# SMALL STREAMS BIOCRITERIA DEVELOPMENT
May 29th, 2009

NORTH CAROLINA DEPARTMENT OF ENVIRONMENT AND NATURAL RESOURCES
DIVISION OF WATER QUALITY
ENVIRONMENTAL SCIENCES SECTION

THIS REPORT HAS BEEN APPROVED FOR RELEASE:

Jimmie Overton
Chief, Environmental Sciences Section

DATE: 29 May, 2009

**May 29, 2009**

**MEMORANDUM**

| | |
|---|---|
| **TO:** | Jimmie Overton |
| **THROUGH:** | Trish MacPherson |
| **FROM:** | Eric Fleek |
| **SUBJECT:** | Benthic Biocriteria for the Small Streams of the North Carolina Mountains and Piedmont: Small Streams Study (2005-2007). |

## (1.0) Introduction

Current North Carolina Division Biological Assessment Unit (BAU) Standard Operating Procedures (SOPs; NCDWQ 2006) prohibit the assignment of bioclassifications (i.e., Excellent, Good, Good-Fair, Fair, or Poor) to perennial streams with drainage areas less than 3 $mi^2$. This deficiency stems from the fact that current bioclassification thresholds are predominately based on data obtained from streams with drainage areas greater than 3.0 $mi^2$ (Lenat 1993). The only instance in which a stream with a drainage area equal to or less than 3.0 $mi^2$ can receive a bioclassification pertains to Small High Quality Mountain Streams (SHQMS) which (after correction factors are applied) can receive one of the five tiered hierarchal bioclassifications (i.e., Poor, Fair, Good-Fair, Good, or Excellent; NCDWQ 2006). Other than this exception, current benthic criteria (NCDWQ 2006) limits the assignment of these bioclassifications to perennial streams with drainage areas equal to or less than 3.0 $mi^2$ as only Not Rated or Not Impaired (NCDWQ 2006).

However, in the last several years, as a result of increased sampling requests associated with the Ecosystem Enhancement Program (EEP), Total Maximum Daily Load (TMDL) studies, Outstanding Resource Waters (ORW)/High Quality Waters (HQW) reclassification studies, and NCDWQ Regional Office requests (e.g., enforcement actions), the Biological Assessment Unit (BAU) has been more frequently tasked with sampling streams with drainage areas equal to or less than 3.0 $mi^2$. The inability to assign one of the five bioclassifications to these small streams can in certain instances decrease the usefulness of this data to end-point users. For example, a Not Impaired rating would not be (in most instances) a sufficient bioclassification for a reclassification of a waterbody to HQW or ORW.

Aside from these important practical management applications associated with this resource, its has been estimated that for a given drainage network small streams (i.e., 1st, and 2nd order) can comprise up to 95% of the total stream channels present (Leopold 1956, Leopold et al. 1964) and 70-80% of total stream length (Leopold 1956, Leopold et al. 1964, Rheinhardt et al. 1999, 2005). Moreover, a recent report published by American Rivers (www.americanrivers.org/site/DocServer/Stream_Miles_Table_FINAL__2_.pdf?docID=4081) ranks North Carolina first among all 50 states in terms of total stream miles (242,691), first in terms of total perennial stream miles (123,772), and fourth in terms of total non-perennial stream miles (118,918). Additional work has shown that small first order headwater streams can

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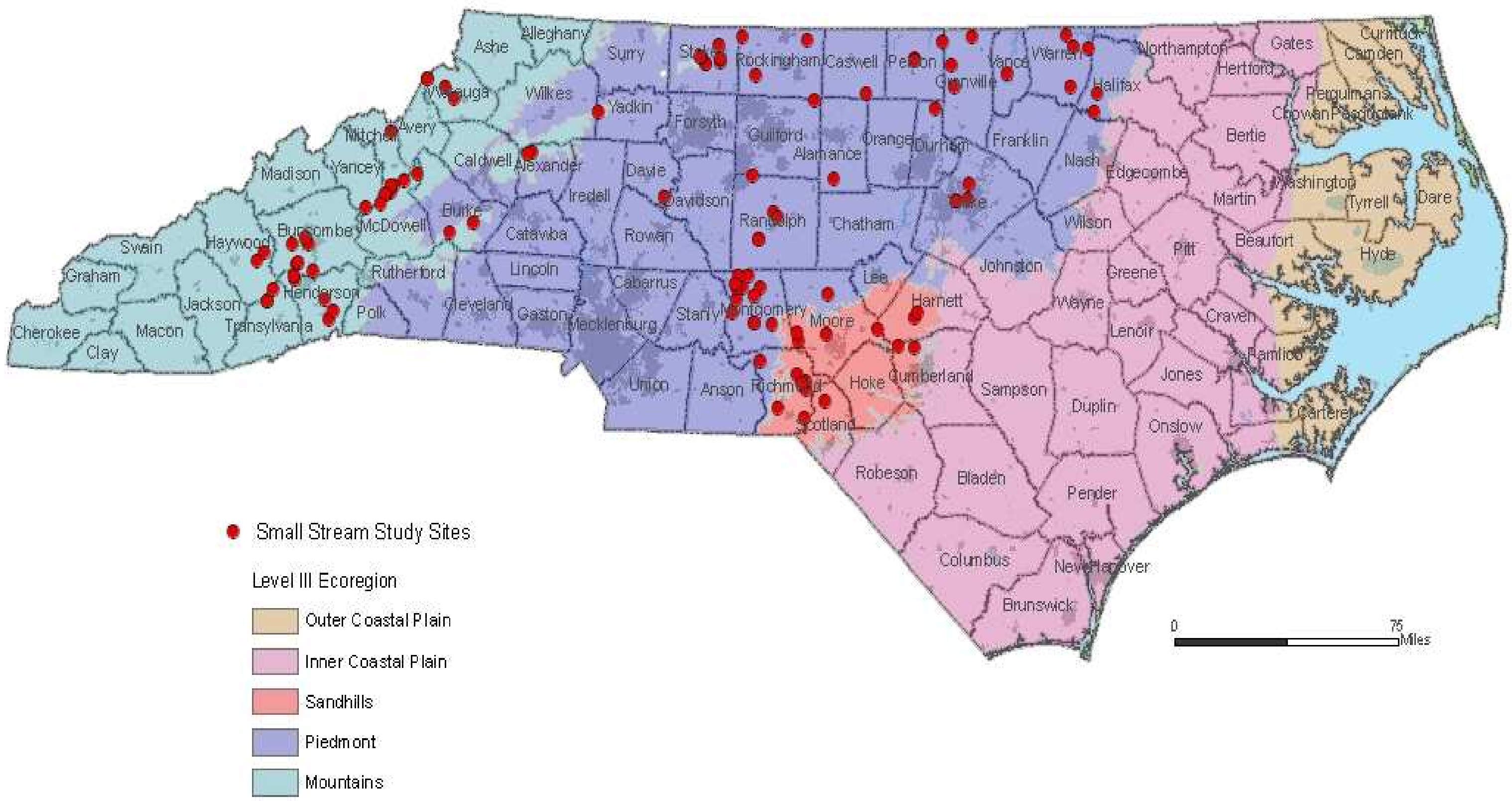



**Figure 1. Small Streams Study Sites: Level III Ecoregions (2005-2007).**

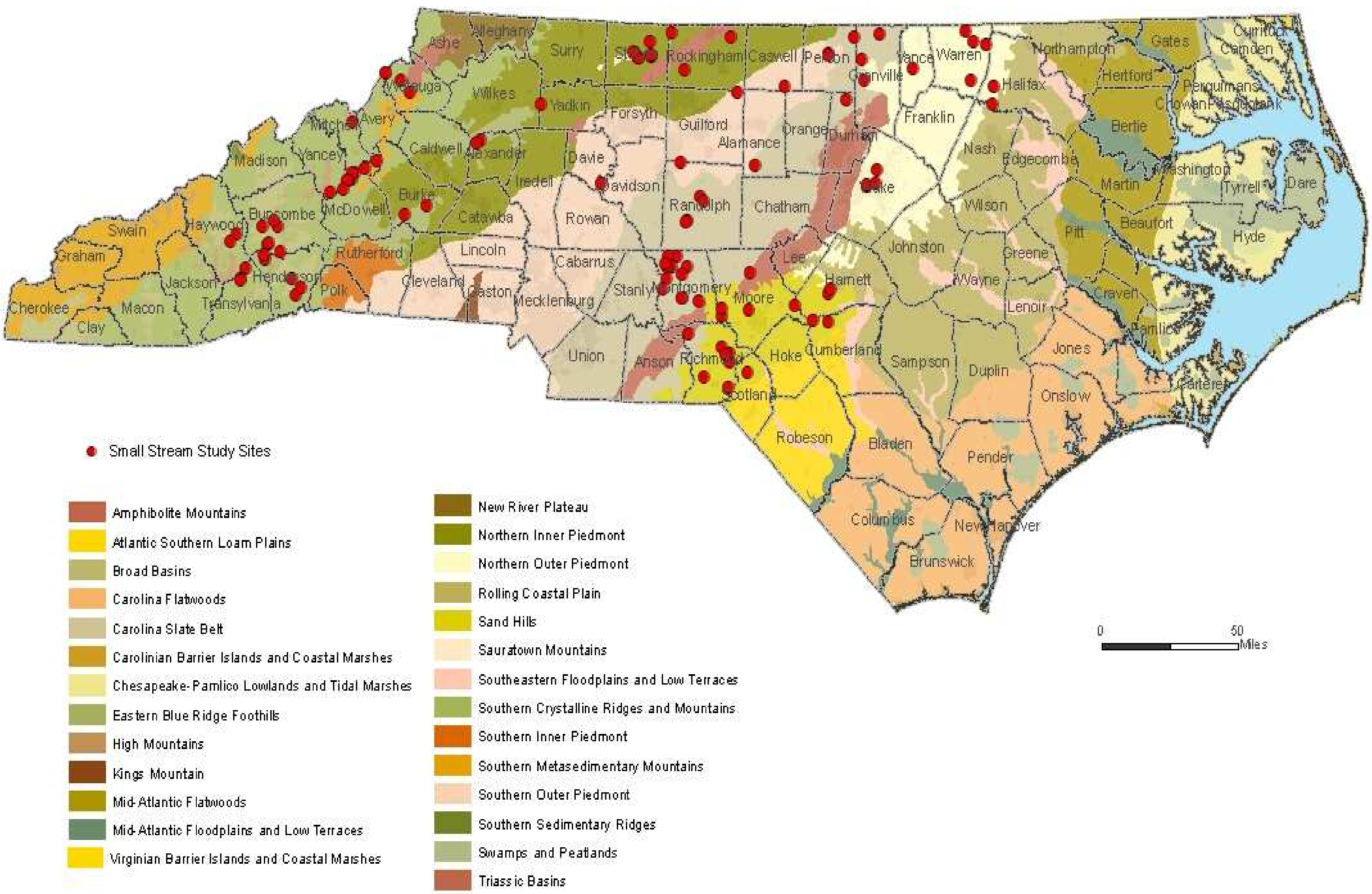



**Figure 2. Small Stream Study Sites: Level IV Ecoregions (2005-2007).**

contribute up to 70% of the mean-annual water volume and 65% of the nitrogen removal to second order streams and 55% of the mean-annual water volume and 40% nitrogen removal to third and higher ordered waterbodies (Alexander et al. 2007) and disproportionately affect water quality in downstream rivers and estuaries (Brinson 1993) since disturbed headwater reaches are in closest contact with sources of non-point pollution (Rheinhardt et al. 1999, 2005). Indeed, non-point source pollution is the major factor affecting poor water quality in most streams and rivers in the agricultural landscape of the southeastern U.S. Coastal Plain (Lowrance et al. 1997). In addition, small streams directly, and positively influence the biodiversity of larger river systems by acting as organic matter sources (Wipfli et al. 2007) as well as diversity sources through mechanisms such as providing unique habitat niches, providing refuge from extremes in both water temperature and discharge, and by providing refugia from competitors, predators, and exotic species (Meyer et al. 2007). The disproportionate influences that small headwater streams exert on a watershed in total magnifies the importance for the need of developing biocriteria whereby these small streams can be assigned water quality bioclassifications thus improving their management potential.

A total of 122 small streams in 25 counties, spanning eight river basins, two level III ecoregions (Figure 1) and nine Level IV ecoregions (Figure 2; Griffith et al. 2002) were sampled between April, May, and June of 2005, 2006, and 2007 and initially included three coarse-scaled, land use-derived disturbance classes: “Reference” sites, “Severe Impact” sites and “Intermediate Impact” sites (sensu Whittier et al. 2007). These coarse-scaled disturbance classes were then subsequently subdivided (again, based on landuse data) into five, fine-scaled disturbance classes (i.e., “Developed”, “Mostly Developed”, “Mixed”, “Mostly Forest”, “Forest”; sensu Bryce et al. 1999, Rheinhardt et al. 2007). This known landuse, impact/test-reference/control based study design has been widely used for both deriving benthic macroinvertebrate biocriteria (Karr et al., 1986, Weisberg et al. 1997, sensu Eaton 2001, Weigel 2002) as well as for measuring the response of invertebrate communities to known disturbance gradients (Bryce et al. 1999, Cuffney et al. 2000, Tate et al. 2005, Herbst and Silldorff 2006, Kratzer et al., 2006, Smith and Lamp 2007, Carlisle et al. 2008). The analysis of this dataset was used to establish a five-tiered bioclassification threshold hierarchy for streams with drainage areas less than or equal to 3.0mi$^2$ in both the Mountain and Piedmont level III ecoregions of North Carolina (Griffith et al. 2002).

## (2.0) Methods: Site Selection

**“Severe Impact” Site Selection.**

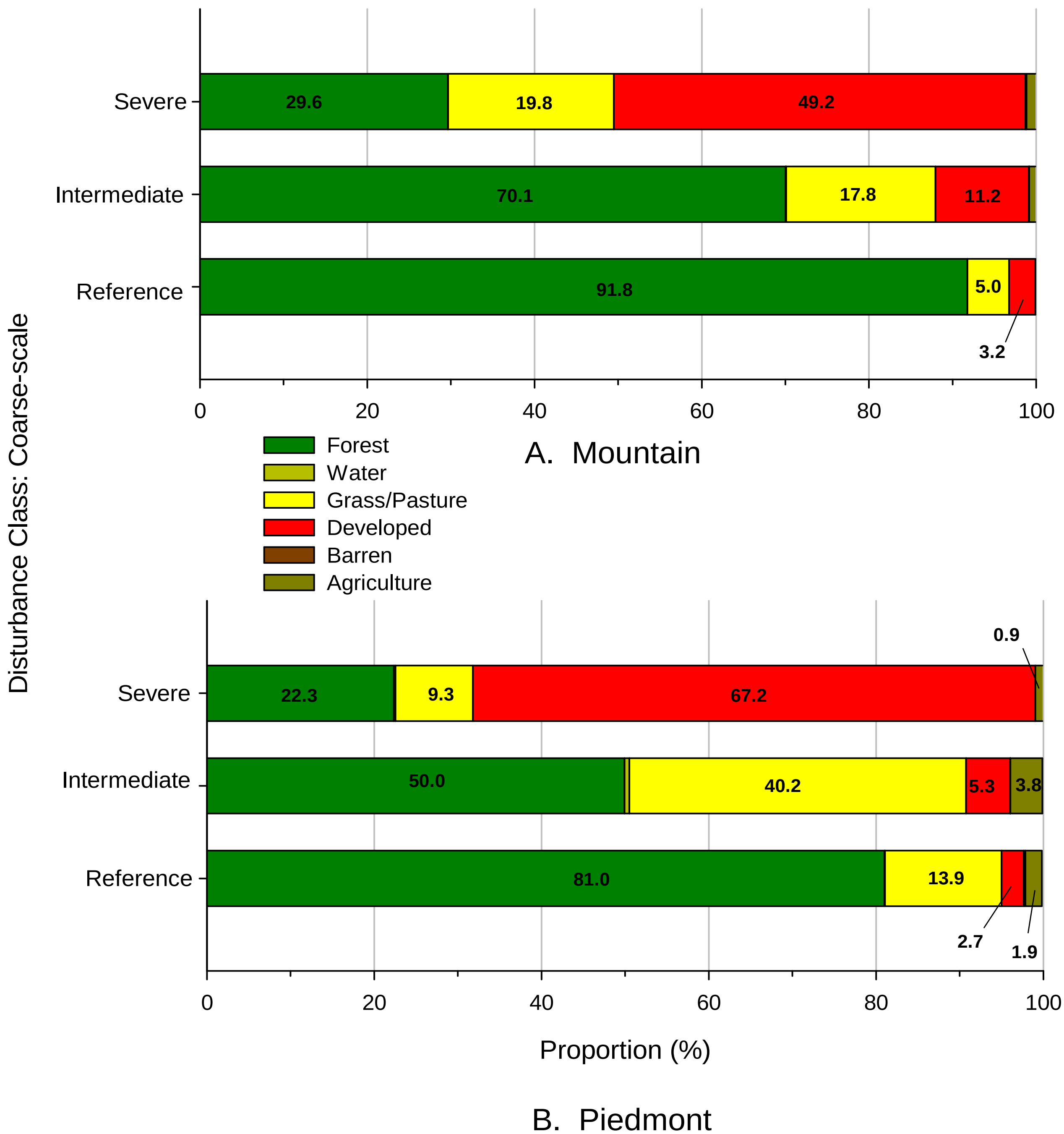
Severe impact disturbance class sites from both the Mountain and Piedmont level III ecoregions were selected a priori and were targeted towards watersheds known to be influenced by several anthropogenic stressors (Cuffney et al. 2000, sensu Eaton 2001, Carlisle et al. 2008). GIS-landuse analysis (2001 National Land Cover Data; NLCD) was conducted using ArcGIS 9.2 (Spatial Analyst—Zonal Statistics Tools) to confirm the validity and accuracy of these selections (Bryce et al. 1999, Cuffney et al. 2000, Maloney et al. 2002, Weigel 2002, Tate et al. 2005, Kratzer et al. 2006, Smith and Lamp 2007, Carlisle et al. 2008). The study sites for the severe impact dataset were selected from catchments composed primarily of a high percentage of impervious surface (i.e., urban—expressed as percent developed for this study), or from catchments with a moderate amount of impervious surface (i.e., suburban), or a combination of the two. In addition, some severe impact sites from both the Piedmont and Mountains included catchments composed of some much smaller percentages of agricultural, pasture/grass, and forest land cover (Figure 3). For the Mountain severe impact sites, percent developed and percent agriculture exceeded suggested minimum thresholds (greater than 25% urban, and less than 33% agriculture) previously established for urbanized Appalachian streams (Bryce et al., 1999, Carlisle et. al. 2008) and Piedmont severe impact sites also met these criteria.

**"Intermediate Impact" Site Selection.**
Intermediate impact disturbance class sites from both the Mountains and Piedmont level III ecoregions were selected a priori and were targeted towards watersheds known to be influenced to varying degrees by anthropogenic activity (Cuffney et al. 2000, Weigel 2002, Tate et al. 2005, Kratzer et al. 2006, Smith and Lamp 2007, Carlisle et al. 2008). GIS-landuse analysis (2001 National Land Cover Data; NLCD) was conducted using ArcGIS 9.2 (Spatial Analyst—Zonal Statistics Tools) to confirm the validity and accuracy of these selections (Cuffney et al. 2000, Maloney et al. 2002, Tate et al. 2005, Carlisle et al. 2008). Intermediate impact sites from both the Mountains and Piedmont were selected from catchments comprised of a mix of agriculture and forest with slightly lesser amounts of percent developed use (Figure 3). Based on 2001 landuse data, the a priori selection process for the Mountain sites resulted in a suite of locations that, if percent agriculture and percent grass/pasture are combined, were comparable to the minimum thresholds (greater than 10% urban, greater than 33% agriculture; Figure 3) established for "mixed" impact Appalachian streams (Bryce et al. 1999, Carlisle et al. 2008), and the intermediate impact sites for the Mountains roughly fell between values for severe impact and reference Mountain sites (Figures 3). Conversely, the Piedmont study sites had a greater proportion of (combined) agricultural and grass/pasture with slightly lesser amounts of developed landuse present relative to the Mountain sites (Figure 3) but were still approximately comparable to the thresholds suggested by Carlisle et al. 2008.

**"Reference" Site Selection.**
Reference sites from both the Mountains and Piedmont level III ecoregions were selected a priori and were targeted from catchments known to be largely protected from most sources of anthropogenic activity (Cuffney et al. 2001, Tate et al. 2005). GIS-landuse analysis (2001 National Land Cover Data; NLCD) was conducted using ArcGIS 9.2 (Spatial Analyst—Zonal Statistics Tools) to confirm the validity and accuracy of these selections (Cuffney et al. 2000, Maloney et al. 2002, Tate et al. 2005, Kratzer et al. 2006, Smith and Lamp 2007, Carlisle et al. 2008). Reference sites from both the Mountains and Piedmont were selected from catchments that were almost entirely protected. These sites included watersheds contained within State Parks, National Forests, State Forests, State Game Lands, and protected lands associated with Fort Bragg. Sites that did not have catchments contained within protected natural areas were selected on the basis of a predominately forested catchment, the presence of no more than a total of two road crossings in the catchment (sensu Herbst and Silldorff, 2006, Whittier et al. 2007) and the presence of no more than two man-made farm ponds in the watershed. Based on 2001 land uses data, reference sites from the Mountains were overwhelmingly composed of forest/wetland landuse with only very minute contributions from developed, agriculture, and grass/pasture practices (Figure 3). Moreover, these sites were well under the percent developed (less than 5%) and percent agriculture (less than 50%) thresholds established by Carlisle et al.,

2008 for reference sites n the Appalachians and they exceeded the 80%-90% forest minima established as optimum for benthic macroinvertebrate communities (Black and Munn, 2004). Likewise, the 2001 Piedmont reference landuse data (Figure 3) were also overwhelmingly composed of forest/wetland and also met relevant criteria for reference stream selection (Bryce et al. 1999, Black and Munn 2004, Carlisle et al. 2008). However, the Piedmont reference sites had more grass/pasture and agricultural uses than did the Mountain data. The slightly higher percentages of grass/pasture and agriculture in the Piedmont were the result of less area protected by National Forests (relative to the Mountain dataset) to select sites from.

**Figure 3. Average Percent Landuse Comparisons Mountain (A) and Piedmont (B) Coarse-Scaled Disturbance Classes:**



## (3.0) Methods: Sampling

Although the BAU can obtain a benthic macroinvertebrate sample at anytime of year, collections are generally concentrated during two major sampling windows. The first of these sampling windows (June-September) is typically reserved for basinwide sampling and sampling of streams greater than 3.0 $mi^2$ in drainage area (NCDWQ 2006). The second major window is reserved for swamp sampling, which is conducted in February and March (NCDWQ, 2006). The winter sampling window is utilized for swamp streams as these waterbodies typically have no (or very little) flow during large portions of the year (NCDWQ unpublished data). In general, this low/no flow period usually occurs during the late spring and extends through summer when evapotranspiration is at its maximum. Typically, flows return to these waterbodies in mid to late fall as evapotranspiration declines. Sampling during late winter and early spring, at times of maximum baseline discharge, has been demonstrated to provide the best opportunity for detecting anthropogenic effects on invertebrate communities in these systems (NCDWQ 2006). Sampling these systems during the February-March window also offers the advantage over the summer sampling window in that confounding influences due to low dissolved oxygen, low pH, high water temperature, and decreased habitat availability due to naturally induced lower summer flows are avoided during the winter months. This is an important point as avoiding confounding effects associated with natural environmental gradients improves both the accuracy and precision of biological assessments (Carlisle et al. 2008) and is an additional argument for sampling small streams (particularly those in the level III Piedmont and level IV Slate Belt; Figure 2) during times of higher discharge (i.e., April and May) in an effort to mitigate the previously detailed confounding effects associated with swamps (i.e., lower dissolved oxygen, higher water temperature, and decreased habitat availability due to lowered summer flows) but also occasionally present in small streams. Indeed, most slate belt streams are sampled by BAU biologists during March-April for this reason.

Therefore, the proposed sampling window for small streams is April, May, and June. This window is proposed for two reasons, one logistical and one for the aforementioned ecological reasons associated with confounding effects and why it is critical to avoid them (Carlisle et al. 2008). Logistically, the April-June timeframe fits between winter swamp sampling and mid-late summer (July-September) basinwide sampling. In general, personnel and property assets are substantially committed to intense field sampling during these two traditional sampling windows and accommodating additional sampling in small streams during these two major windows would prove problematic. This is particularly pronounced during the mid-late summer sampling period as that is when the majority of BAU's benthic samples are collected. Sampling small streams in April, May, and June avoids this conflict.

Future work should include repeat sampling during March from streams used in this research in an effort to extend the small streams sampling window. If March repeat sampling among a subset of sites used in this study is comparable to April, May, and June data, the sampling window could be extended (See Section 13.0). This would be logistically feasible as the vast majority of swamp sampling is traditionally completed by the end of February. In addition, re-sampling a larger subset of the streams used in this study during the month of June may also be useful in an effort to further extend the small streams sampling window towards summer. However, extending the sampling window further into the summer (e.g., July-August) would likely increase the chances of naturally occurring confounding effects (particularly in the Level III Piedmont, and most notably in the level IV Slate Belt Ecoregions (NCDWQ unpublished data, Griffith et al. 2002; Figure 2). Preliminary data suggests little to no seasonal effect between April, May, and June samples. However, the June sample size is low and needs augmentation to make certain this is a valid trend for this month (See Section 11.0 for a discussion of seasonal effects).

