	82 (2.1)

Table 5.5.13. Occurrence of species of fish caught in ANSP surveys of the South Fork and mainstem Holston rivers and Horse Creek from 1965 to 2010. X indicates occurrence in zones sampled in 2010. H indicates occurrence only at Horse Creek. R, O, and 1 indicate that the species was not collected in the current sampling Zones 2-6 during a survey, with R indicating occurrence at 5R, O occurrence at the original Zone 6, and 1 indicating occurrence at Zone 1. (Page 1 of 2)

Scientific Name	1965	1974	1976	1977	1980	1990	1997	2010
Dorosoma cepedianum	1	1	-	-	X	X	X	X
Campostoma anomalum	X	X	H	X	X	X	X	X
Carassius auratus	-	Obs	-	-	-	-	-	-
Cyprinella galactura	1	X	H	1	X	X	X	X
Cyprinella spiloptera	-	X	-	-	-	X	X	X
Cyprinus carpio	X	-	H	-	-	X	X	X
Erimystax dissimilis	X	R	-	X	-	-	-	X
Luxilis chrysocephalus	O	X	H	X	X	X	X	X
Luxilus coccogenis	1	1	H	1	1	-	X	X
Nocomis micropogon	X	X	-	R	X	X	X	-
Notropis amblops	1	X	H	-	1	H	X	X
Notropis ariommus	-	-	-	-	-	-	X	-
Notropis leuciodus	1	-	-	-	R1	X	X	X
Notropis photogenis	-	-	-	1	X	-	X	X
Notropis rubellus	-	X	H	R	R	-	X	X
Notropis stramineus	-	-	-	-	-	H	H	-
Notropis telescopus	1	1	H	X	X	X	X	X
Notropis sp. (sawfin)	-	X	H	-	X	X	X	X
Notropis volucellus	-	-	-	-	-	-	-	X
Phenacobius crassilabrum	-	-	-	-	1	-	-	-
Phenacobius uranops	-	-	-	-	-	-	X	-
Pimephales notatus	O	X	-	X	X	H	H	X
Pimephales promelas	1	1	-	-	-	-	X	-
Rhinichthys atratulus	O1	X	-	-	-	X	X	X
Semotilus atromaculatus	O1	-	-	1	-	-	X	H
Carpiodes carpio	-	-	-	X	X	X	-	-
Carpiodes cyprinus	-	-	-	-	-	-	X	-
Catostomus commersoni	-	X	H	X	X	X	X	X
Hypentelium nigricans	X	X	H	X	X	X	X	X
Moxostoma duquesnei	O	X	-	-	X	-	X	X
Moxostoma erythrurum	-	-	H	-	-	H	X	X
Moxostoma macrolepidotum	-	-	-	-	-	-	-	X
Ameiurus natalis	X	X	H	X	X	X	X	X
Ictalurus punctatus	1	-	-	-	-	-	X	X
Noturus eleutherus	-	-	-	-	-	-	-	X
Pylodictus olivaris	-	R	-	-	-	-	-	-
Onchorhynchus mykiss	X	-	-	-	-	X	-	X
Salmo trutta	-	-	-	-	-	-	X	X

Table 5.5.13 (continued). Occurrence of species of fish caught in ANSP surveys of the South Fork and mainstem Holston rivers and Horse Creek from 1965 to 2010. X indicates occurrence in zones sampled in 2010. H indicates occurrence only at Horse Creek. R, O, and 1 indicate that the species was not collected in the current sampling zones 2-6 during a survey, with R indicating occurrence at 5R, O occurrence at the original Zone 6, and 1 indicating occurrence at the original Zone 1. (Page 2 of 2)

Scientific Name	1965	1974	1976	1977	1980	1990	1997	2010
Gambusia affinis	OR	X	-	-	X	X	X	X
Cottus carolinae	X	X	-	X	X	X	X	X
Morone species	-	-	-	-	-	-	-	X
Ambloplites rupestris	O1	X	-	-	X	X	X	X
Lepomis auritus	X	X	-	-	X	X	X	X
Lepomis cyanellus	-	-	-	-	-	-	X	X
Lepomis gulosus	-	-	-	-	-	-	X	X
Lepomis hybrid	-	-	-	-	-	-	X	X
Lepomis macrochirus	X	X	-	-	X	X	X	X
Lepomis megalotis	-	X	-	-	X	X	X	-
Lepomis microlophus	-	-	-	-	-	X	-	X
Lepomis species	-	-	-	-	-	-	X	-
Micropterus dolomieu	-	X	-	X	X	X	X	X
Micropterus punctulatus	X	-	-	-	-	X	X	-
Micropterus salmoides	-	X	-	-	X	X	X	X
Pomoxis annularis	O	-	-	-	-	-	-	-
Etheostoma blennioides	-	-	H	R	X	X	X	X
Etheostoma camurum	-	-	-	-	-	X	X	X
Etheostoma rufilineatum	1	-	H	-	X	X	X	X
Etheostoma simoterum	X	X	H	X	X	X	X	X
Etheostoma stigmaeum	-	-	-	-	-	-	-	H
Etheostoma zonale	-	-	-	-	R	-	X	X
Percina caprodes	X	-	-	X	1	H	-	X
Percina evides	-	-	-	-	-	-	X	X
Total number of species	30	30	16	21	32	35	46	47
Number of species, without 1, 5U and H	21	26	-	17	28	30	44	45
Number of species, without 1, 5U and H, and excluding boat shock, gill net and trap samples	21	26	-	17	28	29	37	35

Table 5.5.14. Numbers of species of fish caught at different zones in ANSP surveys of the South Fork and mainstem Holston rivers and Horse Creek from 1965 to 2010. (*=count includes both banks.)

Zone	1965	1974	1977	1980	1990	1997	2010
1	19	12	8	18	-	-	-
2	6	4	6	3	12	12	16
3L	-	-	-	5	5	9	13
3R	0	2	2	2	8	11	12
3L+3R	-	-	-	6	9	16	17
4	11	11	3	10	20	17	14
5	3	13	8	23*	19*	28	29
6	15	18	9	16	17	23	35

6. LITERATURE CITED

Academy of Natural Sciences of Philadelphia (ANSP). 1966. Holston River survey, 1965. River survey report for the Tennessee Eastman Company. Acad. Nat. Sci. Phila. 75 pp.

_____. 1967. Cursory survey of the Holston River in the vicinity of Kingsport, Tennessee, 1967 for the Tennessee Eastman Company. Acad. Nat. Sci. Phila. 3 pp.

_____. 1975. Holston River Survey Report 1974 for the Tennessee Eastman Company. Acad. Nat. Sci. Phila. 117 pp.

_____. 1976. Letter report on the fresh-water mussels and fishes in Horse Creek, Kingsport, Tennessee. Acad. Nat. Sci. Phila. 5 pp.

_____. 1978. 1977 Holston River studies for the Tennessee Eastman Company. Report No. 78- 1. Acad. Nat. Sci. Phila. 90 pp.

_____. 1981. 1980 Holston River Studies for the Tennessee Eastman Company. Report No. 81-9F. Acad. Nat. Sci. Phila. 185 pp.

_____. 1992. Aquatic field studies in the vicinity of Kingsport, Tennessee, 1990. Report No. 91-24F. Acad. Nat. Sci. Phila. 382 pp.

_____. 1997. Aquatic field studies in the White River near Batesville, Arkansas. Report No. 96-22F. Acad. Nat. Sci. Phila. 172 pp.

_____. 1998. Aquatic field studies in the vicinity of Kingsport, Tennessee, 1997. Report No. 97-17F. Acad. Nat. Sci. Phila. 213 pp.

_____. 2001. Final Report: Riparian Reforestation in an Urbanizing Watershed: Effects of Upland Conditions on Instream Ecological Benefits. U.S. EPA Grant Number R825798.

American Public Health Association, American Water Works Association and Water Environment Federation (APHA, AWWA and WEF). 1981. Standard Methods for the Examination of Water and Wastewater, 15th ed. United Book Press, Ind. Maryland, USA.

_____. 1992. Standard Methods for the Examination of Water and Wastewater, 18th ed. United Book Press, Ind., Maryland, USA.

_____. 1998. Standard Methods for the Examination of Water and Wastewater, 20th ed. United Book Press, Ind., Maryland, USA.

Barbour, M.T., J. Gerritsen, B.D. Snyder and J.B. Stribling. 1999. Rapid bioassessment protocols for use in streams and wadeable rivers: Periphyton, benthic macroinvertebrates, and fish. EPA 841-B-99-002. US Environmental Protection Agency, Washington, D.C.

Bogan, A.E. and P.W. Parmalee. 1983. Tennessee's rare wildlife. Volume 11: The mollusks. Tennessee Wildlife Resources Agency. 123 pp.

Bray, J.R. and J.T. Curtis. 1957. An ordination of upland forest communities of southern Wisconsin. Ecological Monographs 27:325-349.

Burch, J.B. 1982. Freshwater Snails (Mollusca: Gastropoda) of North America. US Environmental Protection Agency, Cincinnati, OH.

Clarke, A.H. 1981. The Freshwater Mollusks of Canada, National Museum of Natural Sciences, Ottawa, Canada.

Cohen, J. 1988. Statistical Power Analysis for the Behavioral Sciences. 2nd edition. Lawrence Erlbaum Associates, Hillsdale, New Jersey. 567 pp.

Cummins, K.W. 1973. Trophic relations of aquatic insects. Annual Review of Entomology. 18:183-206.

Cummins, K.W. 1974. Structure and Function of stream ecosystems. Bioscience 24:631-641.

Etnier, D.A, and W.C. Starnes. 1993. The Fishes of Tennessee. University of Tennessee Press. Knoxville, TN. 681 pp.

Fonseca, D.M. and D.D. Hart. 1996. Density-dependent dispersal of blackfly neonates is mediated by flow. Oikos. 75:49-58.

Goslee, S.C. and D.L. Urban. 2007. The ecodist package for dissimilarity-based analysis of ecological data. Journal of Statistical Software 22(7):1-19.

Hart, D.D. and C.M. Finelli.1999. Physical-biological coupling in streams: The pervasive effects of flow on benthic organisms. Annual Review of Ecology and Systematics. 30:363-395.

Hill, M.O. and H.G. Gauch. 1980. Detrended correspondence analysis, an improved ordination technique. Vegetatio. 42: 47-58.

Hilsenhoff, W.L. 1987. An improved biotic index of organic stream pollution. Great Lakes Entomol. 20:31-39.

Hobbs, H.H., Jr. 1989. An illustrated checklist of the American crayfishes (Decapoda: Astacidae, Cambaridae, and Parastacidae). Smithsonian Institution Press, Washington, D.C.

Hynes, H.B.N. 1972. The Ecology of Running Waters. University of Toronto Press. 555 pp.

Jongman, R.H.G., C.J.F. ter Braak and O.F.R. van Tongeren. 1987. Data Analysis in Community and Landscape Ecology. Pudoc, Wageningen, The Netherlands.

Karr, J.R. and E.W. Chu. 1999. Restoring life in running waters: Better biological monitoring. Island Press, Washington, D.C.

Karr, J.R., K.D. Fausch, P.L. Angermeier, P.R. Yant and I.J. Schlosser. 1986. Assessing biological integrity in running waters: A method and its rationale. Illinois Natural History Survey, Special Publication 5, Urbana, Illinois.

Klemm, D.J., ed. 1985. Freshwater leeches (Annelida: Hirudinea). Pages 70-173 *in* a Guide to the Freshwater Annelida (Polychaeta, Naidid and Tubificid Oligochaeta, and Hirudinea) of North America. Kendallmunt, Dubuque, IA.

Klemm D.J. 1991. Taxonomy and pollution ecology of the Great Lakes region leeches (Annelida: Hirudinea). Michigan Academician 24:37-103.

Krebs, C.J. 1999. Ecological Methodology, 2nd edition. Benjamin Cummings, Menlo Park, California. 620 pp.

Krebs, C.J. 2009. Ecology: The Experimental Analysis of Distribution and Abundance, 6th edition. Benjamin Cummings, San Francisco. 655 pp.

Lee, D.S., C.R. Gilbert, C.H. Hocutt, R.E. Jenkins, D.E. McAllister and J.R. Stauffer, Jr. 1980. Atlas of North American Freshwater Fishes. North Carolina State Museum Natur. Hist. 854 pp.

Leland, H.V. 1995. Distribution of phytobenthos in the Yakima River Basin, Washington, in relation to geology, land use, and other environmental factors. Canadian Journal of Fisheries and Aquatic Sciences 52:1108–1129.

Lenat, D.R. 1993. A biotic index for the southeastern United States: derivation and list of tolerance values, with criteria for assigning water-quality ratings. Journal of the American Benthological Society. 12:279-290.

Lenat, D.R. and D.L. Penrose. 1996. History of the EPT taxa richness metric. Bulletin of the North American Benthological Society. 13(2).

Lowe, R.L. 1974. Environmental requirements and pollution tolerance of freshwater diatoms. Nat. Environ. Res. Center, U.S. EPA, Cincinnati, OH. 334 pp.

Merritt, R.W., K.W. Cummins and M.B. Berg (eds.). 2008. An Introduction to the Aquatic Insects of North America, 4th edition. Kendall / Hunt, Dubuque, IA.

Miller, D.L., P.M. Leonard, R.M. Hughes, J.R. Karr, P.B. Moyle, L.H. Schrader, B.A. Thompson, R.A. Daniels, K.D. Fausch, G., A. Fitzhugh, J.R. Gammon, D.B. Halliwell, P.L. Angermeier and D.J. Orth. 1988. Regional application of an index of biotic integrity for use in water resource management. Fisheries 13: 12-20.

Millipore. 1972. Biological Analysis of Water and Wastewater, Application Manual AM302. Millipore Corp., Bedford, MA.

Minshall, G.W. 1984. Aquatic insect-substratum relationships. Pages 358-400 *in* Resh, V.H. and D.M. Rosenberg (eds.) 1984. The Ecology of Aquatic Insects. Praeger, N.Y. 625 pp.

Parmalee, P.W. and A.E. Bogan. 1998. The freshwater mussels of Tennessee. University of Tennessee Press, Knoxville, TN.

Peckarsky, B.L. 1996. Alternative predator avoidance syndromes of stream-dwelling mayfly larvae. Ecology 77(6): 1888-1905.

Peckarsky, B.L., P.R. Fraissinet, M.A. Penton and D.J. Conklin. 1990. Freshwater macroinvertebrates of Northeastern North America. Cornell University Press, Ithaca, NY.

Pennak, R.W. 1989. Fresh-water invertebrates of the United States. Wiley and Sons, Inc., New York.

Porter, S.G., T.F. Cuffney, M.E. Gurtz, and M.R. Meador. 1993. Methods for collecting algal samples as part of the national water quality assessment program, U.S. Geological Survey Open-File Report 93-409.

R Development Core Team. 2010. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. http://www.R-project.org.

Resh, V.H. and D.M. Rosenberg (eds.) 1984. The Ecology of Aquatic Insects. Praeger, N.Y. 1984. 625 pp.

Rosenberg, D.M. and V.H. Resh. 1993. Freshwater biomonitoring and benthic macroinvertebrates. Chapman and Hall, New York. 488 pp.

Siddall, M.E. and E. Borda. 2003. Phylogeny and revision of the leech genus *Helobdella* (Glossiphoniidae) based on mitochondrial gene sequences and morphological data and a special consideration of the *triserialis* complex. Zoologica Scripta. 32: 23-33.

Smith, D.G. 2001. Pennak's Freshwater Invertebrates of the United States: Porifera to Crustacea. Fourth Edition. John Wiley & Sons Inc., New York. 638 pp.

Sokal, R.R. and F.J. Rohlf. 1995. Biometry: The Principles and Practice of Statistics in Biological Research, 3rd edition. W. H. Freeman and Co., New York. 887 pp.

Spaulding, S.A. and L. Elwell. 2007 Increase in nuisance blooms and geographic expansion of the freshwater diatom *Didymosphenia geminata*. U.S. Geological Survey Open-File Report 2007-1425, 38 pp.

ter Braak, C.J.F. 1987. CANOCO -A Fortran Program for Canonical Community Ordination. TNO Inst. Appl. Computer Sci., The Netherlands.

ter Braak, C.J.F. and P. Smilauer, P. 2002. CANOCO Reference manual and CanoDraw for Windows User's guide: Software for Canonical Community Ordination (version 4.5). Microcomputer Power (Ithaca, NY, USA), 500 pp.

ter Braak, C.J.F. and P.F.M. Verdonschot. 1995. Canonical correspondence analysis and related multivariate methods in aquatic ecology. Aquatic Sciences 57: 255-289.

Thorp, J.H. and A.P. Covich. 2001. Ecology and classification of North American freshwater invertebrates. p. 1056. Academic Press, San Diego, CA.

Turgeon, D.D., J.F. Quinn, A.E. Bogan, E.V. Coan, F.G. Hochberg, W.G. Lyons, P.M. Mikkelsen, R.J. Neves, C.F.E. Roper, G. Rosenberg, B. Roth, A. Scheltema, F.G. Thompson, M. Vecchione, and J.D. Williams. 1998. Common and scientific names of aquatic invertebrates from the United States and Canada: mollusks. 2nd edition. American Fisheries Society Special Publication 26. pp. 509.

United States Environmental Protection Agency (USEPA). 1983. Methods for chemical analysis of water and wastes. Office of Research and Development, USEPA, Cincinnati, OH. EPA-60014-79-020.430 pp.

_____. 1992. Methods for the determination of chemical substances in marine and estuarine environmental samples. EPA/600®-921121, Office of Research and Development, U.S. EPA, Washington, D.C.

_____. 1993. Methods for the determination of inorganic substances in environmental samples. EPA/600®-931100, Office of Research and Development, U.S. EPA, Washington, D.C.

Vannote R.L., G.W. Minshall, K.W. Cummins, J.R. Sedell, C.E. Cushing. 1980. The River Continuum Concept. Canadian Journal of Fisheries and Aquatic Sciences 37:130-137.

Wentworth, C.K. 1922. A scale of grade and class terms for clastic sediments. Journal of Geology 30:377–392.

Werner, D. ed., 1977. The biology of diatoms. Botanical Monographs, v. 13: Berkeley, Calif., University of California Press, 498 pp.

Wiederholm, T. 1983. Chironomidae of the Holarctic region. Keys and diagnoses. Part 1 Larvae. Entomologica Scandinavica (suppl.) 19: 1-457.

Wilkinson, D.J. 2009. Stochastic modeling for quantitative description of heterogeneous biological systems. Nature Reviews 10:122–133.

Withers, D.I. 2009. A Guide to the rare animals of Tennessee. Division of Natural Areas, Tennessee Department of Environment and Conservation. Tennessee Natural Heritage Program. 61 pp.

Zar, J.H. 1999. Biostatistical analysis, 4th edition. Prentice Hall, Upper Saddle River, NJ.

7. APPENDICES

Appendix 7.1 An Overview of Some Commonly Used Descriptive Methods for Multivariate Ecological Data

7.1.1 Introduction

In this Appendix, we briefly review several descriptive statistical methods for multivariate data, which are employed in the present report but may not be familiar to the reader. By multivariate data, we mean data in which each observation consists of multiple attributes. For example, the data might comprise species lists for ecological samples, with each species found in a given sample being recorded as a '1' (or perhaps as its numerical abundance in the sample) and each species not found in the sample but known to occur in the study area being recorded as a '0'.The list for each sample would represent a single datum or observation, and the presence/absence scores for the various species would be the multiple attributes. If, on the other hand, only the total number of species was recorded for each sample, then each observation would consist of a single attribute (the total number of species for that sample) and we would be faced with univariate rather than multivariate data.

Multivariate ecological datasets typically are complex, making it difficult to identify informative patterns they may contain. For example, there might be data from several different sampling zones, with twenty or more species collected at each. The ecologist is interested in knowing whether the lists of species and their abundances at the different sites show evidence of real differences among sites, and if so, which sites are most similar to one another and which are most different. One approach is to consider only a single property of the data from each zone; e.g., the total number of species, total number individuals, or total biomass. This reduces the data to univariate form, so they can be handled by analysis of variance. But it throws away information, because the identities of the various species are not considered. To take an extreme example, two sites with completely different species but the same total number would be indistinguishable if we only considered the number of species present. More subtly, the main difference between, say, an unpolluted and a mildly polluted site might lie in the relative abundances of a small fraction of the species which are highly sensitive to the pollutant, a difference which is unlikely to be detected if we lump all species together in our statistical analyses. Multivariate statistical methods allow us to include information separately for each species, making it possible to detect complex and unsuspected patterns which may yield insight into key differences between sampling sites.

The foregoing report makes use of two different categories of multivariate methods, traditionally called *classification* and *ordination* methods. Classification methods use simple computational algorithms to create discrete groups within the dataset and (usually) to construct a tree-like diagram showing the estimated degree of similarity among the various groups identified. Cluster analysis is an example of this type of method. Ordination methods do not create discrete groups. Instead, using moderately complex computational algorithms, they rescale and systematically adjust the data in such a way that much of their information content is condensed to a two-dimensional form suitable for graphing. The investigator inspects the resulting graph to determine visually which species or sampling sites are located close together (i.e., are similar) and which are far apart. An attempt may also be made to interpret the associations in terms of underlying gradients in environmental variables (e.g., water chemistry, current speed, substrate type). Correspondence analysis and canonical correspondence analysis are two examples of this type of method. Another example is principal components analysis which, while not employed in the foregoing report, is widely used and may already be familiar to the reader.

In summary, though classification and ordination methods differ in their approaches, they share the goal of reducing the mass of complicated information in multivariate datasets to a simple form which can be interpreted merely by visual inspection of a graph. In addition, both are exploratory methods since they can suggest patterns but cannot confirm them (in the sense that one can confirm patterns at a known level of statistical confidence using, for example, analysis of variance). We now describe these methods in somewhat more detail and explain how to interpret the different types of graph each produces.

7.1.2 Classification Methods: Cluster Analysis

Cluster analysis refers to a diverse set of descriptive methods for exploratory analysis of multivariate data. The basic goal of these methods is to estimate the degree of similarity among the various observations and to use this information to look for natural groupings in the data. The outcome of cluster analysis can be either a set of clusters, representing the "best" grouping of the observations (in which case both the number of clusters and their members are chosen optimally, in some sense), or a dendrogram, which is a diagram that looks like a family tree and shows graphically the estimated degree of similarity among the various observations. Though the former approach is commonly used in certain applications (e.g., associating terrain types with pixel states in digital satellite images), it is the latter approach which is almost always used in ecological applications.

Dendrograms can be constructed in a variety of ways. There is a fundamental distinction between agglomerative and divisive methods. Agglomerative methods start with every observation in its own group, and then merge them step by step until all observations have been placed in a single group. At each step of this process, the two most similar of the remaining groups are merged. The resulting dendrogram shows the entire sequence of merges carried out, which in turn reveals which observations or groups of observations were considered to be most similar at each step of the process. Divisive methods, on the other hand, begin with all observations in a single group and proceed in the opposite direction.