## (4.0) Methods: Collection and Analysis

The BAU currently maintains five sampling methods for the collection of benthic macroinvertebrates in North Carolina: Swamp Method, Full-Scale Qualitative (i.e., “Full-Scale”), EPT (Ephemeroptera, Plecoptera, Trichoptera), Boat, and the Qual-4 (NCDWQ 2006). The Swamp and Boat collection methodologies are not appropriate for small streams. This left the Qual-4, EPT, and “Full-Scale” as potential methods for sampling these systems. Given the small size and sometimes limited habitat associated with small streams, the “Full-Scale” collection method was rejected since this collection method requires three separate “bank sweeps” and two separate “riffle kicks”. During previous work in small streams, BAU staff has often found it difficult to obtain a complete sample using “Full-Scale” collection methodology due to a lack of habitat quantity. This leaves the EPT and the Qual-4 collection methodologies. In general, the EPT collection is typically reserved for streams that are greater than 3.0 $mi^2$, or which may be habitat limited, or for sites that have no, or only low to moderate levels of anthropogenic or other physio-chemical stresses and impacts since the EPT orders are generally the least tolerant to both pollution and physical stress (Crawford and Lenat 1989, Plafkin et al. 1989, Lenat 1993, Kerans and Karr 1994, NCDWQ 2006). However, small streams by their very nature are intrinsically more susceptible to both natural and anthropogenic stresses (i.e., low summer flows, often limited habitat, less volumetric dilution to possible pollutants, etc.), and therefore the use of the EPT method in these systems may not provide enough information (i.e., EPT taxa) to make consistently accurate comparisons between sites (NCDWQ unpublished data, Lenat 1993, NCDWQ 2006). This can be a particularly intractable problem when comparing sites with severe to moderate levels of anthropogenic and/or natural stressors (NCDWQ 2006). Given these limitations, the Qual-4 method was selected for the collection of benthic macroinvertebrates for small stream bioassessment. The Qual-4 has a further advantage over the “Full-Scale” for small streams in that only one “sweep”, one “riffle kick” (plus one leaf pack and three replicates of visuals, NCDWQ 2006) is required and the Qual-4 also has an advantage over the EPT collection method in that all of the taxa collected are retained. Retaining more taxa, including many orders of facultative and pollution tolerant taxa, allows for a more complete comparison between sites with severe to moderate stressors—both natural and anthropogenic (NCDWQ unpublished data, Eaton and Lenat 1991, NCDWQ 2006).

Regardless of sampling technique, there are five major benthic macroinvertebrate (BMI) community metrics that the BAU can employ in the analysis of lotic systems: Total Taxa Richness (S), EPT Taxa Richness (EPTS), EPT Abundance (EPTN), Biotic Index (BI), and EPTBI (NCDWQ 2006). EPT taxa richness (EPTS) is used with NCDWQ biocriteria to assign water quality ratings (i.e., one of the five-tiered bioclassifications, Eaton and Lenat 1991, NCDWQ 2006). EPT are generally intolerant to many types of pollution and physical stresses and higher EPT taxa richness values are indicative of better water quality (Eaton and Lenat 1991). Water quality ratings can also be based on the EPTS in combination with the pollution tolerance of the macroinvertebrate community as summarized by the North Carolina Biotic Index (NCBI; Lenat 1993, NCDWQ 2006). Both tolerance values for individual species and the final biotic index values have a range of 0-10, with higher numbers indicating more tolerant species and more polluted conditions (Lenat 1993). In addition, EPTN, EPTBI, and S metrics can also be used to help examine additional between-site differences in water quality (NCDWQ 2006). In this study, each of these five metrics was evaluated to determine their relative efficacy at discriminating between streams with varying levels of disturbance.

To statistically test the linear relationship between each of BAU's five BMI metrics in response to land use type and disturbance gradient Pearson Correlation Coefficients were calculated. Since some of these correlations were statistically significant, further testing and refining of these relationships were subjected to an ANOVA calculation (F Ratio). In addition, many of the BMI metrics differ substantially in scale (e.g., BI (0.1-10.0) versus EPTN 0-270+). As a result, the Coefficient of Variation (CV) which is independent of measurement magnitude was calculated for each of the five BMI metrics to assess their respective efficacy in response to disturbance gradient. To measure the rate and magnitude of overlap among the five BMI metrics in response to disturbance gradient the Tukey-Kramer test was conducted. All statistical tests were executed in JMP 7.0 (SAS 2008).

## (5.0) Results and Discussion: Piedmont and Mountains (General Analysis)

Benthic macroinvertebrate-based biological assessments can have multiple sources of potential variability including those arising from field sampling, laboratory sample processing, data entry, calculation of indicator variables, site assessments, as well as natural variations (e.g., seasonality and flow regime) intrinsic to biological communities (Narf et al. 1984, Diamond et al. 1996, Carter and Resh 2001, Cao et al. 2003, Clarke and Hering 2006, Clarke et al. 2006, Flotemersch et al. 2006, Herbst and Silldorf 2006, Stribling et al. 2008). Moreover, as Hynes (1970) aptly stated: "rivers are clines in the ecological sense, and such boundaries as do occur in them are ill-defined and are caused by a variety of factors." Given this information, it is not surprising that there were some outliers (Table 1) identified from this research as well as some habitat and specific conductance variability between site groupings (Figures 4-7).

In general, there are certain physical attributes that are typically required for the collection of benthic macroinvertebrates in North Carolina (NCDWQ 2006). For example, in non-swamp streams year-round flow is necessary for routine sampling and generally BAU does not sample streams with extremely low pH values (~4.0) and will not rate any stream with a pH less than or equal to 4.0 (NCDWQ 2006). During the course of this research, some samples were collected that had very low pH values (i.e., 4.0-4.8) and several of these samples had BI scores (as well as other metric scores, Table 1) that were significantly outlying from other samples within their respective disturbance classes. There were 122 total samples collected for this study. Of this total, only two of these samples (1.6%) were classified as "Invalid Outliers" (Table 1) and they were all located in the Sandhills level IV ecoregions (Griffith et al. 2002). These samples were classified as invalid outliers since they were, in effect, “Invalid Samples” as they were essentially collected in violation of NCDWQ benthic macroinvertebrate pH sampling protocols (NCDWQ 2006). More importantly, they were considered invalid samples as the pH value of these two severe impact sites (Jennies Branch and UT Little River) were by far the lowest among that disturbance class as were their respective BI scores despite similar percentages of landuse present at these outlier sites compared to the remaining samples in this disturbance class (Table 1, Table 10). Neither of these invalid outlier samples were used in determining biocriteria thresholds and they were excluded from all data analysis in this report. Additional sampling efforts should be focused on obtaining data from highly impacted sites (with higher pH values) in the Sandhills level IV ecoregions (See Section 13.0).

**Table 1. Justification of “Severe Impact” Piedmont “Invalid Outliers”**

| Stream | Disturbance Class | Justification |
|---|---|---|
| **UT LITTLE R** | “**Severe Impact**” | **1)** This site had a very low pH (4.1) and was far ***below*** the average in this dataset (6.9). The 4.1 pH value was very close to the minimum pH value (4.0) that NCDWQ can use to assign bioclassifications (NCDWQ, 2006). Therefore, UT Little River should be considered an “Invalid Outlier” for this dataset and not suitable for threshold derivation. Although landuse at this site was comparable to other “Severe Impact” sites (percent developed= 57.5%, average for this dataset was 77.4%) the BI (5.13) was far lower than the average for the dataset (7.56). Additional sampling may be necessary to find severely impacted sandhills sites that have slightly higher pH values. |
| **JENNIES BR** | “**Severe Impact**” | **1)** This site had a very low pH (4.4) and was far ***below*** the average in this dataset (6.9) and this value was very close to the minimum pH value (4.0) that NCDWQ can use to assign bioclassifications (NCDWQ, 2006). Therefore, Jennies Branch should be considered an “Invalid Outlier” and is not suitable for threshold derivation. Although landuse at this site was comparable to other “Severe Impact” sites (percent developed= 51.2%, average for this dataset was 57.5%) the BI (5.89) was far lower than the average for the dataset (7.56). Additional sampling may be necessary to find severely impacted sandhills sites that have slightly higher pH values. |

**Piedmont**

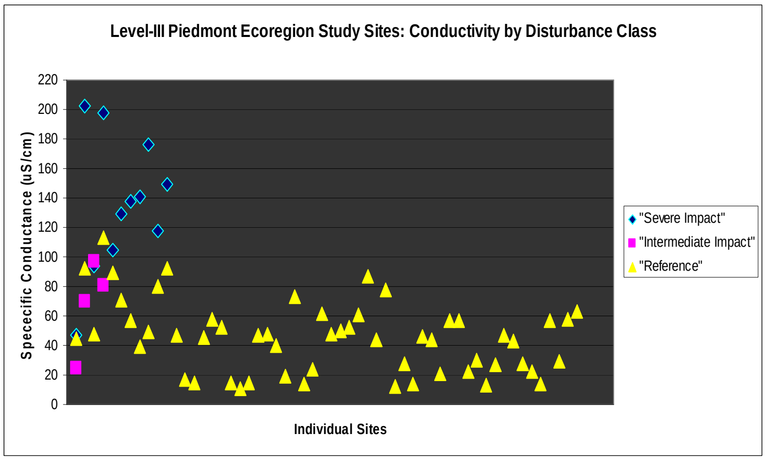
Excluding the two outlier sites, there were 62 streams sampled within the level III Piedmont ecoregions between April, May, and June 2005, 2006, and 2007. Ten severe impact sites were sampled with a mean drainage area of 1.9 $mi^2$, with the largest drainage measured at 2.97 $mi^2$ and the smallest at 0.56 $mi^2$. Seven intermediate impact sites were sampled and mean drainage area for this disturbance class was 2.1 $mi^2$, with the largest drainage measured at 2.7 $mi^2$ and the smallest measured at 1.5 $mi^2$. The remaining 45 samples were composed of reference samples and the mean drainage area for this dataset was 1.75 $mi^2$, with the largest drainage measured at 3.0 $mi^2$ and the smallest calculated at 0.3 $mi^2$. Compared to the Mountain conductivity data (Figure 6) there was more overlap in stream conductivity between the three disturbance classes in the Piedmont (Figure 4) and this was predictably the most pronounced among the intermediate impact data (Figure 4). Nevertheless, the general trend was similar: conductivity was the highest among the severe impact sites and lowest among the reference sites in both ecoregions (Figure 4, Figure 6). The increased overlap of Piedmont conductivity data between disturbance classes relative to the Mountains is likely due in large part to the strong natural variations present in stream conductivity due to the effects of underlying parent geologies (NCDWQ unpublished data, Bryce et al. 1999, Jeff Reid, pers. comm., May

2008) as well as from variation in base flow water chemistry (Feminella 2000). Furthermore, this effect is most pronounced in the Piedmont relative to the Mountain ecoregions (Jeff Reid, pers. comm., May 2008). For example, UT Bear Swamp (Piedmont reference site) had 89.91% forest/wetland land cover, 9.01% grass/pasture and 0% developed, yet the conductivity (92.2 µmhos/cm) was higher or comparable to that measured at some of the most impacted Piedmont sites (e.g., UT Mine Creek; 47 uMhos/cm, UT Richlands Creek; 94 µmhos/cm) despite the fact that both of these highly impacted sites had over 75% developed, less than 5% grass/pasture, and less than 17% forest/wetland landuse (Table 10). This illustrates the hazards of using physicochemical or landuse data alone as a method for determining effects on benthic macroinvertebrate (BMI) communities or as proxies for water quality and is illustrative of results obtained in many previous studies (Yoder and Rankin 1998, Karr and Yoder 2004, Carlisle et al., 2008). In terms of habitat scores, the Piedmont data (Figure 5) were similar to the Mountain dataset (Figures 7) in that habitat scores were somewhat more closely grouped among the three major disturbance classes than were the conductivity data between Piedmont (Figure 4) and Mountain sites (Figure 6). However, the Piedmont data (Figure 5) was less tightly grouped relative to this metric than in the Mountain ecoregions (Figure 7).

**Mountain**

There were 60 sites sampled within the level III Mountain ecoregions between April, May, and June 2005, 2006, and 2007. Ten severe impact sites were sampled and had a mean drainage area of 0.95 $mi^2$. The largest drainage area within the severe impact disturbance class sites was 1.90 $mi^2$ and the smallest was 0.37 $mi^2$. Six intermediate impact sites were sampled and mean drainage area for this disturbance class was 0.97 $mi^2$ with the largest drainage measured at 1.60 $mi^2$ and the smallest at 0.50 $mi^2$. The remaining 44 samples were obtained from reference sites and mean drainage area among these sites was 1.06 $mi^2$ with the largest drainage totaling 2.70 $mi^2$ and the smallest 0.30 $mi^2$. With the exception of a few outlying points, conductivity was generally the highest among the severely impacted sites, lowest among the reference sites, while the conductivity among the intermediate impact study sites was generally distributed between the severe and reference streams (Figure 6). However, habitat scores (Figure 7) grouped more tightly and had slightly less overlap between disturbance classes than did the conductivity data. The slight overlap of conductivity data between disturbance classes is likely due to natural variations present in stream conductivity due to the effects of underlying parent geologies (NCDWQ unpublished data, Bryce et al. 1999, Jeff Reid, pers. comm., May 2008) as well as due to variation in base flow water chemistry (Feminella 2000). For example, Pepper Creek (reference site) had 89.7% forest/wetland land cover, 4.71% grass/pasture and only 5.58% developed (Table 11), yet the conductivity (60 µmhos/cm) was higher or comparable to that measured at some of the most impacted sites (e.g., Moore Branch; 51 µmhos/cm, UT Pigeon River; 68 µmhos/cm, King Creek 45; µmhos/cm) despite the fact that these impacted sites had over 45% developed, less than 8% grass/pasture, and less than 47% forest/wetland landuse (Table 11). Once again, this dramatically illustrates the hazards of using physicochemical or landuse parameters alone as a method for determining effects on BMI communities or as water quality proxies and is consistent with previous results (Yoder and Rankin 1998, Karr and Yoder 2004, Carlisle et al. 2008).

**Figure 4 and Figure 5. Piedmont Conductivity and Habitat Score by Coarse-Scale Disturbance Class.**



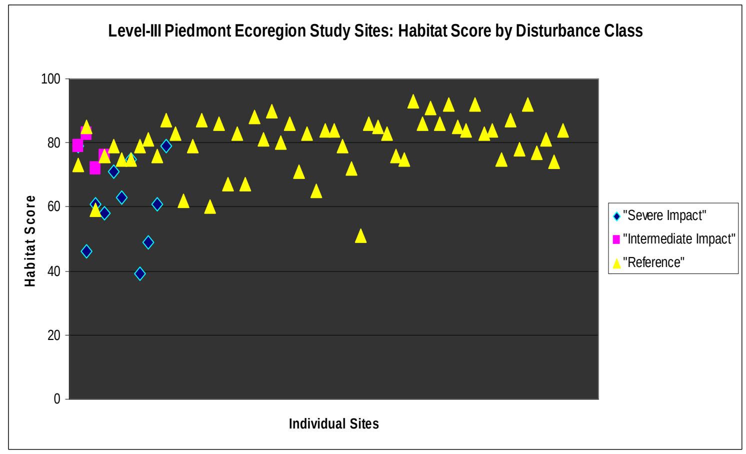
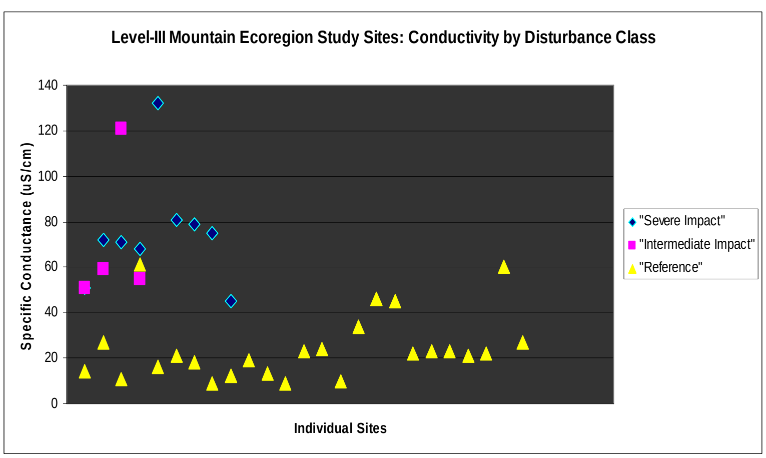
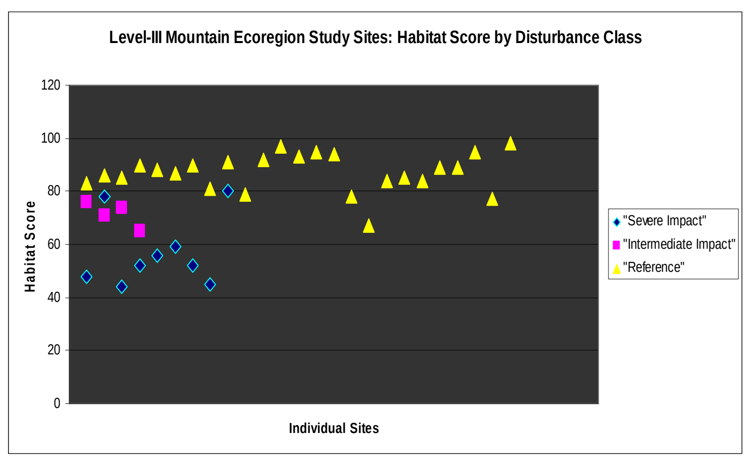




**Figure 6 and Figure 7. Mountain Conductivity and Habitat Score by Coarse-Scale Disturbance Class.**



## (6.0) Results and Discussion: Piedmont and Mountain Sites (Correlation Analysis)

**Piedmont**

Overall, the mean drainage area for the 62 sites sampled in the Piedmont ecoregions was 1.81 $mi^2$ with the largest measured at 3.0 $mi^2$ and the smallest at 0.3$mi^2$. As can be seen in Table 2, drainage area had no significant effect on the BI, EPTN, EPTS, or EPTBI and was only marginally correlated to S. These results are comparable to previous work in North Carolina (Lenat 1993). Among the six different landuse categories (forest/wetland, water, agriculture, barren, and grass/pasture) examined in the Piedmont, the percent developed landuse activity had overall the most powerful effect on the five benthic macroinvertebrate (BMI) metrics analyzed (Table 2). These results are consistent with prior investigations documenting the disproportionate effects of strongly deleterious landuse activities on BMI communities (Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Tate et al. 2005, Carlisle et al. 2008, Bressler et al. 2009). Although percent developed landuse exhibited a statistically significant influence on all five of the benthic macroinvertebrate community metrics, the BI had the strongest response and was followed by the EPTBI, EPTS, EPTN, and then the S respectively (Table 2). The percent forest/wetland landuse activity had the second most powerful overall influence on the five metrics analyzed (Table 2) and the distribution of these data are similar to prior studies that demonstrated an extremely strong negative response in BMI communities with a reduction in percent forest landuse below 70%-80% (Black and Munn 2004, Table 10, Figure 34, Figure 35). Percent forest/wetland landuse exhibited a statistically significant influence on all five of the benthic macroinvertebrate community metrics although the BI exhibited the strongest response to this landuse category and was followed by the EPTN, EPTS, and then the EPTBI, and S respectively (Table 2). Both of these results (i.e., the relative effects of percent developed and percent forest on BMI communities) are comparable with prior investigations examining the comparative impacts of strongly deleterious (e.g., developed) and positive (e.g., forested) landuse types on invertebrate communities (Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Carlisle et al. 2008) and the effectiveness of the BI in measuring water quality (Hilsenhoff 1987) and general stressors (Lenat 1993). Of the remaining landuse types examined in the Piedmont, only percent water had any correlation to the BI (Table 2) while percent grass/pasture, agriculture, agriculture+grass/pasture, barren, and percent water had no statistically significant effect on any of the five metrics evaluated among the Piedmont study sites (Table 2). The lack of BMI correlation to percent agriculture and grass/pasture in particular is consistent with previous findings (Meador and Goldstein 2003, Strayer et al. 2003).

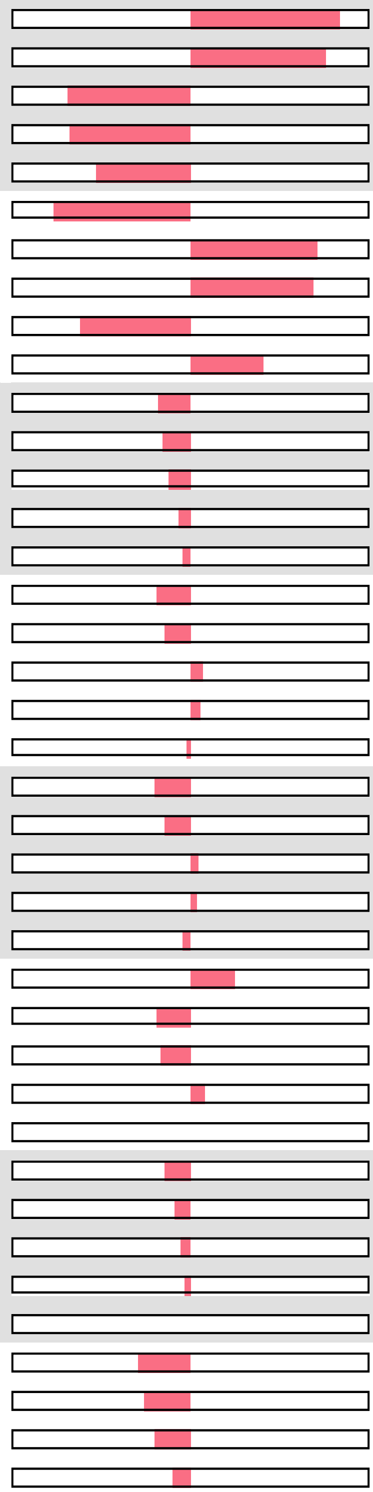
In addition to examining the effect of landuse on NCDWQ's five BMI metrics, the relationship of percent silt, sand, and percent silt+sand to these metrics were also tested. For percent silt, only the EPTN demonstrated any statistically significant effect in response to percent silt in the Piedmont (Table 2). Although this correlation was marginal, it was comparable to previous results (Kondratieff and Voshell 1980, Zweig and Rabeni 2001, Thomson et al. 2004, Cover et al. 2007). All remaining BMI metrics exhibited no notable response to percent silt (Table 2). The EPTBI and EPTN, were also the most sensitive to percent sand and percent silt+sand although this relationship was only moderately correlated (Table 2). Again, these results are generally similar to previous results which have documented various levels of adverse effects on invertebrate communities due to effects of fine sediment (Kondratieff and Voshell 1980, Zweig and Rabeni 2001, Thomson et al. 2004, Cover et al. 2007). The remaining BMI metrics (EPTS, BI, S) showed no statistical relationship to percent sand or percent sand+silt (Table 2).

Habitat scores and specific conductance were also evaluated in this study to assess how they related to the BMI metrics as well as how these parameters correlated with landuse types. Results of this study show that habitat scores exhibited a statistically significant correlation on all five of the benthic macroinvertebrate community metrics in the Piedmont and analysis of the data demonstrate that the BI had the strongest response to habitat score and was followed by the EPTN, EPTS, EPTBI, and the S respectively (Table 2). The ability of the BI to track habitat degradation is a contrary finding from previous investigations (Kerans and Karr 1994, Shields et al. 1995, Larsen et al. 2001) but is consistent with more recent work (Bressler et al. 2009). Although all community metrics (other than S) exhibited statistically significant effects with specific conductance and is similar to recent findings (Bressler et al. 2009), the EPTBI was the most sensitive but was followed closely by the BI (Table 2). The remaining metrics were much less sensitive relative to the EPTBI and BI.

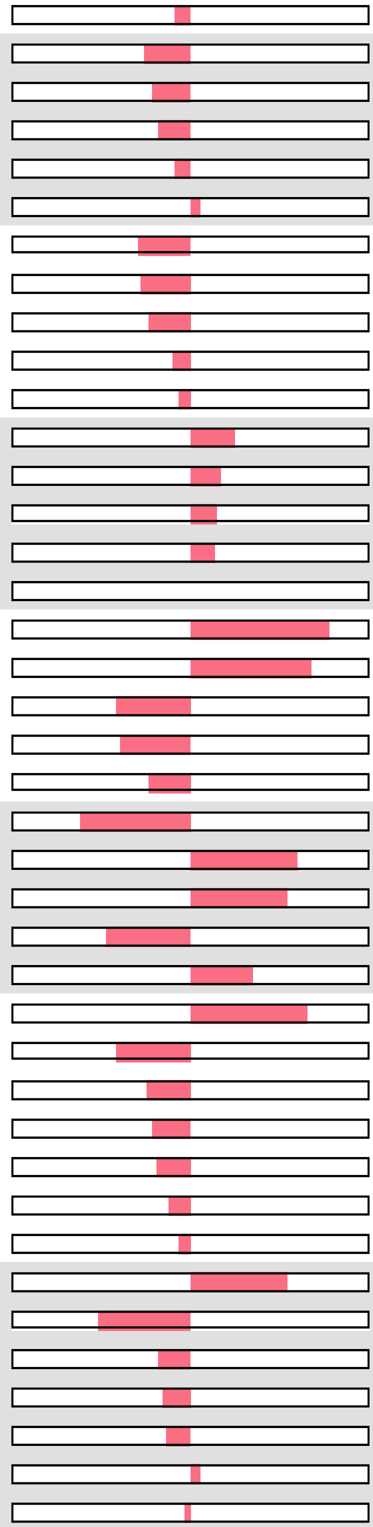
In terms of the six major landuse types tested in this study, percent forest/wetland and percent developed landuse types exerted statistically significant effects on habitat scores in the Piedmont ecoregions with the strongest relationship associated with percent forest/wetland and percent developed (Table 2). These results are consistent with previous findings detailing the negative effects of developed and positive effects of forested landuse types on lotic systems (Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Black and Munn 2004, Carlisle et al. 2008, Bressler et al. 2009). The remaining four landuse types exhibited no statistical effect on habitat scores (Table 2). As was the case for the habitat score dataset, both percent forest/wetland and percent developed had a statistically significant effect on conductivity and the strongest relationship was found with the percent developed and percent forest/wetland respectively (Table 2) and is consistent with previous findings (Bressler et al. 2009). The remaining four land use types had no statistical effect on conductivity (Table 2). These results are consistent with previous findings detailing the biological, physical, and chemical effects of developed and forested landuse types on lotic systems (Smart et al. 1985, Kerans and Karr 1994, Herlihy et al. 1998, Bryce et al. 1999, Cuffney et al. 2000, Paul and Meyer 2001, Black and Munn 2004, Carlisle et al. 2008, Bressler et al. 2009).

**Table 2. Piedmont Correlations: Benthic Macroinvertebrate Metrics, Landuse, Specific Conductance, and Habitat.** **(NOTE: BI and EPTBI scores increase with increasing impacts and are therefore positively (+) correlated to % developed. Conversely, EPTS, EPTN, and S decrease with increasing percent (%) developed landuse and are negatively (-) correlated. For percent (%) forest landuse, these correlations are reversed).**

| Variable | by Variable | Correlation | Count | Signif Prob | Graphical Portrayal of Correlation -1 0 +1 |
|---|---|---|---|---|---|
| BI | Developed % | 0.8386 | 61 | **<.0001** | |
| EPTBI | Developed % | 0.7573 | 62 | **<.0001** | |
| EPTS | Developed % | -0.6808 | 62 | **<.0001** | |
| EPTN | Developed % | -0.6664 | 62 | **<.0001** | |
| S | Developed % | -0.5197 | 62 | **<.0001** | |
| BI | Forest/Wetland % | -0.7548 | 61 | **<.0001** | |
| EPTN | Forest/Wetland % | 0.7152 | 62 | **<.0001** | |
| EPTS | Forest/Wetland % | 0.6885 | 62 | **<.0001** | |
| EPTBI | Forest/Wetland % | -0.6179 | 62 | **<.0001** | |
| S | Forest/Wetland % | 0.4119 | 62 | **0.0009** | |
| EPTN | Ag % | -0.1759 | 62 | 0.1715 | |
| EPTBI | Ag % | -0.1491 | 62 | 0.2474 | |
| EPTS | Ag % | -0.1150 | 62 | 0.3733 | |
| BI | Ag % | -0.0664 | 61 | 0.6110 | |
| S | Ag % | -0.0383 | 62 | 0.7677 | |
| EPTN | Grass/Pasture % | -0.1878 | 62 | 0.1437 | |
| EPTS | Grass/Pasture % | -0.1359 | 62 | 0.2922 | |
| S | Grass/Pasture % | 0.0678 | 62 | 0.6007 | |
| BI | Grass/Pasture % | 0.0563 | 61 | 0.6662 | |
| EPTBI | Grass/Pasture % | -0.0180 | 62 | 0.8895 | |
| EPTN | Ag+Grass/Pasture | -0.2007 | 62 | 0.1178 | |
| EPTS | Ag+Grass/Pasture | -0.1432 | 62 | 0.2670 | |
| S | Ag+Grass/Pasture | 0.0553 | 62 | 0.6692 | |
| BI | Ag+Grass/Pasture | 0.0402 | 61 | 0.7584 | |
| EPTBI | Ag+Grass/Pasture | -0.0414 | 62 | 0.7492 | |
| BI | Water % | 0.2484 | 61 | **0.0536** | |
| EPTN | Water % | -0.1897 | 62 | 0.1398 | |
| EPTS | Water % | -0.1607 | 62 | 0.2122 | |
| EPTBI | Water % | 0.0816 | 62 | 0.5283 | |
| S | Water % | 0.0063 | 62 | 0.9611 | |
| EPTBI | Barren % | -0.1377 | 62 | 0.2859 | |
| BI | Barren % | -0.0823 | 61 | 0.5282 | |
| S | Barren % | -0.0477 | 62 | 0.7130 | |
| EPTS | Barren % | -0.0294 | 62 | 0.8209 | |
| EPTN | Barren % | 0.0009 | 62 | 0.9944 | |
| EPTBI | % Sand | -0.2908 | 62 | **0.0219** | |
| EPTN | % Sand | -0.2505 | 62 | **0.0495** | |
| EPTS | % Sand | -0.2010 | 62 | 0.1173 | |
| BI | % Sand | -0.0911 | 61 | 0.4850 | |



| Variable | by Variable | Correlation | Count | Signif Prob | Graphical Portrayal of Correlation -1 0 +1 |
|---|---|---|---|---|---|
| S | % Sand | -0.0876 | 62 | 0.4983 | |
| EPTN | % Silt | -0.2526 | 62 | **0.0476** | |
| EPTS | % Silt | -0.2063 | 62 | 0.1077 | |
| EPTBI | % Silt | -0.1708 | 62 | 0.1845 | |
| S | % Silt | -0.0824 | 62 | 0.5243 | |
| BI | % Silt | 0.0626 | 61 | 0.6315 | |
| EPTBI | % Sand+Silt | -0.2910 | 62 | **0.0218** | |
| EPTN | % Sand+Silt | -0.2805 | 62 | **0.0272** | |
| EPTS | % Sand+Silt | -0.2261 | 62 | **0.0772** | |
| S | % Sand+Silt | -0.0964 | 62 | 0.4559 | |
| BI | % Sand+Silt | -0.0586 | 61 | 0.6536 | |
| S | Drainage Area | 0.2550 | 62 | **0.0455** | |
| EPTBI | Drainage Area | 0.1753 | 62 | 0.1729 | |
| EPTN | Drainage Area | 0.1565 | 62 | 0.2244 | |
| EPTS | Drainage Area | 0.1448 | 62 | 0.2614 | |
| BI | Drainage Area | 0.0024 | 61 | 0.9852 | |
| Condo | EPTBI | 0.7756 | 62 | **<.0001** | |
| Condo | BI | 0.6802 | 61 | **<.0001** | |
| Condo | EPTS | -0.4095 | 62 | **0.0009** | |
| Condo | EPTN | -0.3834 | 62 | **0.0021** | |
| Condo | S | -0.2259 | 62 | **0.0775** | |
| Habitat | BI | -0.6114 | 61 | **<.0001** | |
| Habitat | EPTN | 0.6021 | 62 | **<.0001** | |
| Habitat | EPTS | 0.5478 | 62 | **<.0001** | |
| Habitat | EPTBI | -0.4718 | 62 | **0.0001** | |
| Habitat | S | 0.3506 | 62 | **0.0052** | |
| Condo | Developed % | 0.6546 | 62 | **<.0001** | |
| Condo | Forest/Wetland % | -0.4135 | 62 | **0.0008** | |
| Condo | Ag % | -0.2379 | 62 | **0.0626** | |
| Condo | Ag+Grass/Pasture | -0.2126 | 62 | 0.0971 | |
| Condo | Grass/Pasture % | -0.1895 | 62 | 0.1402 | |
| Condo | Barren % | -0.1150 | 62 | 0.3735 | |
| Condo | Water % | -0.0618 | 62 | 0.6330 | |
| Habitat | Forest/Wetland % | 0.5405 | 62 | **<.0001** | |
| Habitat | Developed % | -0.5144 | 62 | **<.0001** | |
| Habitat | Ag % | -0.1785 | 62 | 0.1650 | |
| Habitat | Ag+Grass/Pasture | -0.1471 | 62 | 0.2539 | |
| Habitat | Grass/Pasture % | -0.1285 | 62 | 0.3194 | |
| Habitat | Water % | 0.0631 | 62 | 0.6259 | |
| Habitat | Barren % | -0.0266 | 62 | 0.8371 | |



**Mountain**

The mean drainage area for the 60 samples collected in the Mountain ecoregions was 1.02 $mi^2$ with the largest drainage area measured at 2.70$mi^2$ and the smallest at 0.30 $mi^2$. As can be seen in Table 3, drainage area had no significant statistical effect on the BI, EPTN, EPTS, EPTBI, or S. These findings are consistent with previous investigations conducted in North Carolina (NCDWQ unpublished data, Lenat 1993). Among the six different landuse categories (forest/wetland, developed, water, agriculture, barren, and grass/pasture) examined, the percent forest/wetland landuse activity had overall the most powerful influence on the five metrics analyzed in Mountain locations (Table 3). Moreover, the distributions are strikingly similar to prior studies which demonstrated an extremely strong negative response in invertebrate communities with a reduction in percent forest landuse below 70%-80% (Black and Munn 2004; Table 11, Figures 35, 37). Percent forest/wetland landuse exhibited a statistically significant influence on all five of the benthic macroinvertebrate community metrics although the BI had the strongest response to this landuse category followed by the EPTBI, EPTS, EPTN, and S (Table 3). Percent developed landuse exerted the second most powerful influence on the five metrics analyzed among Mountain sites (Table 3). As was the case in the Piedmont, these results are comparable with prior investigations examining the disproportionate effects of strongly deleterious landuse activities (e.g., developed) and positive (e.g., forest) landuse types on invertebrate communities (Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Carlisle et al. 2008, Bressler et al. 2009) and the effectiveness of the BI in measuring water quality (Hilsenhoff 1987) and general stressors (Lenat 1993). Although percent developed landuse exhibited a statistically significant influence on all five of the benthic macroinvertebrate community metrics, the BI had the strongest response followed by the EPTBI, EPTS, EPTN, and S respectively (Table 3). Percent grass/pasture landuse demonstrated the third most powerful influence on the five BMI metrics analyzed from Mountain streams. Unlike results from the Piedmont, percent grass/pasture landuse exhibited a statistically significant influence on all five of the benthic macroinvertebrate community metrics from Mountain sites (Table 3). However, evaluation of the statistical results show that the BI had the strongest response to this landuse category and was followed by the EPTBI, EPTS, EPTN, and S (Table 3). Likewise, percent agriculture in the Mountain ecoregions had a statistical effect on all five BMI metrics although it did not in the Piedmont. The statistical results indicate that the EPTBI had the strongest response to percent agriculture and was followed by the BI, EPTS, EPTN, and S (Table 3). Again, unlike the Piedmont dataset, percent agriculture + grass/pasture exhibited a statistically significant influence on all five of the benthic macroinvertebrate community metrics (Table 3). Analysis of these data demonstrates that the BI had the strongest response to this landuse combination followed by the EPTBI, EPTS, EPTN, and S (Table 3). Although percent water had no effect on any of the five BMI metrics tested, percent barren did show a borderline effect on the BI but not on any of the remaining four metrics (Table 3) and was also a result not detected in the Piedmont (Table 2).

In addition to examining the effect of landuse on BAU’s five BMI metrics, the relationship of percent sand, silt, and percent silt+sand to these metrics were also tested in the Mountain ecoregions. Results of this indicate that for both percent silt, sand, and sand+silt, the EPTBI exhibited the strongest relationship to these stressors, although the BI and EPTN also exhibited statistically significant effects (Table 3). These findings are similar to previous results (Kondratieff and Voshell 1980, Thomson et al. 2004, Cover et al. 2007) but are contrary to the findings of other studies which found no effect on biotic indices in response to sediment (Anagradi 1999, Zweig and Rabeni, 2001, See Section 8.0 for a more detailed discussion). The only BMI metrics that did not demonstrate any statistical significance was the S, EPTS, and EPTN for percent sand.

Habitat scores and specific conductance were also evaluated in this study to assess how they related to the BMI metrics as well as how these parameters correlate with landuse types. As was the case among Piedmont streams, results in the Mountain ecoregions also demonstrated that habitat scores exhibited a statistically significant influence on all five of the BMI community metrics although the EPTBI and BI had the strongest response to habitat score (Table 3). The EPTS and EPTN were the next most sensitive to habitat score followed by the S respectively (Table 3). The ability of the BI and EPTBI to track habitat degradation is a contrary result from prior studies (Kerans and Karr 1994, Shields et al. 1995, Larsen et al. 2001) but is a similar result to more recent work (Bressler et al. 2009). Although all five metrics exhibited a statistically significant relationship to stream conductivity in the Mountains, and was a similar result from recent research (Bressler et al. 2009), the BI exhibited the strongest statistical correlation and was followed by the EPTS, EPTN, EPTBI, and S respectively (Table 3).

In terms of the six major landuse types tested among Mountain streams for this study, percent forest/wetland, percent agriculture+grass/pasture, grass/pasture, developed, and agriculture all showed a statistically significant correlation to habitat score although percent forest/wetland was the most strongly correlated (Table 3). These results are consistent with previous findings detailing the biological, physical, and chemical effects of human induced landuse disruption versus non-disturbing landuse practices on lotic systems (Smart et al. 1985, Kerans and Karr 1994, Herlihy et al. 1998, Bryce et al. 1999, Cuffney et al. 2000, Paul and Meyer 2001, Black and Munn 2004, Carlisle et al. 2008, Bressler et al. 2009). Percent water did not demonstrate any statistical effect although percent barren was borderline significant (Table 3). In terms of conductivity, the Mountain data showed that the most significantly correlated variable to conductivity was percent grass/pasture, Ag+grass/pasture, forest/wetland, agriculture, and percent developed respectively (Table 3). The barren and water landuse categories exhibited no statistically significant effect on stream conductivity.

**Table 3. Mountain Correlations: Benthic Macroinvertebrate Metrics, Landuse, Specific Conductance, and Habitat.** **(NOTE: BI and EPTBI scores increase with increasing impacts and are therefore positively (+) correlated to % developed. Conversely, EPTS, EPTN, and S decrease with increasing percent (%) developed landuse and are negatively (-) correlated. For percent (%) forest landuse, these correlations are reversed).**

| Variable | by Variable | Correlation | Count | Signif Prob | Graphical Portrayal of Correlation -1 0 +1 |
|---|---|---|---|---|---|
| BI | Forest/Wetland % | -0.9079 | 60 | **<.0001** | |
| EPTBI | Forest/Wetland % | -0.8364 | 60 | **<.0001** | |
| EPTS | Forest/Wetland % | 0.7983 | 60 | **<.0001** | |

| Variable | by Variable | Correlation | Count | Signif Prob | Graphical Portrayal of Correlation -1 0 +1 |
|---|---|---|---|---|---|
| EPTN | Forest/Wetland % | 0.7479 | 60 | **<.0001** | |
| S | Forest/Wetland % | 0.5941 | 60 | **<.0001** | |
| BI | Developed % | 0.7459 | 60 | **<.0001** | |
| EPTBI | Developed % | 0.6754 | 60 | **<.0001** | |
| EPTS | Developed % | -0.6734 | 60 | **<.0001** | |
| EPTN | Developed % | -0.6306 | 60 | **<.0001** | |
| S | Developed % | -0.4843 | 60 | **<.0001** | |
| BI | Ag+Grass/Pasture | 0.5964 | 60 | **<.0001** | |
| EPTBI | Ag+Grass/Pasture | 0.5709 | 60 | **<.0001** | |
| EPTS | Ag+Grass/Pasture | -0.4927 | 60 | **<.0001** | |
| EPTN | Ag+Grass/Pasture | -0.4627 | 60 | **0.0002** | |
| S | Ag+Grass/Pasture | -0.3966 | 60 | **0.0017** | |
| BI | Grass/Pasture % | 0.5929 | 60 | **<.0001** | |
| EPTBI | Grass/Pasture % | 0.5642 | 60 | **<.0001** | |
| EPTS | Grass/Pasture % | -0.4840 | 60 | **<.0001** | |
| EPTN | Grass/Pasture % | -0.4556 | 60 | **0.0003** | |
| S | Grass/Pasture % | -0.3897 | 60 | **0.0021** | |
| EPTBI | Ag % | 0.5362 | 60 | **<.0001** | |
| BI | Ag % | 0.5096 | 60 | **<.0001** | |
| EPTS | Ag % | -0.5043 | 60 | **<.0001** | |
| EPTN | Ag % | -0.4576 | 60 | **0.0002** | |
| S | Ag % | -0.4043 | 60 | **0.0014** | |
| BI | Barren % | 0.2349 | 60 | **0.0708** | |
| EPTN | Barren % | -0.2193 | 60 | 0.0923 | |
| EPTS | Barren % | -0.2004 | 60 | 0.1247 | |
| EPTBI | Barren % | 0.1929 | 60 | 0.1398 | |
| S | Barren % | -0.1170 | 60 | 0.3732 | |
| EPTBI | Water % | 0.0415 | 60 | 0.7530 | |
| BI | Water % | -0.0148 | 60 | 0.9108 | |
| EPTS | Water % | 0.0121 | 60 | 0.9268 | |
| S | Water % | 0.0108 | 60 | 0.9348 | |
| EPTN | Water % | -0.0105 | 60 | 0.9366 | |
| EPTBI | %Sand+Silt | 0.6416 | 60 | **<.0001** | |
| BI | %Sand+Silt | 0.5429 | 60 | **<.0001** | |
| EPTN | %Sand+Silt | -0.3366 | 60 | **0.0085** | |
| EPTS | %Sand+Silt | -0.3310 | 60 | **0.0098** | |
| S | %Sand+Silt | -0.0881 | 60 | 0.5034 | |
| EPTBI | %Sand | 0.5548 | 60 | **<.0001** | |
| BI | %Sand | 0.4693 | 60 | **0.0002** | |
| S | %Sand | 0.0377 | 60 | 0.7748 | |
| EPTS | %Sand | -0.2121 | 60 | 0.1037 | |
| EPTN | %Sand | -0.2086 | 60 | 0.1097 | |
| EPTBI | % Silt | 0.4882 | 60 | **<.0001** | |

| Variable | by Variable | Correlation | Count | Signif Prob | Graphical Portrayal of Correlation -1 0 +1 |
|---|---|---|---|---|---|
| EPTN | % Silt | -0.4220 | 60 | **0.0008** | |
| BI | % Silt | 0.4132 | 60 | **0.0010** | |
| EPTS | % Silt | -0.4008 | 60 | **0.0015** | |
| S | % Silt | -0.2962 | 60 | **0.0216** | |
| S | Drainage Area | 0.1835 | 60 | 0.1604 | |
| EPTS | Drainage Area | 0.1825 | 60 | 0.1628 | |
| EPTN | Drainage Area | 0.1644 | 60 | 0.2094 | |
| EPTBI | Drainage Area | 0.1446 | 60 | 0.2704 | |
| BI | Drainage Area | -0.0074 | 60 | 0.9554 | |
| BI | Condo | 0.6139 | 60 | **<.0001** | |
| EPTS | Condo | -0.5538 | 60 | **<.0001** | |
| EPTN | Condo | -0.5336 | 60 | **<.0001** | |
| EPTBI | Condo | 0.4794 | 60 | **0.0001** | |
| S | Condo | -0.4069 | 60 | **0.0013** | |
| EPTBI | Habitat | -0.7937 | 60 | **<.0001** | |
| BI | Habitat | -0.7683 | 60 | **<.0001** | |
| EPTS | Habitat | 0.7032 | 60 | **<.0001** | |
| EPTN | Habitat | 0.6652 | 60 | **<.0001** | |
| S | Habitat | 0.4900 | 60 | **<.0001** | |
| Habitat | Forest/Wetland % | 0.7531 | 60 | **<.0001** | |
| Habitat | Ag+Grass/Pasture | -0.6098 | 60 | **<.0001** | |
| Habitat | Grass/Pasture % | -0.6067 | 60 | **<.0001** | |
| Habitat | Developed % | -0.5536 | 60 | **<.0001** | |
| Habitat | Ag % | -0.5149 | 60 | **<.0001** | |
| Habitat | Barren % | -0.2358 | 60 | **0.0697** | |
| Habitat | Water % | 0.0347 | 60 | 0.7922 | |
| Condo | Grass/Pasture % | 0.6878 | 60 | **<.0001** | |
| Condo | Ag+Grass/Pasture | 0.6791 | 60 | **<.0001** | |
| Condo | Forest/Wetland % | -0.6433 | 60 | **<.0001** | |
| Condo | Ag % | 0.3990 | 60 | **0.0016** | |
| Condo | Developed % | 0.3815 | 60 | **0.0026** | |
| Condo | Barren % | 0.1808 | 60 | 0.1668 | |
| Condo | Water % | -0.0681 | 60 | 0.6054 | |

## (7.0) Results and Discussion:
## Piedmont and Mountain (Analysis by Disturbance Class)

In terms of biocriteria selection for the severe impact disturbance class, the BI had the lowest Coefficient of Variation (CV) of all the other metrics for both the Piedmont and the Mountains (Figure 8, Figure 9). Within the intermediate impact disturbance class dataset, the BI again had a substantially lower CV relative to all the other BMI metrics in both the Piedmont and Mountains (Figure 8, Figure 9). Similarly, the reference dataset also demonstrated that the BI had the lowest CV of all the other metrics in the Piedmont and Mountains (Figure 8, Figure 9).The evaluation of this is crucial as the CV has repeatedly been shown to be a highly effective tool for selecting metrics and bioassessment methods that are the most effective in discriminating between reference and impaired streams (Gebler 2004, Black et al. 2004, Herbst and Silldorff 2006, Stribling et al. 2008) and has been used frequently to compare the relative precision among various indicators, data sets, and metrics (Gomez and Gomez 1976, Diamond et al. 1996, Feminella 2000, Zweig and Rabeni, 2001, Schloesser and Nalepa 2002, Tullos et al. 2006, Rehn et al. 2007, Cuffney et al. 2007). In addition, this method is particularly useful when comparing metrics with differing units of magnitude (SAS 2006) and is an extremely useful test for comparing the raw discriminatory efficacy of a given metric (Dr. Catherine Truxillo, pers. comm., April 2008). Based on this analysis, only the BI had a CV that was small enough to provide useful information for establishing water quality biocriteria thresholds in the Piedmont and Mountains and was the least variable of all the other metrics (Figures 8-10). The only instance where the BI failed to outperform the other BMI metrics occurred when the reference foothills data was combined with the reference Mountains data (Figure 10). However, even in this solitary instance the difference in the CV between the top performing (S) metric relative to the BI (1.72 for the coarse-scale disturbance data, and 2.34 for fine-scale) were the smallest differences measured between the top performing BI and the next best CV metric for the entire Mountain dataset. The only such difference that was smaller (1.58) occurred between the BI and the EPTS from the mostly forest disturbance class (Figure 12). Indeed, the remaining differences in the CV of the BI to the next best metric ranged from a low of 2.61 to a high of 24.53 and thus solidify the overall stability of the BI relative to the EPTBI, EPTS, EPTN, and S.

In addition to the CV measurement, the S, EPTS, EPTN and EPTBI demonstrated significant overlap (see Figure 12A) between all disturbance classes in both ecoregions with the largest differences measured among the coarse-scale disturbance classes (e.g., overlap between reference sites with severe impact sites as well as overlap between intermediate impact sites with severe and reference sites (Figures 13-17, Figures 23-27). In addition, the S, EPTN, EPTBI, and EPTS also exhibited significant rates of overlap between more narrowly separated, finer-scaled disturbance classes (e.g., overlap between forest and mixed, forest with mostly forest, mostly forest with mixed, as well as developed with mixed, developed with mostly developed and mostly developed with mixed; Figures 18-22, Figures 28-32) in the Piedmont and Mountains. As a result, the EPTBI, S, EPTS, and EPTN are not optimal for use in the establishment of biocriteria in streams less than or equal to 3.0 $mi^2$ in North Carolina's Piedmont and Mountains as these metrics consistently failed to detect both large and small differences in disturbance gradients within the benthic macroinvertebrate community. These findings are consistent with previous investigations (Herbst and Silldorff 2006, Stribling et al. 2008).

These data suggest that the BI is the most effective metric for reliably and consistently discriminating between reference, intermediate impact and severe impact sites in both ecoregions (Figures 13-17, Figures 23-27), as well as discriminating between finer-scaled disturbance gradients (Figures 18-22, Figures 28-32). Indeed, only the BI had the "power" to reliably and consistently discern between reference, intermediate impact and severe impact sites in both ecoregions. Specifically, "power" is measured empirically as the rate of overlap between "Test" sites (i.e., classified as severe impact, intermediate impact, developed, mostly developed, and mixed sites in this study; sensu Herbst and Silldorff, 2006) with "Control" sites (i.e., sites classified as reference, forest, and mostly forest in this study; sensu Herbst and Silldorff, 2006) while "overlap" is measured as the proportion of test sites that exceed the reference distribution values for that metric (Herbst and Silldorff, 2006). In effect, overlap evaluates the signal-to noise ratio by considering the rate and magnitude of separation between the test and reference site distributions (Herbst and Silldorff, 2006). Therefore, the lower the power and higher the rate of overlap, the more frequently one will misidentify "Test" sites as "Reference" sites and "Reference" sites with "Test" sites (Herbst and Silldorff 2006). In other words, this situation could result in misidentifying impaired streams with those in reference, or near-reference condition. In summary, the effectiveness of the BI, and relative ineffectiveness of the EPTS, EPTBI, EPTN, and S in discriminating between both large and small scale disturbance gradients in small Piedmont and Mountain streams can be seen in Figures 13-32.