Regardless of whether an agglomerative or a divisive method is used, cluster analysis requires the investigator to choose a specific method of measuring similarity between observations and also a specific method of measuring similarity between groups of observations. (It is sometimes convenient to think in terms of distances between objects instead of similarity, but there is no essential difference.) At each step of an agglomerative analysis, for example, numerical similarities are computed for all remaining pairs of objects (observations and previously formed clusters), and the two most similar are merged.

Numerous indices are available for measuring similarity between observations. Two of the more common ones are the Jaccard index and the Simple Matching index, both of which are scaled to range between 0 (minimum similarity) and 1 (maximum similarity). The Jaccard index is based on the number of positive matches between two ecological samples; that is, the number of species shared by the samples. The Simple Matching index is like the Jaccard index, except that both positive and negative matches are counted, negative matches being species which are known to occur in the study area but are absent from both samples. Another commonly used measure of similarity is the Bray-Curtis index, which differs from the Jaccard and Simple Matching indices by making use of each species' observed abundance rather than simply scoring it as present ('1') or absent ('0'). Any of these similarity indices can be subtracted from 1 to produce a corresponding measure of distance.

There are also numerous methods for measuring the similarity of clusters. In the Nearest Neighbor method, for example, the similarity between clusters is taken to be the greatest similarity or "shortest distance" between any pair of observations spanning the two clusters, while the Farthest Neighbor method employs the least similarity or "greatest distance" between pairs of observations. Another common method is the Arithmetic Average method, which uses the arithmetic average of all pairs of observations spanning the two clusters. Any such method can also be applied to measure the similarity between a cluster and a single observation.

In summary, three basic choices must be made in order to generate a dendrogram: the clustering method, the similarity index for pairs of observations, and the method of measuring similarity between clusters. Each of these choices is largely subjective and is made primarily on the basis of previous experience and personal preference. The dendrogram resulting from a particular set of choices can be quite different from that resulting from another set, so it is a good idea to investigate the robustness of the dendrogram by trying different choices. Assessing robustness is all the more important because none of the traditional methods yields anything analogous to statistical confidence intervals. It is important to remember that even if one creates a set of purely random multivariate data, cluster analysis will still construct a dendrogram. The pattern of similarities will of course be purely coincidental and therefore meaningless, but traditional cluster analysis provides no way of determining this because it does not assess statistical confidence. For these reasons, cluster analysis should be viewed as an exploratory method whose usefulness lies primarily in suggesting patterns to be more-rigorously investigated by other methods.

7.1.2.1 How to Interpret a Dendrogram

Dendrograms are used to assess the similarity of observations in much the same way a family tree is used to determine how closely related various surviving family members are. The first step in interpreting a dendrogram is to locate the observations--the branch tips of the tree. These are normally displayed in such a way that the most similar are closest together. The two most similar observations can be located by working from the branch tips toward the base of the trunk, looking for the pair of observations whose branches join first. After joining, this pair of observations becomes a single cluster and is represented in the dendrogram by a single branch. The next most similar pair of objects is then located by continuing to work toward the base of the trunk, looking for the next pair of branches that join. And so on.

Observations that join near the branch tips are most similar, while those that remain in different clusters until nearly reaching the base of the trunk are least similar. Computer programs that construct dendrograms usually provide a scale for reading off the estimated degree of similarity (or distance, if measures of distance rather than similarity were used) between objects at any level of the dendrogram. This scale is represented as an axis running from the base of the trunk (least similarity or greatest distance) to the branch tips (greatest similarity or least distance). In constructing the diagram, each time two objects are merged, the point at which the corresponding branches of the dendrogram join is placed so its position on the similarity axis equals the numerical similarity between the objects. It can be shown mathematically that, at least for the measures of similarity or distance in current use, numerical similarity cannot increase with successive merges, and in practice it nearly always decreases. Thus, the positions on the similarity axis at which branches of the dendrogram join start at high similarity and proceed successively toward low similarity.

A hypothetical example with four observations is shown in Figure 7.1.1. The observations are labeled a, b, c, and d. Starting at the branch tips, b and d join first and are therefore the most similar. These two branches join at a position of about 0.8 on the similarity axis. Working toward the base of the trunk, the next two branches to join correspond to observations a and c, and this merge occurs at about 0.7 on the similarity axis. The final merge occurs between the branches corresponding to clusters bd and ac at about 0.2 on the similarity axis. Thus, observations b and d are the most similar, while observations a and c are the next most similar objects. Moreover, a and c are almost as similar as b and d, while the two clusters, bd and ac, are substantially less similar to one another. This suggests there are two natural groupings in the data, a conclusion which should be further investigated by trying a different index of similarity among observations and a different method of measuring similarity among clusters.

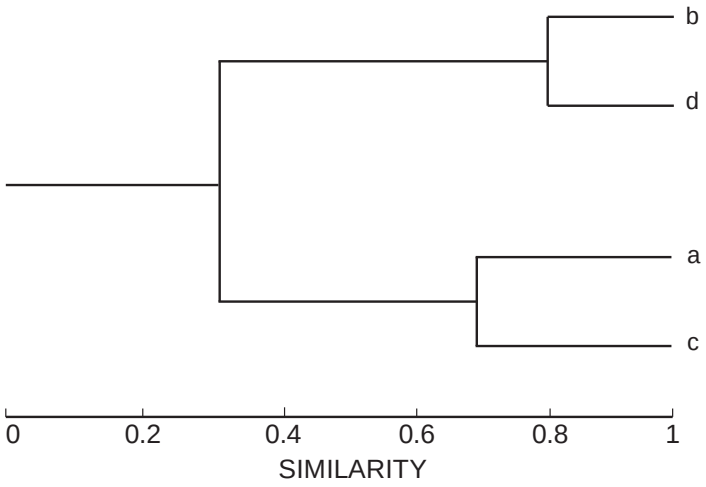


Fig. 7.1.1. A simple dendrogram of the type produced by cluster analysis. Observations are labeled *a-d*.

7.1.3 Ordination Methods: Correspondence Analysis and Canonical Correspondence Analysis

A fundamental tenet of ecology is that different species have distinct properties, and that their optimal conditions for survival and growth therefore differ. Thus, if sampling sites in an ecological study differ with respect to a key environmental variable, we expect to see related differences in species abundances. Put another way, if we ordered the samples from different sites with respect to the key environmental parameter (e.g., increasing water depth or increasing phosphorus concentration), then we would expect to see a related ordering in the abundances of different species, with some being most abundant at one end of the gradient, some in the middle, and some at the other end. Ordination methods are statistical techniques that help us look for such natural orderings of species or samples when we do not know the key environmental gradient.

7.1.3.1 A Simple Example with Two Species

Ordination methods are designed to help us make inferences about possible but unknown environmental gradients, based on an ecological dataset. But to appreciate the basic idea underlying these methods, it is useful to proceed in the opposite direction. Suppose, then, that we already know the key environmental gradient, and we collect samples at various points along it to document the relationship between the gradient and the abundances of two species. Thus, aside from its location on the gradient, each sample has two attributes: the abundance of Species 1 and the abundance of Species 2.

Suppose we graph the abundance of each species against its position on the gradient and obtain the relationships shown in Fig. 7.1.2A. Now take the two abundances at each sampling location and plot one against the other, the x-coordinate of each point being the abundance of Species 1 and the y-coordinate the abundance of Species 2. Then we get the graph shown in Fig. 7.1.2B. Unlike the graph in Fig. 7.1.2A, this graph could have been constructed from our dataset even if we had no knowledge of the underlying environmental gradient; we simply need to know the abundance of each species in each sample. But clearly there is a relationship between this graph and the gradient-related pattern of abundances in Fig.7.1.2A, raising the possibility that we could make useful inferences about the gradient patterns in Fig.7.1.2A if only the data in Fig. 7.1.2B were at our disposal. This is essentially what ordination methods are designed to do.

To illustrate, let us apply a simple ordination method to the data in Fig. 7.1.2B. Draw a straight line through the data, fitting it "by eye" (line I in Fig. 7.1.2C). Then draw a second line that is perpendicular to the first and passes through roughly the midpoint of the data (line II in Fig. 7.1.2C). Now treat these two perpendicular lines as a new set of coordinate axes. The new coordinates of each sample point are found by moving from the point to line I along a path parallel to line II (giving the new x-coordinate), then moving from the point to line II along a path parallel to line I (giving the new y-coordinate). Plotting all the points in this new coordinate system gives the graph shown in Fig. 7.1.2D; the axes of this graph are commonly called ordination axes. Note that the various samples now appear to be ordered along axis I, with little variation in the direction of axis II. This suggests that a single source of variation is capable of explaining almost all the variation in the data. We therefore hypothesize the existence of a

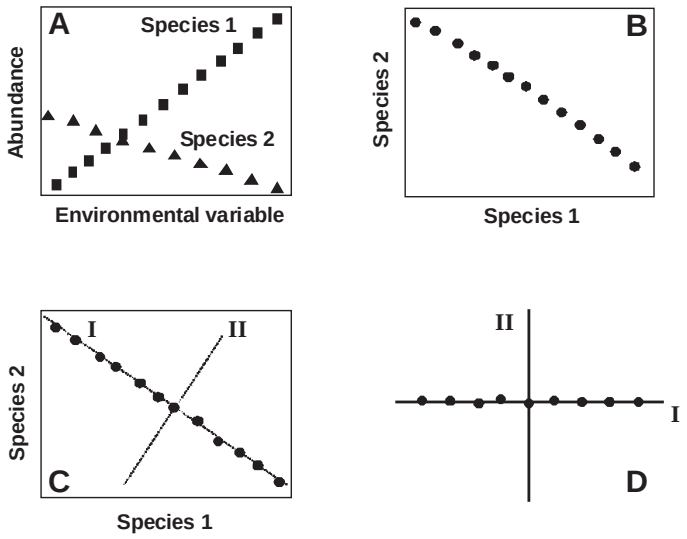


Fig. 7.1.2. A simple example of ordination analysis applied to a hypothetical dataset with 2 species, and with sampling sites spread along a known environmental gradient. A. Abundances of Species 1 and Species 2 plotted against the key environmental variable. B. Abundance of Species 2 plotted against that of Species 1. C. Adding straight lines to describe variation in two (perpendicular) directions. Line I describes the main direction of variation; Line II describes the second direction. D. Plotting the samples in the coordinate system defined by Lines I and II, which are now the two ordination axes.

single, dominant environmental gradient. Of course, we already knew this was the case in our example, but the point is that a simple ordination method led us to the correct conclusion. (The method we used is, in fact, a graphical version of principal components analysis.)

With real datasets, we usually do not know in advance what environmental gradients species are responding to; we simply have data collected from different sampling locations, typically with many species. Ordination methods are applied to such data with the goal of finding one or two ordination axes which account for most of the variation. These are the axes along which the species "sort out", and each ordination method constructs them in a different way.

7.1.3.2 Extension to Many Species

Real datasets normally contain many more than two species. One can imagine, however, a plot of samples from an ecological dataset in a notional "species-space" with as many coordinate axes as there are species. In the species-space of Fig. 7.1.2A, there are two species and therefore two axes. If there were three species, then the species-space would have three axes, one for each of the three attributes (species abundances) of each sample. And so on. Ordination techniques use computer algorithms (typically based on standard numerical methods of linear algebra) to construct two coordinate axes along which most of the variation in the multivariate data occurs. In this way, the many dimensions of species-space are reduced to only two. Using the two new coordinate axes, the adjusted data can be plotted in a two-dimensional graph, much like Fig. 7.1.2D, despite the fact that many species are present. We then can visually inspect this graph exactly as in the two-species case, looking for natural orderings of the samples.

7.1.3.3 Types of Ordination Techniques

Numerous ordination techniques exist, and each has its own strengths and weaknesses. One important way in which ordination techniques differ is in how inferences are made regarding the identity of the key gradients. Most methods are designed to be used with ecological datasets that do not include measurements of potentially important environmental variables (e.g., current speed, temperature, pH, nitrogen). With these methods, gradient analysis is indirect in the sense that inferences about the possible physicochemical nature of the key gradients must be made by examining an ordination plot that includes no explicit information on environmental variables. Other methods exist, however, that make use of such information in constructing the ordination plot. Gradient analysis is then said to be direct, since the resulting ordination plot allows explicit assessment of the degree to which various measured parameters account for the orderings of samples or species along the ordination axes. The term "constrained ordination analysis" is also used for such methods, because the ordination axes are constructed from notional gradient variables that are constrained to be weighted sums (i.e., linear combinations) of the measured environmental parameters. In contrast, indirect methods of gradient analysis also create ordination axes based on notional gradient variables, but these are not constrained in any way. Principal components analysis and correspondence analysis are examples of indirect gradient analysis or unconstrained ordination, while redundancy analysis (yet another ordination technique) and canonical correspondence analysis are examples of direct gradient analysis or constrained ordination.

Another important difference among ordination techniques is the type of gradient each is designed to detect. Some perform best when the relationship between species abundance and environmental gradients is approximately linear (as in Fig. 7.1.2A), some when the response is nonlinear but monotonic, and others when it is hump-shaped (as in Fig. 7.1.2A). Unfortunately, we normally do not know which case holds for the range of sampling sites in a particular study, so we must make an assumption. The most common assumption is that the abundance of each species varies in a hump-shaped fashion along key environmental gradients; that is, there exists an intermediate position on the gradient which is optimal for growth, with each species having a different optimum. Principal components analysis is best suited to cases with linear responses to gradients, and this is one of the reasons it is less popular in ecological studies than are some other methods. Correspondence analysis and canonical correspondence analysis, on the other hand, are best suited to cases with hump-shaped responses, and these methods are widely used.

To illustrate the consequences of applying an ordination technique based on linear gradient responses to data from a system where these responses are distinctly nonlinear, consider the example in Fig. 7.1.3A (and compare Fig. 7.1.2A). Plotting the samples as in Fig. 7.1.2B yields Fig. 7.1.3B. Following the graphical ordination procedure of Fig. 7.1.2D, we arrive at the ordination plot shown in Fig. 7.1.3D. Note the pronounced curve in the cluster of datapoints, with substantial variation along both ordination axes (compare Fig. 7.1.2D). This suggests there are two key environmental gradients along which the species sort out. But we happen to know there is only one key gradient in this case, because we set the example up that way. The curve in

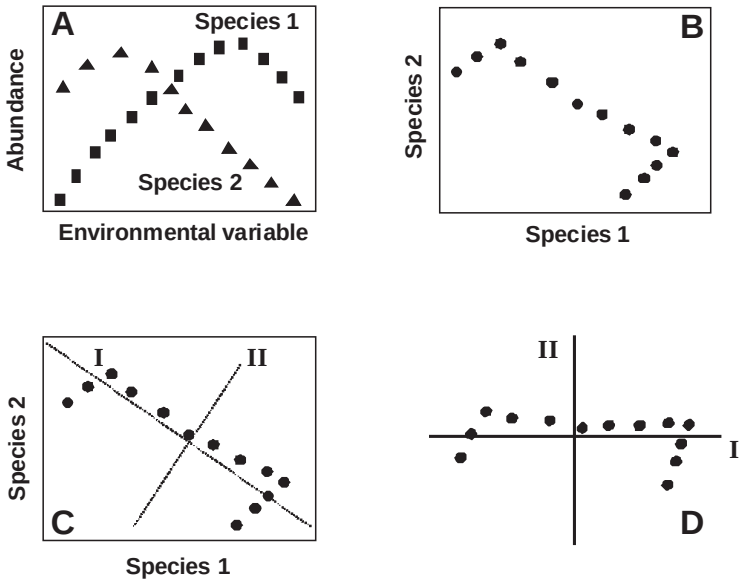


Fig. 7.1.3. Consequences of applying a linear ordination technique to data where the underlying relationship between species abundance and key environmental gradients is nonlinear and unimodal. See Fig. 7.1.2 for an explanation of panels A-D.

the ordination plot is an artifact of applying a linear ordination technique to data generated by nonlinear responses to a gradient. Clearly this could be misleading, in practice.

7.1.3.4 Arch Effects and Detrending

Other types of so-called *arch effects* occur in ordination plots for quite different reasons, even when using an appropriate ordination method. Though the causes differ, the consequence is the same: we are led to overestimate the number of key environmental gradients. Several heuristic techniques have been developed to ameliorate this problem, especially for correspondence and canonical correspondence analysis. These are usually referred to as detrending techniques, and we speak of detrended correspondence analysis and detrended canonical correspondence analysis. Unfortunately, detrending techniques can introduce new artifacts into a dataset, so caution is necessary in interpreting the results.

7.1.3.5 Correspondence Analysis

Correspondence analysis is an unconstrained ordination technique, a method of indirect gradient analysis, and is best suited to analyzing patterns produced by hump-shaped (unimodal) responses of species abundances to environmental gradients. In its pure form, it suffers from the arch effect and therefore is commonly applied in conjunction with a detrending technique. Correspondence analysis is also known to be unduly influenced by rare species and by unusual sampling sites (outliers), meaning that the presence of either of these features in a dataset can have a pronounced, distorting effect on the resulting ordination. Rare species and outlier sites are therefore sometimes removed from a dataset before analysis.

The result of correspondence analysis is an ordination plot. Two types of plots are commonly produced: one showing *samples* plotted against the two most important ordination axes (i.e., the two along which most of the variation in the dataset occurs), and the other showing *species* plotted in the same way. The sample plot is useful for making inferences about the degree of similarity or difference among various sampling locations, while the species plot is useful in making such inferences about the various species in a dataset.

To illustrate the process of making these inferences, Fig. 7.1.4 shows an ordination which was produced by (detrended) correspondence analysis. Multiple samples were collected from each of four sampling zones, the zone being identified in the plot by a number from 1 to 4. To begin, note that samples from each zone tend to form a distinct cluster, suggesting that the species composition is more similar in samples from the same zone than in samples from different zones, as one would expect. Note also that the samples exhibit substantial variation along both ordination axes, suggesting that at least two important environmental gradients underlie the sample differences. Note further that samples from Zones 1, 2 and 4 differ little from each along the second ordination axis (i.e., the vertical one) but do differ from Zone 3. All zones, however, appear to differ along the first ordination axis. These patterns suggest there is a primary environmental gradient along which all zones differ, and a secondary gradient along which Zone 3 differs from the rest. Unfortunately, the ordination plot provides no direct information regarding the physicochemical nature of these putative environmental gradients. This is a key property in which the plots produced by canonical correspondence analysis differ.

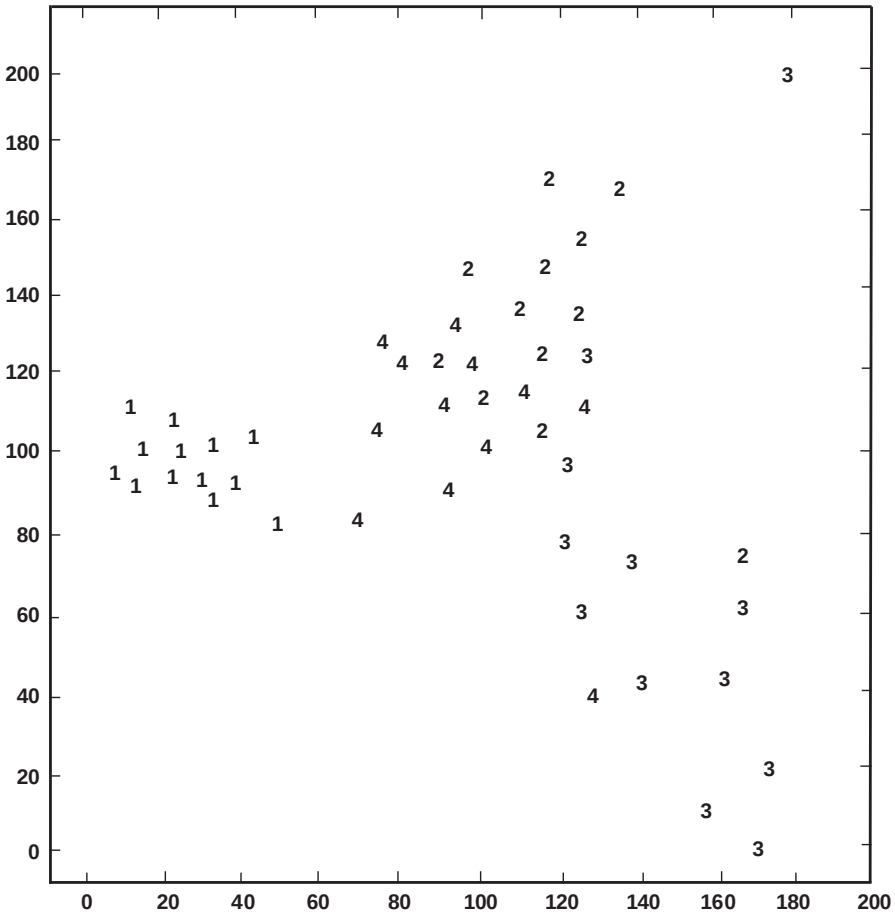


Fig. 7.1.4. An ordination plot produced by (detrended) correspondence analysis. Each sample is represented by its zone number (1-4).

As with cluster analysis, no tests of statistical significance are produced by correspondence analysis. Various artifacts are possible, as well (e.g., effects of rare species or unusual samples). Moreover, the orderings of samples or species produced by this technique may be quite different from those produced by a different ordination technique. For these reasons, correspondence analysis should be viewed as an exploratory technique whose results are most useful when corroborated by other lines of evidence.

7.1.3.6 Canonical Correspondence Analysis

Canonical correspondence analysis is a constrained ordination technique, a method of direct gradient analysis, and is best suited to analyzing patterns produced by hump-shaped (unimodal) responses of species abundance to environmental gradients. Like correspondence analysis, it suffers from the arch effect and therefore is commonly applied in conjunction with a detrending technique. Because the underlying numerical methods are very similar to those of correspondence analysis, canonical correspondence analysis also is expected to be unduly sensitive to rare species and unusual samples (outliers), so datasets should be examined for the presence of these properties. (The computer program CANOCO, used to perform the canonical correspondence analyses in the foregoing report, has options which attempt to reduce problems created by rare species and outlier samples.)

The result of canonical correspondence analysis is an ordination plot, which can include either samples or species plotted against the two most important ordination axes. A key difference from the plots produced by correspondence analysis is that information about the relative contribution of each measured environmental variable to the ordination can be displayed on the same graph. Thus, in addition to enabling us to make inferences about the degree of similarity among sampling locations or species, these plots also allow us to make inferences about the relative contribution of each environmental variable to the ordination.

An example of an ordination plot generated by canonical correspondence analysis is shown in Fig. 7.1.5. Samples are designated by dots, while information about the various measured environmental variables is conveyed by arrows pointing outward from the origin. Each arrow can be thought of as an additional coordinate axis, with the arrowhead indicating the direction of increase in the environmental variable and the length indicating the relative importance or influence of the variable in the ordination. Thus, the most influential variables are those with the longest arrows. (Note: the absolute lengths of the arrows are meaningless; only the relative lengths matter.)

The significance of the orientation (or angle) of each environmental arrow can be seen in the following way. Pick out a particular arrow (e.g., the one representing temperature) and form an axis by extending the arrow as a straight line in both directions. From each sample point, draw a line perpendicular to this axis. The points at which these perpendiculars cross the axis (that is, the projections of the sample points onto the temperature axis) indicate the ordering of samples along the temperature gradient. Samples whose projections lie near the arrowhead come from areas with relatively high temperatures, while those lying at the opposite end of the axis come from areas with relatively low temperatures. Moreover, samples whose projections lie close together come from areas with similar temperatures, while those whose projections lie far apart come from areas with dissimilar temperatures. Clearly, the orientation of each environmental axis is crucial to determining the arrangement of the sample projections onto it. In addition, the orientation and relative length of the arrows helps us interpret the two most important ordination axes (i.e., the horizontal and vertical axes of the ordination plot): an ordination axis is heavily influenced by a particular environmental variable if the arrow corresponding to that variable is relatively long and nearly parallel to the ordination axis. Thus, temperature and pH appear to

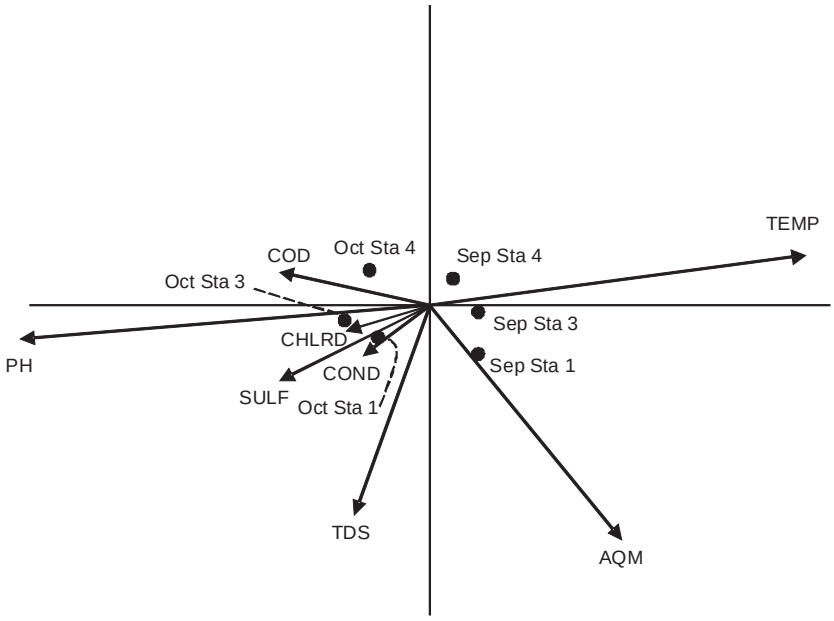


Fig. 7.1.5. An ordination plot produced by (detrended) canonical correspondence analysis. Samples are represented by dots and are labeled by month and station of origin. Measured environmental variables are represented by arrows and labeled as follows: AQM -aquatic macrophytes, CHLRD -chloride, COD -chemical oxygen demand, COND -conductivity, PH -pH, SULF -sulfate, TDS -total dissolved solids, TEMP -temperature.

contribute strongly to the first ordination axis (i.e., the horizontal one) and weakly to the second, while total dissolved solids and aquatic macrophytes appear to contribute strongly to the second.

Returning to Fig. 7.1.5, there is significant variation along both ordination axes, suggesting that two different environmental gradients are important. (The gradients represented by the ordination axes are constrained to be linear combinations of the measured environmental variables, with each axis corresponding to a different combination.) The first gradient appears to be strongly influenced by temperature and pH. Noting that the September samples lie on one side of the axis and the October samples on the other, these temperature and pH differences are probably associated with calendar date rather than station location. Projecting the samples onto the temperature and pH axes, the September samples are associated with higher temperature and lower pH, while the October samples are associated with lower temperature and higher pH.

The second gradient appears to be strongly influenced by aquatic macrophytes and total dissolved solids. Moreover, differences in these environmental variables appear to be related to station location, with both Station 1 samples (i.e., September and October) lying toward one end of the second ordination axis, both Station 4 samples toward the other end, and both Station 3 samples in the middle. Projecting the samples onto the total dissolved solids and aquatic macrophyte axes, Station 1 is associated with the highest abundances of aquatic macrophytes

and the highest concentrations of total dissolved solids, while Station 4 is associated with the lowest abundances and. concentrations.

Finally, note that samples differ more along the first ordination axis than along the second, so the main differences appear to be related to calendar date rather than to station location.

As with cluster analysis and correspondence analysis, canonical correspondence analysis does not produce tests of statistical significance. Various artifacts are possible, and the orderings of samples or species produced may be different from those produced by a different ordination technique. Once again, then, we reach the familiar conclusion that canonical correspondence analysis should be viewed as an exploratory technique whose results are most useful when corroborated by other lines of evidence.

Appendix 7.2. Tolerance values, functional feeding groups (FFG) and insect habits assigned to the invertebrate taxa collected in quantitative samples during the 2010 Holston River survey. (Page 1 of 3)

		Tolerance	FFG*	Habit*
Ephemeroptera				
Baetidae		4.52	CG	Sw
	Acentrella	3.6	CG	Sw
	Acerpenna	3.7	CG	Sw
	Baetis	5.4	CG	Sw
	Plauditus	4.52	CG	Sw
	Heterocleon	3.6	CG	Sw
	Centroptilum	6.3	CG	Sw
Caenidae		7.6		Cb
	Caenis	7.6	CG	Cb
Isonychidae		3.8		Sw
	Isonychia	3.8	CF	Sw
Heptageniidae		4.2		Cl
	Maccaffertium	2.9	SC	Cl
	Leucrocuta	2.5	SC	Cl
	Stenacron	3.9	SC	Cl
	Stenonema femoratum	7.5	SC	Cl
Ephemerellidae		2.3		Cl
	Serratella	2.3	CG	Cl
Leptohyphidae		5.4	CG	Sp
	Tricorythodes	5.4	CG	Sp
Plecoptera				Cl
Capniidae		0	SH	Cl
Leuctidae		1	SH	Cl
	Leuctra	0.7	SH	Cl
Perlidae		1	PR	Cl
	Hansonoperla appalachia	1	PR	Cl
Odonata				
Calopterygidae			PR	Cb
	Hetaerina americana	6.2	PR	Cb
Coenagrionidae		6	PR	Cb
	Argia	4	PR	Cl
Gomphidae		5.55	PR	Bu
	Stylogomphus	4.8	PR	Bu
	Dromogomphus	6.3	PR	Bu
Hemiptera				
Veliidae		undet	PR	O
	Rhagoveliia	undet	PR	O
Megaloptera				
Corydalidae		6	PR	Cl
	Corydalus cornutis	5.6	PR	Cl
Lepidoptera				
	Petrophila	1.8	SC	Cl
Coleoptera				
Dytiscidae			PR	Sw
Elmidae		4	SC	Cl
	Dubiraphia	6.4	SC	Cl
	Macronychus	4.7	SC	Cl
	Optioservus	2.7	SC	Cl
	Oulimnius	1.8	SC	Cl
	Stenelmis	5.4	CG	Cl
	Heterelmis	4	SC	Cl

Appendix 7.2 (continued). Tolerance values, functional feeding groups (FFG) and insect habits assigned to the invertebrate taxa collected in quantitative samples during the 2010 Holston River survey. (Page 2 of 3)

		Tolerance	FFG*	Habit*
Hydraenidae		6	CG	Cl
Hydrophilidae		6	CG	Sp
	Berosus	8.6	CG	Sp
Scirtidae		6	CG	Sp
Psephenidae		3.4	SC	Cl
	Psephenus herricki	2.5	SC	Cl
	Ectopria	4.3	SC	Cl
Trichoptera				
Helicopsychidae		5	SC	Cl
	Helisopsyche	5	SC	Cl
Brachycentridae			CF	Cl
	Micrasema	1.8	CF	Cl
	Brachycentrus lateralis	0.4	CF	Cl
Hydroptilidae			O	O
	Hydroptila	6.2	O	O
	Leucotrichia	4.3	SC	Cl
Hydropsychidae		5.55	CF	Cl
	Hydropsyche	4.5	CF	Cl
	Cheumatopsyche	6.6	CF	Cl
Psychomyiidae		2.7	CG	Cl
	Psychomyia	2.7	CG	Cl
Glossosomatidae		3	SC	Cl
	Protoptila	2.8	SC	Cl
Polycentropodidae		5	CF	Cl
Leptoceridae		4.8	SH	Cl
	Oecetis	4.8	SH	Cb
Philopotamidae			CF	Cl
	Chimarra	2.8	CF	Cl
Diptera				
Tipulidae		5.7	SH	Bu
	Antocha	4.6	CG	Bu
	Hexatoma	4.7	PR	Bu
	Tipula	7.7	SH	Cl
Simuliidae		6	CF	Cl
	Simulium	6.8	CF	Cl
Empididae		7	PR	Cl
	Hemerodromia	7	PR	Cl
Ceratopogonidae			PR	Bu
	Atrichopogon	6.8	PR	Sp
Stratiomyidae		7	CG	Bu
Annelida				
Oligochaeta		9	CG	Bu
Hirudinea		8	PR	Bu
Tricladida		8	PR	Sp
	Acari	8	PR	Cb
Gastropoda		6	SC	Cl
	Ferrisia	6.9	SC	Cl
	Corbicula	6.3	CF	Bu
Cambaridae		6	CG	Cb

Appendix 7.2 (continued). Tolerance values, functional feeding groups (FFG) and insect habits assigned to the invertebrate taxa collected in quantitative samples during the 2010 Holston River survey. (Page 3 of 3)

		Tolerance	FFG*	Habit*
Amphipoda		8	CG	Sw
	Hyalella	7.9	CG	Sw
	Spherium	7.7	CF	Bu
Harpactacoida		7	CG	Bu
Cladocera		8	CF	Sw
Hydra		8	PR	Cl
Isopoda		8	CG	Cb
Nematoda		6	PR	Bu
Chironomidae		6	CG	Sp
Tanypodinae		8.2	PR	Sp
	Ablabesmyia	6.4	PR	Sp
	Natarsia	10	PR	Sp
	Theinemannimyia grp	8.2	PR	Sp
	Orthocladinae	6	CG	Bu
	Theinemanniella	6	CG	Bu
	Cricotopus	8.1	CG	Bu
	Orthocladius	6.4	CG	Bu
	Eukeifferella	3.3	O	Sp
	Lopescladius	2.2	CG	Sp
	Nanocladius	4.9	PR	Sp
	Tvetenia	4	CG	Sp
	Synorthocladius	4.7	CG	Bu
	Parametriocnemus	3.7	CG	Sp
	Cricotopus/Orthocladius	7.1	CG	Bu
Chrironominae		6	CG	
Chironomini		6	CG	Sp
	Dicrotendipes	9.1	CG	Bu
	Microtendipes	6.2	CF	Cl
	Polypedilium	6.7	SH	Bu
	Cryptochrionomus	7.4	PR	Sp
	Psuedochironomus	4.2	CG	
	Paratendipes	5.3	CG	Bu
	Stenochironomus	6.4	CG	Bu
	Phaenospectra	6.9	SC	Cl
	Xestochironomus	6.4	CG	Bu
Tanytarsini		6	CF	Bu
	Tanytarsus	6.7	CG	Cl
	Rheotanytarsus	6.4	CF	Cl
	Micropsectra	1.4	CG	Cb
	Stempellinella	5.3	CG	Sp
	Paratanytarsus	7.7	CG	Sp
	Sublettea	1.7	CG	Sp
	Cladotanytarsus	3.7	CG	Bu

*Functional feeding groups are: CF=collector-filterer; CG=collector-gatherer; O=other; PR=predator; SC=scraper; SH=shredder. Habits are: Bu=burrower; Cb=climber; Cl=clinger; O=other; Sp=sprawler; Sw=swimmer.

Appendix 7.3. Summary of effort in 2010, 1997 and 1990 South Fork and mainstem Holston rivers surveys. NR= not measured in field.

	5 X 5 m			Shore Shock									Total Area Backpack	
	2010	1997	1990	2010			1997			1990			2010	1997
	#	#	#	#	Length (m)	Area (m^2)	#	Length (m)	Area (m^2)	#	Length (m)	Area (m^2)	Area (m^2)	Area (m^2)
2	6	5	3	1	70	210	1	125	375	3	106		360	500
3L	3	3	1	1	67	201	2	87	414	1	127		276	489
3LR	2						1	67	402				50	402
3R	1		2	1	67	268	2	124	497	2	100		293	497
3Rlower		1					1	67	335					360
4M	7	6	1				1	113	281	1	NR		175	431
4D (ledges)			1	3	137	420	1	30	120	1	97		420	120
4U		0												
5 (all 5L)	9	8	2	1	82	246	2	225	825	2	194, NR		471	1025
5U			2							2	195, NR			
6	8	7	1	2	190	570	3	216	778	1	406	972	770	953
HC1			1	1: 2 passes		941	1: 2 passes	89	939	1	212	581.6	941	939
HC2			1	1: 2 passes	125	957	1: 2 passes	101	723	1	234	2340	957	723
KU				3	115	345							345	
KL				2	100	300							300	

	Boat electrofish						Traps	Gill nets	Seine/Otherbackpack			Dip Net		
	2010			1997			1990	1990	2010	1997	1990	2010	1997	1990
	#	Duration (m)	Length	#	Duration									
2	7	95	1823	4	65		8	6				x	x	x
3L							3					x	x	
3LR														
3R							3	1						x
3Rlower									1 S					
4M										1 BP	1 BP (75 m)			
4D (ledges)							8	1					x	x
4U										1 S				
5 (all 5L)													x	x
5U	3	52	987	3	45		12	7						x
6	3	45	614	4	53		3	3				x	x	
HC1							4					x	x	x
HC2												x	x	x
KU														
KL														

Appendix 7.4.1. List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 1 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Phylum Bacillariophyta (Diatoms)								
Class Bacillariophyceae								
Order Centrales								
Family Aulacoseiraceae								
Aulacoseira granulata (Ehrenberg) Simonsen	X	-	-	-	-	-	-	-
Aulacoseira subarctica (Müller) Haworth	X	X	-	X	-	X	-	-
Aulacoseira subborealis (Nygaard) Denys, Muylaert et Krammer in Denys et al.	-	-	-	-	-	-	-	X
Family Melosiraceae								
Melosira varians Agardh	X	X	X	X	X	X	X	X
Family Stephanodiscaceae								
Cyclostephanos tholiformis Stoermer, Håkansson et Theriot	X	-	-	-	-	-	-	-
Cyclotella michiganiana Skvortzow	X	X	X	X	X	-	-	-
Cyclotella ocellata Pantocsek	X	X	X	X	X	X	-	-
Stephanodiscus hantzschii Grunow in Cleve and Grunow	X	-	-	-	-	X	-	-
Stephanodiscus minutulus (Kützing) Cleve et Möller	X	-	-	-	-	-	-	-
Family Thalassiosiraceae								
Discostella pseudostelligera (Hustedt) Houk et Klee	X	X	-	-	-	X	-	-
Family Triceratiaceae								
Pleurosira laevis (Ehrenberg) Compère	-	X	-	-	-	-	-	-
Order Pennales								
Family Achnanthaceae								
Achnanthes minutissima var. *jackii* (Rabenhorst) Lange-Bertalot et Ruppel	X	X	-	-	-	X	-	X
Achnanthes subhudsonis var. *kraeuselii* (Cholnoky) Cholnoky	-	X	-	-	-	-	-	-
Cocconeis pediculus Ehrenberg	X	X	X	X	X	X	X	X
Cocconeis placentula var. *lineata* (Ehrenberg) Van Heurck	X	X	X	X	X	X	X	X
Karayevia clevei (Grunow in Cleve and Grunow) Bukhtiyarova	X	-	X	X	X	-	-	-
Karayevia laterostrata (Hantzsch) Bukhtiyarova	-	X	X	X	X	-	X	X

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 2 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Planothidium delicatulum (Kützing) Round et Bukhtiyarova	-	-	X	-	-	-	-	-
Planothidium frequentissimum (Lange-Bertalot in Krammer and Lange-Bertalot) Lange-Bertalot	X	X	X	X	-	-	X	X
Planothidium lanceolatum (Brébisson ex Kützing) Lange-Bertalot	-	-	-	-	-	X	-	-
Planothidium minutissimum (Krasske) Morales	-	-	X	-	-	-	-	-
Planothidium rostratum (Østrup) Lange-Bertalot	-	X	X	X	X	-	X	-
Platessa conspicua (Mayer) Lange-Bertalot in Krammer and Lange-Bertalot	-	-	X	X	X	-	X	X
Platessa hustedtii (Krasske) Lange-Bertalot in Krammer and Lange-Bertalot	-	X	-	-	-	-	-	-
Achnanthidium affine (Grunow in Cleve and Grunow) Czarnecki	-	-	X	X	X	-	X	-
Achnanthidium atomus (Hustedt) Monnier, Lange-Bertalot et Ector in Monnier et al.	-	-	-	-	-	-	X	X
Achnanthidium catenatum (Bily et Marvan) Lange-Bertalot	X	X	X	X	X	X	-	-
Achnanthidium deflexum (Reimer in Patrick and Reimer) Kingston	X	X	X	X	X	X	X	X
Achnanthidium eutrophilum (Lange-Bertalot in Lange-Bertalot and Metzeltin) Lange-Bertalot	X	X	X	X	X	-	-	-
Achnanthidium exiguum (Grunow in Cleve and Grunow) Czarnecki	-	X	-	X	X	-	-	-
Achnanthidium minutissimum (Kützing) Czarnecki	X	X	X	X	X	X	X	X
Achnanthidium pyrenaicum (Hustedt) Kobayashi	X	X	-	X	X	X	-	-
Achnanthidium rivulare Potapova et Ponader	X	X	X	X	-	X	X	X
Family Amphipleuraceae								
Frustulia vulgaris (Thwaites) De Toni	-	-	-	X	X	-	-	-
Family Bacillariaceae								
Denticula subtilis Grunow	-	X	-	-	-	-	-	-
Nitzschia acicularioides Hustedt	-	-	X	-	-	-	-	-
Nitzschia acidoclinata Lange-Bertalot	-	-	-	X	-	-	-	-
Nitzschia amphibia Grunow	X	X	X	X	X	-	X	X
Nitzschia archibaldii Lange-Bertalot	-	X	X	X	-	X	-	X
Nitzschia biacrula Hohn et Hellerman	-	X	-	-	-	-	-	-
Nitzschia brevissima Grunow ex Van Heurck	-	-	-	X	-	-	-	-

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 3 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Nitzschia capitellata Hustedt in A. Schmidt	-	-	-	-	-	-	X	X
Nitzschia clausii Hantzsch	-	X	-	X	X	-	-	-
Nitzschia communis Rabenhorst in Grunow	X	-	-	-	-	-	-	-
Nitzschia dissipata (Kützing) Grunow	X	X	X	X	X	-	X	X
Nitzschia fonticola (Grunow) Grunow in Van Heurck	X	X	X	X	X	X	-	-
Nitzschia frustulum (Kützing) Grunow in Cleve and Grunow	X	X	-	X	X	-	-	X
Nitzschia gracilis Hantzsch in Rabenhorst	-	-	-	X	-	-	X	-
Nitzschia heufleriana Grunow	X	-	-	-	-	-	X	X
Nitzschia inconspicua Grunow	-	X	-	X	-	-	-	-
Nitzschia intermedia Hantzsch ex Cleve et Grunow	X	X	X	X	X	-	X	X
Nitzschia palea (Kützing) Smith	X	-	X	X	X	-	X	X
Nitzschia palea var. *debilis* (Kützing) Grunow in Cleve and Grunow	X	X	X	X	X	-	X	X
Nitzschia paleacea Grunow in Van Heurck	-	-	-	X	-	-	-	-
Nitzschia recta Hantzsch ex Rabenhorst	-	-	-	-	-	X	X	X
Nitzschia sigmoidea (Nitzsch) Smith	-	-	-	-	-	-	X	X
Nitzschia sinuata var. *delognei* (Grunow in Van Heurck) Lange-Bertalot	-	-	-	X	-	-	-	-
Nitzschia sinuata var. *tabellaria* (Grunow) Grunow in Van Heurck	X	-	-	-	-	-	-	-
Nitzschia sociabilis Hustedt	X	-	X	-	-	-	X	X
Nitzschia subtilis (Kützing) Grunow in Cleve and Grunow	-	-	X	-	-	-	X	X
Nitzschia supralitorea Lange-Bertalot	X	-	-	X	-	-	X	-
Nitzschia valdecostata Lange-Bertalot et Simonsen	-	-	-	X	X	-	X	X
Simonsenia delognei (Grunow) Lange-Bertalot	X	-	-	-	X	-	X	X
Tryblionella apiculata Gregory	-	-	-	-	-	-	X	X
Family Catenulaceae								
Amphora montana Krasske	-	-	X	X	X	-	X	X
Amphora ovalis (Kützing) Kützing	X	-	-	-	-	-	X	X

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 4 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Amphora pediculus (Kützing) Grunow in A. Schmidt	X	X	X	X	X	X	X	X
Family Cymbellaceae								
Cymbella affinis Kützing	X	-	X	-	-	-	X	-
Cymbella delicatula Kützing	-	-	-	X	-	-	-	-
Cymbella hustedtii Krasske	X	X	-	-	-	-	-	-
Cymbella tumida (Brébisson ex Kützing) Van Heurck	-	-	X	-	-	-	-	X
Cymbella turgidula Grunow in Schmidt	X	-	X	-	-	-	-	-
Encyonema auerswaldii Rabenhorst	X	-	-	-	-	-	-	-
Encyonema hebridicum Grunow ex Cleve	-	-	-	-	-	-	X	X
Encyonema minutum (Hilse in Rabenhorst) Mann in Round, Crawford and Mann	X	X	-	-	-	X	-	-
Encyonema prostratum (Berkeley) Ralfs	-	-	-	X	-	-	-	-
Encyonema reichardtii (Krammer in Krammer and Lange-Bertalot) Mann in Round, Crawford and Mann	X	-	-	-	-	-	-	-
Encyonema silesiacum (Bleisch in Rabenhorst) Mann in Round, Crawford and Mann	X	X	X	X	X	X	X	X
Encyonopsis subminuta Krammer et Reichardt in Krammer	X	-	-	-	-	-	-	-
Family Diadesmidaceae								
Diadesmis confervacea Kützing	-	X	-	X	X	-	-	-
Diadesmis contenta (Grunow ex Van Heurck) Mann in Round, Crawford and Mann	-	X	-	-	-	-	-	X
Luticola goeppertiana (Bleisch in Rabenhorst) Mann in Round, Crawford and Mann	-	X	X	X	-	-	-	-
Luticola undulata (Hilse in Rabenhorst) Mann in Round, Crawford and Mann	-	-	-	X	-	-	-	-
Luticola ventricosa (Kützing) Mann in Round, Crawford and Mann	-	X	-	-	-	-	-	-
Family Diploneidaceae								
Diploneis oculata (Brébisson in Desmazières) Cleve	-	-	-	-	-	-	X	-
Family Fragilariaceae								
Ctenophora pulchella (Ralfs ex Kützing) Williams et Round	-	X	-	X	X	-	-	-
Diatoma moniliformis Kützing	-	-	-	-	-	-	X	-
Diatoma vulgaris Bory	X	X	X	X	X	X	X	X
Fragilaria capucina Desmazières	-	X	X	-	-	-	-	X