Combined, these data suggest that the Biotic Index (BI) is the most powerful and precise metric (among the five BMI metrics analyzed) for the establishment of biocriteria for streams with drainage areas less than or equal to 3.0 $mi^2$ in North Carolina's Piedmont and Mountains. In summary, the BI had no overlap between the three major (landuse derived; Figure 3, Table 10, Table 11) coarse-scale disturbance classes (i.e., severe impact, intermediate impact, reference) while the remaining four remaining metrics (EPTS, EPTN, EPTBI, and S) did demonstrate overlap (Figures 13-17, Figures 23-27, Table 10, Table 11). Importantly, when these three coarse-scale disturbance classes were further subdivided into five fine-scaled disturbance classes (also based on landuse; Figure 33, Table 10, Table 11) in the Piedmont the BI again demonstrated no overlap between forest, mostly forest, mixed, mostly developed, and developed landuse disturbance gradients (Figure 17, Figure 22, Table 10) and the BI in the Mountains also exhibited no instances of overlap between either the coarse-scale or fine-scale disturbance classes (Figure 27, Figure 32, Table 11). Indeed, despite the fact that the slightly more variable foothills reference data were included with the Mountain reference data, the BI still exhibited no overlap between the two closest reference categories (forest and mostly forest; Figure 27, Figure 32, Table 11). These results further bolster the robustness of the BI relative to the S, EPTS, EPTN, and EPTBI.

In total, the BI exhibited no overlap among all of the disturbance gradients (both coarse and fine-scale) in the Piedmont and Mountain ecoregions (Figure 17, Figure 22, Figure 27, Figure 32). Conversely, the S, EPTS, EPTN, and EPTBI did exhibit overlap between not only the fine-scale disturbance class categories (Figures 18-21, Figures 28-31), but the S, EPTS, EPTN, and EPTBI demonstrated overlap between the worst, and near-worst sites (developed/mostly developed and severe impact) with the best, and near-best sites (forest/mostly forest and reference, Figures 13-16, Figures 23-26). Therefore, the EPTN, EPTBI, EPTS, and S are not optimal for use in biocriteria development in streams with drainage areas less than or equal to 3.0$mi^2$ in the Piedmont and Mountains of North Carolina as frequent overlap of metrics between impaired (e.g., severe impact) and non-impaired (e.g., intermediate impact and reference) sites leads to misclassification of reference sites with impaired sites and vice versa (Herbst and Silldorff, 2006, Stribling et al. 2008). This condition would have severe negative consequences for water resource managers (EPA 2000). Moreover, establishing a gradient of disturbance also aids in interpreting results of sampling as well as guiding the calibration of metrics composing the qualitative and/or quantitative indices of biological assemblage response (Bryce et al. 1999). Further, the comparison of these metrics to disturbance gradients tests whether a given metric's response covers the entire spectrum of water quality, habitat quality, and which metrics are most (or least) sensitive to extremes in impact, and which taxa disappear or become more prevalent with the accumulation of stressors (Bryce et al. 1999). Therefore, testing benthic macroinvertebrate metrics against subtle disturbance gradients are crucial in selecting the metric(s) which provides the best overall signal (Bryce et al. 1999).

These data demonstrate the power of the BI as a reliable method to not only discriminate between the best and worst sites but also to distinguish between varying levels of subtler land-use based disturbance gradients in both the Piedmont and Mountains. Distinguishing gradations in biological structure and function is a key underpinning of the regulatory process of assigning streams to different categories of aquatic life use attainment (Jackson 2004) and the clarity and accuracy with which different metrics and/or methods permit identification of thresholds and intermediate subdivisions of impairment is another key feature that should be considered when comparing metric and method performance (Herbst and Silldorff 2006). This is important since effective water quality management requires tools that can detect degrees and gradients of degradation to biological communities since a decision of simply "degraded" or "not degraded" can often be accurately inferred from examining discharger locations, land use trends, and previous investigations (EPA 2000, Eaton 2001). In both the Piedmont and Mountains, the S, EPTN, EPTBI, and EPTS were not as effective in detecting gradations in disturbance gradients. Conversely, the BI was highly effective at detecting gradients in disturbance.

The BI also demonstrated the lowest Coefficient of Variation (CV) relative to the other four metrics in both ecoregions, which further bolsters the use of this metric in biocriteria (Gomez and Gomez 1976, Diamond et al. 1996, Herbst and Silldorff 2006, Tullos et al. 2006, SAS 2006 Dr. Catherine Truxillo, pers. comm., April 2008, Stribling et al. 2008,). In fact, the CV of the BI among the Piedmont severe impact, intermediate impact, and reference disturbance classes (Figure 8) were well within the range (10-15) of previously recommended (precision) measurement quality CV objectives (Stribling et al., 2008). All of the remaining metrics (EPTN, EPTS, EPTBI, and S) within the three major coarse-scale disturbance classes fell beyond this recommended range (Stribling et al. 2008) and without exception were all higher than the BI for each disturbance class in the Piedmont (Figure 8). In addition, when the CVs by metric are examined among the five, finer-scaled landuse categories in the Piedmont (Figure 11) the BI was lower than all the other metrics for each of the five fine-scale disturbance categories and all of the BI CV values met the recommended precision CV objective (Stribling et al. 2008). Conversely, only the EPTBI CV for the mostly developed and forest landuse categories met these thresholds (Figure 11).

In the Mountains, the BI CV among the severe impact, intermediate impact, and reference sites without "Foothills" streams (Figure 9; See Section 9.0 for a detailed discussion of the foothills dataset) were well within the range (10-15) of previously recommended (precision) measurement quality CV objectives (Stribling et al. 2008). Conversely, only the reference EPTBI CV fell within this range in the Mountains (Figure 9). Remaining metrics among all of the three major, coarse-scale disturbance classes fell beyond this recommended range (Stribling et al. 2008) and without exception they were all higher than the BI for each disturbance class among Mountain sites (Figure 9). Moreover, in the Mountains, the BI CV (excluding reference foothills sites) was lower than any of the other metrics within the five, finer-scaled (forest, mostly forest, mixed, mostly developed, and developed) landuse categories (Figure 12) and the BI CV (excluding the forest+foothills forest category) were well below the recommended precision (10-15) CV requirements (Stribling et al. 2008). Conversely, only the mostly developed EPTBI CV and mostly forest EPTS CV met these thresholds (Figure 12).

When the foothills sites are added into the Level III Mountain ecoregions reference disturbance class dataset, in both the coarse-scale (Figure 10) and fine scale land-use derived disturbance gradients (Figure 12; See Section 9.0 for a detailed discussion of the foothills dataset), the CVs of the five BMI metrics shifted somewhat as the S (16.17) and EPTS (17.89) outperformed the BI (18.51) in the coarse-scale dataset (Figure 10), and all three CV values associated with these metrics failed to meet the minimum precision requirements (Stribling et al. 2008). However, when examined singularly, the CV values for metrics contained within just the foothills dataset show that only the BI (15.85, Figure 10) met the Stribling et al. 2008 precision thresholds (10-15) while the CVs for the S, EPTS, EPTN, and EPTBI exceeded these minimum precision values (Figure 10). While all of the foothills sites contained core Mountain taxa, some of these sites had slightly higher BI values than some of the Mountain reference streams even though the landuse trends between these two groups were comparable (Table 11). The slightly higher BI scores associated with the foothills dataset, are responsible for the increased BI CV for the combined coarse-scale and fine-scale foothills/Mountains reference dataset (Figure 10, Figure 12). Furthermore, if the foothills reference data are grouped with the Piedmont reference dataset, the BI CV of that combination increases from 12.13 to 17.77 and helps illustrate the transitional nature of the foothills data. See section 9.0 for a detailed treatment of the foothills dataset.

Of all the metrics examined in the Piedmont and Mountains, the BI was the most strongly correlated to the percent forest/wetland and percent developed landuse categories (Table 2, Table 3). This is particularly significant as these two landuse types have consistently been demonstrated to exert the strongest negative (developed) and positive (forest) effect on benthic macroinvertebrate communities (Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Weigel 2002, Black and Munn 2004, Moore and Palmer 2005, Kratzer et al. 2006, Smith and Lamp 2007, Carlisle et al. 2008). Therefore it is crucial to select a metric that best tracks these relationships (Karr et al. 1986, Lenat 1988, Weisburg et al. 1997). Furthermore, the BI has the additional advantage over the other benthic macroinvertebrate metrics evaluated in this study as the BI has been shown to be an effective measure of water quality (Hilsenhoff 1987) and of general stressors (Lenat 1993).

Indeed, these two landuse types combined to have the strongest effect on all five of the benthic metrics in the Piedmont (Table 2) and Mountains (Table 3) and the percent forest/wetland and percent developed landuse types were also shown to have the strongest correspondence to habitat score and conductivity in the Piedmont (Table 2). In the Mountains, percent forest/wetland had the strongest overall correlation to habitat score although every other landuse type (excluding barren and water) were also statistically significant (Table 3). In the Mountains, the conductivity was significantly correlated to percent grass/pasture and forest followed by percent agriculture and developed (Table 3). Although some of the other non-BI metrics were also significantly related to percent developed and percent forest/wetland, these non-BI metrics, in every instance had a much higher CV than did the BI in the Piedmont (Table 2), and in all but one instance, the BI has a lower CV than all other BMI metrics in the Mountains (Table 3). In instances where parameters have both a high degree of correlation and a high CV (i.e., are inconsistent) the strength of the correlation is weakened and therefore parameters that are both highly correlated and highly consistent (i.e., have a low CV) should be given the greater weighting (Cuffney et al. 2007). This would indicate the BI is superior to the other non-BI metrics in this study.

In the Mountains, percent sand and percent sand+silt had the strongest statistical effect on the EPTBI and BI respectively (Table 3), while percent silt exhibited the strongest statistical correlation to the EPTBI, EPTN, and BI (Table 3). These findings are contrary to previous work which showed that biotic indices have little or no correlation to sedimentation (Anagradi 1999, Zweig and Rabeni, 2001). However, the Anagradi study artificially manipulated sedimentation rates and therefore those results may not have been representative of natural conditions (Zweig and Rabeni, 2001). Moreover, the Zweig and Rabeni study assessed naturally sandy streams in central Missouri. These streams and their invertebrate communities are adapted to robust, naturally occurring sediment regimes and therefore would be expected to be less sensitive to small and moderate increases in sedimentation (Zweig and Rabeni, 2001). Indeed, the differential response in BMI communities to sediment from mountain areas (i.e., invertebrates sensitive to sediment) to lowlands (i.e., invertebrates less sensitive to sediment) has been demonstrated previously (Connolly and Pearson, 2007). Therefore the present findings in the Mountain ecoregion are consistent with many other studies which have demonstrated the deleterious effects of fine sediment on lotic benthic macroinvertebrates (Kondratieff and Voshell, 1980, Thomson et al. 2004, Cover et al. 2007). The fact that the BI and EPTBI were significantly correlated to sand, silt, and sand+silt in the Mountains is likely the result of the fact that the invertebrate communities in Mountain streams are not as well adapted to the higher rates of naturally occurring sedimentation in the Piedmont and are therefore more sensitive to these stressors and again is consistent with Connolly and Pearson (2007).

In the Piedmont, only the EPTBI and EPTN had any significant statistical correlation to percent sand+silt and it was only marginally so (Table 2). In addition, only the EPTBI and EPTN had any correlation to percent sand, while only the EPTN demonstrated any correlation to percent silt (Table 2). All remaining BMI metrics demonstrated no significant relationship to percent silt, percent sand, or percent sand+silt in the Piedmont (Table 2). Unlike the findings in the Mountains, the Piedmont data are more consistent with Anagradi 1999 and Zweig and Rabeni, 2001 who demonstrated that measures of abundance are significantly correlated to increasing rates of sedimentation among naturally sediment laden central Missouri streams. Again, the findings in the Piedmont are likely related to the higher amounts of naturally occurring sand and silt found in the level III Piedmont ecoregions and most of its level IV ecoregions relative to the level III Mountains and its associated level IV ecoregions (Griffith et al. 2002). Indeed, differences among the mean values of percent silt, sand, and sand+silt ($p$=0.0071, $p$=0.0515, and $p$=0.0174, respectively) between the Piedmont and Mountains were all statistically significant even though the Piedmont sites with the highest percentages of naturally occurring sand and silt (level IV Sandhills ecoregions, Griffith et al. 2002) were removed from the analysis. Had sandhills sites been included in this analysis the significance of this statistical effect would have been much larger. Sandhills Level IV ecoregions data were removed from consideration as these sites are naturally composed nearly entirely of all sand (Griffith et al. 2002) and would therefore artificially skew the analysis.

In the Mountains, although the EPTBI was slightly more related to habitat score, percent sand, silt, sand+silt, and percent agriculture relative to the BI (Table 3) the EPTBI (in every instance and dataset excluding the Mountains+foothills sites) had a much higher CV than did the BI (Figure 9, Figure 10, Figure 12). As noted previously, in instances where parameters have both a high degree of correlation and a high CV (i.e., are inconsistent) the strength of the correlation is weakened and therefore parameters that are both highly correlated and highly consistent (i.e., have a low CV) should be given the greater weighting (Cuffney et al. 2007). This would indicate the BI is superior to the EPTBI in this dataset. Moreover, the difference between the EPTBI and BI among these metrics was trivial and therefore the performance of the EPTBI and BI for these variables is relatively comparable in the Mountains. For example, in the Mountain ecoregions, ANOVA (F-Ratio) scores for the EPTBI and BI were 66.07 and 61.57 for habitat, 13.71 and 12.38 for percent agriculture, 16.34 and 16.24 for percent grass/pasture, and 16.56 and 16.34 for percent grass/pasture+agriculture respectively. Conversely, of all the metrics and specifically relative to the EPTBI, the BI was the most strongly associated with percent forest/wetland and percent developed. For example, for percent forest, the ANOVA (F-Ratio) values for the BI and EPTBI respectively were 306.33 and 162.58 and for percent developed they were 85.36 and 62.50. The fact that the BI had a stronger statistical correlation to the forest/wetland and developed landuse types in both ecoregions is significant as these two landuse types have consistently been demonstrated to exert the strongest negative and/or positive effect on benthic macroinvertebrate communities (Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Weigel 2002, Black and Munn 2004, Moore and Palmer 2005, Kratzer et al. 2006, Smith and Lamp 2007, Carlisle et al. 2008). Therefore it is crucial to select a metric that best tracks this relationship (Karr et al. 1986, Lenat 1988, Weisburg et al. 1997). Indeed, as previously seen, these two landuse types combined to have the strongest statistical effect on all five of the benthic metrics in the Piedmont and Mountains and the percent forest/wetland and percent developed landuse types were also shown to have the strongest correlation to habitat score and conductivity in the Piedmont. In the Mountains, habitat score was most strongly correlated to percent forest/wetland although every other landuse type (other than percent water) was also highly correlated (Table 3). In terms of conductivity, percent grass/pasture, percent grass/pasture+Ag, and percent forest/wetland were most correlated to conductivity although every remaining landuse type (other than barren and water) were also statistically significant (Table 3).

In total, these data strongly suggest the BI is the best performing, efficacious, and precise metric for use in the establishment of biocriteria for small streams in North Carolina's Piedmont and Mountain ecoregions. In addition, using the BI is advantageous relative to the EPTBI, EPTS, and EPTN since in extremely anthropogenically disturbed (or naturally variable) streams there will likely be a very reduced EPT fauna present since these taxa are generally the most sensitive to pollution and other forms of physicochemical stress (Crawford and Lenat 1989, Plafkin et al. 1989, Lenat 1993, Kerans and Karr 1994, NCDWQ 2006). As a result, in highly disturbed streams (either disturbed naturally or anthropogenically) there can often be zero, or at best too few EPT (and non-EPT taxa present; Maloney et al. 2002) to generate a meaningful EPTBI, EPTS, or EPTN metric and therefore comparing amongst highly (or intermediately impacted sites) using these metrics will be highly limited or effectively impossible (Crunkilton and Duchrow 1991, NCDWQ 2006). Moreover, whenever the EPTN is at or below 30 (as was the case in eight of the Piedmont sites; Table 10, and five of the Mountain sites; Table 11), the EPTBI and EPTS metrics have been found to have little interpretive meaning (NCDWQ 2006). In fact, this is the primary reason why in streams with known or suspected (natural or anthropogenic) impacts, the EPT method is generally not recommended (NCDWQ 2006). This situation is analogous to BAU's current prohibition on sampling streams with pH values less than or equal to 4.0 as these sites will have such low EPT and total taxa richness values (as well as low EPT and non-EPT abundances) that the calculation of community metrics will largely lose meaning and context (NCDWQ 2006). This will reduce the efficacy at which these sites can be compared to not only other low pH systems, but will also limit these streams from being effectively compared to sites that have higher pH values since it may not be possible to separate the deleterious effects of low pH with those of anthropogenic impact (NCDWQ 2006). Moreover, the EPTBI and EPTS are less effective if the diversity and abundance of more tolerant invertebrate groups need to be evaluated (NCDWQ 2006). In addition, the BI has been shown to be more reliable than the EPTS metric when assessing small streams (Lenat 1993, Lenat and Barbour 1994, Stewart and Loar 1994) and biotic indices, multi-assemblage (Carlisle et al. 2008) and composite (Watzin and McIntosh 1999) measures have repeatedly demonstrated their superior utility and accuracy relative to other benthic macroinvertebrate community metrics (Chutter 1972, Jones et al. 1981, Hilsenhoff 1982, 1987, 1988, Narf et al. 1984). Moreover, biotic indices have proven to be accurate measures of water quality (Hilsenhoff 1987), ecosystem health (Karr 1991, 1993), and of general stressors (Leant 1993).

For example, immediately after clear-cutting, increases in EPT taxa occurred in streams draining the clear-cut catchment, compared with that of a nearby reference stream draining a protected forested catchment (Swift 1983, Wallace et al. 1996). Several EPT taxa normally confined to larger downstream segments colonized the stream draining the clear-cut watershed without a significant loss of headwater EPT taxa (Swift 1983, Wallace et al. 1996). With forest regrowth, the number of EPT taxa exhibited successive declines compared to that of the reference stream. In contrast, compared to the reference stream, the BI correctly indicated decreased biological integrity immediately following clear-cutting, which subsequently improved following forest regrowth (Swift 1983, Wallace et al. 1996). Furthermore, other types of disturbance (e.g., low intensity agriculture, riparian thinning, or pasture operations) may also inflate EPT taxa richness metrics as well as total taxa richness (S) in small streams by increasing productivity (NCDWQ unpublished data, Quinn 2000) and this response is consistent with the subsidy-stress response (Odum et al. 1979). Conversely, in these situations the BI will in most cases correctly reflect the decrease in biological integrity (NCDWQ unpublished data). Indeed, recent work in North Carolina from small headwater streams ranging in size from 14 to 816 acres (640 acres equals 1.0 $mi^2$) has shown higher taxa richness scores in impacted catchments relative to reference watersheds due to a disproportionate increase in tolerant taxa (Burton 2004) while other studies have shown that metrics limited to only measures of EPT (e.g., EPTS, EPTN, EPTBI) are often lower in headwater streams relative to larger downstream reaches and therefore may compromise its use for biological monitoring in these smaller streams (Lenat and Barbour 1994, Stewart and Loar 1994). These data demonstrate the relative effectiveness of the BI versus measures of taxa richness for accurately assessing the integrity of benthic macroinvertebrate communities (Burton 2004) and lends additional support for its use in the establishment of biocriteria for streams with drainage areas less than or equal to 3.0 $mi^2$ in North Carolina's Piedmont and Mountain ecoregions.

**Figure 8. Coefficient of Variation (CV): Piedmont Coarse-Scale Disturbance Classes.**

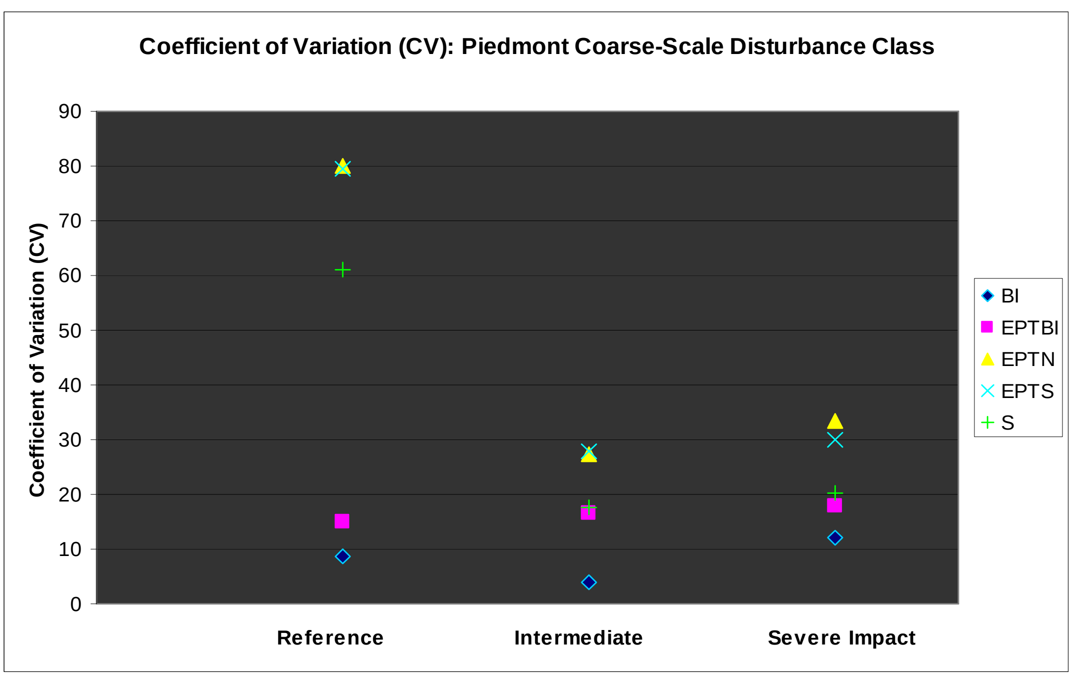


**Figure 9. Coefficient of Variation (CV): Mountain Coarse-Scale Disturbance Classes.**

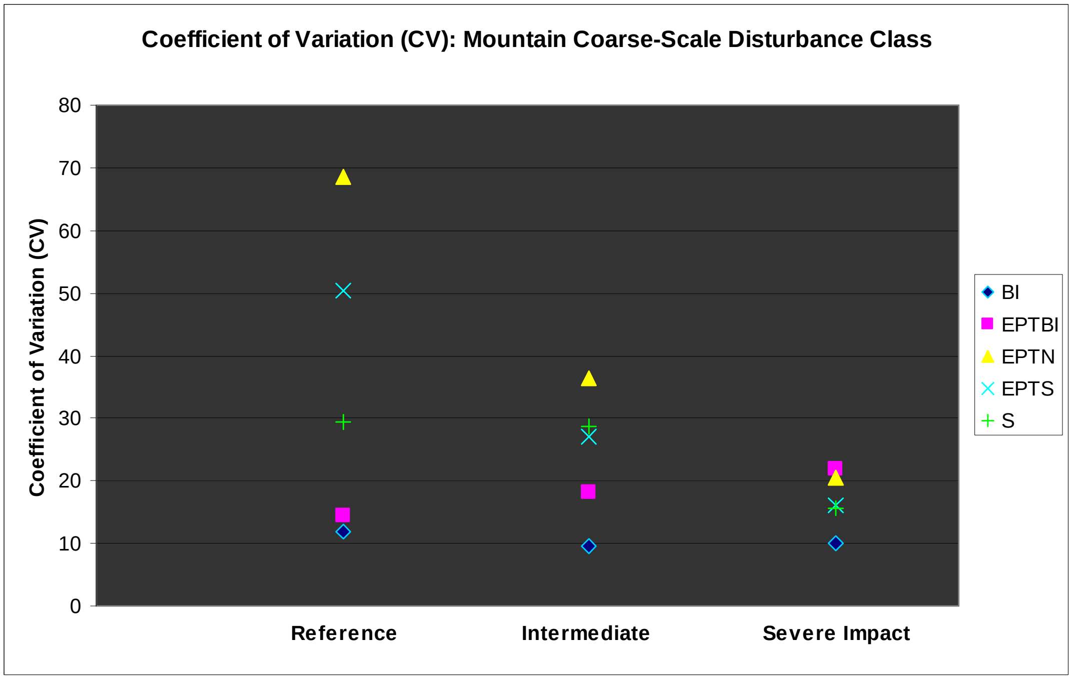



**Figure 10. Coefficient of Variation (CV): Foothills and Mountain Coarse-Scale Disturbance Classes.**

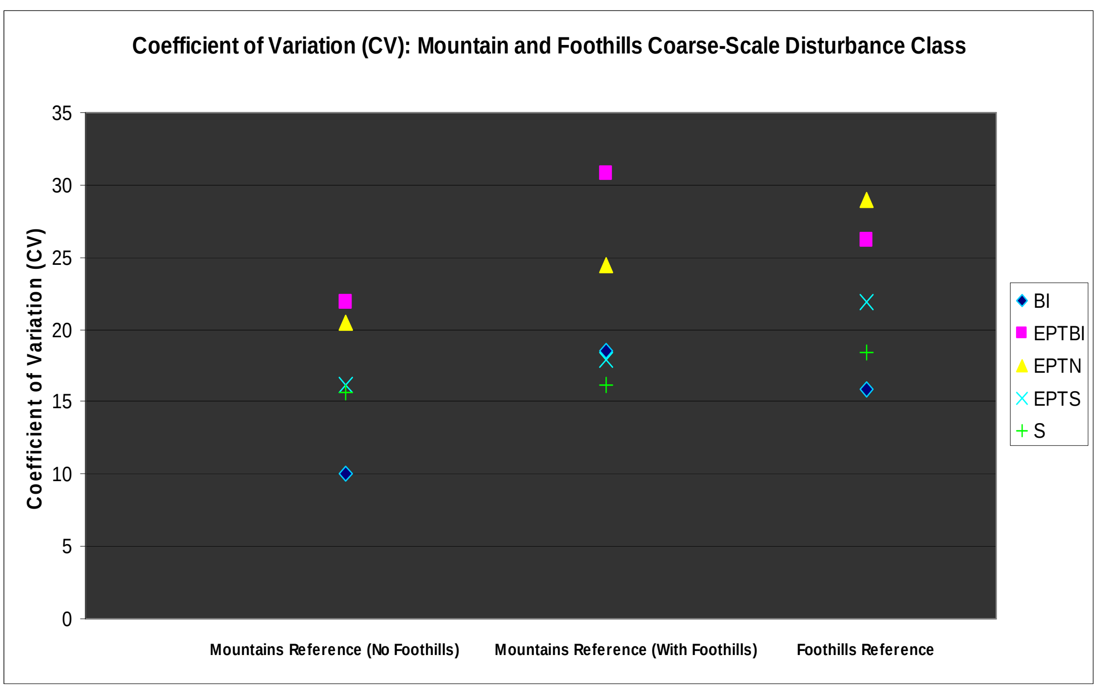


**Figure 11. Coefficient of Variation (CV): Piedmont Fine-Scale Disturbance Classes.**

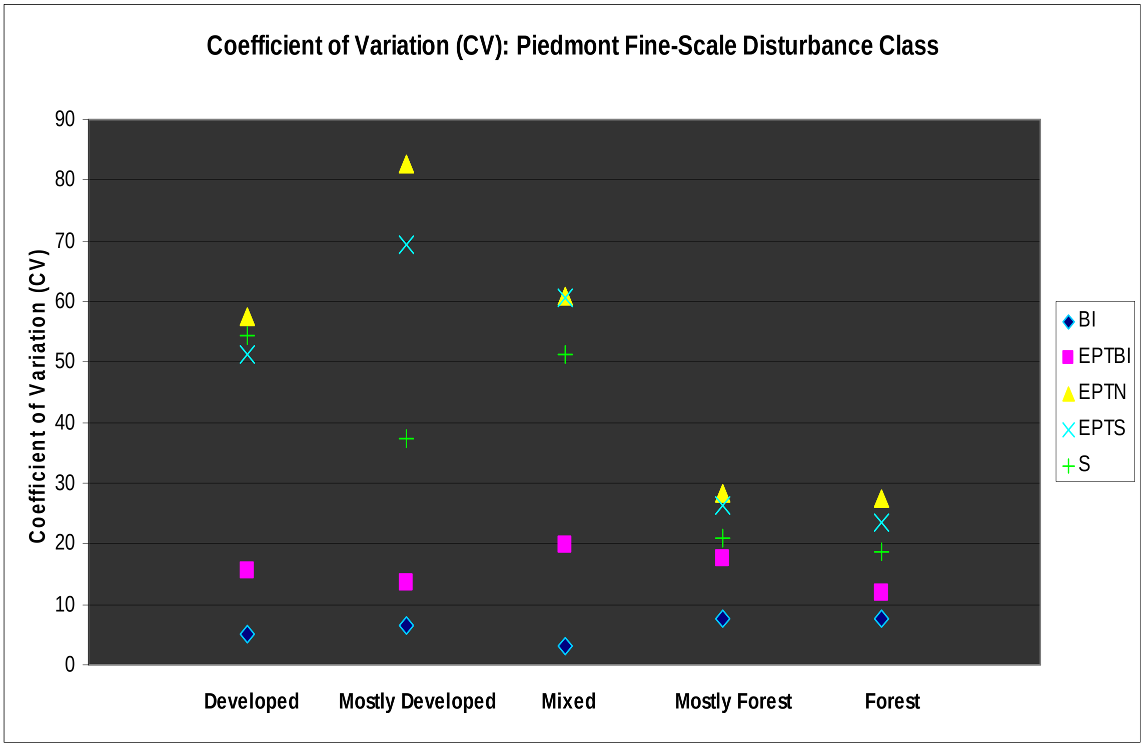
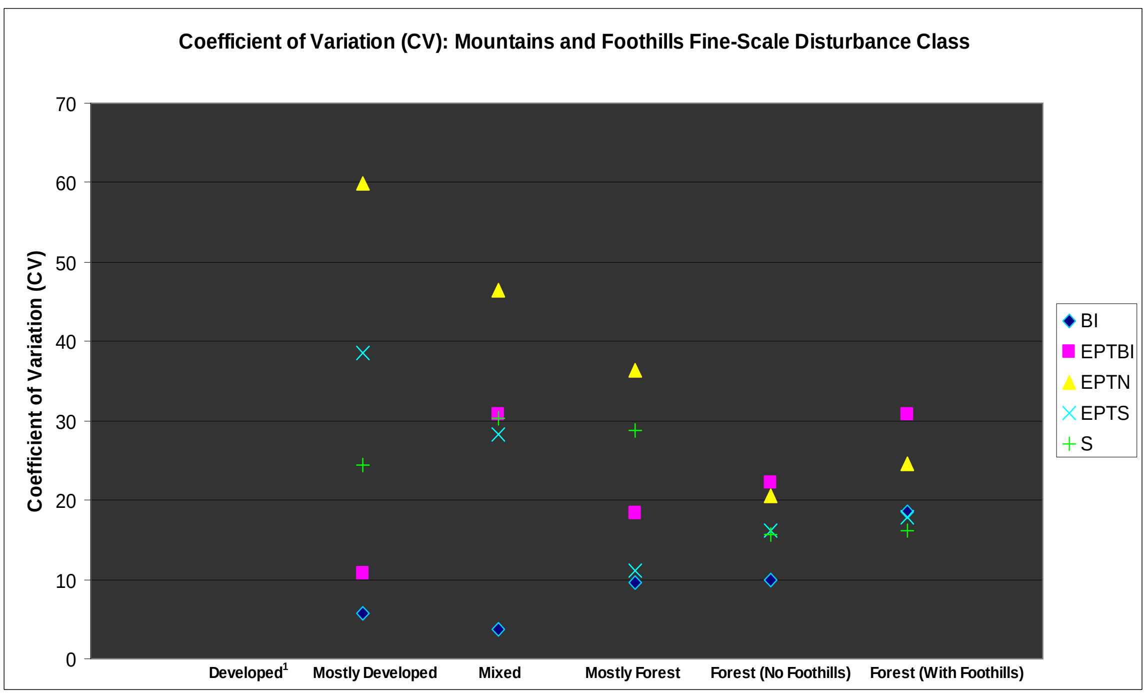
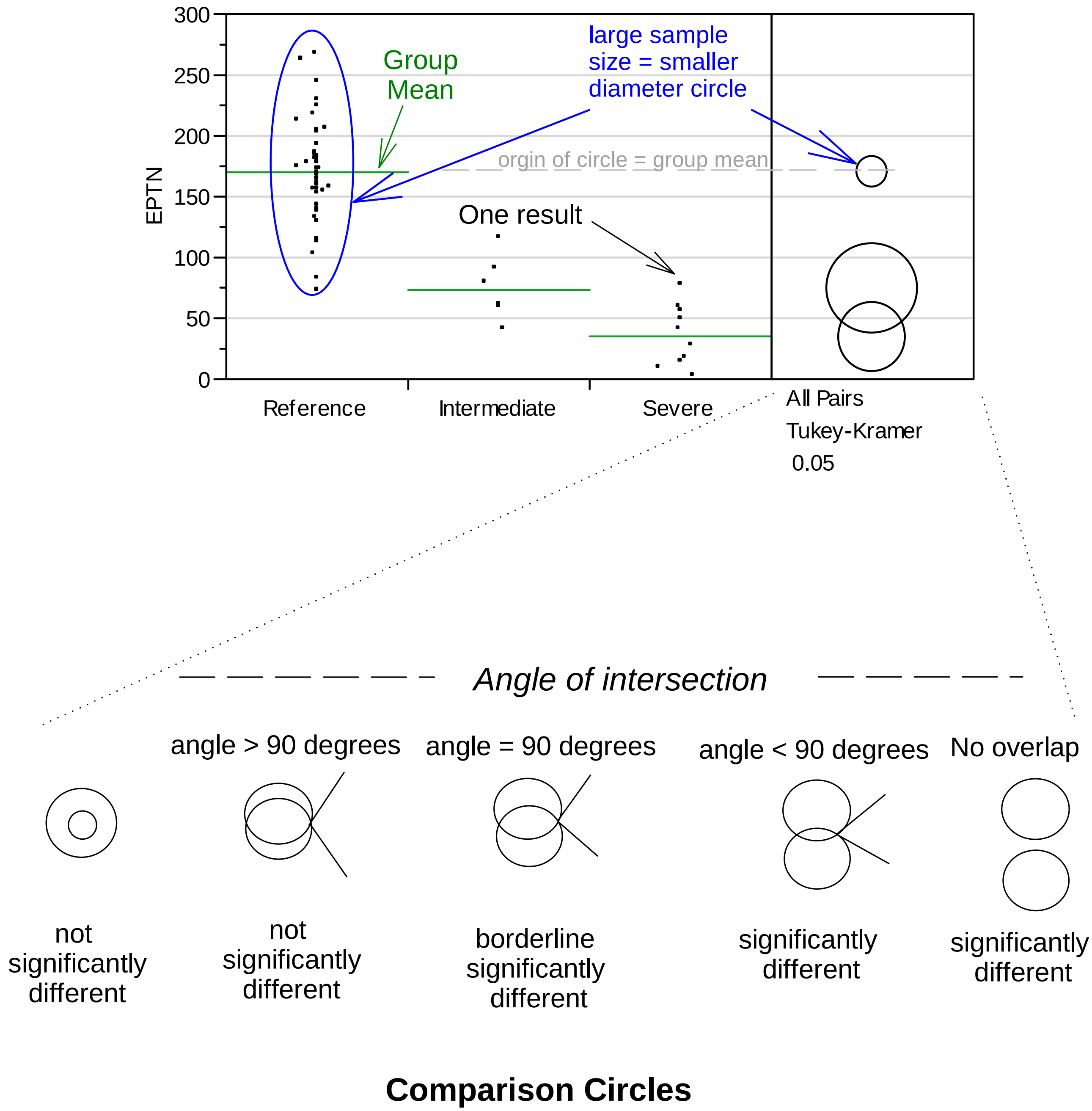



**Figure 12. Coefficient of Variation (CV): Piedmont Fine-Scale Disturbance Classes.**




[1] Only one sample obtained from "Developed" disturbance class and a CV could not be calculated. Additional data from the "Developed" disturbance class will be collected spring 2009 (See Section 13.0).