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 5 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Fragilaria capucina var. *mesolepta* (Rabenhorst) Rabenhorst	-	-	X	-	-	-	-	-
Fragilaria crotonensis Kitton	X	X	X	X	-	X	-	-
Fragilaria pinnata var. a*cuminata* Mayer	-	X	-	X	-	-	-	-
Fragilaria radians (Kützing) Williams et Round	X	X	-	X	X	-	X	-
Fragilaria sepes Ehrenberg	X	-	-	-	-	X	-	-
Fragilaria tenera (Smith) Lange-Bertalot	X	-	-	-	-	-	-	-
Fragilaria vaucheriae (Kützing) Petersen	X	X	-	X	-	X	X	X
Opephora martyi Héribaud	X	-	-	-	-	-	-	-
Pseudostaurosira brevistriata (Grunow in Van Heurck) Williams et Round	X	X	X	X	-	X	-	-
Staurosira construens Ehrenberg	-	-	-	X	-	-	-	-
Staurosira construens var. *venter* (Ehrenberg) Hamilton in Hamilton, Poulin, Charles and Angell	X	-	X	X	X	-	-	-
Staurosirella pinnata (Ehrenberg) Williams et Round	X	X	-	-	-	-	-	-
Synedra delicatissima var. *angustissima* Grunow in Van Heurck	X	-	-	-	-	-	-	-
Synedra rumpens Kützing	X	-	-	-	-	-	-	-
Synedra sp. 2 NAWQA MORALES	X	X	X	X	-	-	-	X
Synedra sp. 8 NAWQA MORALES	X	X	X	X	X	X	X	-
Synedra ulna var. *contracta* Østrup	-	X	X	X	-	-	-	-
Synedra ulna var. *oxyrhynchus fo. mediocontracta* (Fonti) Hustedt	-	-	X	X	-	-	-	-
Tabularia fasciculata (Agardh) Williams et Round	-	X	-	-	-	-	-	-
Tabularia tabulata (Agardh) Snoeijs	-	X	-	-	-	-	-	-
Family Gomphonemataceae								
Gomphonema angustatum (Kützing) Rabenhorst	-	X	X	-	-	-	-	X
Gomphonema insigne Gregory	-	X	-	X	-	-	-	X
Gomphonema kobayasii Kociolek et Kingston	X	X	X	X	X	-	X	X
Gomphonema lagenula Kützing	-	X	X	-	X	-	X	X
Gomphonema micropus Kützing	-	-	-	-	-	-	-	X
Gomphonema minusculum Krasske	X	-	-	-	-	-	-	-

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 6 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Gomphonema minutum (Agardh) Agardh	X	X	X	X	X	X	X	X
Gomphonema olivaceum (Lyngbye) Kützing	X	-	X	-	X	-	X	X
Gomphonema parallelistriatum Lange-Bertalot et Reichardt in Lange-Bertalot	-	X	X	X	X	-	-	-
Gomphonema pumilum var. *rigidum* Reichardt et Lange-Bertalot in Reichardt	X	-	X	X	X	X	X	X
Reimeria uniseriata Sala, Guerrero et Ferrario	-	-	X	-	X	-	X	X
Family Naviculaceae								
Adlafia bryophila (Petersen) Moser, Lange-Bertalot et Metzeltin	-	-	-	-	-	-	X	-
Geissleria acceptata (Hustedt) Lange-Bertalot et Metzeltin	X	-	-	-	-	-	-	-
Navicula absoluta Hustedt	-	-	-	-	-	-	X	X
Navicula aff. subminuscula ANS NAWQA EAM Manguin	-	X	X	X	X	-	-	-
Navicula antonii Lange-Bertalot in U. Rumrich, Lange-Bertalot and M. Rumrich	X	X	X	X	X	-	X	X
Navicula canalis Patrick	-	-	-	-	-	-	X	X
Navicula capitatoradiata Germain	X	X	X	X	X	X	X	X
Navicula caterva Hohn et Hellerman	X	-	X	-	-	-	X	X
Navicula cryptocephala Kützing	X	-	X	X	-	-	X	-
Navicula cryptotenella Lange-Bertalot in Krammer and Lange-Bertalot	X	X	X	X	X	X	X	X
Navicula cryptotenelloides Lange-Bertalot	-	X	-	-	-	-	-	X
Navicula erifuga Lange-Bertalot in Krammer and Lange-Bertalot	-	X	X	-	X	-	X	X
Navicula germainii Wallace	X	-	X	-	X	-	X	X
Navicula glomus Carter et Bailey-Watts	-	-	-	-	-	-	X	X
Navicula gregaria Donkin	X	X	X	X	X	X	X	X
Navicula incertata Lange-Bertalot in Krammer and Lange-Bertalot	-	X	-	-	-	-	-	-
Navicula ingenua Hustedt	-	X	-	-	-	-	-	-
Navicula kotschyi Grunow	-	-	-	-	-	-	-	X
Navicula lanceolata (Agardh) Kützing	X	-	-	-	-	-	-	X
Navicula menisculus Schumann	-	-	X	X	-	-	X	X
Navicula minima Grunow in Van Heurck	X	X	X	X	X	X	X	X

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 7 of 11)

	Zone							
	2	3	4	5	6	BTS	HC1	HC2
Navicula reichardtiana Lange-Bertalot in Lange-Bertalot and Krammer	X	-	X	X	X	X	X	X
Navicula rostellata Kützing	X	X	X	X	X	-	X	X
Navicula ruttneri var. *capitata* Hustedt	-	-	-	-	-	-	X	-
Navicula schroeteri v ar. *escambia* Patrick	-	-	X	-	-	-	X	-
Navicula subminuscula Manguin	-	-	X	X	X	-	-	-
Navicula submuralis Hustedt	-	-	-	-	-	-	X	-
Navicula symmetrica Patrick	-	X	X	X	X	-	X	X
Navicula tenelloides Hustedt	-	X	-	X	X	-	X	X
Navicula tripunctata (Müller) Bory	X	X	X	X	X	X	X	X
Navicula trivialis Lange-Bertalot	-	-	-	-	-	-	-	X
Navicula vaucheriae Petersen	-	-	-	-	-	-	X	-
Navicula veneta Kützing	-	-	-	X	X	-	X	X
Navicula vilaplanii (Lange-Bertalot et Sabater) Lange-Bertalot et Sabater in U. Rumrich, Lange-	-	-	-	X	X	-	X	X
Nupela vitiosa (Schimanski) Siver et Hamilton	-	-	X	X	-	-	X	-
Family Pinnulariaceae								
Caloneis bacillum (Grunow) Cleve	-	-	-	-	-	-	X	X
Family Pleurosigmataceae								
Gyrosigma scalproides (Rabenhorst) Cleve	-	-	-	X	-	-	X	X
Gyrosigma spencerii (Smith) Griffith et Henfrey	-	-	X	-	-	-	X	X
Family Rhoicospheniaceae								
Rhoicosphenia abbreviata (Agardh) Lange-Bertalot	X	X	X	X	X	X	X	X
Gomphosphenia lingulatiformis (Lange-Bertalot et Reichardt in Lange-Bertalot) Lange-Bertalot	-	-	X	-	-	-	-	X
Family Sellaphoraceae								
Fallacia lenzii (Hustedt) Lange-Bertalot in Werum and Lange-Bertalot	-	-	-	-	-	-	-	X
Fallacia monoculata (Hustedt) Mann in Round, Crawford and Mann	-	-	-	X	X	-	-	X
Fallacia subhamulata (Grunow in Van Heurck) Mann in Round, Crawford and Mann	-	-	-	-	-	-	X	-
Sellaphora pupula (Kützing) Meresckowsky	-	-	-	-	-	-	X	X
Sellaphora seminulum (Grunow) Mann	-	X	X	X	X	-	X	X

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 8 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Family Stauroneidaceae								
Craticula molestiformis (Hustedt) Mayama	-	-	-	-	-	-	-	X
Stauroneis smithii Grunow	-	-	-	-	-	-	X	X
Family Surirellaceae								
Surirella minuta Brébisson	X	-	-	-	-	-	X	X
Phylum Cyanophyta (Blue-Green Algae)								
Class Myxophyceae								
Order Chroococcales								
Family Merismopediaceae								
Merismopedia glauca (Ehrenberg) Kützing	-	X	-	-	-	-	-	-
Family Microcystaceae								
Gloeocapsa sp.	-	-	-	-	-	X	-	-
Family Chamaesiphon								
Chamaesiphon incrustans Grunow ex Rabenhorst	X	-	-	-	-	-	-	-
Family Hyellaceae								
Pleurocapsa minor Hansgirg	X	-	X	X	X	-	-	-
Order Oscillatoriales								
Family Pseudanabaenaceae								
Heteroleibleinia sp.	-	X	X	-	X	X	-	-
Homoeothrix (Tapinothrix) *janthina* (Bornet et Flahault) Starmach	-	-	X	X	X	-	X	X
Homoeothrix (Tapinothrix) *varians* Geitler	X	-	X	X	-	-	X	-
Leptolyngbya sp.	X	-	X	X	-	X	X	-
Pseudanabaena sp.	-	X	X	-	-	-	-	-
Family Phormidiaceae								
Phormidium amoenum Kützing	X	-	X	X	-	-	-	-

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 9 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Phormidium autumnale (Agardh) Trevisan ex Gomont	X	-	X	X	-	X	X	-
Phormidium granulatum (Gardner) Anagnostidis	X	X	X	X	-	-	-	-
Phormidium sp.	X	X	X	X	X	X	X	X
Family Oscillatoriaceae								
Homoeothrix juliana (Bornet et Flahault) Kirchner	-	-	X	-	-	-	X	X
Lyngbya martensiana Meneghini ex Gomont	-	-	X	-	-	X	X	-
Plectonema sp.	-	-	X	X	X	-	-	-
Order Nostocales								
Family Microchaetoideae								
Tolypothrix sp.	-	-	X	-	-	-	-	-
Family Rivulariaceae								
Calothrix sp.	-	X	-	-	X	-	-	-
Phylum Rhodophyta (Red Algae)								
Class Rhodophyceae								
Undermined *Chantransia* -stage	-	-	-	X	-	-	X	X
Phylum Chrysophyta (Yellow-Green Algae)								
Class Xanthophyceae								
Order Vaucheriales								
Family Vaucheriaceae								
Vaucheria sp.	-	-	-	-	X	-	-	-
Phylum Euglenophyte (Euglenoids)								
Class Euglenophyceae								
Order Euglenales								
Family Euglenaceae								
Euglena sp.	-	-	-	-	-	-	X	-

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 10 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Phylum Chlorophyta (Green Algae)								
Class Chlorophyceae								
Order Chlorococcales								
Family Chlorococcaceae								
Characium sp.	-	X	-	-	-	X	-	-
Tetraedron minimum (A. Braun) Hansgirg	X	-	-	-	-	-	-	-
Family Hydrodictyaceae								
Hydrodictyon reticulatum (L.) Lagerheim	-	X	-	-	X	-	-	-
Pediastrum boryanum (Turpin) Meneghini	X	-	-	-	-	-	-	-
Pediastrum duplex var. *clathratum* (A. Braun) Lagerheim	X	-	-	-	-	-	-	-
Pediastrum simplex (Meyen) Lemmermann	X	-	-	-	-	-	-	-
Family Scenedesaceae								
Coelastrum pulchrum Schmidle	X	-	X	-	-	-	-	-
Scenedesmus acutus Meyen	X	-	-	X	-	-	-	-
Scenedesmus denticulatus Kirchner	X	-	-	-	-	-	-	-
Scenedesmus ecornis (Ralfs) Chodat	-	X	-	-	-	-	-	-
Scenedsmus spinosus Chodat	X	-	-	X	-	-	-	-
Order Ulotrichales								
Family Microsporaceae								
Microspora sp.	X	X	X	X	-	-	X	-
Family Ulotrichaceae								
Ulothrix zonata (Weber et Mohr) Kützing	-	-	-	X	X	X	-	-
Order Ulvales								
Family Schizomeridaceae								
Schizomeris leibleinii Kützing	-	X	-	-	-	-	-	-

Appendix 7.4.1 (continued). List of taxa of algae collected in zones on the South Fork and mainstem Holston rivers (including Big Tree Spring [BTS] within Zone 2), Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present) (Page 11 of 11)

	Zone							
	2	**3**	**4**	**5**	**6**	**BTS**	**HC1**	**HC2**
Order Tetrasporales								
Family Tetrasporaceae								
Tetraspora gelatinosa (Vaucher) Desvaux	-	-	X	-	-	-	-	-
Order Chaetophorales								
Family Aphanochaetaceae								
Aphanochaete repens Braun	-	-	-	X	-	-	-	-
Family Chaetophoraceae								
Gongrosira debaryana Rabenhorst	X	X	X	-	-	X	-	-
Stigeoclonium lubricum (Dillwyn) Kützing	X	-	X	-	-	-	-	X
Order Oedogoniales								
Family Oedogoniaceae								
Oedogonium sp.	X	X	X	X	-	X	X	-
Order Siphonocladales								
Family Cladophoraceae								
Cladophora glomerata (L.) Kützing	X	X	X	X	X	X	X	-
Rhizoclonium hieroglyphicum (Agardh) Kützing	-	-	X	-	X	-	-	-
Order Zygnematales								
Family Zygnemataceae								
Spirogyra sp.	-	X	-	-	X	-	-	-
Family Desmidiaceae								
Closterium lunula (Müller) Nitzsch	-	-	X	-	X	X	X	-
Closterium moniliferum Ehrenberg	-	-	X	X	X	-	-	-
Cosmarium sp.	X	-	X	-	-	X	X	-
Staurastrum sp.	X	-	-	-	-	-	-	-

Appendix 7.4.2. List of taxa of aquatic macrophytes collected in zones on the South Fork and mainstem Holston rivers, Big Sluice and Horse Creek (HC) near Kingsport, TN in 2010. (X = present; - = not present).

Taxa	Zone						
	2	3	4	5	6	HC1	HC2
Phylum Spermatophyta							
Subdivision Angiospermae							
Class Monocotyledoneae							
Family Zosteraceae							
Potamogeton crispus L.	X	X	X	-	X	-	-
P. nodosus Poiret	X	X	X	X	X	-	-
P. pectinatus L.	-	X	X	X	X	-	-
Family Hydrocharitaceae							
Elodea canadensis Michx.	X	X	-	-	X	-	-
Vallisneria americana Michx.	-	-	-	X	X	-	-
Family Cyperaceae							
Eleocharis erythropoda Steud.	-	-	-	-	X	-	-
Schoenoplectus tabernaemontanii (Gmel.) Palla	X	-	-	-	-	-	-
Family Pontederiaceae							
Heteranthera dubia (Jacq.) MacM.	X	X	-	-	X	-	-
Class Dictyledoneae							
Family Polygonaceae							
Polygonum sp.	-	-	-	-	-	X	-
Subclass Metachlamydeae							
Family Acanthaceae							
Justica americana (L.) Vahl	X	-	X	-	-	X	X

Appendix 7.4.3. List of taxonomic changes for Holston River (Tennessee) algal taxa. Names used previously were reported for studies of Holston River algal assemblages during surveys conducted in 1977, 1980, 1990 and 1997 (see ANSP 1978; ANSP 1981; ANSP 1992; ANSP 1998). The status "Change" refers to a taxonomic change in the literature; "Lump" refers to a change made to combine data from different years (and different analysts). (Page 1 of 5)

Original ANS Taxon ID	Original Taxon Name	Current ANS Taxon ID	Current Taxon Name	Status	Algal Group
1023	*Achnanthidium pyrenaicum* (Hustedt) Kobayashi	1038	*Achnanthidium deflexum* (Reimer in Patrick and Reimer) Kingston	Lump	Diatom
1030	*Achnanthidium catenatum* (Bily et Marvan) Lange-Bertalot	1010	*Achnanthidium minutissimum* (Kützing) Czarnecki	Lump	Diatom
1037	*Achnanthidium atomus* (Hustedt) Monnier, Lange-Bertalot et Ector in Monnier et al.	1010	*Achnanthidium minutissimum* (Kützing) Czarnecki	Lump	Diatom
1046	*Achnanthidium eutrophilum* (Lange-Bertalot in Lange-Bertalot and Metzeltin) Lange-Bertalot	1010	*Achnanthidium minutissimum* (Kützing) Czarnecki	Lump	Diatom
2001	Achnanthes affinis Grunow	1011	*Achnanthidium affine* (Grunow in Cleve and Grunow) Czarnecki	Change	Diatom
2004	*Achnanthes clevei* Grunow	125001	*Karayevia clevei* (Grunow in Cleve and Grunow) Bukhtiyarova	Change	Diatom
2007	*Achnanthes exigua* Grunow	1024	*Achnanthidium exiguum* (Grunow in Cleve and Grunow) Czarnecki	Change	Diatom
2012	*Achnanthes hauckiana* Grunow	155015	*Planothidium hauckianum* (Grunow) Round et Bukhtiyarova	Change	Diatom
2015	*Achnanthes lanceolata* (Brébisson ex Kützing) Grunow	155003	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot	Change	Diatom
2016	*Achnanthes lanceolata* var. *dubia* Grunow	155021	*Planothidium dubium* (Grunow) Round et Bukhtiyarova	Change	Diatom
2019	*Achnanthes lapidosa* Krasske	92006	*Nupela lapidosa* (Krasske) Lange-Bertalot	Change	Diatom
2024	*Achnanthes linearis* (Smith) Grunow	1036	*Achnanthidium rivulare* Potapova et Ponader	Change	Diatom
2025	*Achnanthes linearis* fo. *curta* Smith	1036	*Achnanthidium rivulare* Potapova et Ponader	Change	Diatom
2029	*Achnanthes lanceolata* var. *dubia* Grunow	155021	*Planothidium dubium* (Grunow) Round et Bukhtiyarova	Change	Diatom
2033	*Achnanthes peragalli* Brun et HJribaud	155005	*Planothidium peragalli* (Brun et Héribaud) Round et Bukhtiyarova	Change	Diatom
2034	*Achnanthes pinnata* Hustedt	2508001	*Platessa conspicua* (Mayer) Lange-Bertalot in Krammer and Lange-Bertalot	Change	Diatom
2049	*Achnanthes bioreti* Germain	186001	*Psammothidium bioretii* (Germain) Bukhtiyarova et Round	Change	Diatom
2121	*Achnanthes biporoma* Hohn et Hellerman	155006	*Planothidium biporomum* (Hohn et Hellerman) Lange-Bertalot	Change	Diatom
2126	*Achnanthes deflexa* Reimer	1038	*Achnanthidium deflexum* (Reimer in Patrick and Reimer) Kingston	Change	Diatom
2127	*Achnanthes hauckiana* var. *rostrata* Schulz	155018	*Planothidium rostratum* (Ostrup) Lange-Bertalot	Change	Diatom
2136	*Achnanthes* cf. *grana* ROBERTS Hohn et Hellerman	155014	*Planothidium granum* (Hohn et Hellerman) Lange-Bertalot	Change	Diatom
2153	*Achnanthes nollii* Bock	2245	*Achnanthes reimeri* Camburn	Change	Diatom
2176	*Achnanthes minutissima* var. *jackii* (Rabenhorst) Lange-Bertalot et Ruppel	1010	*Achnanthidium minutissimum* (Kützing) Czarnecki	Lump	Diatom
7004	*Amphora perpusilla* (Grunow) Grunow	7043	*Amphora pediculus* (Kützing) Grunow in A. Schmidt	Change	Diatom
7010	*Amphora inariensis* Krammer	7043	*Amphora pediculus* (Kützing) Grunow in A. Schmidt	Lump	Diatom
7019	*Amphora submontana* Hustedt	7042	*Amphora montana* Krasske	Lump	Diatom
8007	*Anomoeoneis vitrea* (Grunow) Ross	18013	*Brachysira microcephala* (Kützing) Compère	Change	Diatom
11002	*Biddulphia laevis* Ehrenberg	158001	*Pleurosira laevis* (Ehrenberg) Compère	Change	Diatom
16020	*Cocconeis cf. neodiminuta* Krammer	16019	*Cocconeis neodiminuta* Krammer	Lump	Diatom
20010	*Cyclotella stelligera* (Cleve et Grunow) Van Heurck	2506003	*Discostella stelligera* (Cleve et Grunow in Cleve) Houk et Klee	Change	Diatom

Appendix 7.4.3 (continued). List of taxonomic changes for Holston River (Tennessee) algal taxa. Names used previously were reported for studies of Holston River algal assemblages during surveys conducted in 1977, 1980, 1990 and 1997 (see ANSP 1978; ANSP 1981; ANSP 1992; ANSP 1998). The status "Change" refers to a taxonomic change in the literature; "Lump" refers to a change made to combine data from different years (and different analysts). (Page 2 of 5)