**Figure 12(A). Explanatory Graphic for Interpreting Overlap Diagrams: (Figures 13-32, Pages 33-36).**



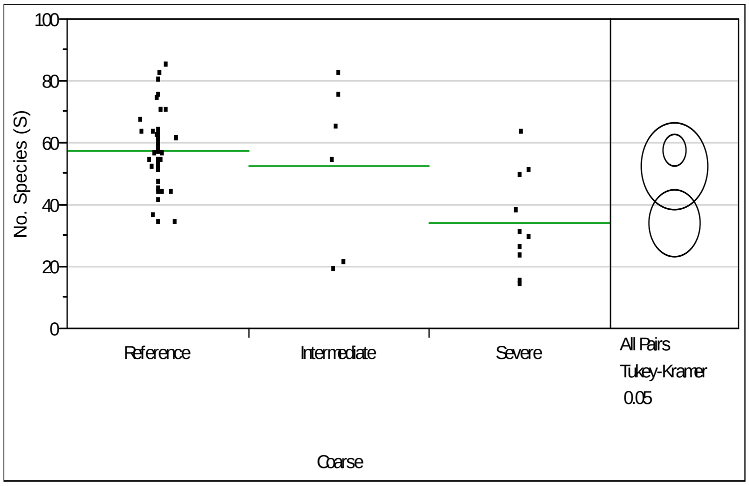



**Figure 13.** S by Coarse-Scale Disturbance Class (PIEDMONT)

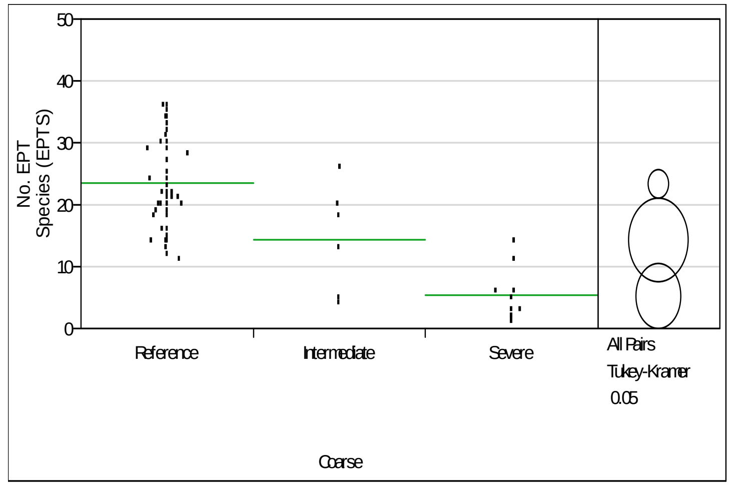



**Figure 14.** EPTS by Coarse-Scale Distribution Class (PIEDMONT)

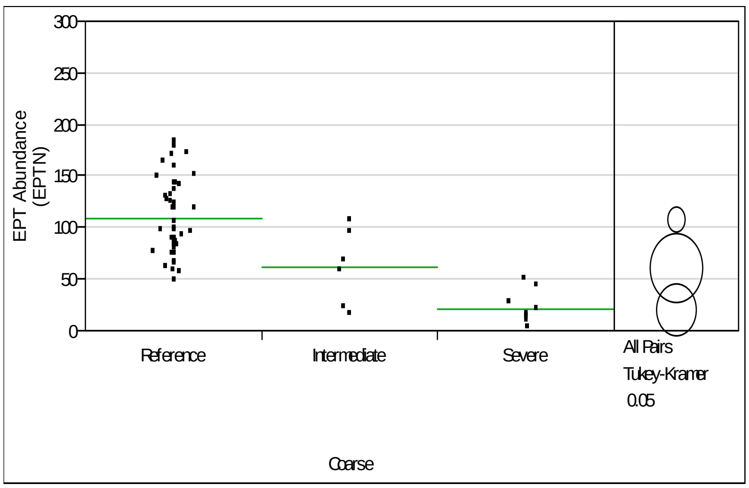



**Figure 15.** EPTN by Coarse-Scale Disturbance Class (PIEDMONT)

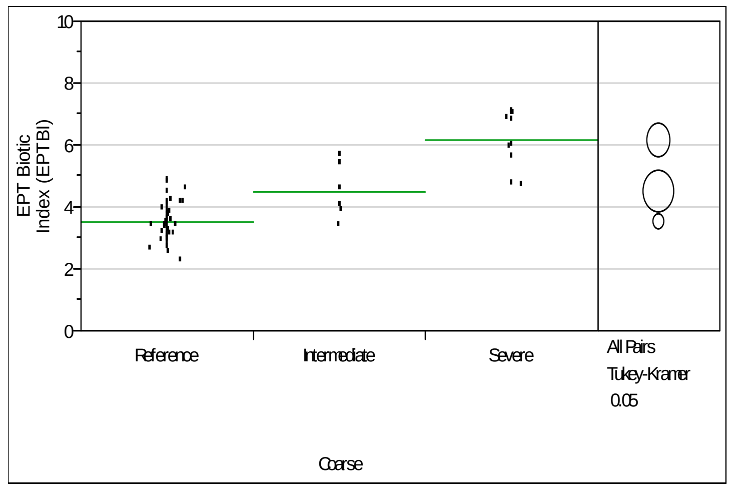



**Figure 16.** EPTBI by Coarse-Scale Disturbance Class (PIEDMONT)

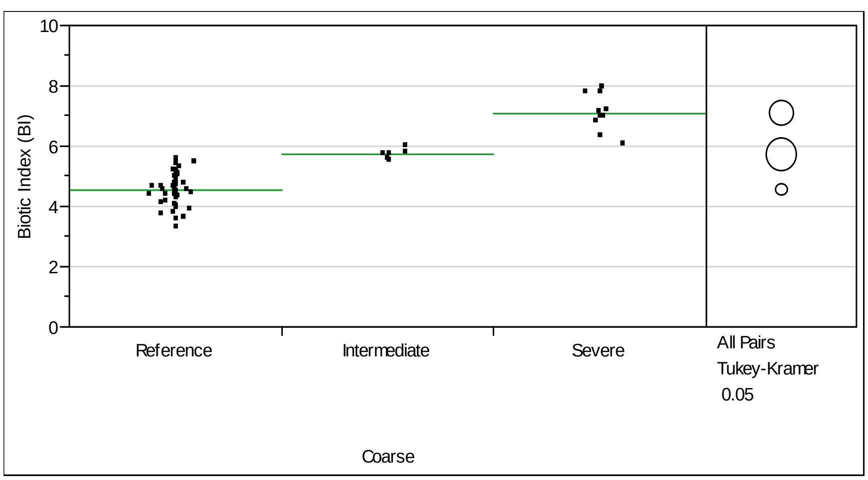



**Figure 17.** BI by Coarse-Scale Disturbance Class (PIEDMONT)

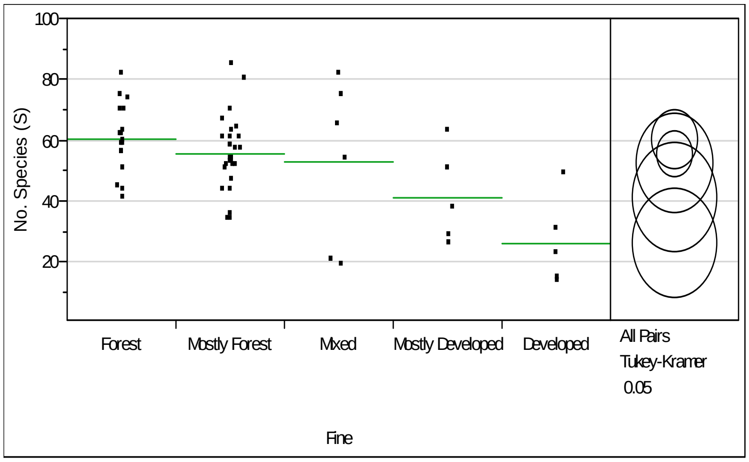



**Figure 18.** S by Fine-Scale Disturbance Class (PIEDMONT)

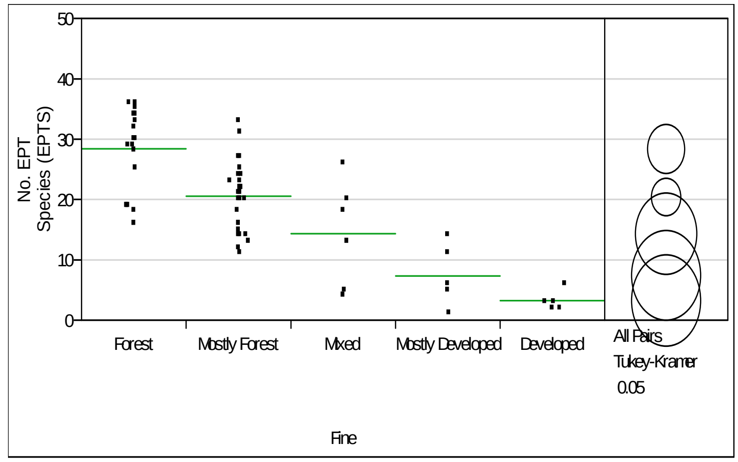



**Figure 19.** EPTS by Fine-Scale Disturbance Class (PIEDMONT)

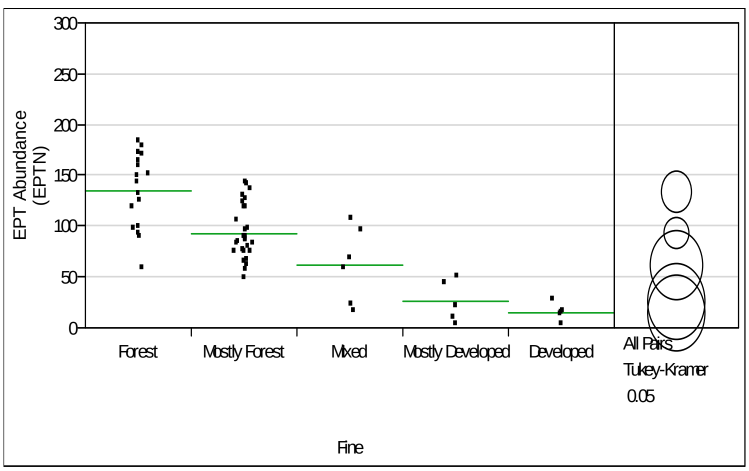



**Figure 20.** EPTN by Fine-Scale Disturbance Class (PIEDMONT)

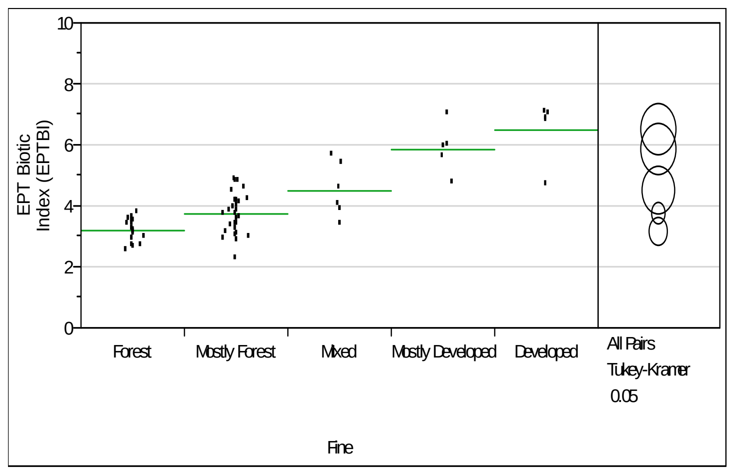



**Figure 21.** EPTBI by Fine-Scale Disturbance Class (PIEDMONT)

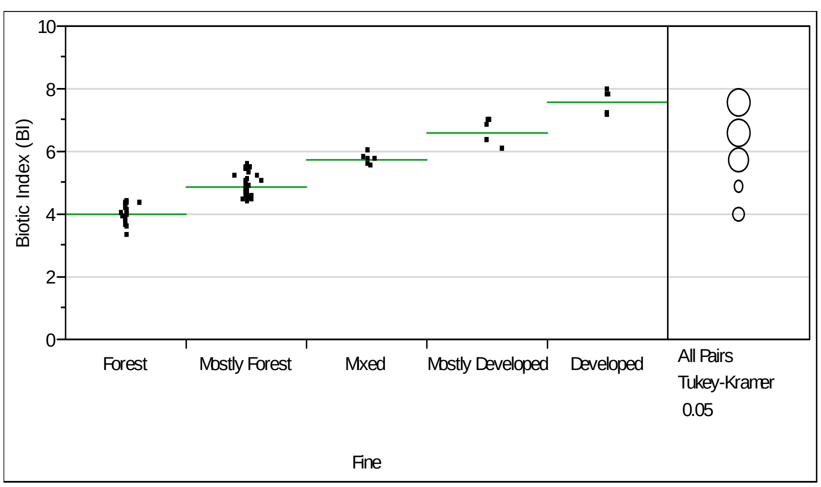



**Figure 22.** BI by Fine-Scale Disturbance Class (PIEDMOMT)

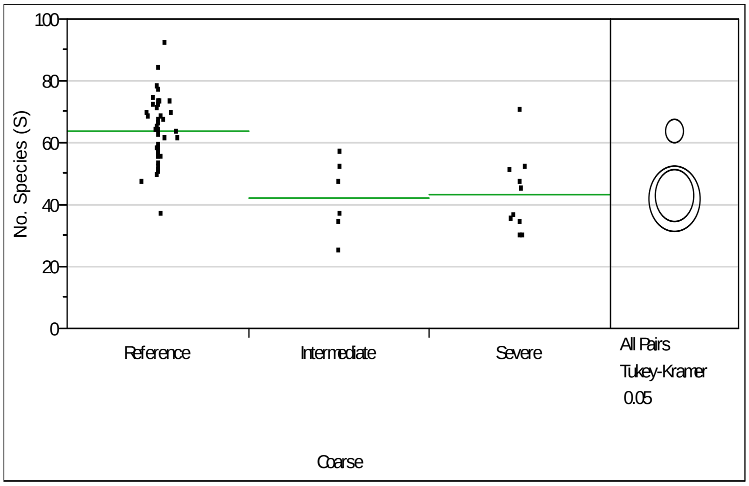



**Figure 23.** S by Coarse-Scale Disturbance Class (MOUNTAINS)

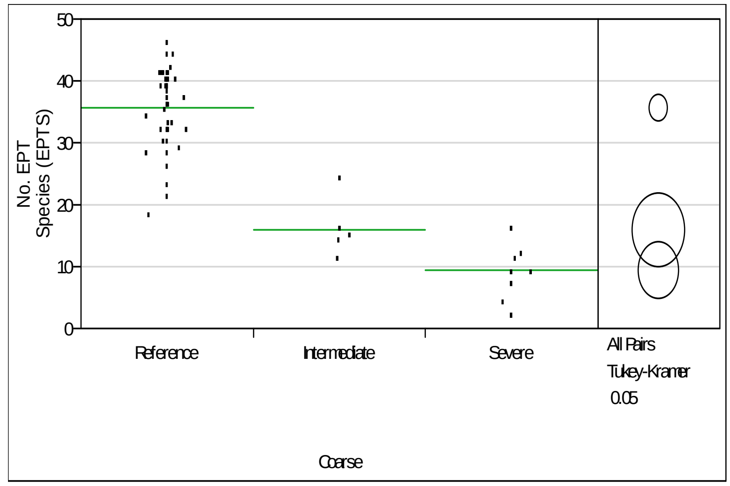



**Figure 24.** EPTS by Coarse-Scale Disturbance Class (MOUNTAINS)

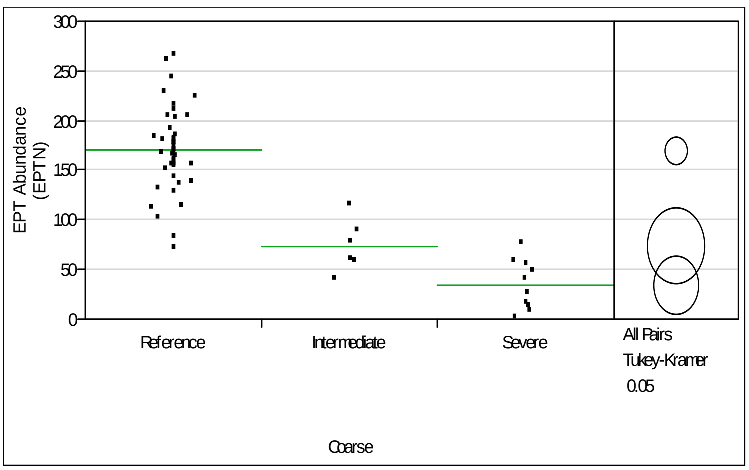



**Figure 25.** EPTN by Coarse Scale-Disturbance Class (MOUNTAINS)

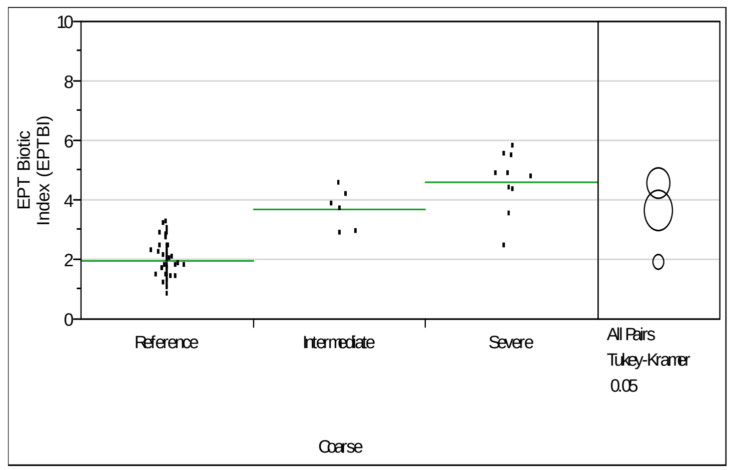



**Figure 26.** EPTBI by Coarse-Scale Disturbance Class (MOUNTAINS)

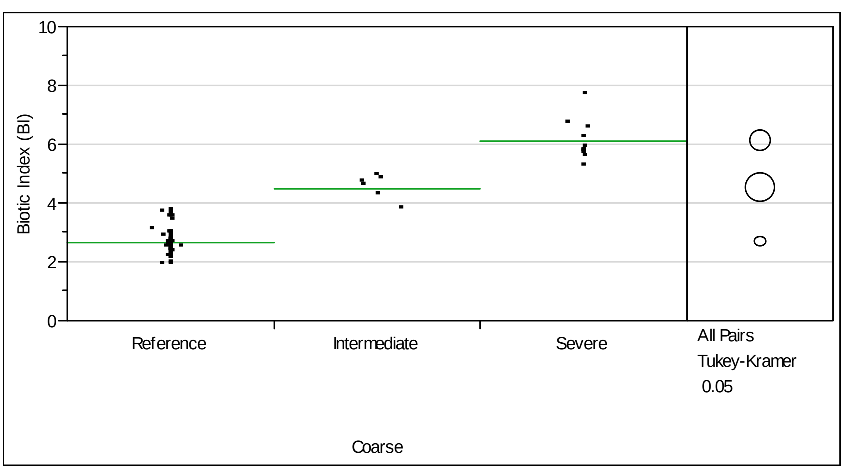



**Figure 27.** BI by Coarse-Scale Disturbance Class (MOUNTAINS)

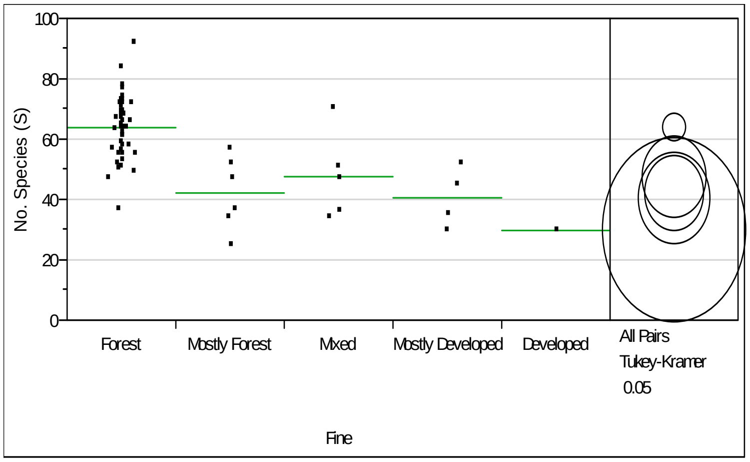



**Figure 28.** S by Fine-Scale Disturbance Class (MOUNTAINS)

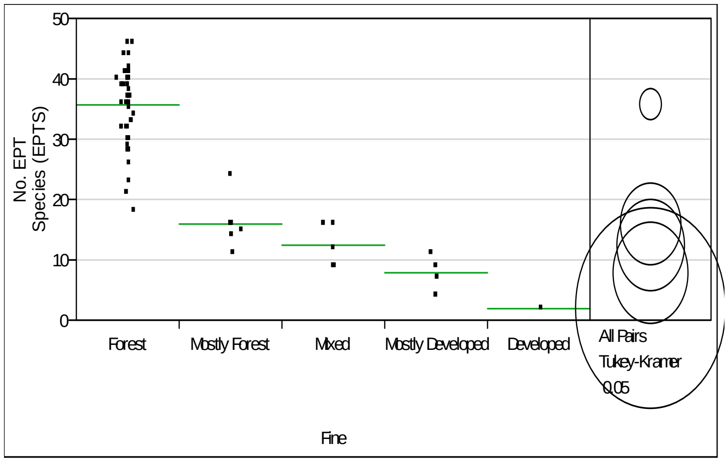



**Figure 29.** EPTS by Fine-Scale Disturbance Class (MOUNTAINS)

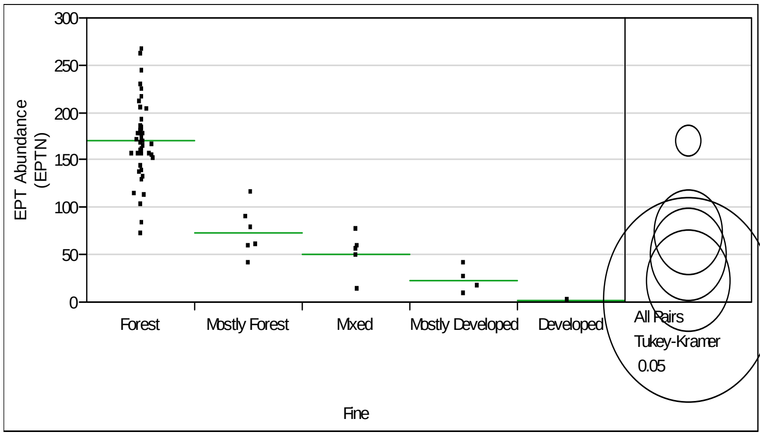



**Figure 30.** EPTN by Fine-Scale Disturbance Class (MOUNTAINS)

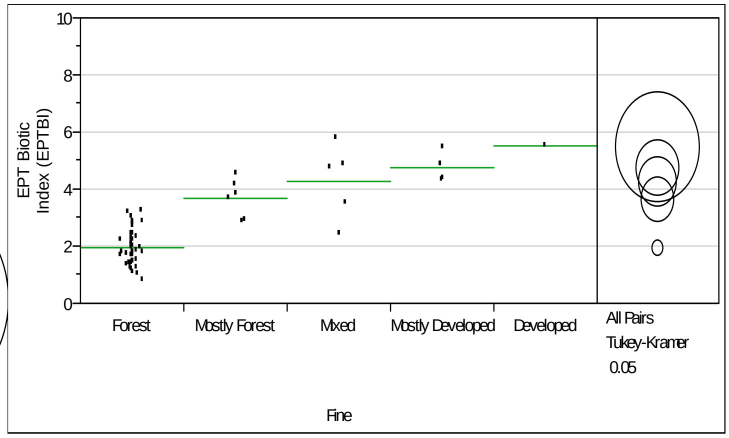



**Figure 31.** EPTBI by Fine-Scale Disturbance Class (MOUNTAINS)

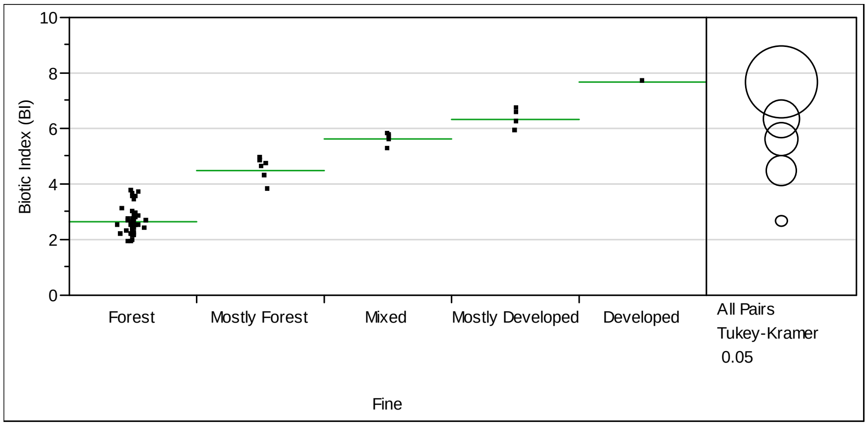



**Figure 32.** BI by Fine-Scale Disturbance Class (MOUNTAINS)

## (8.0) Development of Piedmont and Mountain Biocriteria Thresholds

Based on this information, the BI was selected for use in the development of biocriteria and a five-tiered bioclassification hierarchy (based on the BI) and derived from the landuse data was developed from the originally (a priori) selected (coarse-scale) reference, intermediate, and severe impact study sites from both the Piedmont and Mountains (sensu Karr et al. 1986, Lenat 1988, Weisburg et al. 1997, sensu Eaton 2001, Weigel 2002, Tate et al. 2005, Kratzer et al. 2006, Smith and Lamp 2007, Carlisle et al. 2008). The sites comprising these coarse-scale disturbance classes were further subdivided into five fine-scaled disturbance categories (based on landuse data; sensu Carlisle et al. 2008, Table 10 and Table 11) and were then sorted based on percent forest, percent developed, percent grass/pasture, and percent agriculture. Percent water and percent barren were excluded from this process as these landuse types exhibited no effect on any of the five BMI metrics tested in this study. As a result of this sorting into disturbance gradients based on the landuse data, the corresponding BI scores (Table 10, Table 11) were then used as the basis for the five-tiered bioclassification thresholds (i.e., "Excellent", "Good", "Good-Fair", "Fair", and "Poor") that NCDWQ currently uses to assign water quality (bioclassification) ratings to streams greater than 3.0 $mi^2$ (NCDWQ 2006). These small stream thresholds are presented below for both the Piedmont (Table 4) and Mountains (Table 5).

**Table 4. Piedmont Biocriteria Thresholds.**

| PIEDMONT Bioclassification | BI Values | Number of Samples (*n*) | Number of Samples Falling Outside of Thresholds |
|---|---|---|---|
| **Excellent** | **</= 4.36** | **17** | **1** |
| **Good** | **4.37-5.48** | **29** | **0** |
| **Good-Fair** | **5.49-6.02** | **6** | **0** |
| **Fair** | **6.03-6.98** | **5** | **0** |
| **Poor** | **>/= 6.99** | **5** | **0** |

**Table 5. Mountain Biocriteria Thresholds.**

| MOUNTAIN Bioclassification | BI Values | Number of Samples (*n*) | Number of Samples Falling Outside of Thresholds |
|---|---|---|---|
| **Excellent** | **</= 3.75** | **44** | **0** |
| **Good** | **3.76-4.99** | **6** | **0** |
| **Good-Fair** | **5.00-5.80** | **5** | **0** |
| **Fair** | **5.81-6.70** | **4** | **0** |
| **Poor** | **>/= 6.71** | **1** | **0** |

The results of this process indicate that for the Piedmont and the Mountains the BI is not only a powerful predictor of primarily deleterious or positive landuse types (e.g., developed and forest) with corresponding disturbance gradients (e.g., severe impact with reference, Table 10 and Table 11) but the BI is also very effective at predicting smaller gradients of response in the invertebrate assemblage that are associated with subtler (and evenly or nearly evenly mixed landuse types) and corresponding subtle disturbance gradients (Figures 13-32, Table 10 and Table 11). These findings are comparable to previous work (Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Black and Munn 2004, Moore and Palmer 2005, Carlisle et al. 2008) Indeed, not only were (most) of the mean landuse percentages, habitat scores, conductivity, BI (and to a lesser extent) most of the other four benthic macroinvertebrate metrics significantly different between landuse-derived disturbance gradients and associated bioclassifications (separated by two bioclassification steps) in the Piedmont (Table 6) and Mountains (Table 8) but many of these parameters also exhibited significant differences among the five (landuse-derived) fine-scale disturbance categories and associated five-tiered bioclassifications separated by only one step (Table 7, Table 9). Specifically, 72.7% of the means among the variables in the Piedmont and Mountains (separated by two bioclassification steps and disturbance classes) were significantly different (Table 6 and Table 8) while 43.2% of the means were significantly different in the Piedmont and 60.6% of the means significantly different in the Mountains (when separated by only one bioclassification and disturbance class step; Table 7, Table 9). The fact that the differences in the means of these variables were often statistically significantly between the various disturbance categories demonstrates the presence of “real” physical, chemical, and biological differences among these sites and this technique has been used previously to demonstrate the same point (Carlisle et al. 2008). In short, the statistically significant differences in the means between disturbance gradients strongly support the creation of a five-tiered bioclassification system. Conversely, if most of these parameters lacked statistically significant differences between disturbance gradients and associated bioclassifications, then a five-tiered bioclassification hierarchy would not be supported. Of particular note, relative to the S, EPTS, EPTN, and EPTBI, the BI consistently exhibited not only the strongest statistical difference (often with $p$ values $<0.0001$) between the disturbance classes, but the BI was statistically significant in every instance whereas the other metrics frequently were not (Tables 6-9). . This further supports the selection of the BI as the preferred metric for use in biocriteria development in small streams.

## Table 6. Piedmont Mean Landuse Differences by Fine-Scale Disturbance Class: Separation by Two Bioclassification Steps and Two Disturbance Classes.

| Disturbance Class & BIOCLASSIFICATION | S | EPTS | EPTN | EPTBI | BI | Habitat | Conductivity | % Forest | % Developed | % Grass/Pasture | % Ag |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference: Forest (EXCELLENT) **TO** Intermediate: Mixed Forest (GOOD-FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | $p=0.7309$ | **$p=0.0744$** | **$p=0.0989$** | **$p=0.0059$** | **$p<0.0001$** | $p=0.2144$ | $p=0.2889$ | **$p=0.0544$** | $p=0.4557$ | **$p=0.0210$** | $p=0.8973$ |
| Reference: Mostly Forest (GOOD) **TO** Impact: Mostly Developed (FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | $p=0.1817$ | **$p=0.0124$** | **$p=0.0238$** | **$p=0.0462$** | **$p<0.0050$** | $p=0.1854$ | **$p=0.0320$** | **$p=0.0004$** | **$p=0.0008$** | **$p=0.0006$** | $p=0.8950$ |
| Intermediate: Mixed (GOOD-FAIR) **TO** Impact: Developed (POOR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | **$p=0.0491$** | **$p=0.0138$** | **$p=0.0270$** | **$p=0.0381$** | **$p<0.0001$** | **$p=0.0730$** | **$p=0.0566$** | **$p=0.0251$** | **$p<0.0001$** | $p=0.8440$ | **$p=0.0223$** |

**Significant *p* values highlighted in yellow.**
**Borderline significant *p* values highlighted in orange.**

## Table 7. Piedmont Mean Landuse Differences by Fine-Scale Disturbance Class: Separation by One Bioclassification Step and One Disturbance Class.

| Disturbance Class & BIOCLASSIFICATION | S | EPTS | EPTN | EPTBI | BI | Habitat | Conductivity | % Forest | % Developed | % Grass/Pasture | % Ag |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference: Forest (EXCELLENT) **TO** Reference: Mostly Forest (GOOD) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | $p=0.2227$ | **$p=0.0007$** | **$p=0.0004$** | **$p=0.0032$** | **$p<0.0001$** | **$p=0.0377$** | **$p=0.0435$** | $p=0.2162$ | $p=0.3621$ | $p=0.1057$ | $p=0.4908$ |
| Reference: Mostly Forest (GOOD) **TO** Intermediate: Mixed (GOOD-FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | $p=0.9752$ | $p=0.4347$ | $p=0.2913$ | $p=0.2992$ | **$p=0.0179$** | $p=0.7809$ | $p=0.2047$ | **$p=0.0092$** | **$p=0.0133$** | **$p=0.0186$** | **$p=0.0470$** |
| Intermediate: Mixed (GOOD-FAIR) **TO** Impact: Mostly Developed (FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | $p=0.3386$ | $p=0.1960$ | $p=0.1480$ | $p=0.1042$ | **$p=0.0091$** | **$p=0.0874$** | **$p=0.0056$** | **$p=0.0856$** | **$p=0.0010$** | **$p=0.0069$** | $p=0.1123$ |
| Impact: Mostly Developed (FAIR) **TO** Impact: Developed (POOR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | $p=0.2445$ | $p=0.2103$ | $p=0.4021$ | $p=0.3401$ | **$p=0.0200$** | $p=0.5370$ | $p=0.6945$ | $p=0.1681$ | **$p=0.0318$** | $p=0.2261$ | $p=0.9502$ |

**Significant *p* values highlighted in yellow.**
**Borderline significant *p* values highlighted in orange.**

**Table 8. Mountain Mean Landuse Differences by Fine-Scale Disturbance Class: Separation by Two Bioclassification Steps and Two Disturbance Classes.**

| Disturbance Class & BIOCLASSIFICATION | S | EPTS | EPTN | EPTBI | BI | Habitat | Conductance | % Forest | % Developed | % Grass/Pasture | % Ag |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference: Forest (EXCELLENT) **TO** Intermediate: Mixed Forest (GOOD-FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | ***p*=0.0928** | ***p*=0.0014** | ***p*=0.0005** | ***p*=0.0138** | ***p*<0.0001** | ***p*=0.0101** | ***p*=0.0094** | ***p*=0.0020** | ***p*=0.0139** | ***p*=0.0267** | *p*=0.1956 |
| Reference: Mostly Forest (GOOD) **TO** Impact: Mostly Developed (FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | *p*=0.7314 | ***p*=0.0056** | ***p*=0.0022** | ***p*=0.0433** | ***p*=0.0070** | *p*=0.3446 | *p*=0.2566 | ***p*=0.0057** | ***p*=0.0813** | *p*=0.5651 | *p*=0.6250 |
| Intermediate: Mixed: Developed (GOOD-FAIR) **TO** Impact: Developed (POOR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | NA[1] | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

**Significant *p* values highlighted in yellow.**
**Borderline significant *p* values highlighted in orange.**
[1] **Not enough data to generate a meaningful t-test. See Section 13 For Further Information.**

**Table 9. Mountain Mean Landuse Differences by Fine-Scale Disturbance Class: Separation by One Bioclassification Step and One Disturbance Class.**

| DisturbanceClass & BIOCLASSIFICATION | S | EPTS | EPTN | EPTBI | BI | Habitat | Conductivity | % Forest | % Developed | % Grass/Pasture | % Ag |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Reference: Forest (EXCELLENT) **TO** Reference: Mostly Forest (GOOD) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | ***p*=0.0201** | ***p*=0.0013** | ***p*=0.0005** | ***p*=0.0009** | ***p*=0.0001** | ***p*=0.0220** | *p*=0.1218 | ***p*=0.0042** | *p*=0.2375 | ***p*=0.0307** | ***p*=0.0061** |
| Reference: Mostly Forest (GOOD) **TO** Intermediate: Mixed (GOOD-FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | *p*=0.7984 | ***p*=0.0427** | ***p*=0.0117** | *p*=0.2599 | ***p*=0.0014** | *p*=0.5290 | *p*=0.1631 | ***p*=0.0105** | ***p*=0.0459** | ***p*=0.0973** | *p*=0.5930 |
| Intermediate: Mixed (GOOD-FAIR) **TO** Impact: Mostly Developed (FAIR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | ***p*=0.0917** | ***p*=0.0819** | ***p*=0.0368** | *p*=0.4716 | ***p*=0.0626** | *p*=0.8747 | *p*=0.1518 | ***p*=0.0155** | *p*=0.1401 | *p*=0.5888 | *p*=0.9001 |
| Impact: Mostly Developed (FAIR) **TO** Impact: Developed (POOR) | | | | | | | | | | | |
| **MEAN DIFFERENCE** | NA[1] | NA | NA | NA | NA | NA | NA | NA | NA | NA | NA |

**Significant *p* values highlighted in yellow.**
**Borderline significant *p* values highlighted in orange.**
[1] **Not enough data to generate a meaningful t-test. See Section 13 For Further Information.**

Although there is some variation in the landuse percentages between sites grouped into a particular disturbance classes (Figure 33), the general BI trends are tightly coupled to the relative proportions of percent developed, percent forest/wetland, percent agriculture, and percent grass/pasture in both ecoregions (Figure 35, Figure 37, Table 10, Table 11). For example, based on the BI scores and resultant biocriteria thresholds, as you progress "downward" from landuse percentages associated with an Excellent bioclassification to landuse patterns corresponding to a Good bioclassification the relative proportions of percent development, agriculture, and grass/pasture will increase while relative proportions of percent forest/wetland will decrease. Interestingly, as you progress downward from a Good bioclassification to a Good-Fair bioclassification, while the percent forest/wetland continues a decreasing trend, and percent developed continues an increased trend, the proportion of their relative changes is less and this holds true for both ecoregions (Figure 35, Figure 37, Table 10 and Table 11). As the rate of change in these two landuse types lessens, the rate of change in the percent agriculture and percent grass/pasture proportionately increases. It is only once you continue "downward" from the Good-Fair bioclassification and associated landuse trends towards the Fair and Poor bioclassifications do the percent agriculture, and percent grass/pasture landuse percentages resume their downward progression, while percent forest continues to decrease and percent developed continues to increase (Figure 35, Figure 37, Table 10 and Table 11). These landuse trends are consistent with the character of the resulting land use disturbances and are reflected by the BI-derived bioclassifications.