Original ANS Taxon ID	Original Taxon Name	Current ANS Taxon ID	Current Taxon Name	Status	Algal Group
20012	*Cyclotella pseudostelligera* Hustedt	2506002	*Discostella pseudostelligera* (Hustedt) Houk et Klee	Change	Diatom
20035	*Cyclotella radiosa* (Grunow) Lemmermann	208002	*Puncticulata radiosa* (Lemmermann) Håkansson	Change	Diatom
23010	*Cymbella microcephala* Grunow	203002	*Encyonopsis microcephala* (Grunow) Krammer	Change	Diatom
23012	*Cymbella minuta* Hilse ex Rabenhorst	110004	*Encyonema minutum* (Hilse in Rabenhorst) Mann in Round, Crawford and Mann	Change	Diatom
23013	*Cymbella minuta* fo. *latens* (Krasske) Reimer	110017	*Encyonema latens* (Krasske) Mann	Change	Diatom
23015	*Cymbella minuta* var. *silesiaca* (Bleisch ex Rabenhorst) Reimer	110005	*Encyonema silesiacum* (Bleisch in Rabenhorst) Mann in Round, Crawford and Mann	Change	Diatom
23031	*Cymbella muelleri* Hustedt	110011	*Encyonema muelleri* (Hustedt) Mann	Change	Diatom
23047	*Cymbella sinuata* fo. *antiqua* (Grunow) Reimer in Patrick and Reimer	55004	*Reimeria uniseriata* Sala, Guerrero et Ferrario	Change	Diatom
23051	*Cymbella sinuata* Gregory	55002	*Reimeria sinuata* (Gregory) Kociolek et Stoermer	Change	Diatom
23079	*Cymbella prostrata* (Berkeley) Cleve	110013	*Encyonema prostratum* (Berkeley) Ralfs	Change	Diatom
23080	*Cymbella prostrata* var. *auerswaldii* (Rabenhorst) Reimer	110018	*Encyonema auerswaldii* Rabenhorst	Change	Diatom
23084	*Cymbella* sp. 4 ANS NAR STROUD	110035	*Encyonema hebridicum* Grunow ex Cleve	Change	Diatom
27006	*Diatoma tenuis* var. *elongatum* Lyngbye	27008	*Diatoma moniliformis* Kützing	Lump	Diatom
30005	*Diploneis oculata* (Brébisson in DesmaziPres) Cleve	30008	*Diploneis petersenii*	Change	Diatom
34003	*Fragilaria brevistriata* Grunow	73001	*Pseudostaurosira brevistriata* (Grunow in Van Heurck) Williams et Round	Change	Diatom
34012	*Fragilaria construens* (Ehrenberg) Grunow	172001	*Staurosira construens* Ehrenberg	Change	Diatom
34013	*Fragilaria construens* var. *binodis* (Ehrenberg) Grunow	172005	*Staurosira construens* var. *binodis* (Ehrenberg) Hamilton in Hamilton, Poulin, Charles and Angell	Change	Diatom
34014	*Fragilaria construens* var. *pumila* Grunow	172006	*Staurosira construens* var. *venter* (Ehrenberg) Hamilton in Hamilton, Poulin, Charles and Angell	Change	Diatom
34022	*Fragilaria leptostauron* (Ehrenberg) Hustedt	175001	*Staurosirella leptostauron* (Ehrenberg) Williams et Round	Change	Diatom
34025	*Fragilaria pinnata* Ehrenberg	175005	*Staurosirella pinnata* (Ehrenberg) Williams et Round	Change	Diatom
34038	*Fragilaria pinnata* var. *acuminata* Mayer	34027	*Fragilaria pinnata* var. *lancettula* (Schumann) Hustedt in Schmidt	Lump	Diatom
34210	*Fragilaria radians* (Kützing) Williams et Round	66016	*Synedra rumpens* Kützing	Lump	Diatom
36004	*Gomphoneis herculeanum* var. *robusta* (Grunow) Cleve	36003	*Gomphoneis herculeana* (Ehrenberg) Cleve	Lump	Diatom
36010	*Gomphoneis minuta* (Stone in McLaughlin and Stone) Kociolek et Stoermer	36003	*Gomphoneis herculeana* (Ehrenberg) Cleve	Lump	Diatom
36990	*Gomphoneis* sp. 1 ?	36003	*Gomphoneis herculeana* (Ehrenberg) Cleve	Lump	Diatom
37022	*Gomphonema truncatum* Ehrenberg	37178	*Gomphonema minutum* (Agardh) Agardh	Change	Diatom
37060	*Gomphonema clevei* Fricke in Schmidt et al.	37197	*Gomphonema kobayasii* Kociolek et Kingston	Change	Diatom
37067	*Gomphonema abbreviatum* Agardh	209003	*Gomphosphenia lingulatiformis* (Lange-Bertalot et Reichardt in Lange-Bertalot) Lange-Bertalot	Change	Diatom
37074	*Gomphonema grovei* Schmidt	209001	*Gomphosphenia grovei* (Schmid) Lange-Bertalot	Change	Diatom
37109	*Gomphonema* sp. 2 ANS LAP	37197	*Gomphonema kobayasii* Kociolek et Kingston	Change	Diatom
37156	*Gomphonema insigne* Gregory	37002	*Gomphonema affine* Kützing	Lump	Diatom
37278	*Gomphonema lagenula* Kützing	37010	*Gomphonema parvulum* (Kützing) Kützing	Lump	Diatom
38011	*Gyrosigma nodiferum* (Grunow) Reimer in Patrick and Reimer	38012	*Gyrosigma scalproides* (Rabenhorst) Cleve	Change	Diatom
44001	*Melosira ambigua* (Grunow) Müller	10008	*Aulacoseira ambigua* (Grunow) Simonsen	Change	Diatom
44005	*Melosira granulata* (Ehrenberg) Ralfs	10018	*Aulacoseira granulata* (Ehrenberg) Simonsen	Change	Diatom

Appendix 7.4.3 (continued). List of taxonomic changes for Holston River (Tennessee) algal taxa. Names used previously were reported for studies of Holston River algal assemblages during surveys conducted in 1977, 1980, 1990 and 1997 (see ANSP 1978; ANSP 1981; ANSP 1992; ANSP 1998). The status "Change" refers to a taxonomic change in the literature; "Lump" refers to a change made to combine data from different years (and different analysts). (Page 3 of 5)

Original ANS Taxon ID	Original Taxon Name	Current ANS Taxon ID	Current Taxon Name	Status	Algal Group
44010	*Melosira italica* (Ehrenberg) Kützing	10019	*Aulacoseira italica* (Ehrenberg) Simonsen	Change	Diatom
46005	*Navicula atomus* (Kützing) Grunow	211003	*Mayamaea atomus* (Kützing) Lange-Bertalot	Change	Diatom
46007	*Navicula biconica* Patrick	21015	*Craticula molestiformis* (Hustedt) Mayama	Change	Diatom
46013	*Navicula contenta* var. *biceps* (Grunow) Van Heurck	197002	*Diadesmis contenta* (Grunow ex Van Heurck) Mann in Round, Crawford and Mann	Change	Diatom
46025	*Navicula halophila* (Grunow) Cleve	21005	*Craticula halophila* (Grunow) Mann	Change	Diatom
46041	*Navicula muralis* Grunow	204003	*Adlafia minuscula* var. *muralis* (Grunow) Lange-Bertalot	Change	Diatom
46042	*Navicula mutica* Kützing	130002	*Luticola mutica* (Kützing) Mann in Round, Crawford and Mann	Change	Diatom
46046	*Navicula paucivisitata* Patrick	46017	*Navicula difficillima* Hustedt	Change	Diatom
46047	*Navicula pelliculosa* Hilse	218001	*Fistulifera pelliculosa* (Brébisson ex Kützing) Lange-Bertalot	Change	Diatom
46051	*Navicula pupula* Kützing	170006	*Sellaphora pupula* (Kützing) Meresckowsky	Change	Diatom
46053	*Navicula pupula* var. *mutata* (Krasske) Hustedt	170012	*Sellaphora mutata* (Krasske) Lange-Bertalot	Change	Diatom
46055	*Navicula pygmaea* Kützing	115006	*Fallacia omissa* (Hustedt) Mann	Change	Diatom
46057	*Navicula radiosa* var. *parva* Wallace	46527	*Navicula cryptotenella* Lange-Bertalot in Krammer and Lange-Bertalot	Change	Diatom
46058	*Navicula radiosa* var. *tenella* (Brébisson ex Kützing) Grunow	46527	*Navicula cryptotenella* Lange-Bertalot in Krammer and Lange-Bertalot	Change	Diatom
46070	*Navicula seminulum* Grunow	170014	*Sellaphora seminulum* (Grunow) Mann	Change	Diatom
46077	*Navicula submolesta* Hustedt	21007	*Craticula submolesta* (Hustedt) Lange-Bertalot	Change	Diatom
46102	*Navicula cohnii* (Hilse) Lange-Bertalot	130005	*Luticola cohnii* (Hilse) Mann	Change	Diatom
46105	*Navicula omissa* Hustedt	115006	*Fallacia omissa* (Hustedt) Mann	Change	Diatom
46107	*Navicula tantula* Hustedt	46039	*Navicula minima* Grunow in Van Heurck	Change	Diatom
46144	*Navicula mutica* var. *tropica* Hustedt	130006	*Luticola goeppertiana* (Bleisch in Rabenhorst) Mann in Round, Crawford and Mann	Change	Diatom
46155	*Navicula salinarum* var. *intermedia* (Grunow) Cleve	46661	*Navicula capitatoradiata* Germain	Change	Diatom
46170	*Navicula cryptocephala* var. *veneta* (Kützing) Rabenhorst	46504	*Navicula veneta* Kützing	Change	Diatom
46255	*Navicula cryptocephala* var. *exilis* Grunow	46774	*Navicula trivialis* Lange-Bertalot	Change	Diatom
46257	*Navicula decussis* Ostrup	210003	*Geissleria decussis* (Ostrup) Lange-Bertalot et Metzeltin	Change	Diatom
46268	*Navicula hustedtii* Krasske	170033	*Sellaphora hustedtii* (Krasske) Lange-Bertalot	Change	Diatom
46272	*Navicula capitata* Ehrenberg	213001	*Hippodonta capitata* (Ehrenberg) Lange-Bertalot, Metzeltin et Witkowski	Change	Diatom
46299	*Navicula elginensis* (Gregory) Ralfs	194005	*Placoneis elginensis* (Gregory) Cox	Change	Diatom
46303	*Navicula mutica* var. *ventricosa* (Kützing) Cleve et Grunow	130003	*Luticola ventricosa* (Kützing) Mann in Round, Crawford and Mann	Change	Diatom
46309	*Navicula accomoda* Hustedt	21003	*Craticula accomoda* (Hustedt) Mann	Change	Diatom
46316	*Navicula bryophila* Petersen	204001	*Adlafia bryophila* (Petersen) Moser, Lange-Bertalot et Metzeltin	Change	Diatom
46320	*Navicula capitata* var. *lueneburgensis* (Grunow) Patrick	213003	*Hippodonta lueneburgensis* (Grunow) Lange-Bertalot, Metzeltin et Witkowski	Change	Diatom
46325	*Navicula cincta* var. *rostrata* Reimer	46504	*Navicula veneta* Kützing	Change	Diatom
46354	*Navicula grimmei* Krasske	46269	*Navicula kotschyi* Grunow	Change	Diatom

Appendix 7.4.3 (continued). List of taxonomic changes for Holston River (Tennessee) algal taxa. Names used previously were reported for studies of Holston River algal assemblages during surveys conducted in 1977, 1980, 1990 and 1997 (see ANSP 1978; ANSP 1981; ANSP 1992; ANSP 1998). The status "Change" refers to a taxonomic change in the literature; "Lump" refers to a change made to combine data from different years (and different analysts).(Page 4 of 5)

Original ANS Taxon ID	Original Taxon Name	Current ANS Taxon ID	Current Taxon Name	Status	Algal Group
46388	*Navicula rhynchocephala* var. *germainii* (Wallace) Patrick	46616	*Navicula germainii* Wallace	Change	Diatom
46395	*Navicula secreta* var. *apiculata* Patrick	46023	*Navicula gregaria* Donkin	Change	Diatom
46402	*Navicula tenera* Hustedt	115008	*Fallacia tenera* (Hustedt) Mann	Change	Diatom
46406	*Navicula tripunctata* var. *schizonemoides* (Van Heurck) Patrick	46648	*Navicula erifuga* Lange-Bertalot in Krammer and Lange-Bertalot	Change	Diatom
46410	*Navicula viridula* var. *rostellata* (Kützing) Cleve	46896	*Navicula rostellata* Kützing	Change	Diatom
46421	*Navicula agrestis* Hustedt	211001	*Mayamaea agrestis* (Hustedt) Lange-Bertalot	Change	Diatom
46469	*Navicula viridula* var. *avenacea* (Brébisson ex Grunow) Van Heurck	46034	*Navicula lanceolata* (Agardh) Kützing	Change	Diatom
46472	*Navicula minuscula* Grunow	204002	*Adlafia minuscula* (Grunow in Van Heurck) Lange-Bertalot in Lange-Bertalot and Genkal	Change	Diatom
46518	*Navicula monoculata* var. *omissa* (Hustedt) Lange-Bertalot	115006	*Fallacia omissa* (Hustedt) Mann	Change	Diatom
46523	*Navicula ignota* var. *acceptata* (Hustedt) Lange-Bertalot	210001	*Geissleria acceptata* (Hustedt) Lange-Bertalot et Metzeltin	Change	Diatom
46537	*Navicula schadei* Krasske	46494	*Navicula absoluta*	Correction EAM	Diatom
46813	*Navicula monoculata* Hustedt	115005	*Fallacia monoculata* (Hustedt) Mann in Round, Crawford and Mann	Change	Diatom
46842	*Navicula lenzii* Hustedt in A.S.	115016	*Fallacia lenzii* (Hustedt) Lange-Bertalot in Werum and Lange-Bertalot	Change	Diatom
46893	*Navicula antonii* Lange-Bertalot in U. Rumrich, Lange-Bertalot and M. Rumrich	46527	*Navicula cryptotenella* Lange-Bertalot in Krammer and Lange-Bertalot	Change	Diatom
48133	*Nitzschia biacrula* Hohn et Hellerman	48099	*Nitzschia dissipata* var. *media* (Hantzsch) Grunow in Van Heurck	Lump	Diatom
48157	*Nitzschia linearis* var. *tenuis* (Smith) Grunow in Cleve et Grunow	48023	*Nitzschia linearis* (Agardh) Smith	Lump	Diatom
48179	*Nitzschia tryblionella* var. *debilis* (Arnott) Hustedt	185002	*Tryblionella debilis* Arnott	Change	Diatom
48180	*Nitzschia tryblionella* var. *levidensis* (Smith) Grunow in Cleve et Grunow	185026	*Tryblionella levidensis* Smith	Change	Diatom
48228	*Nitzschia palea* var. *debilis* (Kützing) Grunow in Cleve and Grunow	48025	*Nitzschia palea* (Kützing) Smith	Lump	Diatom
48231	*Nitzschia calida* Grunow in Cleve and Grunow	185021	*Tryblionella calida* (Grunow in Cleve and Grunow) Mann in Round, Crawford and Mann	Change	Diatom
48268	*Nitzschia constricta* (Kützing) Ralfs in Pritchard	185023	*Tryblionella apiculata* Gregory	Change	Diatom
48294	*Nitzschia* cf. *supralitorea* Lange-Bertalot	48312	*Nitzschia supralitorea* Lange-Bertalot	Change	Diatom
48296	*Nitzschia* cf. *archibaldii* CODY Lange-Bertalot	48417	*Nitzschia archibaldii* Lange-Bertalot	Lump	Diatom
48297	*Nitzschia* cf. *acidoclinata* Lange-Bertalot	48347	*Nitzschia acidoclinata* Lange-Bertalot	Lump	Diatom
57001	*Rhoicosphenia curvata* (Kützing) Grunow ex Rabenhorst	57002	*Rhoicosphenia abbreviata* (Agardh) Lange-Bertalot	Change	Diatom
64012	*Stephanodiscus invisitatus* Hohn et Hellerman	64012	*Cyclostephanos invisitatus* (Hohn et Hellerman) Theriot, Stoermer et Håkansson	Change	Diatom
65049	*Surirella moelleriana* Grunow ex Möller	65069	*Surirella amphioxys* Smith	Change	Diatom
66014	*Synedra parasitica* (Smith) Hustedt	73010	*Pseudostaurosira parasitica* (Smith) Morales	Change	Diatom
66018	*Synedra rumpens* var. *familiaris* (Kützing) Hustedt	34098	*Fragilaria capucina* var. *gracilis* (Ostrup) Hustedt	Change	Diatom
66023	*Synedra tenera* Smith	34105	*Fragilaria tenera* (Smith) Lange-Bertalot	Change	Diatom

Appendix 7.4.3 (continued). List of taxonomic changes for Holston River (Tennessee) algal taxa. Names used previously were reported for studies of Holston River algal assemblages during surveys conducted in 1977, 1980, 1990 and 1997 (see ANSP 1978; ANSP 1981; ANSP 1992; ANSP 1998). The status "Change" refers to a taxonomic change in the literature; "Lump" refers to a change made to combine data from different years (and different analysts). (Page 5 of 5)

Original ANS Taxon ID	Original Taxon Name	Current ANS Taxon ID	Current Taxon Name	Status	Algal Group
66027	*Synedra ulna* var. *oxyrhynchus* fo. *mediocontracta* (Fonti) Hustedt	66024	*Synedra ulna* (Nitzsch) Ehrenberg	Lump	Diatom
66036	*Synedra pulchella* Ralfs ex Kützing	201001	*Ctenophora pulchella* (Ralfs ex Kützing) Williams et Round	Change	Diatom
66053	*Synedra delicatissima* var. *angustissima* Grunow in Van Heurck	66046	*Synedra delicatissima* Smith	Lump	Diatom
66054	*Synedra fasciculata* var. *truncata* (Greville) Patrick	200002	*Tabularia fasciculata* (Agardh) Williams et Round	Change	Diatom
66056	*Synedra pulchella* var. *lacerata* Hustedt	201001	*Ctenophora pulchella* (Ralfs ex Kützing) Williams et Round	Change	Diatom
66078	*Synedra* sp. 1 ANS LAP	34212	*Fragilaria sepes* Ehrenberg	Change	Diatom
66814	*Synedra* sp. 8 NAWQA MORALES	66024	*Synedra ulna* (Nitzsch) Ehrenberg	Lump	Diatom
70002	*Thalassiosira fluviatilis* Hustedt	70008	*Thalassiosira weissflogii* (Grunow) Fryxell et Hasle	Change	Diatom
73012	*Pseudostaurosira subsalina* (Hustedt) Morales	172006	*Staurosira construens* var. *venter* (Ehrenberg) Hamilton in Hamilton, Poulin, Charles and Angell	Lump	Diatom
73013	*Pseudostaurosira trainorii* Morales	172006	*Staurosira construens* var. *venter* (Ehrenberg) Hamilton in Hamilton, Poulin, Charles and Angell	Lump	Diatom
125002	*Karayevia laterostrata* (Hantzsch) Bukhtiyarova	94022	*Achnanthes ploenensis* var. *gessneri*	Change	Diatom
155003	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot	155015	*Planothidium hauckianum* (Grunow) Round et Bukhtiyarova	Change	Diatom
155024	*Planothidium lanceolatum* var. *omissum* (Reimer in Patrick and Reimer) Andresen, Stoermer et Kreis	155003	*Planothidium lanceolatum* (Brébisson ex Kützing) Lange-Bertalot	Lump	Diatom
200001	*Tabularia tabulata* (Agardh) Snoeijs	200002	*Tabularia fasciculata* (Agardh) Williams et Round	Lump	Diatom
801000	*Agmenellum quadruplicatum* (Meneghini) BrJbisson	875002	*Merismopedia punctata* Meyen	Lump	Blue-Green
801001	*Agmenellum thermale* (Kützing) Drouet et Daily	875003	*Merismopedia glauca* (Ehrenberg) Kützing	Change	Blue-Green
835000	*Entophysalis rivularis* (Kützing) Drouet	893002	*Pleurocapsa minor* Hansgirg	Change	Blue-Green
835002	*Entophysalis lemaniae* (Agardh) Drouet et Daily	819001	*Chamaesiphon incrustans* Grunow ex Rabenhorst	Lump	Blue-Green
878000	*Microcoleus vaginatus* Gomont ex Gomont	890033	*Phormidium amoenum* Kützing in Anagnostidis et Kom rek	Change	Blue-Green
878006	*Microcoleus lyngbyaceus* Kützing ex Rabenhorst	890028	*Phormidium autumnale* (Agardh) Trevisan ex Gomont	Change	Blue-Green
896000	*Porphyrosiphon splendidus* (Greville ex Gomont) Drouet	847001	*Geitlerinema spendidum* (Greville ex Gomont) Anagnostidis	Change	Blue-Green
896002	*Porphyrosiphon notarisii* Kützing ex Gomont	890025	*Phormidium* sp.	Lump	Blue-Green
903003	*Schizothrix calcicola* Gomont	863016	*Leptolyngbya* sp.	Lump	Blue-Green

Appendix 7.5.1. List of non-insect macroinvertebrate taxa collected July 2010 at Zones 2, 3, 4, 5 and 6 on the South Fork and mainstem Holston rivers and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, Tennessee R = Rare [1 individual], UC = Uncommon [2-3 individuals], MC = Moderately Common [4-15 individuals], C = Common [16-30 individuals], A = Abundant [31+ individuals]). (Page 1 of 2)

		Holston					Horse	
Order	Taxon	2	3	4	5	6	1	2
Phylum Porifera								
Class Demospongiae								
Order Haplosclerina								
Spongillidae	Undetermined	C	C	-	R	UC	UC	UC
Phylum Platyhelminthes								
Class Turbellaria								
Order Tricladida								
Dugesiidae	*Dugesia tigrina* (Girard)	A	A	A	A	A	A	A
Phylum Ectoprocta								
Class Phylactolaemata								
Order Ctenostomata								
Plumatellidae	*Plumatella repens* (Linnaeus)	-	C	-	C	-	-	UC
Phylum Annelida								
Class Oligochaeta								
Order Tubificida								
Tubificidae	*Branchiura sowerbyi* Beddard	-	-	R	-	-	-	R
Tubificidae	Undetermined	MC	MC	MC	A	-	R	-
Naididae	*Stylaria lacustris* (Linnaeus)	UC	-	-	-	UC	-	-
Order Opisthopora								
Lumbricidae	*Eiseniella cf. tetraedra* (Savigny)	A	A	A	MC	UC	-	UC
Class Hirudinea								
Order Arhynchobdellida								
Erpobdellidae	*Erpobdella punctata* (Leidy)	-	A	-	MC	R	-	-
Erpobdellidae	*Mooreobdella microstoma* (Moore)	MC	-	UC	MC	-	-	-
Order Rhynchobdellida								
Glossiphoniidae	*Helobdella triserialis* (Blanchard)	R	C	-	MC	MC	-	-
Glossiphoniidae	*Gloiobdella elongata* (Castle)	UC	MC	-	C	-	-	-
Glossiphoniidae	*Helobdella stagnalis* (Linnaeus)	-	UC	-	UC	-	-	-
Glossiphoniidae	*Placobdella papillifera* (Verrill)	-	UC	UC	UC	-	-	-
Glossiphoniidae	*Placobdella parasitica* (Say)	-	-	-	-	R	-	-
Piscicolidae	*Piscicolaria reducta* Meyer	-	-	-	R	MC	-	R