For example, BI scores associated with Excellent bioclassifications should have mostly all forest, with little to no input from other landuse types that are related to disturbance (Black and Munn 2004). Biotic index scores related to Good bioclassifications should have slightly less forest with some of the difference being increasingly comprised of landuse types associated with disturbance (e.g., developed, agriculture, and grass/pasture). BI scores related to the Good-Fair bioclassification should include quantities of landuse that have less percent developed and less percent forest categories, but rather there should be more "intermediate" effects (e.g., grass/pasture and agriculture) present (Carlisle et al. 2008). Conversely, as the BI scores increase and associated bioclassifications decrease to Fair and Poor, the landuse types that lack deleterious effects on invertebrate communities (percent forest/wetland; Black and Munn 2004), or that have lessened deleterious effects (grass/pasture and agriculture; Carlisle et al. 2008) should continue to decrease while the landuse type most deleterious to invertebrate communities (percent developed; Kerans and Karr 1994, Cuffney et al. 2000, Paul and Meyer 2001, Black and Munn 2004, Moore and Palmer 2005, Carlisle et al. 2008) should increase. As can be seen in Figure 35, Figure 37, Table 10, and Table 11, this is the trend in both ecoregions. Although the S, EPTS, EPTBI, and EPTN also tracked these trends, (Figure 34, Figure 36), their much higher CV values (Figures 8-12) and higher rates of overlap (Figure 13-14, Figure 16, Figures 23-26, Figures 28-31) between all disturbance gradients (both coarse-scale and fine-scale) make them less optimum relative to the BI for use in biocriteria development for small streams.

**Figure 33. Average Percent Landuse Comparisons Mountain (A) and Piedmont (B)**

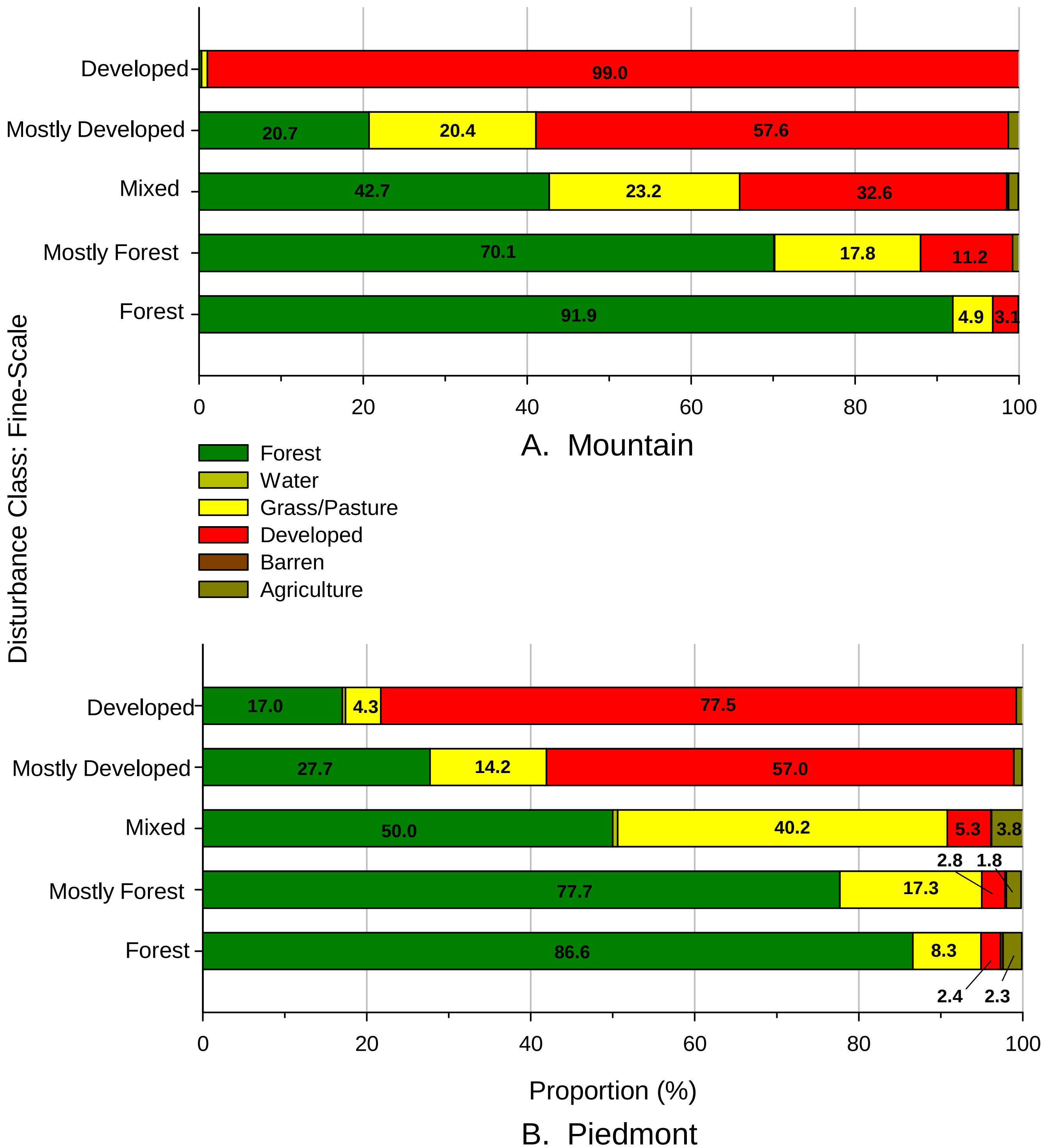


**Figure 34. Piedmont Total Taxa Richness (S), EPTS, EPT Abundnace (EPTN), Habitat, Conductivity and Landuse by Fine-Scale Disturbance Class.**

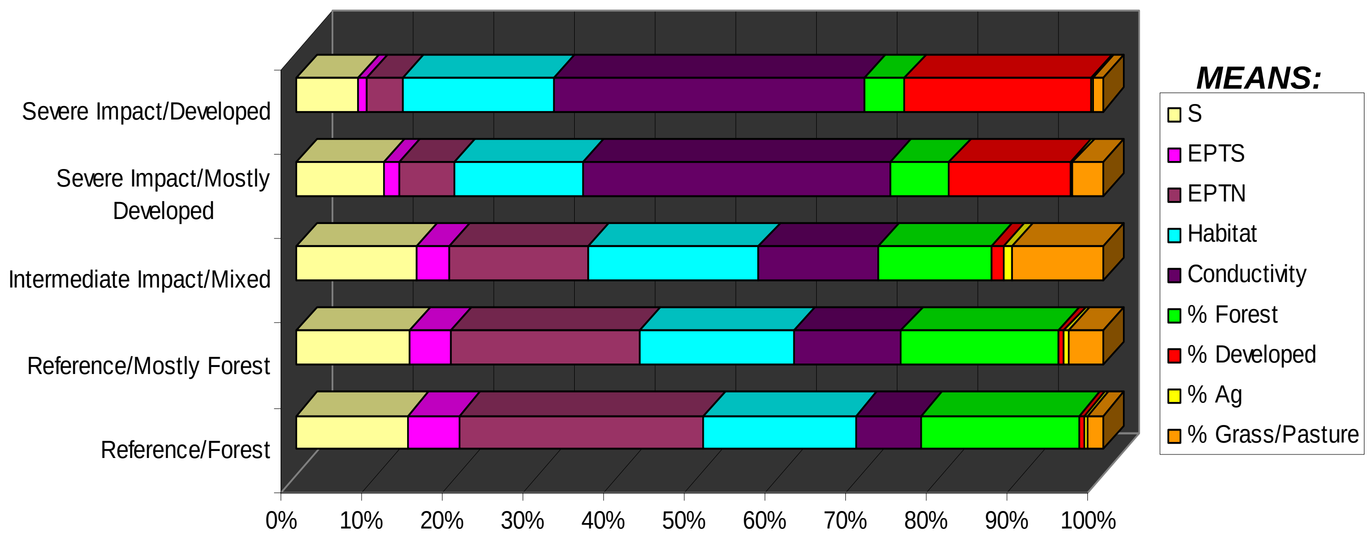



**Figure 35. Piedmont Biotic Index (BI), EPTBI, and Percent Landuse by Fine-Scale Disturbance Class.**

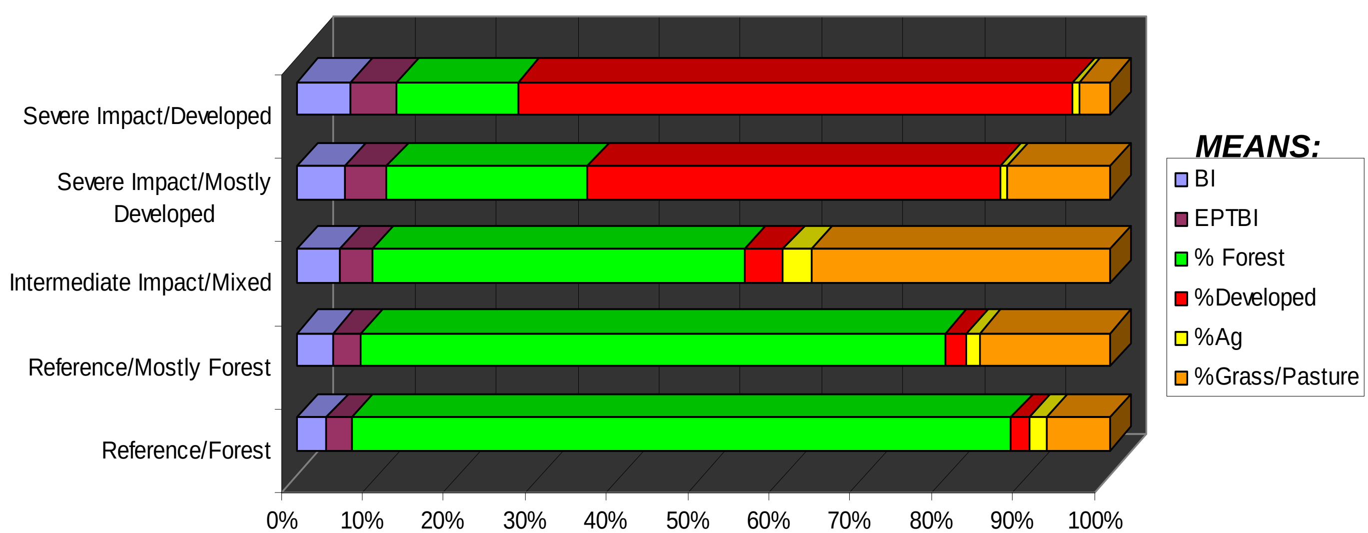


**Figure 36. Mountain Total Taxa Richness (S), EPTS, EPT Abundance (EPTN), Habitat, Conductivity and Landuse by Fine-Scale Disturbance Class.**

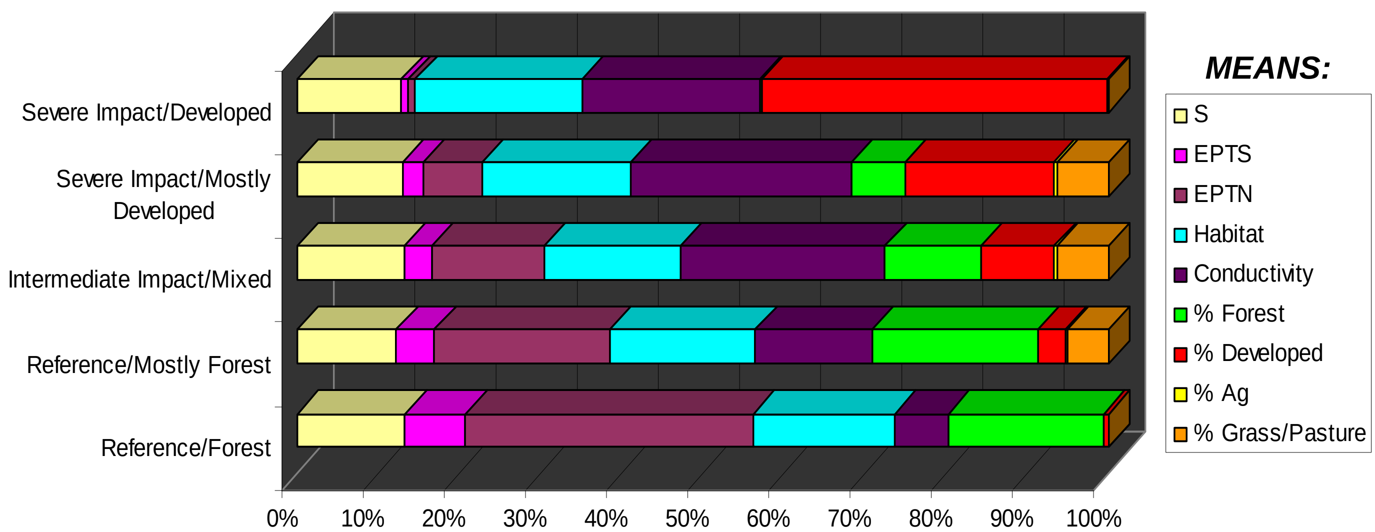



**Figure 37. Mountain Biotic Index (BI), EPTBI, and Percent Landuse by Fine-Scale Disturbance Class.**

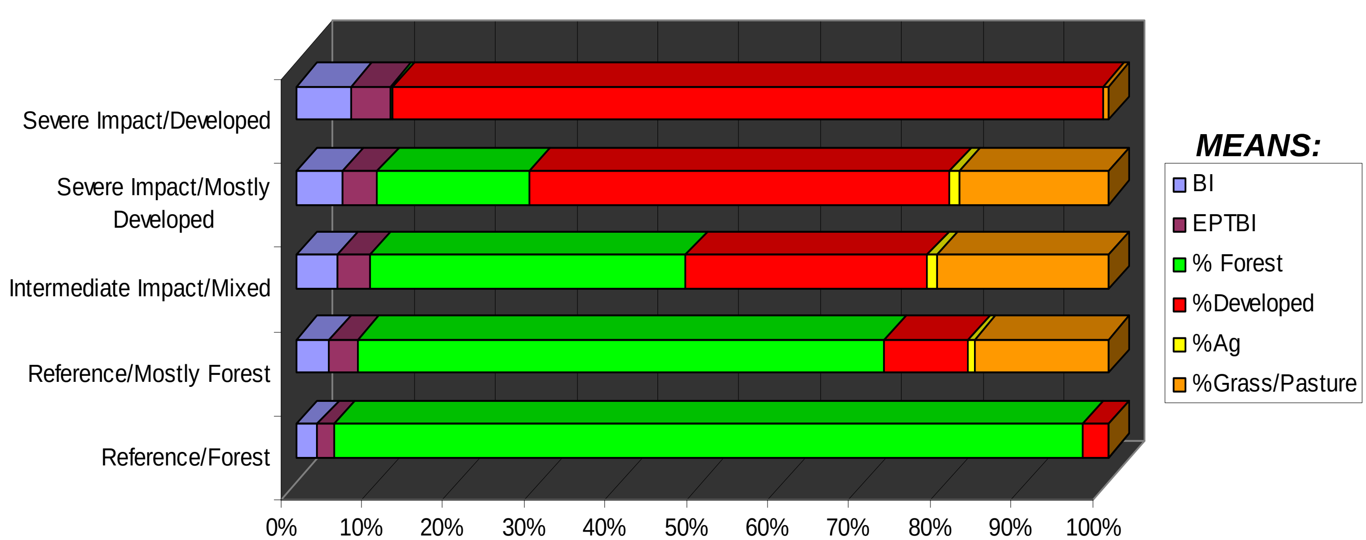


**Table 10. Piedmont Study Sites: Raw Data. Five-Tiered Biotic Index Ranges Based on Sorting by Landuse: % Forest, % Developed, % Grass/Pasture, and % Agriculture.** **Note Overlap of the S, EPTS, EPTN, and EPTBI Between Disturbance Classes.**

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Severe Impact/Developed** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | BI | S | EPTS | EPTBI | EPTN | DA | Condo | Habitat |
| Ut Mine Cr | 16.49 | 1.89 | 4.95 | 75.63 | 0 | 1.04 | 7.12 | 23 | 2 | 4.7 | 4 | 0.56 | 47 | 79 |
| Tanyard Br | 26.93 | 0 | 8.57 | 61.79 | 0 | 2.71 | 7.78 | 15 | 3 | 6.88 | 14 | 1.16 | 202 | 46 |
| Ut Richlands Cr | 17.22 | 0 | 3.2 | 79.59 | 0 | 0 | 7.94 | 14 | 2 | 6.81 | 13 | 1.38 | 94 | 61 |
| Nutbush Cr | 19.91 | 0 | 4.36 | 74.83 | 0.11 | 0.79 | 7.19 | 49 | 6 | 7.09 | 28 | 2.28 | 198 | 58 |
| Beaverdam Cr | 4.25 | 0 | 0.22 | 95.53 | 0 | 0 | 7.79 | 31 | 3 | 7 | 16 | 2.97 | 105 | 71 |
| **MEANS** | **17.0** | **0.37** | **4.3** | **77.5** | **0.02** | **0.90** | **7.564** | **26** | **3.2** | **6.49** | **15** | **1.67** | **129.2** | **63** |
| **Severe Impact/Mostly Developed** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | BI | S | EPTS | EPTBI | EPTN | DA | Condo | Habitat |
| Ut Richlands Cr | 18.06 | 0 | 42.45 | 38.65 | 0 | 0.84 | 6.32 | 38 | 6 | 5.98 | 21 | 0.61 | 138 | 75 |
| Marlowe Cr | 39.04 | 0 | 9.55 | 50.48 | 0.23 | 0.69 | 6.95 | 26 | 5 | 5.62 | 9 | 1.74 | 141 | 39 |
| UT Muddy Fk | 9.65 | 0 | 10.69 | 76.54 | 0 | 3.12 | 6.81 | 63 | 11 | 5.92 | 44 | 2.56 | 176 | 49 |
| Hasketts Cr | 38.51 | 0 | 5.27 | 56.04 | 0 | 0.17 | 6.98 | 29 | 1 | 7 | 3 | 2.9 | 118 | 61 |
| Penwood Br | 33.36 | 0.11 | 3.25 | 63.28 | 0 | 0 | 6.03 | 51 | 14 | 4.76 | 51 | 2.9 | 149 | 79 |
| **MEANS** | **27.7** | **0.02** | **14.2** | **57.0** | **0.04** | **0.96** | **6.618** | **41** | **7.4** | **5.85** | **25.6** | **2.14** | **144.4** | **60.6** |
| **Intermediate Impact/Mixed** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | BI | S | EPTS | EPTBI | EPTN | DA | Condo | Habitat |
| Ut Haw R | 35.2 | 0.8 | 54.5 | 3.4 | 0.2 | 5.8 | 5.54 | 75 | 18 | 4.6 | 96 | 0.5 | 25 | 79 |
| Birch Fk | 33.8 | 0.8 | 56.3 | 2.6 | 0 | 6.4 | 5.76 | 65 | 20 | 5.4 | 68 | 2.4 | 70 | 83 |
| Wells Cr | 54.6 | 0.3 | 38.6 | 4.3 | 0.1 | 2 | 6.02 | 54 | 13 | 5.7 | 59 | 2.7 | 97 | 72 |
| Brushy Cr | 49.1 | 1.2 | 41.6 | 6.3 | 0 | 1.6 | 5.55 | 82 | 26 | 3.9 | 107 | 2.1 | 81 | 76 |
| L Rocky Run | 61.67 | 0 | 28.23 | 8.36 | 0 | 1.7 | 5.71 | 19 | 5 | 4.05 | 16 | 0.3 | 24 | 80 |
| UT Nicks Cr | 65.35 | 0.34 | 22.25 | 6.6 | 0 | 5.5 | 5.74 | 21 | 4 | 3.41 | 22 | 1 | 18 | 63 |
| **MEANS** | **50.0** | **0.57** | **40.2** | **5.3** | **0.05** | **3.8** | **5.72** | **53** | **14.3** | **4.51** | **61.3** | **1.5** | **52.5** | **75.5** |
| **Reference/Mostly Forest** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | BI | S | EPTS | EPTBI | EPTN | DA | Condo | Habitat |
| Ut Tar R | 89.87 | 0 | 7.58 | 1.98 | 0.57 | 0 | 5.29 | 36 | 14 | 2.97 | 67 | 0.4 | 44.3 | 73 |
| Talbots Br | 91.49 | 0 | 6.93 | 0.72 | 0 | 0.87 | **5.57*** | 51 | 20 | 4.88 | 127 | 0.5 | 92 | 85 |
| L Stonehouse Cr | 73.71 | 0.06 | 20.48 | 3.18 | 0.03 | 2.54 | 5.01 | 61 | 22 | 3.86 | 89 | 1.2 | 48 | 59 |
| Ut Powells Cr | 93.4 | 0 | 4.79 | 1.01 | 0.02 | 0.77 | 5.08 | 44 | 14 | 2.85 | 56 | 1.75 | 113 | 76 |
| Rattlesnake Cr | 75.53 | 0.14 | 21.9 | 1.99 | 0 | 0.44 | 5.42 | 57 | 16 | 4.5 | 74 | 1.8 | 89 | 79 |
| Parkwood Br | 80.85 | 0.1 | 15.5 | 1.49 | 1.14 | 0.92 | 5.39 | 70 | 24 | 4.61 | 119 | 2.9 | 71 | 75 |
| Little Rocky Cr | 88.49 | 0 | 7.59 | 3.55 | 0.03 | 0.34 | 5.18 | 85 | 31 | 4.13 | 143 | 2.9 | 56.6 | 75 |
| Jordan Cr | 87.13 | 0 | 8.08 | 4.24 | 0.14 | 0.41 | 5.18 | 61 | 15 | 4.83 | 79 | 2.8 | 39 | 79 |
| Jordan Cr | 87.13 | 0 | 8.08 | 4.24 | 0.14 | 0.41 | 4.85 | 57 | 22 | 3.75 | 86 | 2.8 | 49 | 81 |
| Ut L Buffalo Cr | 88.53 | 0 | 9.85 | 1.62 | 0 | 0 | 5.03 | 52 | 18 | 4.81 | 82 | 2.9 | 80 | 86 |
| Ut Bear Swp | 89.91 | 0 | 9.01 | 0 | 0.09 | 0.99 | 4.65 | 58 | 24 | 4.01 | 130 | 0.7 | 92.2 | 87 |
| N Pr Anderson Cr | 11.68 | 0 | 73.31 | 8.56 | 0 | 6.45 | 4.68 | 54 | 20 | 3.13 | 75 | 0.88 | 17 | 62 |
| N Pr Anderson Cr | 11.68 | 0 | 73.31 | 8.56 | 0 | 6.5 | 4.42 | 44 | 12 | 2.92 | 65 | 0.88 | 15 | 79 |
| Tanyard Cr | 77.76 | 0.04 | 19.57 | 2.27 | 0 | 0.37 | 4.64 | 67 | 27 | 3.1 | 124 | 0.95 | 58 | 60 |
| Arnett Br | 90.84 | 0 | 7.1 | 1.9 | 0 | 0.17 | 4.5 | 64 | 33 | 3.6 | 141 | 1.2 | 52 | 86 |
| Big Br | 82.91 | 0 | 8.61 | 8.07 | 0 | 0.41 | 4.75 | 53 | 20 | 3.35 | 90 | 1.3 | 15 | 67 |
| W Pr Juniper Cr | 82.19 | 0 | 10.71 | 5.93 | 0 | 1.17 | 4.39 | 34 | 11 | 3.38 | 48 | 1.4 | 11 | 83 |
| W Br Maclean Cr | 95.28 | 0 | 4.12 | 0.36 | 0 | 0.24 | 4.45 | 58 | 23 | 3.57 | 97 | 1.7 | 47 | 88 |
| W Br Maclean Cr | 95.28 | 0 | 4.12 | 0.36 | 0 | 0.2 | 4.54 | 54 | 21 | 3.73 | 105 | 1.7 | 48 | 81 |
| Bones Fk Cr | 77.19 | 0 | 8.15 | 7.63 | 0 | 0.39 | 5.45 | 47 | 13 | 3.41 | 61 | 2.2 | 14 | 78 |
| Bones Fk Cr | 77.19 | 0 | 8.15 | 7.63 | 0 | 0.39 | 4.37 | 63 | 21 | 2.27 | 82 | 2.2 | 19 | 80 |
| N Pr Anderson Cr | 34.55 | 0 | 57.99 | 4.48 | 0 | 2.98 | 5.48 | 61 | 22 | 4.16 | 77 | 2.82 | 25 | 62 |
| Dial Cr | 73.66 | 0.22 | 21.54 | 3.03 | 0.06 | 1.49 | 4.89 | 52 | 23 | 3.85 | 85 | 2.9 | 73 | 86 |
| Ut Drowning Cr | 58.25 | 0.34 | 33.46 | 0.98 | 0 | 6.97 | 4.52 | 53 | 20 | 3.01 | 88 | 1.23 | 24 | 80 |
| Hubquarter Cr | 83.37 | 0 | 13.09 | 2.21 | 0 | 1.33 | 4.96 | 80 | 27 | 4.2 | 119 | 2.9 | 61.8 | 65 |
| Crooked Fk | 70.76 | 0 | 25.51 | 1.35 | 1.96 | 0.41 | 4.76 | 34 | 14 | 3.27 | 90 | 2.52 | 52 | 79 |
| Little Cr | 81.87 | 0 | 8.13 | 0.56 | 0 | 9.44 | 4.78 | 54 | 21 | 4.18 | 95 | 2.6 | 60.5 | 72 |
| Negro Cr | 75.08 | 0.05 | 19.48 | 2.88 | 0 | 2.5 | 4.66 | 54 | 20 | 4.17 | 74 | 2.62 | 87 | 51 |
| Ut Talbots Cr | 88.41 | 0.04 | 10.75 | 0.79 | 0 | 0 | 4.56 | 52 | 25 | 3.94 | 136 | 2.4 | 78 | 85 |
| **MEANS** | **77.7** | **0.03** | **17.3** | **2.8** | **0.14** | **1.8** | **4.87** | **56** | **20.4** | **3.73** | **93.2** | **1.9** | **52.81** | **75.82** |
| **Reference/Forest** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | BI | S | EPTS | EPTBI | EPTN | DA | Condo | Habitat |
| Horsepen Cr | 98.26 | 0 | 1.65 | 0.09 | 0 | 0 | 4.18 | 59 | 28 | 3.07 | 99 | 0.8 | 47 | 83 |
| Moccasin Cr | 98.32 | 0 | 0.92 | 0.76 | 0 | 0 | 4.05 | 56 | 29 | 3.54 | 151 | 2.5 | 50 | 84 |
| Iron Hill Br | 80.29 | 0 | 17.21 | 0.97 | 1.53 | 0 | 4.32 | 70 | 29 | 3.78 | 149 | 0.9 | 45.2 | 87 |
| Ut Hitchcock Cr | 47.85 | 0 | 19.1 | 10.76 | 2.98 | 19.31 | 4.35 | 45 | 16 | 2.72 | 58 | 1.64 | 15 | 67 |
| L Dumas Cr | 79.32 | 0 | 17.78 | 1.33 | 0.07 | 1.5 | 4.28 | 62 | 30 | 3.62 | 163 | 2 | 40 | 90 |
| Millstone Cr | 70.25 | 0.37 | 15 | 9.25 | 0 | 5.13 | 4.35 | 51 | 18 | 2.93 | 93 | 3 | 13.9 | 71 |
| Poison Fk | 92.01 | 0.06 | 7.49 | 0.44 | 0 | 0 | 4.11 | 70 | 35 | 3.57 | 172 | 2.8 | 48 | 84 |
| Joes Cr | 78.49 | 0 | 14.42 | 4.47 | 0 | 2.62 | 3.79 | 44 | 19 | 3.13 | 97 | 1.8 | 14 | 75 |
| Joes Cr | 78.49 | 0 | 14.42 | 4.47 | 0 | 2.6 | 4.36 | 41 | 19 | 3.39 | 90 | 1.8 | 12 | 83 |
| Ut Dobbins Cr | 97.16 | 0 | 1.56 | 1.28 | 0 | 0 | 3.59 | 82 | 36 | 2.56 | 142 | 0.7 | 28 | 76 |
| Wood Run | 98.52 | 0 | 0.64 | 0.79 | 0 | 0 | 4 | 59 | 25 | 3.4 | 170 | 2.2 | 44 | 86 |
| Wood Run | 98.52 | 0 | 0.64 | 0.79 | 0 | 0.04 | 3.62 | 63 | 36 | 3.24 | 179 | 2.2 | 46 | 93 |
| Wood Run | 98.52 | 0 | 0.64 | 0.79 | 0 | 0 | 3.94 | 56 | 30 | 3.18 | 131 | 2.2 | 44 | 86 |
| Ut Drowning Cr | 63.24 | 0.23 | 25.53 | 3.3 | 0 | 7.7 | 3.9 | 75 | 32 | 3 | 125 | 2.4 | 21 | 91 |
| Dutchmans Cr | 98.04 | 0 | 1.26 | 0.63 | 0 | 0.07 | 3.3 | 60 | 34 | 2.71 | 159 | 2.8 | 57 | 86 |
| Dutchmans Cr | 98.04 | 0 | 1.26 | 0.63 | 0 | 0.1 | 3.97 | 74 | 34 | 3.51 | 184 | 2.8 | 57 | 92 |
| Hog Pen Br | 99.9 | 0 | 0 | 0 | 0.1 | 0 | 3.75 | 62 | 33 | 2.65 | 118 | 0.5 | 22 | 85 |
| **MEANS** | **86.6** | **0.03** | **8.3** | **2.4** | **0.27** | **2.3** | **3.99** | **61** | **28.4** | **3.17** | **134** | **1.94** | **35.54** | **83.47** |