Appendix 7.5.1. (cont.) List of non-insect macroinvertebrate taxa collected July 2010 at Zones 2, 3, 4, 5 and 6 on the South Fork and mainstem Holston rivers and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, Tennessee R = Rare [1 individual], UC = Uncommon [2-3 individuals], MC = Moderately Common [4-15 individuals], C = Common [16-30 individuals], A = Abundant [31+ individuals]). (Page 2 of 2)

		Holston					Horse	
Order	Taxon	2	3	4	5	6	1	2
Phylum Mollusca								
Class Gastropoda								
Order Mesogastropoda								
Viviparidae	*Campeloma decisum* (Say)	-	-	A	A	A	A	-
Pleuroceridae	*Pleurocera uncialis* (Anthony)	A	A	A	A	A	A	A
Pleuroceridae	*Leptoxis praerosa* (Say)	-	-	-	A	A	-	-
Order Basommatophora								
Lymnaeidae	*Fossaria obrussa* (Say)	A	A	-	UC	R	R	R
Planorbidae	*Gyraulus parvus* (Say)	A	A	-	-	R	-	-
Planorbidae	*Micromenetus dilatatus* (Gould)	-	R	-	-	-	-	-
Planorbidae	*Helisoma anceps* (Menke)	A	MC	MC	MC	-	-	-
Physidae	*Physella heterostropha* (Conrad)	A	A	R	A	A	A	A
Ancylidae	*Laevapex diaphanus* (Haldeman)	-	-	-	-	A	-	-
Ancylidae	*Ferrissia rivularis* (Say)	-	MC	A	A	A	A	A
Class Bivalvia								
Order Veneroida								
Sphaeriidae	*Pisidium* sp.	A	A	A	A	UC	UC	-
Sphaeriidae	*Musculium securis* (Prime)	-	-	-	A	-	-	-
Sphaeriidae	*Sphaerium fabale* (Prime)	-	-	-	A	-	UC	-
Sphaeriidae	*Sphaerium striatinum* (Lamarck)	-	-	-	A	-	A	A
Corbiculidae	*Corbicula fluminea* (Muller)	A	A	A	A	A	A	A
Phylum Arthropoda								
Subphylum Crustacea								
Order Isopoda								
Asellidae	*Caecidotea* sp.	C	-	-	UC	UC	-	-
Order Amphipoda								
Hyalellidae	*Hyalella azteca* (Saussure)	-	-	-	-	UC	-	-
Crangonyctidae	*Crangonyx* sp.	C	R	MC	A	R	-	-
Order Decapoda								
Cambaridae	*Orconectes rusticus* (Girard)	R	A	A	A	A	A	A
Cambaridae	*Cambarus bartonii cavatus* Hay	MC	R	-	-	-	-	UC
Cambaridae	*Cambarus girardianus* Faxon	MC	R	R	MC	MC	MC	MC
Cambaridae	*Cambarus striatus* Hay	-	-	R	-	-	-	-
Class Arachnida								
Order Trombidiformes								
Lebertiidae	*Lebertia* sp.	R	UC	-	-	UC	-	-
Hydrachnidae	*Hydrachna* sp.	-	-	-	-	R	-	-
	Total Richness = 39	21	24	17	28	25	14	15

Appendix 7.5.2. Presence and absence of non-insect macroinvertebrate taxa collected during the 1965, 1974, 1977, 1980, 1990, 1997, and 2010 surveys at Zones 2, 3, 4, 5 and 6 on the South Fork and mainstem Holston rivers and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, TN. (Page 1 of 2)

		Holston					Horse	
Order	Taxon	2	3	4	5	6	1	2
Phylum Porifera								
Class Demospongiae								
Order Haplosclerina								
Spongillidae	Undetermined	X	X	X	X	X	X	X
Phylum Platyhelminthes								
Class Turbellaria								
Order Tricladida								
Dugesiidae	*Dugesia tigrina* (Girard)	X	X	X	X	X	X	X
Phylum Ectoprocta								
Class Phylactolaemata								
Order Ctenostomata								
Plumatellidae	*Plumatella repens* (Linnaeus)	X	X	X	X	X	X	X
Paludicellidae	*Paludicella articulata* (Ehrenberg)	X	X	X	-	-	-	-
Phylum Annelida								
Class Oligochaeta								
Order Tubificida								
Tubificidae	*Branchiura sowerbyi* Beddard	X	-	X	X	-	-	X
Tubificidae	Undetermined	X	X	X	X	X	X	-
Naididae	*Stylaria lacustris* (Linnaeus)	X	-	-	-	X	-	-
Order Opisthopora								
Lumbricidae	*Eiseniella cf. tetraedra* (Savigny)	X	X	X	X	X	X	X
Order Lumbriculida								
Lumbriculidae	*Lumbriculus variegatus* (Mueller)	X	X	X	X	X	-	-
Class Hirudinea								
Order Arhynchobdellida								
Erpobdellidae	*Erpobdella punctata* (Leidy)	-	X	X	X	X	-	X
Erpobdellidae	*Mooreobdella microstoma* (Moore)	X	-	X	X	X	-	-
Erpobdellidae	*Nephelopsis obscura* Verrill	-	X	-	-	-	-	-
Erpobdellidae	Erpobdellidae	-	-	-	-	-	-	X
Order Rhynchobdellida								
Glossiphoniidae	*Desserobdella phalera* (Graf)	-	-	-	-	-	X	X
Glossiphoniidae	*Helobdella triserialis* (Blanchard)	X	X	X	X	X	-	X
Glossiphoniidae	*Gloiobdella elongata* (Castle)	X	X	-	X	X	-	-
Glossiphoniidae	*Helobdella stagnalis* (Linnaeus)	-	X	-	X	X	-	-
Glossiphoniidae	*Placobdella papillifera* (Verrill)	X	X	X	X	-	-	-
Glossiphoniidae	*Placobdella parasitica* (Say)	-	-	-	-	X	-	-
Glossiphoniidae	Undetermined	-	X	-	-	-	-	-
Piscicolidae	*Myzobdella lugubris* Leidy	-	-	X	-	-	-	-
Piscicolidae	*Piscicolaria reducta* Meyer	-	-	-	X	X	-	X
Piscicolidae	Undetermined	-	X	X	-	-	-	-

Appendix 7.5.2. (cont.) Presence and absence of non-insect macroinvertebrate taxa collected during the 1965, 1974, 1977, 1980, 1990, 1997, and 2010 surveys at Zones 2, 3, 4, 5, and 6 on the South Fork and mainstem Holston rivers and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, TN. (Page 2 of 2)

		Holston					Horse	
Order	Taxon	2	3	4	5	6	1	2
Phylum Mollusca								
Class Gastropoda								
Order Mesogastropoda								
Viviparidae	*Campeloma decisum* (Say)	-	-	X	X	X	X	-
Pleuroceridae	*Pleurocera uncialis* (Anthony)	X	X	X	X	X	X	X
Pleuroceridae	*Leptoxis praerosa* (Say)	-	-	X	X	X	-	-
Pomatiopsidae	*Pomatiopsis lapidaria* (Say)	X	-	-	-	X	-	-
Order Basommatophora								
Lymnaeidae	*Fossaria obrussa* (Say)	X	X	X	X	X	X	X
Planorbidae	*Gyraulus parvus* (Say)	X	X	-	-	X	-	-
Planorbidae	*Micromenetus dilatatus* (Gould)	X	X	X	X	X	-	X
Planorbidae	*Planorbella trivolvis* (Say)	-	-	X	-	X	-	-
Planorbidae	*Helisoma anceps* (Menke)	X	X	X	X	-	-	-
Physidae	*Physella heterostropha* (Conrad)	X	X	X	X	X	X	X
Ancylidae	*Laevapex diaphanus* (Haldeman)	-	-	-	-	X	-	-
Ancylidae	*Ferrissia rivularis* (Say)	X	X	X	X	X	X	X
Class Bivalvia								
Order Unionida								
Unionidae	*Lampsilis teres* (Rafinesque)	-	-	X	-	-	-	-
Order Veneroida								
Sphaeriidae	*Pisidium* sp.	X	X	X	X	X	X	X
Sphaeriidae	*Musculium securis* (Prime)	-	X	X	X	X	-	-
Sphaeriidae	*Sphaerium fabale* (Prime)	-	-	X	X	-	X	-
Sphaeriidae	*Sphaerium striatinum* (Lamarck)	-	-	X	X	-	X	X
Corbiculidae	*Corbicula fluminea* (Muller)	X	X	X	X	X	X	X
Phylum Arthropoda								
Subphylum Crustacea								
Order Isopoda								
Asellidae	*Caecidotea* sp.	X	-	X	X	X	-	-
Asellidae	*Lirceus* sp.	X	-	X	-	-	-	-
Order Amphipoda								
Hyalellidae	*Hyalella azteca* (Saussure)	-	-	X	-	X	-	-
Crangonyctidae	*Crangonyx* sp.	X	X	X	X	X	-	-
Order Decapoda								
Cambaridae	*Orconectes rusticus* (Girard)	X	X	X	X	X	X	X
Cambaridae	*Orconectes forceps* (Faxon)	-	-	-	X	X	-	-
Cambaridae	*Cambarus bartonii cavatus* Hay	X	X	X	X	X	X	X
Cambaridae	*Cambarus girardianus* Faxon	X	X	X	X	X	X	X
Cambaridae	*Cambarus striatus* Hay	-	-	X	-	-	-	-
Class Arachnida								
Order Trombidiformes								
Lebertiidae	*Lebertia* sp.	X	X	X	X	X	-	-
Hygrobatidae	*Hygrobates* sp.	-	X	-	-	X	-	-
Limnesiidae	*Tyrellia* sp.	-	X	-	-	-	-	-
Hydrachnidae	*Hydrachna* sp.	-	-	-	-	X	-	-

Appendix 7.5.3A. Bray-Curtis dissimilarity matrix for non-insect macroinvertebrate taxa collected from 1965 through 2010 surveys at Zones 2, 3, 4, 5 and 6 on the South Fork and mainstem Holston rivers and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, TN. Bold values are the average Bray-Curtis dissimilarity for Holston River sites for each survey year. (Page 1 of 3)

		2010							1997							1990						
		2	3	4	5	6	HC1	HC2	2	3	4	5	6	HC1	HC2	2	3	4	5	6	HC1	HC2
2010	2					**0.33**																
	3	0.20																				
	4	0.37	0.37																			
	5	0.31	0.23	0.33																		
	6	0.30	0.35	0.48	0.32																	
	HC1	0.43	0.42	0.35	0.33	0.44																
	HC2	0.44	0.38	0.44	0.40	0.45	0.31															
1997	2	0.25	0.26	0.50	0.45	0.36	0.45	0.41					**0.29**									
	3	0.44	0.19	0.43	0.30	0.44	0.38	0.39	0.35													
	4	0.29	0.29	0.32	0.27	0.30	0.37	0.33	0.30	0.33												
	5	0.35	0.26	0.33	0.24	0.36	0.39	0.41	0.37	0.30	0.26											
	6	0.29	0.29	0.37	0.27	0.22	0.37	0.44	0.21	0.33	0.20	0.26										
	HC1	0.45	0.39	0.45	0.45	0.51	0.23	0.19	0.42	0.40	0.33	0.47	0.44									
	HC2	0.52	0.39	0.45	0.45	0.46	0.23	0.33	0.48	0.33	0.39	0.41	0.39	0.17								
1990	2	0.39	0.33	0.44	0.40	0.45	0.45	0.27	0.24	0.33	0.28	0.46	0.33	0.33	0.48					**0.28**		
	3	0.41	0.20	0.39	0.32	0.41	0.40	0.42	0.31	0.12	0.30	0.26	0.30	0.43	0.36	0.29						
	4	0.33	0.24	0.37	0.18	0.30	0.20	0.33	0.30	0.23	0.19	0.21	0.20	0.33	0.27	0.33	0.19					
	5	0.42	0.32	0.41	0.33	0.38	0.42	0.31	0.33	0.37	0.21	0.28	0.27	0.38	0.38	0.25	0.33	0.21				
	6	0.46	0.45	0.39	0.36	0.27	0.40	0.35	0.43	0.47	0.24	0.37	0.25	0.43	0.50	0.29	0.44	0.30	0.21			
	HC1	0.50	0.49	0.43	0.54	0.56	0.36	0.23	0.53	0.52	0.44	0.52	0.54	0.22	0.30	0.38	0.56	0.44	0.36	0.41		
	HC2	0.56	0.54	0.50	0.59	0.61	0.36	0.31	0.60	0.59	0.50	0.58	0.60	0.22	0.30	0.46	0.63	0.50	0.43	0.48	0.09	

Appendix 7.5.3B. Bray Curtis dissimilarity matrix for non-insect macroinvertebrate taxa collected from 1965 through 2010 surveys at Zones 2, 3, 4, 5 and 6 on the South Fork and mainstem Holston rivers and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, TN. Bold values are the average Bray-Curtis dissimilarity for Holston River sites for each survey year. (Page 2 of 3)

		2010							1997							1990						
		2	3	4	5	6	HC1	HC2	2	3	4	5	6	HC1	HC2	2	3	4	5	6	HC1	HC2
1980	2	0.67	0.64	0.69	0.62	0.71	0.65	0.67	0.50	0.63	0.60	0.61	0.58	0.71	0.81	0.50	0.52	0.53	0.54	0.52	0.80	0.80
	3	0.63	0.54	0.64	0.54	0.67	0.60	0.69	0.53	0.52	0.63	0.52	0.54	0.57	0.57	0.62	0.41	0.50	0.57	0.70	0.82	0.82
	4	0.45	0.44	0.61	0.49	0.45	0.52	0.59	0.42	0.51	0.45	0.46	0.49	0.55	0.61	0.53	0.43	0.40	0.56	0.49	0.73	0.73
	5	0.44	0.38	0.63	0.40	0.40	0.59	0.53	0.47	0.52	0.39	0.35	0.44	0.56	0.56	0.47	0.42	0.39	0.38	0.35	0.62	0.62
	6	0.40	0.30	0.59	0.36	0.40	0.56	0.62	0.37	0.45	0.40	0.27	0.35	0.59	0.53	0.57	0.37	0.35	0.38	0.47	0.70	0.70
1977	2	0.60	0.58	0.77	0.62	0.65	0.65	0.50	0.43	0.63	0.47	0.68	0.58	0.52	0.71	0.33	0.52	0.53	0.46	0.52	0.70	0.70
	3	0.66	0.56	0.76	0.61	0.58	0.55	0.57	0.63	0.46	0.59	0.60	0.63	0.60	0.50	0.57	0.42	0.52	0.60	0.67	0.68	0.68
	4	0.56	0.54	0.63	0.40	0.50	0.59	0.73	0.65	0.64	0.50	0.46	0.54	0.70	0.70	0.67	0.55	0.39	0.44	0.48	0.85	0.85
	5	0.52	0.56	0.66	0.55	0.51	0.62	0.56	0.55	0.67	0.45	0.53	0.50	0.58	0.58	0.48	0.57	0.52	0.38	0.43	0.57	0.57
	6	0.50	0.49	0.56	0.44	0.40	0.52	0.53	0.53	0.58	0.50	0.51	0.49	0.56	0.48	0.47	0.48	0.44	0.38	0.42	0.62	0.62
1974	2	0.67	0.70	0.85	0.73	0.76	0.83	0.67	0.50	0.78	0.60	0.81	0.64	0.62	0.81	0.42	0.68	0.67	0.62	0.68	0.80	0.80
	3	0.66	0.63	0.76	0.67	0.70	0.82	0.83	0.78	0.69	0.72	0.67	0.75	0.80	0.70	0.74	0.58	0.66	0.68	0.75	0.79	0.79
	4	0.59	0.57	0.67	0.51	0.53	0.56	0.64	0.50	0.61	0.47	0.54	0.51	0.60	0.68	0.50	0.52	0.41	0.40	0.45	0.75	0.75
	5	0.39	0.44	0.59	0.45	0.51	0.54	0.70	0.55	0.53	0.52	0.53	0.50	0.58	0.50	0.63	0.43	0.39	0.59	0.64	0.74	0.74
	6	0.44	0.44	0.69	0.44	0.55	0.66	0.67	0.53	0.58	0.56	0.62	0.59	0.63	0.63	0.53	0.55	0.56	0.50	0.68	0.77	0.77
1965	2	0.64	0.68	0.67	0.71	0.81	0.71	0.55	0.54	0.68	0.64	0.66	0.68	0.58	0.79	0.55	0.65	0.64	0.58	0.65	0.67	0.67
	3	0.78	0.73	0.83	0.82	0.87	0.80	0.81	0.76	0.75	0.85	0.79	0.80	0.78	0.78	0.81	0.73	0.78	0.74	0.91	0.76	0.76
	4	0.63	0.71	0.64	0.59	0.61	0.52	0.77	0.67	0.66	0.56	0.70	0.66	0.74	0.74	0.69	0.63	0.50	0.71	0.63	0.82	0.82
	5	0.84	0.79	0.81	0.81	0.86	0.78	0.89	0.83	0.73	0.92	0.77	0.79	0.88	0.75	0.89	0.70	0.76	0.81	0.90	0.87	0.87
	6	0.66	0.56	0.84	0.61	0.76	0.73	0.74	0.70	0.54	0.72	0.67	0.75	0.70	0.70	0.74	0.58	0.66	0.68	0.92	0.79	0.79

Appendix 7.5.3C. Bray Curtis dissimilarity matrix for non-insect macroinvertebrate taxa collected from 1965 through 2010 surveys at Zones 2, 3, 4, 5 and 6 on the South Fork and mainstem Holston rivers and Zones 1 and 2 on Horse Creek, Hawkins and Sullivan counties, TN. Bold values are the average Bray-Curtis dissimilarity for Holston River sites for each survey year. (Page 3 of 3)

		1980					1977					1974					1965				
		2	3	4	5	6	2	3	4	5	6	2	3	4	5	6	2	3	4	5	6
1980	2					**0.41**															
	3	0.50																			
	4	0.36	0.40																		
	5	0.42	0.62	0.35																	
	6	0.48	0.52	0.32	0.19																
1977	2	0.33	0.60	0.43	0.50	0.55					**0.47**										
	3	0.65	0.58	0.48	0.48	0.53	0.41														
	4	0.67	0.54	0.41	0.47	0.41	0.58	0.57													
	5	0.62	0.65	0.55	0.33	0.41	0.43	0.50	0.48												
	6	0.58	0.54	0.47	0.40	0.46	0.50	0.48	0.40	0.33											
1974	2	0.56	0.60	0.57	0.67	0.68	0.22	0.65	0.67	0.52	0.67					**0.46**					
	3	0.76	0.58	0.63	0.48	0.53	0.65	0.38	0.57	0.50	0.65	0.65									
	4	0.55	0.50	0.44	0.50	0.49	0.36	0.52	0.21	0.36	0.50	0.45	0.52								
	5	0.62	0.39	0.35	0.41	0.35	0.52	0.40	0.41	0.42	0.48	0.52	0.30	0.44							
	6	0.67	0.46	0.53	0.53	0.46	0.42	0.48	0.40	0.41	0.33	0.50	0.48	0.36	0.33						
1965	2	0.50	0.56	0.62	0.64	0.66	0.50	0.73	0.73	0.58	0.73	0.50	0.73	0.50	0.58	0.64					**0.57**
	3	0.73	0.65	0.84	0.81	0.71	0.73	0.71	0.90	0.67	0.81	0.73	0.71	0.68	0.67	0.71	0.54				
	4	0.70	0.64	0.53	0.69	0.70	0.70	0.58	0.54	0.74	0.62	0.70	0.68	0.50	0.48	0.69	0.56	0.76			
	5	0.69	0.60	0.74	0.79	0.69	0.69	0.50	0.79	0.63	0.79	0.69	0.50	0.65	0.50	0.68	0.64	0.40	0.73		
	6	0.76	0.47	0.70	0.74	0.67	0.65	0.50	0.74	0.70	0.65	0.65	0.63	0.71	0.50	0.48	0.60	0.43	0.58	0.50	

Appendix 7.6.1. Occurrence of different fish species caught on the South Fork and mainstem Holston rivers and Horse Creek in July 2010. Occurrence is indicated by technique with which the fish was caught (Bp=backpack electrofishing, Bs=boat electrofishing, H=hand, S=seine, Pl=PIBS sampler). Bp is shore backpack electrofishing and 5 x 5 electrofishing.

	Zone								
Species	**2**	**3L**	**3R**	**3L+3R**	**4**	**5**	**6**	**HC1**	**HC2**
Dorosoma cepedianum	-	-	-	-	-	Bs	Bs	-	-
Campostoma anomalum	-	Bp	Bp	Bp	Bp	Bp	BpH	Bp	Bp
Cyprinella galactura	-	-	-	-	-	Bp	Bp	-	-
Cyprinella spiloptera	Bp	-	-	-	-	Bs	Bs	-	-
Cyprinus carpio	Bs	-	-	-	-	Bs	Bs	-	-
Erimystax dissimilis	-	-	-	-	-	-	Bs	-	-
Luxilus chrysocephalus	-	-	S	S	Bp	Bp	BpBs	Bp	Bp
Luxilus coccogenis	-	-	-	-	Bp	Bp	Bp	Bp	-
Notropis amblops	-	-	-	-	-	Bs	BsBp	Bp	Bp
Notropis leuciodus	-	-	-	-	-	-	Bp	-	-
Notropis photogenis	-	-	-	-	-	-	BsBp	-	Bp
Notropis rubellus	-	Bp	-	Bp	-	-	-	-	Bp
Notropis sp. (sawfin)	-	-	S	S	-	Bp	BpBs	-	-
Notropis telescopus	BpBs	Bp	SBp	BpS	Bp	BpBs	BpBs	Bp	Bp
Notropis volucellus	-	-	-	-	-	Bs	Bs	Bp	Bp
Pimephales notatus	-	-	-	-	-	-	BsBp	-	Bp
Rhinichthys atratulus	H	-	-	-	-	-	-	Bp	Bp
Semotilus atromaculatus	-	-	-	-	-	-	-	-	Bp
Catostomus commersoni	BsH	-	-	-	-	-	-	-	Bp
Hypentelium nigricans	-	Bp	-	Bp	Bp	BpBs	BpBs	Bp	Bp
Moxostoma duquesnei	Bs	-	-	-	-	Bs	BsHBp	-	-
Moxostoma erythrurum	Bs	-	Bp	Bp	-	Bs	BsBp	-	-
Moxostoma macrolepidotum	-	-	-	-	Bp	-	BsBp	-	-
Moxostoma species	-	-	-	-	-	Bs	-	-	-
Ameiurus natalis	-	-	-	-	-	-	HBs	Bp	-
Ictalurus punctatus	-	-	-	-	-	Bs	-	-	-
Noturus eleutherus	-	-	-	-	-	Bp	BpH	-	-
Oncorhynchus mykiss	BsBp	-	-	-	-	-	-	-	-
Salmo trutta	Bs	-	-	-	-	-	-	-	-
Gambusia affinis	-	HBp	-	HBp	-	-	Bp	Bp	BpH
Cottus carolinae	Bp	-	-	-	Bp	Bp	Bp	BpPl	BpPl
Morone species	Bs	-	-	-	-	-	-	-	-
Ambloplites rupestris	Bs	H	-	H	Bp	BsBp	BsHBp	Bp	BpH
Lepomis auritus	Bs	H	Bp	HBp	Bp	Bs	Bs	Bp	Bp
Lepomis cyanellus	-	-	-	-	-	Bs	Bp	-	Bp
Lepomis gulosus	Bs	-	-	-	-	Bs	-	-	-
Lepomis hybrid	-	-	-	-	-	-	Bp	-	-
Lepomis macrochirus	Bs	-	-	-	-	Bs	Bs	Bp	Bp
Lepomis microlophus	-	-	-	-	-	Bs	-	-	-
Micropterus dolomieu	-	BpH	Bp	BpH	Bp	BpBs	BsH	Bp	Bp
Micropterus salmoides	-	Bp	Bp	Bp	-	Bs	Bp	-	Bp
Etheostoma blennioides	-	Bp	Bp	Bp	Bp	BpBs	Bp	Bp	Bp
Etheostoma camurum	-	-	-	-	-	-	BpBs	-	-
Etheostoma rufilineatum	-	Bp	Bp	Bp	Bp	Bp	BpH	BpPl	Bp
Etheostoma simoterum	HBpBs	BpH	BpS	BpHS	Bp	Bp	BpHBs	Bp	BpH
Etheostoma stigmaeum	-	-	-	-	-	-	-	Bp	-
Etheostoma zonale	-	Bp	-	Bp	Bp	Bp	BpH	Bp	-
Percina caprodes	-	-	-	-	-	-	Bp	-	-
Percina evides	-	-	Bp	Bp	-	-	-	-	-
Total Number of Species	16	13	12	17	14	29	35	20	23

Appendix 7.6.2. Fish caught using shore backpack electrofishing at Zone HC1 during ANSP July 2010 survey. (n.e. = could not be estimated.)

			Total			Density	
Species	**Pass 1**	**Pass 2**	**Raw**	**Estimated**	**p**	**Raw**	**Estimated**
Cyprinidae	86	62	148	>119	-	3.94	>3.16
Campostoma anomalum	41	24	65	92	0.08	1.73	2.45
Luxilus chrysocephalus	5	7	12	n.e.	0.00	0.32	n.e
Luxilus coccogenis	5	0	5	n.e.	0.00	0.13	n.e
Notropis amblops	14	6	20	22	0.29	0.53	0.58
Notropis telescopus	18	23	41	n.e.	0.00	1.09	n.e
Notropis volucellus	0	1	1	1	0.00	0.03	0.03
Rhinichthys atratulus	3	1	4	4	0.22	0.11	0.11
Hypentelium nigricans	0	2	2	n.e.	0.00	0.05	n.e
Ameiurus natalis	1	0	1	n.e	0.00	0.03	n.e
Gambusia affinis	9	2	11	11	0.24	0.29	0.29
Cottus carolinae	213	111	324	440	0.01	8.61	11.70
Centrachidae	27	13	40	46	-	1.06	1.22
Ambloplites rupestris	10	4	14	15	0.26	0.37	0.40
Lepomis auritus	2	1	3	3	0.38	0.08	0.08
Lepomis macrochirus	1	0	1	n.e.	0.00	0.03	n.e
Micropterus dolomieu	14	8	22	28	0.18	0.58	0.74
Percidae	133	77	210	345	-	5.59	9.17
Etheostoma blennioides	32	11	43	47	0.12	1.14	1.25
Etheostoma rufilineatum	30	15	45	56	0.11	1.20	1.49
Etheostoma simoterum	66	49	115	236	0.04	3.06	6.27
Etheostoma stigmaeum	1	0	1	n.e.	0.00	0.03	n.e
Etheostoma zonale	4	2	6	6	0.76	0.16	0.16
Total	469	267	736	1082	0.01	19.56	28.76
Area (m^2)	940.5						

Appendix 7.6.3. Fish caught using shore backpack electrofishing at Zone HC2 during ANSP July 2010 survey.

Species	Pass 1	Pass 2	Total			Density	
			Raw	Estimated	p	Raw	Estimated
Cyprinidae	66	29	96	>88	-	2.51	>2.30
Campostoma anomalum	38	14	52	57	0.14	1.36	1.49
Luxilus chrysocephalus	0	6	6	n.e.	0.00	0.16	n.e
Notropis amblops	22	6	28	29	0.22	0.73	0.76
Notropis photogenis	0	1	1	n.e.	0.00	0.03	n.e
Notropis rubellus	1	0	1	n.e.	0.00	0.03	n.e
Notropis telescopus	1	1	2	2	0.81	0.08	0.05
Notropis volucellus	3	0	3	n.e.	0.00	0.08	n.e
Pimephales notatus	1	0	1	n.e.	0.00	0.03	n.e
Rhinichthys atratulus	0	1	1	n.e.	0.00	0.03	n.e
Semotilus atromaculatus	1	0	1	n.e.	0.00	0.03	n.e
Catostomus commersoni	1	0	1	n.e.	0.00	0.03	n.e
Hypentelium nigricans	3	0	3	n.e.	0.00	0.08	n.e
Gambusia affinis	9	3	12	12	0.67	0.31	0.31
Cottus carolinae	93	42	135	166	0.04	3.53	4.34
Centrarchidae	43	24	67	86	-	1.75	2.25
Ambloplites rupestris	11	9	20	35	0.24	0.52	0.91
Lepomis auritus	6	3	9	9	1.14	0.24	0.24
Lepomis cyanellus	1	1	2	2	0.81	0.05	0.05
Lepomis macrochirus	9	3	12	12	0.67	0.31	0.31
Micropterus dolomieu	15	8	23	28	0.22	0.60	0.73
Micropterus salmoides	1	0	1	n.e.	0.00	0.03	n.e
Percidae	110	53	163	510	-	4.26	13.33
Etheostoma blennioides	26	7	33	34	0.31	0.86	0.89
Etheostoma rufilineatum	20	11	31	40	0.13	0.81	1.05
Etheostoma simoterum	64	35	99	436	0.04	2.59	11.40
Total	322	151	473	602	0.01	12.49	15.73
Area (m^2)	956.5						

Appendix 7.6.4. Catch Per Unit Effort (fish per 100 m) in shore backpack electrofishing.

	Zone										
Species	**2**	**3L**	**3R**	**4**	**5**	**6**	**HC1**	**HC2**	**KL**	**KU**	**T2**
Ambloplites rupestris	-	-	-	5.08	6.10	0.53	5.83	8.00	1.00	-	-
Ameiurus natalis	-	-	-	-	-	-	0.42	-	2.00	-	-
Campostoma anomalum	-	16.42	2.99	8.39	14.63	2.63	27.08	20.80	23.00	7.11	6.00
Catostomus commersoni	-	-	-	-	-	-	-	0.40	-	-	-
Cottus carolinae	-	-	-	0.61	1.22	-	135.00	54.00	3.00	3.33	-
Cyprinella galactura	-	-	-	-	1.22	-	-	-	-	-	-
Cyprinella spiloptera	1.43	-	-	-	-	-	-	-	-	-	-
Etheostoma blennioides	-	13.43	-	31.82	8.54	4.21	17.92	13.20	22.00	33.33	-
Etheostoma rufilineatum	-	1.49	-	9.30	-	0.53	18.75	12.40	2.00	-	-
Etheostoma simoterum	1.43	92.54	20.90	163.31	69.51	15.79	47.92	39.60	224.00	179.33	12.00
Etheostoma stigmaeum	-	-	-	-	-	-	0.42	-	-	-	-
Etheostoma zonale	-	7.46	-	5.57	9.76	3.68	2.50	-	2.00	1.33	-
Gambusia affinis	-	1.49	-	-	-	0.53	4.58	4.80	0.00	-	-
Hypentelium nigricans	-	2.99	-	0.51	2.44	2.11	0.83	1.20	1.00	4.44	-
Lepomis auritus	-	-	13.43	0.51	-	0.00	1.25	3.60	-	-	-
Lepomis cyanellus	-	-	-	-	-	4.74	-	0.80	-	-	-
Lepomis hybrid	-	-	-	-	-	0.53	-	-	-	-	-
Lepomis macrochirus	-	-	-	-	-	-	0.42	4.80	-	-	-
Luxilus chrysocephalus	-	-	-	-	4.88	3.16	5.00	2.40	-	0.67	-
Luxilus coccogenis	-	-	-	-	8.54	-	2.08	-	-	-	-
Micropterus dolomieu	-	2.99	25.37	9.36	19.51	-	9.17	9.20	4.00	10.67	-
Micropterus salmoides	-	2.99	2.99	-	-	0.53	-	0.40	-	-	-
Moxostoma duquesnei	-	-	-	-	-	1.05	-	-	-	-	-
Moxostoma erythrurum	-	-	1.49	-	-	1.58	-	-	-	-	-
Moxostoma macrolepidotum	-	-	-	0.51	-	2.63	-	-	-	-	-
Notropis amblops	-	-	-	-	-	0.53	8.33	11.20	-	-	4.00
Notropis atherinoides	-	-	-	-	-	-	-	-	-	-	-
Notropis photogenis	-	-	-	-	-	-	-	0.40	-	-	-
Notropis rubellus	-	-	-	-	-	-	-	0.40	-	-	-
Notropis telescopus	21.43	-	-	-	2.44	1.05	17.08	1.20	2.00	0.67	234.00
Notropis volucellus	-	-	-	-	-	-	0.42	1.20	2.00	-	-
Pimephales notatus	-	-	-	-	-	0.53	-	0.40	-	-	-
Rhinichthys atratulus	-	-	-	-	-	-	1.67	0.40	-	-	12.00
Semotilus atromaculatus	-	-	-	-	-	-	-	0.40	-	-	-
Total	24.29	141.79	67.16	234.98	148.78	46.32	306.67	191.20	288.00	240.89	268.00
Total Number of Fish	17	95	45	343	122	88	736	478	288	345	134
Average Number of Species	3.0	9.0	6.0	7.7	12.0	11.0	17.0	18.0	9.0	5.3	5.0
Total Number of Species	3	9	6	11	12	18	20	22	12	9	5
Number of Samples	1	1	1	3	1	2	2	2	2	3	1
Total Shoreline Shocked (m)	70	67	67	140	82	190	240	250	100	115	50

Appendix 7.6.5. Catch per unit effort (fish per 100 m fished) in shore back-pack electrofishing samples from zones on the Holston River in 1997. (Page 1 of 2)

	Zone									
Species	**2**	**3L**	**3R**	**3RU**	**4M**	**4L**	**5**	**6**	**HC1**	**HC2**
Campostoma anomalum	24.00	5.05	-	-	7.11	86.67	37.67	6.19	655.37	39.53
Cyprinella spiloptera	-	-	-	-	-	-	0.33	-	2.26	-
Cyprinus carpio	-	-	-	-	-	-	-	-	2.26	-
Luxilus chrysocephalus	-	-	-	-	-	-	-	0.95	18.08	1.98
Luxilus coccogenis	-	-	-	-	-	-	2.67	1.43	-	0.99
Nocomis micropogon	-	-	-	-	-	3.33	1.67	-	-	-
Notropis amblops	-	-	-	-	0.89	-	-	1.50	-	-
Notropis photogenis	-	-	-	-	-	-	-	0.95	-	-
Notropis stramineus	-	-	-	-	-	-	-	-	13.56	0.99
Notropis telescopus	-	-	-	0.68	-	-	0.67	0.48	-	-
Notropis sp. (sawfin)	-	-	1.49	-	-	-	2.00	-	-	-
Pimephales notatus	-	-	-	-	-	-	-	-	-	0.99
Pimephales promelas	0.80	-	-	-	-	-	-	-	32.77	1.98
Rhinichthys atratulus	11.20	-	-	-	-	-	5.67	-	2.26	0.99
Semotilus atromaculatus	-	-	-	-	-	-	0.67	-	-	-
Catostomus commersoni	3.20	9.17	-	0.98	-	-	5.33	-	5.65	0.99
Hypentelium nigricans	-	1.55	-	-	-	-	2.33	0.95	6.78	0.99
Moxostoma duquesnei	-	-	-	-	-	-	-	-	1.13	-
Moxostoma erythrurum	-	-	-	-	-	-	-	0.48	-	-
Ameiurus natalis	-	-	-	-	-	-	-	-	3.39	-
Ictalurus punctatus	-	-	-	-	-	-	-	0.48	-	-

Appendix 7.6.5 (continued). Catch per unit effort (fish per 100 m fished) in shore back-pack electrofishing samples from zones on the Holston River in 1997. (Page 2 of 2)

	Zone									
Species	**2**	**3L**	**3R**	**3RU**	**4M**	**4L**	**5**	**6**	**HC1**	**HC2**
Gambusia affinis	-	1.27	-	-	-	-	-	-	96.05	31.62
Cottus carolinae	-	-	-	-	2.67	16.67	8.67	14.92	302.82	55.34
Ambloplites rupestris	-	-	-	-	5.33	13.33	6.00	5.43	6.78	7.91
Lepomis auritus	-	-	-	3.03	1.78	-	0.67	1.23	14.69	12.85
Lepomis cyanellus	-	-	-	1.95	16.89	6.67	4.67	-	132.20	14.82
Lepomis gulosus	-	-	-	-	-	-	-	-	1.13	-
Lepomis hybrid	-	-	-	-	3.56	-	-	-	2.26	3.95
Lepomis macrochirus	-	-	-	-	-	-	1.33	1.10	31.64	3.95
Lepomis megalotis	-	-	-	-	-	-	-	-	-	1.98
Lepomis species	-	-	-	-	-	-	-	-	-	0.99
Micropterus dolomieu	-	-	-	2.93	0.89	-	0.67	0.28	1.13	-
Micropterus punctulatus	-	2.05	1.49	5.38	-	-	-	-	-	-
Micropterus salmoides	-	-	-	1.95	-	-	-	0.75	2.26	-
Etheostoma blennioides	-	0.50	11.94	-	7.11	46.67	1.67	14.80	25.99	8.89
Etheostoma camurum	-	-	-	-	-	-	-	1.10	-	-
Etheostoma rufilineatum	-	-	-	-	-	-	-	5.65	32.77	39.53
Etheostoma simoterum	-	25.10	29.85	15.83	25.78	80.00	53.67	70.22	279.10	30.63
Etheostoma zonale	-	-	-	-	-	-	0.33	0.83	-	-
Total	39.20	44.69	44.78	32.73	72.00	253.33	136.67	129.72	1672.32	261.86
Total Number of Fish	49	65	30	38	81	76	296	207	1480	265
Total Number of Species	4	7	4	8	9	7	19	20	23	19
Avg. Number of Species	4.0	4.7	4.0	5.5	9.0	7.0	14.5	10.3	23.0	19.0
Number of Samples	1	3	1	2	1	1	2	3	1	1
Total Shoreline Shocked (m)	125	154.1	67	124.2	112.5	30	225	216	88.5	101.2

Appendix 7.6.6. Occurrence in ANSP sampling by technique at Zone 1 on the Holston River from 1965 to 1980. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; C, cast nets; A, angling; and Obs., observed. Minor techniques are indicated in lowercase letters. (Page 1 of 2)

Species	1965	1974	1977	1980
Dorosoma cepedianum	R	Rs	-	B
Campostoma anomalum	R	Rs	S	BS/C
Carassius auratus	-	-	-	-
Cyprinella galactura	R	Rs	S	BS/C
Cyprinella spiloptera	-	-	-	-
Cyprinus carpio	-	-	-	-
Erimystax dissimilis	-	-	-	-
Luxilus chrysocephalus	-	-	-	-
Luxilus coccogenis	R	Rs	S	BS/C
Nocomis micropogon	R	-	-	B
Notropis amblops	R	Rs	-	B
Notropis ariommus	-	-	-	-
Notropis leuciodus	R	-	-	B
Notropis photogenis	-	-	S	S/C
Notropis rubellus	-	-	-	-
Notropis stramineus	-	-	-	-
Notropis telescopus	R	Rs	S	BS/C
Notropis species	R	Rs	-	-
Notropis sp. (sawfin shiner)	-	-	-	-
Phenacobius crassilabrum	-	-	-	S/C
Phenacobius uranops	-	-	-	-
Pimephales notatus	-	-	-	-
Pimephales promelas	R	Rs	-	-
Rhinichthys atratulus	R	-	-	-
Semotilus atromaculatus	R	-	S	-
Carpiodes carpio	-	-	-	-
Carpiodes cyprinus	-	-	-	-
Catostomus commersoni	-	Rs	-	B
Hypentelium nigricans	R	Rs	S	B
Moxostoma duquesnei	-	-	-	-
Moxostoma erythrurum	-	-	-	-
Moxostoma species	-	-	-	-
Ameiurus natalis	-	-	-	-
Ictalurus punctatus	R	-	-	-
Pylodictis olivaris	-	-	-	-
Oncorhynchus mykiss	-	-	-	-
Salmo trutta	-	-	-	-

Zone 1 was not sampled after 1980.

Appendix 7.6.6 (continued). Occurrence in ANSP sampling by technique at Zone 1 on the South Fork Holston River from 1965 to 1980. Techniques are R, rotenone; S, seining; B, backpack eletrofishing; G, gill nets; T, traps; E, boat electrofishing; C, cast nets; A, angling; and Obs., observed. Minor techniques are indicated in lowercase letters. (Page 2 of 2)

Species	1965	1974	1977	1980
Gambusia affinis	-	-	-	-
Cottus carolinae	R	Rs	S	B
Ambloplites rupestris	R	Rs	-	B
Lepomis auritus	-	-	-	-
Lepomis cyanellus	-	-	-	-
Lepomis gulosus	-	-	-	-
Lepomis hybrid	-	-	-	-
Lepomis macrochirus	R	-	-	B
Lepomis megalotis	-	-	-	-
Lepomis microlophus	-	-	-	-
Lepomis species	-	-	-	-
Micropterus dolomieu	-	-	-	-
Micropterus punctulatus	-	-	-	-
Micropterus salmoides	-	-	-	-
Pomoxis annularis	-	-	-	-
Etheostoma blennioides	-	-	-	-
Etheostoma camurum	-	-	-	-
Etheostoma rufilineatum	R	-	-	B
Etheostoma simoterum	R	Rs	-	B
Etheostoma zonale	-	-	-	-
Etheostoma species	-	-	-	-
Percina caprodes	R	-	-	BS/C
Percina evides	-	-	-	-
Number of Species	19	12	8	18

Zone 1 was not sampled after 1980.

Appendix 7.6.7. Occurrence in ANSP sampling by technique at Zone 2 on the South Fork Holston River, from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; H, hand sampling; T, traps; E, boat electrofishing; A, angling; and Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1990 was done at Cliffside. In 1990 and 1997 location of sampling is indicated in parentheses after the technique (L: lower; U: upper; and C: cliffside). No location indicates a fish caught in all locations with the exception of Cliffside in 1997. (Page 1 of 2)

Scientific Name	1965	1974	1977	1980	1990	1997	2010
Dorosoma cepedianum	-	-	-	-	G(L)	-	-
Campostoma anomalum	-	R	S	-	B(U)	B(U)	-
Carassius auratus	-	Obs	-	-	-	-	-
Cyprinella galactura	-	-	-	-	-	-	-
Cyprinella spiloptera	-	-	-	-	-	E(U)	B(U)
Cyprinus carpio	-	-	-	-	-	-	E(U)
Erimystax dissimilis	-	-	-	-	-	-	-
Luxilis chrysocephalus	-	R	-	-	TG(L)	-	-
Luxilus coccogenis	-	-	-	-	-	-	-
Nocomis micropogon	Rs	-	-	-	-	-	-
Notropis amblops	-	-	-	-	-	E(U)	-
Notropis ariommus	-	-	-	-	-	-	-
Notropis leuciodus	-	-	-	-	-	-	-
Notropis photogenis	-	-	-	-	-	-	-
Notropis rubellus	-	-	-	-	-	-	-
Notropis stramineus	-	-	-	-	-	-	-
Notropis telescopus	-	-	-	-	-	-	BE(U)
Notropis species	Rs	-	-	-	-	-	-
Notropis undes	-	-	-	-	-	-	-
Notropis volucellus	-	-	-	-	-	-	-
Phenacobius crassilabrum	-	-	-	-	-	-	-
Phenacobius uranops	-	-	-	-	-	-	-
Pimephales notatus	-	-	-	-	-	-	-
Pimephales promelas	-	-	-	-	-	B(U)	-
Rhinichthys atratulus	-	-	-	-	-	B(U)	H(U)
Semotilus atromaculatus	-	-	-	-	-	-	-
Carpiodes carpio	-	-	-	-	-	-	-
Carpiodes cyprinus	-	-	-	-	-	-	-
Catostomus commersoni	-	-	S	-	HGB	BE	EH(CLU)
Hypentelium nigricans	Rs	-	S	-	-	-	-
Moxostoma duquesnei	-	R	-	-	-	-	E(U)
Moxostoma erythrurum	-	-	-	-	-	-	E(U)

Appendix 7.6.7 (continued). Occurrence in ANSP sampling by technique at Zone 2 on the South Fork Holston River, from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; H, hand sampling; T, traps; E, boat electrofishing; A, angling; and Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1990 was done at Cliffside. In 1990 and 1997 location of sampling is indicated in parentheses after the technique (L: lower; U: upper; and C: cliffside). No location indicates a fish caught in all locations with the exception of Cliffside in 1997. (Page 2 of 2)

Scientific Name	1965	1974	1977	1980	1990	1997	2010
Moxostoma macrolepidotum	-	-	-	-	-	-	-
Moxostoma species	-	-	-	-	-	-	-
Ameiurus natalis	-	-	-	-	-	-	-
Ictalurus punctatus	-	-	-	-	-	-	-
Noturus eleutherus	-	-	-	-	-	-	-
Pylodictus olivaris	-	-	-	-	-	-	-
Onchorhynchus mykiss	A	-	-	-	A(U)	-	EB(U)
Salmo trutta	-	-	-	-	-	E(U)	E(U)
Gambusia affinis	-	-	-	-	-	-	-
Cottus carolinae	Rs	R	S	BR	B(UC)	BE	B(U)
Morone species	-	-	-	-	-	-	E(U)
Ambloplites rupestris	-	-	-	R	-	E(U)	E(CU)
Lepomis auritus	-	-	-	-	-	-	E(U)
Lepomis cyanellus	-	-	-	-	-	-	-
Lepomis gulosus	-	-	-	-	-	-	E(U)
Lepomis hybrid	-	-	-	-	-	-	-
Lepomis macrochirus	-	-	-	-	B(U)	-	E(U)
Lepomis megalotis	-	-	-	G	GB(CL)	E(L)	-
Lepomis microlophus	-	-	-	-	-	-	-
Lepomis species	-	-	-	-	-	-	-
Micropterus dolomieu	-	-	-	-	G(L)	-	-
Micropterus punctulatus	-	-	-	-	G(L)	-	-
Micropterus salmoides	-	-	-	-	B(U)	E(L)	-
Pomoxis annularis	-	-	-	-	-	-	-
Etheostoma blennioides	-	-	-	-	-	-	-
Etheostoma camurum	-	-	-	-	-	-	-
Etheostoma rufilineatum	-	-	-	-	-	-	-
Etheostoma simoterum	Rs	-	S	-	HB(U)	H(C)	HBE(UC)
Etheostoma stigmaeum	-	-	-	-	-	-	-
Etheostoma zonale	-	-	-	-	-	-	-
Percina caprodes	Rs	-	S	-	-	-	-
Percina evides	-	-	-	-	-	-	-
Number of Species	6	4	6	3	12	12	16
Upper	-	-	-	-	7	9	16
Cliffside	6	4	6	3	3	1	3
Lower	-	-	-	-	6	5	1
Excluding boat electrofishing, gill nets and traps	6	4	6	3	8	6	7

Appendix 7.6.8. Occurrence in ANSP sampling by technique at Zone 3L on the South Fork Holston River from 1980 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; A, angling; H, hand collections; and Obs., observed. Minor techniques are indicated in lowercase letters.

Species	1980	1990	1997	2010
Dorosoma cepedianum	-	-	-	-
Campostoma anomalum	B	-	B	B
Cyprinella galactura	-	-	-	-
Cyprinella spiloptera		-	-	-
Cyprinus carpio	-	-	-	-
Erimystax dissimilis	-	-	-	-
Luxilus chrysocephalus	-	-	-	-
Luxilus coccogenis	-	-	-	-
Notropis amblops	-	-	-	-
Notropis leuciodus	-	-	-	-
Notropis photogenis	-	-	-	-
Notropis rubellus	-	-	-	B
Notropis sp (sawfin)	-	-	-	-
Notropis telescopus	-	-	-	B
Notropis volucellus	-	-	-	-
Pimephales notatus	-	-	-	-
Rhinichthys atratulus	-	-	-	-
Semotilus atromaculatus	-	-	-	-
Catostomus commersoni	RB	B	B	-
Hypentelium nigricans	-	B	B	B
Moxostoma duquesnei	-	-	-	-
Moxostoma erythrurum	-	-	-	-
Moxostoma macrolepidotum	-	-	-	-
Moxostoma species	-	-	-	-
Ameiurus natalis	R	BT	H	-
Ictalurus punctatus	-	-	-	-
Noturus eleutherus	-	-	-	-
Oncorhynchus mykiss	-	-	-	-
Salmo trutta	-	-	-	-
Gambusia affinis	-	-	B	HB
Cottus carolinae	-	-	-	-
Morone species	-	-	-	-
Ambloplites rupestris	-	B	-	H
Lepomis auritus	-	-	-	H
Lepomis cyanellus	-	-	-	-
Lepomis gulosus	-	-	-	-
Lepomis hybrid	-	-	-	-
Lepomis macrochirus	RB	-	B	-
Lepomis microlophus	-	-	-	-
Lepomis species	R	-	-	-
Micropterus dolomieu	-	-	-	BH
Micropterus punctulatus	-	-	B	-
Micropterus salmoides	-	-	-	B
Etheostoma blennioides	-	-	B	B
Etheostoma camurum	-	-	-	-
Etheostoma rufilineatum	-	-	-	B
Etheostoma simoterum	-	B	BH	BH
Etheostoma stigmaeum	-	-	-	-
Etheostoma zonale	-	-	-	B
Percina caprodes	-	-	-	-
Percina evides	-	-	-	-
Total Number of Species	5	5	9	13

Appendix 7.6.9. Occurrence in ANSP sampling by technique at Zone 3R on the South Fork Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; A, angling; H, hand collections; and Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1990 was done at the lower site. In 1990, sampling was done at the upper site. In 1997 both sites were sampled, and location is indicated in parentheses after the techniques (L: lower, U: upper). No location indicates a fish caught in all locations. (Page 1 of 2)

Species	1965	1974	1977	1980	1990	1997	2010
Dorosoma cepedianum	-	-	-	-	-	-	-
Campostoma anomalum	-	-	S	B	-	-	B
Cyprinella galactura	-	-	-	-	-	-	-
Cyprinella spiloptera	-	-	-	-	-	-	-
Cyprinus carpio	-	-	-	-	H	-	-
Erimystax dissimilis	-	-	-	-	-	-	-
Luxilus chrysocephalus	-	-	-	-	-	-	S
Luxilus coccogenis	-	-	-	-	-	B(L)	-
Notropis amblops	-	-	-	-	-	-	-
Notropis leuciodus	-	-	-	-	-	-	-
Notropis photogenis	-	-	-	-	-	-	-
Notropis rubellus	-	-	-	-	-	-	-
Notropis sp (sawfin)	-	-	-	-	-	B(L)	S
Notropis telescopus	-	-	-	-	-	B	SB
Notropis volucellus	-	-	-	-	-	-	-
Pimephales notatus	-	-	-	-	-	-	-
Rhinichthys atratulus	-	-	-	-	-	-	-
Semotilus atromaculatus	-	-	-	-	-	-	-
Catostomus commersoni	-	R	-	-	B	B(U)	-
Hypentelium nigricans	-	-	-	-	-	-	-
Moxostoma duquesnei	-	-	-	-	-	-	-
Moxostoma erythrurum	-	-	-	-	-	-	B
Moxostoma macrolepidotum	-	-	-	-	-	-	-
Moxostoma species	-	-	-	-	-	-	-

Appendix 7.6.9 (continued). Occurrence in ANSP sampling by technique at Zone 3R on the South Fork Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; A, angling; H, hand collections; and Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1990 was done at the lower site. In 1990, sampling was done at the upper site. In 1997 both sites were sampled, and location is indicated in parentheses after the techniques (L: lower, U: upper). No location indicates a fish caught in all locations. (Page 2 of 2)

Species	1965	1974	1977	1980	1990	1997	2010
Ameiurus natalis	-	-	S	B	HB	-	-
Ictalurus punctatus	-	-	-	-	-	-	-
Noturus eleutherus	-	-	-	-	-	-	-
Oncorhynchus mykiss	-	-	-	-	-	-	-
Salmo trutta	-	-	-	-	-	-	-
Gambusia affinis	-	R	-	-	HB	-	-
Cottus carolinae	-	-	-	-	-	-	-
Morone species	-	-	-	-	-	-	-
Ambloplites rupestris	-	-	-	-	-	-	-
Lepomis auritus	-	-	-	-	-	B(U)	B
Lepomis cyanellus	-	-	-	-	-	B(U)	-
Lepomis gulosus	-	-	-	-	-	-	-
Lepomis hybrid	-	-	-	-	-	-	-
Lepomis macrochirus	-	-	-	-	B	-	-
Lepomis megalotis	-	-	-	-	B	-	-
Lepomis microlophus	-	-	-	-	T	-	-
Micropterus dolomieu	-	-	-	-	-	B(U)	B
Micropterus punctulatus	-	-	-	-	-	B	-
Micropterus salmoides	-	-	-	-	-	B(U)	B
Etheostoma blennioides	-	-	-	-	-	B(L)	B
Etheostoma camurum	-	-	-	-	-	-	-
Etheostoma rufilineatum	-	-	-	-	-	-	B
Etheostoma simoterum	-	-	-	-	HB	B	B
Etheostoma stigmaeum	-	-	-	-	-	-	-
Etheostoma zonale	-	-	-	-	-	-	-
Percina caprodes	-	-	-	-	-	-	-
Percina evides	-	-	-	-	-	-	B
Total Number of Species	0	2	2	2	8	11	12
Upper	-	-	-	0	8	8	-
Lower	0	2	2	2	-	6	-

Appendix 7.6.10. Occurrence in ANSP sampling by technique at Zone 4 on the South Fork Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; A, angling; and H, hand collections; Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1990 was done at the lower site. In 1990, sampling was done at the upper site. In 1997 both sites were sampled, and location is indicated in parentheses after the techniques (L: lower, U: upper). No location indicates a fish caught in all locations. Seine sampling was done only at an upper zone in 1990, and fish collected there are indicated solely with the S for technique. (Page 1 of 2)

Species	1965	1974	1977	1980	1990	1997	2010
Dorosoma cepedianum	-	-	-	B	B(M)	-	-
Campostoma anomalum	R	R	S	RB	BS	B	B
Cyprinella galactura	-	R	-	B	B(L)	-	-
Cyprinella spiloptera	-	-	-	-	-	-	-
Cyprinus carpio	R	-	-	-	-	-	-
Erimystax dissimilis	-	-	-	-	-	-	-
Luxilus chrysocephalus	R	R	-	-	S	-	B
Luxilus coccogenis	-	-	-	-	-	-	B
Nocomis micropogon	R	-	-	-	-	B(L)	-
Notropis amblops	-	R	-	-	-	B(M)	-
Notropis leuciodus	-	-	-	-	-	-	-
Notropis photogenis	-	-	-	-	-	-	-
Notropis rubellus	-	-	-	-	-	-	-
Notropis sp (sawfin)	-	R	-	-	-	-	-
Notropis telescopus	-	-	S	-	BS(L)	B(M)	B
Notropis volucellus							-
Notropis species	-	R	S	-	-	-	-
Pimephales notatus	-	-	-	-	S	-	-
Rhinichthys atratulus	-	R	-	-	B(M)	H(L)	-
Semotilus atromaculatus	-	-	-	-	-	-	-
Catostomus commersoni	-	R	-	RB	S	-	-
Hypentelium nigricans	R	R	-	B	BS(M)	B(M)	B
Moxostoma duquesnei	-	-	-	-	-	-	-
Moxostoma erythrurum	-	-	-	-	-	-	-
Moxostoma macrolepidotum	-	-	-	-	-	-	B
Moxostoma species	-	-	-	-	-	-	-
Ameiurus natalis	R	R	-	-	HB	H(L)	-
Ictalurus punctatus	-	-	-	-	-	-	-
Noturus eleutherus	-	-	-	-	-	-	-

Appendix 7.6.10 (continued). Occurrence in ANSP sampling by technique at Zone 4 on the South Fork Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; A, angling; H, hand collections; and Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1990 was done at the lower site. In 1990, sampling was done at the upper site. In 1997 both sites were sampled, and location is indicated in parentheses after the techniques (L: lower, U: upper). No location indicates a fish caught in all locations. Seine sampling was done only at an upper zone in 1990, and fish collected there are indicated solely with the S for technique. (Page 2 of 2)

Species	1965	1974	1977	1980	1990	1997	2010
Oncorhynchus mykiss	-	-	-	-	-	-	-
Salmo trutta	-	-	-	-	-	-	-
Gambusia affinis	-	-	-	-	-	H(L)	-
Cottus carolinae	R	-	-	-	HB	BH	B
Morone species	-	-	-	-	-	-	-
Ambloplites rupestris	-	-	-	B	HTBS	BH	B
Rhinichthys atratulus	R	R	-	B	T(L)	B(M)	B
Lepomis cyanellus	-	-	-	-	-	B	-
Lepomis gulosus	-	-	-	-	-	-	-
Lepomis hybrid	-	-	-	-	-	B(M)	-
Lepomis macrochirus	R	-	-	B	B(L)	-	-
Lepomis megalotis	-	-	-	B	B	-	-
Lepomis microlophus	-	-	-	-	-	-	-
Micropterus dolomieu	-	-	-	-	B(M)	B(M)	B
Micropterus punctulatus	R	-	-	-	S	-	-
Micropterus salmoides	-	-	-	-	-	-	-
Etheostoma blennioides	-	-	-	-	B	B	B
Etheostoma camurum	-	-	-	-	-	B(M)	-
Etheostoma rufilineatum	-	-	-	-	BS	B(M)	B
Etheostoma simoterum	R	R	-	RB	BS	BH	B
Etheostoma stigmaeum	-	-	-	-	-	-	-
Etheostoma zonale	-	-	-	-	-	-	B
Etheostoma species	-	-	S	-	-	-	-
Percina caprodes	-	-	-	-	-	-	-
Percina evides	-	-	-	-	-	-	-
Total Number of Species	11	11	3	10	20	17	14
Middle and lower	-	-	-	-	16	17	-
Middle	-	-	-	-	12	13	-
Lower	11	11	3	10	12	10	-
Upper	-	-	-	-	10	-	-

Appendix 7.6.11. Occurrence in ANSP sampling by technique at Zone 5 on the South Fork Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; L, trot lines; A, angling; H, hand collections; and Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1980 was done on the right bank. In 1980 and 1990, the bank is indicated at the top of the column. In 1997 location of sampling is indicated in parentheses after the techniques (L: lower, U: upper). No location indicates a fish caught in all locations. An asterisk indicates an ambiguity in total numbers in the 1980 report. In 1997 and 2010, samples were taken at 5U and 5L, but only on the right bank. (Page 1 of 2)

Species	1965 5R	1974 5R	1977 5R	1980 5R	1980 5L	1990 5L	1990 5U	1997 5	2010 5
Dorosoma cepedianum	-	-	-	-	-	-	-	H(L)	E
Campostoma anomalum	-	Rs	S	B	B*	BH	BT	B(L)	B
Cyprinella galactura	-	Rs	-	B	B	-	-	-	B
Cyprinella spiloptera	-	-	-	-	-	-	-	B(L)	E
Cyprinus carpio	R	-	-	-	-	-	E	E(R)	E
Erimystax dissimilis	-	Rs	-	-	-	-	-	-	-
Luxilus chrysocephalus	-	Rs	S	B	B	-	THG	-	B
Luxilus coccogenis	-	Rs	S	B	-	-	-	B(L)	B
Nocomis micropogen	-	Rs	S	B	B	BH	-	B(L)	-
Notropis amblops	-	-	-	-	-	-	-	-	E
Notropis leuciodus	-	-	-	B	-	-	-	-	-
Notropis photogenis	-	-	-	B	-	-	-	-	-
Notropis rubellus	-	Rs	S	B	-	-	-	-	-
Notropis sp. (sawfin)	-	-	-	B	B	BH	-	BE(L)	B
Notropis telescopus	-	-	S	B	-	BH	J	B(L)	BE
Notropis volucellus	-	-	-	-	-	-	-	-	E
Pimephales notatus	-	-	-	-	-	-	-	-	-
Rhinichthys atratulus	-	-	-	-	-	H	BT	B(L)	-
Semotilus atromaculatus	-	-	-	-	-	-	-	B(L)	-
Carpoides carpio	-	-	-	-	-	-	EG	-	-
Carpoides cyprinus	-	-	-	-	-	-	-	E(L)	-
Catostomus commersoni	-	-	-	B	B	-	B	BE(L)	-
Hypentelium nigricans	-	Rs	-	B	B*	BH	B	B(L)	BE
Moxostoma duquesnei	-	-	-	-	B	-	-	-	E
Moxostoma erythrurum	-	-	-	-	-	-	-	E(L)	E
Moxostoma macrolepidotum	-	-	-	-	-	-	-	-	-
Moxostoma species	-	-	S	-	-	-	-	-	E
Ameiurus natalis	-	Rs	-	B	B	BH	BGL	E(R)	-
Ictalurus punctatus	-	-	-	-	-	-	-	-	E
Noturus eleutherus	-	-	-	-	-	-	-	-	B

Appendix 7.6.11 (continued). Occurrence in ANSP sampling by technique at Zone 5 on the South Fork Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; L, trot lines; H, hand collections; A, angling; and Obs., observed. Minor techniques are indicated in lowercase letters. All sampling prior to 1980 was done on the right bank. In 1980 and 1990, the bank is indicated at the top of the column. In 1997 location of sampling is indicated in parentheses after the techniques (L: lower, U: upper). No location indicates a fish caught in all locations. An asterisk indicates an ambiguity in total numbers in the 1980 report. In 1997 and 2010, samples were taken at 5U and 5L, but only on the right bank. (Page 2 of 2)

Species	1965 5R	1974 5R	1977 5R	1980 5R	1980 5L	1990 5L	1990 5U	1997 5	2010 5
Pylodictis olivaris	-	Rs	-	-	-	-	-	-	-
Oncorhynchus mykiss	-	-	-	-	-	-	-	-	-
Salmo trutta	-	-	-	-	-	-	-	-	-
Gambusia affinis	R	-	-	-	-	-	H	-	-
Cottus carolinae	-	-	-	B	-	B	B	BH(L)	B
Morone species	-	-	-	-	-	-	-	-	-
Ambloplites rupestris	-	Rs	-	B	B	BH	BLT	BE	EB
Lepomis auritus	R	-	-	-	-	-	BT	BE	E
Lepomis cyanellus	-	-	-	-	-	-	-	BE	E
Lepomis gulosus	-	-	-	-	-	-	-	E(L)	E
Lepomis hybrid	-	-	-	-	-	-	-	-	-
Lepomis macrochirus	-	-	-	-	B	-	ET	BE	E
Lepomis megalotis	-	-	-	B	-	B	AE	E(R)	-
Lepomis microlophus	-	-	-	-	-	-	-	-	E
Micropterus dolomieu	-	Rs	-	B	B	BH	B	BE	BE
Micropterus salmoides	-	-	-	-	-	-	-	E(L)	E
Etheostoma blennioides	-	-	S	B	-	-	-	BH(L)	BE
Etheostoma camurum	-	-	-	-	-	-	-	-	-
Etheostoma rufilineatum	-	-	-	B	-	-	-	B(L)	B
Etheostoma simoterum	-	Rs	-	-	B	B	B	BEH(L)	B
Etheostoma stigmaeum	-	-	-	-	-	-	-	-	-
Etheostoma zonale	-	-	-	B	-	-	-	B(L)	B
Percina caprodes	-	-	-	-	-	-	-	-	-
Percina evides	-	-	-	-	-	-	-	-	-
Total Number of Species	3	13	8	20	13	12	17	28	29
Left Bank	-	-	-	-	13	12	-	25	-
Right Bank	3	13	8	20	-	-	-	8	-
Upper	-	-	-	-	-	-	17	-	-
Left, Excluding boat electro-shocking, gill nets, traps, and trot lines	-	-	-	-	13	12	-	21	15

Appendix 7.6.12. Occurrence in ANSP sampling by technique at South Fork Zone 6 on the Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; A, angling; and H, hand collections; Obs., observed. Minor techniques are indicated in lowercase letters. In 1965 Zone 6 was at a different location. (Page 1 of 2)

Species	1965	1974	1977	1980	1990	1997	2010
Dorosoma cepedianum	-	-	-	-	-	-	E
Campostoma anomalum	R	R	S	-	B	B	BH
Cyprinella galactura	-	R	-	BC	-	E	B
Cyprinella spiloptera	-	R	-	-	B	E	E
Cyprinus carpio	R	-	-	-	-	E	E
Erimystax dissimilis	-	-	-	-	-	-	E
Luxilus chrysocephalus	R	-	S	B	T	BE	BE
Luxilus coccogenis	-	-	-	-	-	BE	B
Nocomis micropogon	R	-	-	-	B	E	-
Notropis amblops	-	-	-	-	-	B	EB
Notropis ariommus	-	-	-	-	-	E	-
Notropis leuciodus	-	-	-	-	B	E	B
Notropis photogenis	-	-	-	R	-	BE	EB
Notropis rubellus	-	R	-	-	-	E	-
Notropis sp. (sawfin)	-	R	-	-	-	E	BE
Notropis telescopus	-	-	-	CR	-	BE	BE
Notropis volucellus	-	-	-	-	-	-	E
Notropis species	R	R	-	R	-	-	-
Phenacobius uranops	-	-	-	-	-	E	
Pimephales notatus	R	R	S	R	-	-	EB
Rhinichthys atratulus	R	R	-	-	B	-	-
Semotilus atromaculatus	R	-	-	-	-	-	-
Carpiodes carpio	-	-	S	B	-	-	-
Catostomus commersoni	R	-	-	-	-	-	-
Hypentelium nigricans	-	R	S	-	B	BE	BE
Moxostoma duquesnei	R	R	-	-	-	E	EHB
Moxostoma erythrurum	-	-	-	-	-	B	EB
Moxostoma macrolepidotum	-	-	-	-	-	-	EB
Moxostoma species	-	-	S	-	-	-	-
Ameiurus natalis	R	R	S	RB	TB	H	HE
Ictalurus punctatus	-	-	-	-	-	B	-
Noturus eleutherus	-	-	-	-	-	-	BH
Oncorhynchus mykiss	-	-	-	-	-	-	-

Appendix 7.6.12 (continued). Occurrence in ANSP sampling by technique at Zone 6 on the South Fork Holston River from 1965 to 2010. Techniques are R, rotenone; S, seining; B, backpack electrofishing; G, gill nets; T, traps; E, boat electrofishing; A, angling; H, hand collections; and Obs., observed. Minor techniques are indicated in lowercase letters. In 1965 Zone 6 was at a different location. (Page 2 of 2)

Species	1965	1974	1977	1980	1990	1997	2010
Salmo trutta	-	-	-	-	-	-	-
Gambusia affinis	R	R	-	R	-	H	B
Cottus carolinae	-	R	-	R	B	B	B
Morone species	-	-	-	-	-	-	-
Ambloplites rupestris	R	R	-	B	GTB	BEH	EHB
Lepomis auritus	R	R	-	-	B	BE	E
Lepomis cyanellus	-	-	-	-	-	-	B
Lepomis gulosus	-	-	-	-	-	-	-
Lepomis hybrid	-	-	-	-	-	-	B
Lepomis macrochirus	R	R	-	-	-	BE	E
Lepomis megalotis	-	R	-	B	B	-	-
Lepomis microlophus	-	-	-	-	-	-	-
Micropterus dolomieu	-	R	S	R	B	BE	EH
Micropterus salmoides	-	R	-	R	-	B	B
Etheostoma blennioides	-	-	-	B	B	BE	B
Etheostoma camurum	-	-	-	-	B	B	BE
Etheostoma rufilineatum	-	-	-	RB	B	B	BH
Etheostoma simoterum	-	-	S	C	B	BH	BHE
Etheostoma species	-	-	S	-	-	-	-
Etheostoma stigmaeum	-	-	-	-	-	-	-
Etheostoma zonale	-	-	-	-	-	B	BH
Percina caprodes	-	-	-	-	-	-	B
Percina evides	-	-	-	-	-	B	-
Total Number of Species	15	18	9	16	17	33	35
Excluding boat electro-fishing, gill nets and traps	15	18	9	16	16	23	28