***The Talbots Branch BI (5.57) Exceeded the Minimum Excellent Threshold for the Level III Piedmont Ecoregions (Table 4).**

**Table 11. Mountain Study Sites: Raw Data. Raw Data. Five-Tiered Biotic Index Ranges Based on Sorting by Landuse: % Forest, % Developed, % Grass/Pasture, and % Agriculture.** **Note Overlap of the S, EPTS, EPTN, and EPTBI Between Disturbance Classes.**

| | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Severe Impact/Developed** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | S | EPTSs | EPTN | EPTBI | BI | DA | Condo | Habitat |
| Moore Br | 0.33 | 0 | 0.66 | 99.01 | 0 | 0 | 30 | 2 | 2 | 5.5 | 7.67 | 0.63 | 51 | 48 |
| **MEANS** | **0.33** | **0** | **0.66** | **99.01** | **0** | **0** | **30** | **2** | **2** | **5** | **7.67** | **0.63** | **51** | **48** |
| **Severe Impact/Mostly Developed** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | S | EPTs | EPTN | EPTBI | BI | DA | Condo | Habitat |
| Ut French Broad R | 17.56 | 0 | 15.27 | 66.66 | 0.07 | 0.45 | 35 | 7 | 17 | 4.34 | 6.55 | 0.63 | 72 | 78 |
| Ut Mud Cr | 27.83 | 0 | 2.14 | 69.82 | 0 | 0.21 | 52 | 11 | 40 | 5.45 | 6.24 | 0.9 | 71 | 44 |
| Ut Pigeon R | 6.55 | 0 | 5.35 | 88.1 | 0 | 0 | 45 | 9 | 26 | 4.39 | 5.88 | 0.43 | 68 | 52 |
| Ut Pigeon R | 30.66 | 0 | 59.01 | 5.92 | 0 | 4.4 | 30 | 4 | 8 | 4.86 | 6.7 | 0.37 | 132 | 56 |
| **MEANS** | **20.7** | **0** | **20.4** | **57.6** | **0.02** | **1.3** | **41** | **7.8** | **23** | **4.8** | **6.34** | **0.58** | **85.8** | **57.5** |
| **Intermediate Impact/Mixed** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | S | EPTs | EPTN | EPTBI | BI | DA | Condo | Habitat |
| Ut French Broad R | 37.28 | 0 | 22.47 | 37 | 0.94 | 2.31 | 51 | 16 | 55 | 3.51 | 5.27 | 1 | 81 | 59 |
| Moore Cr | 61.41 | 0 | 20.16 | 18.43 | 0 | 0 | 70 | 16 | 77 | 4.75 | 5.79 | 1.1 | 79 | 52 |
| Bat Fk | 21.73 | 0.19 | 30.71 | 43.54 | 0.13 | 3.7 | 47 | 9 | 58 | 5.78 | 5.7 | 1.65 | 75 | 45 |
| George Br | 46.9 | 0 | 34.8 | 18 | 0 | 0 | 34 | 9 | 13 | 2.42 | 5.75 | 0.3 | 175 | 69 |
| King Cr | 46.18 | 0 | 7.82 | 45.8 | 0 | 0.2 | 36 | 12 | 49 | 4.87 | 5.56 | 1.9 | 45 | 80 |
| **MEANS** | **42.7** | **0.04** | **23.2** | **32.6** | **0.21** | **1.2** | **48** | **12** | **50** | **4.3** | **5.61** | **1.19** | **91** | **61** |
| **Reference/Mostly Forest** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | S | EPTs | EPTN | EPTBI | BI | DA | Condo | Habitat |
| Ross Cr | 63.92 | 0 | 3.31 | 32.68 | 0 | 0.08 | 34 | 15 | 90 | 2.94 | 3.76 | 1.7 | 93 | 84 |
| Mud Cr | 78.53 | 0.33 | 12.61 | 7.51 | 0.04 | 0.98 | 52 | 24 | 115 | 3.65 | 4.25 | 2.4 | 48 | 70 |
| Peter Weaver Cr | 74.68 | 0 | 12.81 | 11.72 | 0 | 0.79 | 37 | 16 | 78 | 4.15 | 4.58 | 0.9 | 32 | 56 |
| Clear Cr | 65.05 | 0 | 28.46 | 5.32 | 0.03 | 1.14 | 47 | 14 | 60 | 3.82 | 4.71 | 2 | 41 | 50 |
| Cox Cr | 83.48 | 0 | 12.27 | 3.53 | 0 | 0.73 | 57 | 16 | 58 | 2.84 | 4.82 | 2.7 | 30 | 73 |
| Mill Cr | 54.67 | 0 | 37.55 | 6.34 | 0.09 | 1.35 | 25 | 11 | 40 | 4.54 | 4.9 | 1.5 | 52 | 37 |
| **MEANS** | **70.1** | **0.06** | **17.8** | **11.2** | **0.03** | **0.8** | **42** | **16** | **74** | **3.7** | **4.5** | **1.87** | **49.3** | **61.7** |
| **Reference/Forest** | Forest/Wetland % | Water % | Grass/Pasture % | Developed % | Barren % | Ag % | S | EPTs | EPTN | EPTBI | BI | DA | Condo | Habitat |
| Ut Wash Cr | 97.79 | 0 | 2.01 | 0.2 | 0 | 0 | 67 | 39 | 160 | 1.81 | 2.46 | 0.34 | 14 | 83 |
| Ledford Br | 96.96 | 0 | 2.21 | 0.84 | 0 | 0 | 58 | 30 | 177 | 1.77 | 2.48 | 0.45 | 27 | 86 |
| Bearwallow Br | 94.15 | 0 | 0 | 5.85 | 0 | 0 | 78 | 42 | 204 | 1.44 | 2.64 | 0.5 | 11 | 85 |
| Buuchanan Cr | 87.26 | 0 | 2.01 | 10.73 | 0 | 0 | 69 | 35 | 171 | 1.65 | 2.39 | 0.8 | 61 | 90 |
| Reedy Br | 88.31 | 0 | 8.3 | 3.16 | 0 | 0.23 | 57 | 36 | 159 | 1.67 | 2.17 | 0.9 | 16 | 88 |
| Reedy Br | 88.31 | 0 | 8.3 | 3.16 | 0 | 0.23 | 55 | 39 | 184 | 2.01 | 2.53 | 0.9 | 21 | 87 |
| UT Stone Mountain Cr | 86.2 | 0 | 10 | 3.5 | 0 | 0 | 55 | 32 | 155 | 1.7 | 2.76 | 0.5 | 51 | 76 |
| Upper Laurel Fk | 69.8 | 0 | 17.6 | 12.5 | 0 | 0 | 61 | 38 | 181 | 2.44 | 2.71 | 1.1 | 59 | 71 |
| George Gap Br | 79.7 | 0 | 12.2 | 8 | 0 | 0 | 64 | 34 | 142 | 2.05 | 2.87 | 0.7 | 121 | 74 |
| Greene Cr | 88.8 | 0 | 9.6 | 1.6 | 0 | 0 | 73 | 41 | 229 | 2.42 | 3.07 | 1.6 | 55 | 65 |
| Harper Cr | 84.87 | 0 | 7.69 | 7.05 | 0.14 | 0.25 | 56 | 26 | 82 | 2.68 | 3.62 | 1.3 | 38 | 80 |
| Ut Double Br | 99.51 | 0 | 0.49 | 0 | 0 | 0 | 47 | 21 | 102 | 2.21 | 2.93 | 0.3 | 30 | 84 |
| Cascade Cr | 97.52 | 0 | 1.68 | 0.42 | 0 | 0.38 | 37 | 18 | 72 | 1.18 | 2.8 | 1 | 13 | 92 |
| Poplar Cr | 98.3 | 0 | 0.6 | 1.11 | 0 | 0 | 84 | 40 | 185 | 2.13 | 2.98 | 1.2 | 27 | 83 |
| Hickory Cr | 89.71 | 0 | 8.18 | 2.1 | 0 | 0 | 72 | 39 | 163 | 2.89 | 3.49 | 1.2 | 47 | 84 |
| Hickory Cr | 89.71 | 0 | 8.18 | 2.1 | 0 | 0 | 69 | 37 | 173 | 3.18 | 3.54 | 1.2 | 43 | 75 |
| Ut Mill Cr | 92.96 | 0 | 5.1 | 1.84 | 0.09 | 0 | 72 | 46 | 244 | 2.79 | 2.99 | 1.4 | 28 | 87 |
| Ut Lower Little R | 95.3 | 0 | 2.37 | 2.33 | 0 | 0 | 68 | 37 | 176 | 2.34 | 2.97 | 1.5 | 22 | 78 |
| Indian Cr | 96.4 | 0.83 | 1.1 | 1.61 | 0 | 0.06 | 64 | 37 | 156 | 1.79 | 2.39 | 1.7 | 14 | 92 |
| Wood Benton Cr | 66.91 | 0 | 30.25 | 2.15 | 0 | 0.68 | 74 | 40 | 205 | 2.87 | 3.51 | 1.8 | 57 | 77 |
| Lambert Fk | 98.67 | 0 | 1.15 | 0.18 | 0 | 0 | 65 | 29 | 128 | 2.45 | 3.42 | 1.8 | 29 | 81 |
| Lynn Br | 83.46 | 0 | 14.38 | 2.14 | 0.02 | 0 | 73 | 39 | 155 | 3.25 | 3.75 | 2 | 58 | 74 |
| Racoon Cr | 76.21 | 0.03 | 21.83 | 1.79 | 0 | 0.13 | 73 | 41 | 223 | 3.03 | 3.66 | 2.1 | 63 | 84 |
| Garrison Cr | 96.73 | 0 | 1.85 | 1.41 | 0 | 0 | 72 | 41 | 176 | 2.29 | 2.72 | 1.4 | 18 | 86 |
| Garrison Cr | 96.73 | 0 | 1.85 | 1.41 | 0 | 0 | 55 | 36 | 112 | 1.46 | 2.12 | 1.4 | 16 | 80 |
| Reedy Br | 88.31 | 0 | 8.3 | 0 | 0 | 0.23 | 63 | 32 | 167 | 1.78 | 2.67 | 0.9 | 18 | 90 |
| Laurel Br | 98.86 | 0 | 0.17 | 0.96 | 0 | 0 | 51 | 28 | 136 | 1.47 | 2.5 | 1 | 9 | 81 |
| Big Bearpen Br | 96.97 | 0 | 0 | 3.03 | 0 | 0 | 92 | 44 | 266 | 1.24 | 2.51 | 1 | 12 | 91 |
| Boyd Br | 99.35 | 0 | 0 | 0.65 | 0 | 0 | 53 | 33 | 154 | 2.2 | 2.64 | 1 | 19 | 79 |
| Bad Fk | 95.21 | 0 | 0 | 4.79 | 0 | 0 | 64 | 40 | 180 | 2.08 | 2.5 | 2.7 | 13 | 92 |
| Lower Cr | 98.84 | 0 | 0 | 1.16 | 0 | 0 | 77 | 46 | 261 | 1.94 | 2.29 | 1.6 | 9 | 97 |
| Singe Cat Br | 100 | 0 | 0 | 0 | 0 | 0 | 68 | 33 | 166 | 1.1 | 2.16 | 0.8 | 23 | 93 |
| Singe Cat Br | 100 | 0 | 0 | 0 | 0 | 0 | 49 | 32 | 131 | 1.51 | 1.9 | 0.8 | 24 | 95 |
| Log Hollow Br | 96.45 | 0 | 0.35 | 3.19 | 0 | 0 | 71 | 44 | 211 | 1.22 | 1.91 | 0.8 | 10 | 94 |
| Stone Mountain Cr | 91.4 | 0 | 5.3 | 3 | 0 | 0 | 62 | 39 | 216 | 1.96 | 2.38 | 0.9 | 34 | 78 |
| Bartlett Cr | 84.13 | 0 | 2.16 | 13.7 | 0 | 0 | 61 | 28 | 138 | 0.83 | 1.94 | 0.3 | 46 | 67 |
| Bartlett Cr | 84.13 | 0 | 2.16 | 13.7 | 0 | 0 | 50 | 23 | 113 | 1.05 | 2.16 | 0.3 | 45 | 84 |
| Roses Cr | 98.79 | 0 | 0.02 | 1.19 | 0 | 0 | 64 | 41 | 169 | 1.75 | 2.71 | 1.8 | 22 | 85 |
| Roses Cr | 98.79 | 0 | 0.02 | 1.19 | 0 | 0 | 52 | 30 | 151 | 1.85 | 2.35 | 1.8 | 23 | 84 |
| Bee Rock Cr | 98.51 | 0 | 0.16 | 1.33 | 0 | 0 | 59 | 36 | 191 | 1.33 | 2.12 | 1.6 | 23 | 89 |
| Bee Rock Cr | 98.51 | 0 | 0.16 | 1.33 | 0 | 0 | 66 | 40 | 180 | 2.1 | 2.6 | 1.6 | 21 | 89 |
| Bee Rock Cr | 98.51 | 0 | 0.16 | 1.33 | 0 | 0 | 67 | 41 | 202 | 1.66 | 2.35 | 1.6 | 22 | 95 |
| Pepper Cr* | 89.7 | 0 | 4.71 | 5.58 | 0 | 0 | 58 | 32 | 155 | 1.4 | 2.23 | 1 | 60 | 77 |
| Cow Cr | 98.18 | 0 | 0 | 1.82 | 0 | 0 | 66 | 37 | 171 | 1.39 | 2.38 | 1.2 | 27 | 98 |
| **MEANS** | **91.9** | **0.02** | **4.9** | **3.1** | **0** | **0** | **64** | **36** | **170** | **1.9** | **2.67** | **1.18** | **31.8** | **84.1** |

**Sites in Blue Indicate "Foothills" data. See Section 13.0.**

**(9.0) Results and Discussion: "Foothills" Versus Piedmont, and Mountain Data**

One of the only disadvantages in using level III and level IV ecoregion data occurs when the coordinates for a given stream physically places it in a particular level III (e.g., Piedmont) and level IV (e.g., Northern Inner Piedmont) ecoregion yet the vast majority of its watershed actually lies in a different level III (e.g., Mountain) and level IV (e.g., Southern Crystalline Ridges and Mountains) ecoregion. This scenario often causes difficulty in choosing the correct biocriteria thresholds for assessing the stream as defensible arguments can be made for using either Mountain or Piedmont criteria in this instance (NCDWQ 2006). A similar dilemma has been found for a small subset of sites during the course of this study. Unfortunately, this can be a crucial decision since in North Carolina benthic macroinvertebrate metrics have been demonstrated to vary substantially between ecoregions (Lenat 1993).

A small portion of the reference streams sampled for this study were arbitrarily deemed as "foothills" sites for the fact that they were located very close to the level III Mountain ecoregion even though the actual sampling point was located in a level III Piedmont ecoregion (Figure 38). Similarly, other sites were physically located in a level III Mountain ecoregion but were surrounded almost entirely by level III Piedmont (Figure 38). Additionally, some of these foothills sites were physically located in the level III Piedmont ecoregion (level IV Northern Inner Piedmont) but portions (to varying degrees) of these streams' respective watersheds actually included a different level III (Mountain) and level IV ecoregion (Sauratown Mountains; Figure 39). These streams include Cascade Creek and Indian Creek. The remaining foothills sites (Garrison Creek, UT Double Branch, Lambert Fork, Poplar Creek, and Robinette Creek) are both physically located (and whose entire catchment is contained within) the Mountain level III ecoregion (Eastern Blue Ridge Foothills level IV ecoregion) but which are actually encompassed almost entirely by level III Piedmont ecoregion and also have lower elevations relative to other Mountain level III and level IV ecoregion (e.g., Southern Crystalline Ridges and Mountains; Figure 38, Figure 39, Table 12). While these sites are technically located in the level III Mountain ecoregion their extremely close proximity to the level III Piedmont ecoregion make these sites function as ecotonal transitional zones between the Piedmont and Mountains. For example, all of the foothills sites contained core Mountain taxa, and yet some of their BI scores group closer to the Piedmont reference BI data, while others group closer to the Mountain reference data (Figure 40, Table 12). In addition, the foothill reference sites (level IV Ecoregions: Sauratown Mountains, Eastern Foothills, Northern Inner Piedmont; Griffith et al. 2002) all have lower elevations versus sites located within the (level III Mountain) level IV Southern Crystalline Mountains and Ridges, and Southern Metasedimentary Mountains (Figure 40, Table 12; Griffith et al. 2002). This elevation difference suggests why most of the foothills (Sauratown Mountains, Northern Inner Piedmont, Eastern Foothills) reference level IV Piedmont sites have higher BI scores relative to the Southern Crystalline Mountains and Ridges, and Southern Metasedimentary Mountains level IV Mountain reference sites (Figure 40, Table 12), while their close proximity to these level III Mountain ecoregions explain the presence of core Mountain taxa. This elevation gradient also explains why the foothills sites have lower BI scores relative to other (non-foothills) level III and level IV Piedmont data (Figure 42, Table 12). This hypothesis is strongly supported by the data presented in Figure 42, which demonstrates a strong relationship between elevation and biotic index and is consistent with past findings (Eaton and Lenat 1991, Lenat 1993, Cuffney et al. 1997, Cuffney et al. 2000, NCDWQ 2006, Carlisle et al. 2008).

Although valid arguments can be made for placing reference foothills sites with either the Piedmont or Mountains reference dataset, the most logical place for these transitional data is with the Mountains reference data. This is recommended primarily due to the fact that if core Mountain taxa are present in a sample, regardless of where that sample was obtained, it is (in nearly all instances) currently rated using Mountain criteria (NCDWQ 2006). The only exceptions to this practice are extreme disjunct occurrences of *Drunella walkeri* in Coastal Plain reaches of the lower Tar River (Edgecombe and Nash Counties) and *Ceratopsyche sparna* and *Epeorus rubidus* from streams located in close proximity to the Uwharrie Mountains in Montgomery and Stanly Counties in the Piedmont. Therefore, since the foothills sites evaluated for this research all contained core Mountain taxa, were all located very close to or actually within level III Mountain ecoregions (and would therefore be rated using Mountain criteria as per NCDWQ 2006), and had BI values most similar to the Mountain values, the foothills data were ultimately deposited within the Mountain dataset.

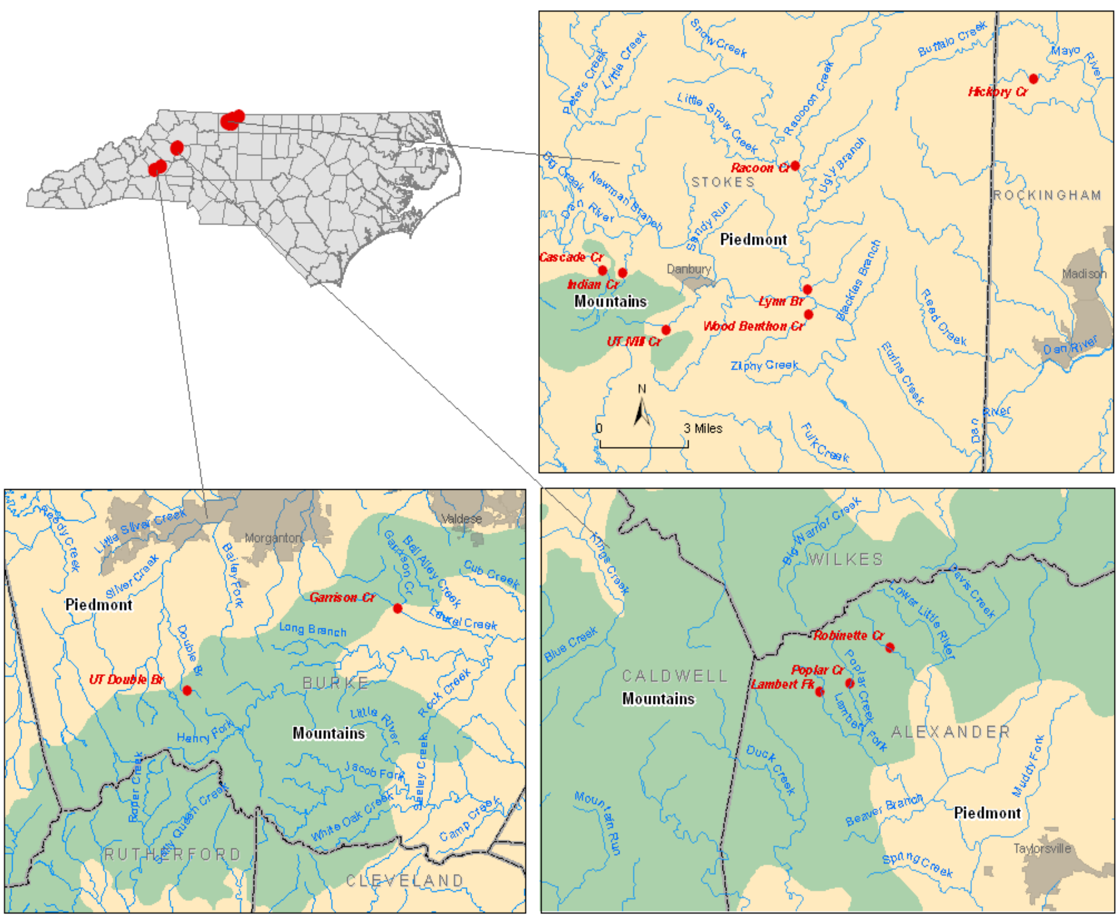



**Figure 38. Level III Ecoregions and "Foothills" Small Stream Study Sites.**

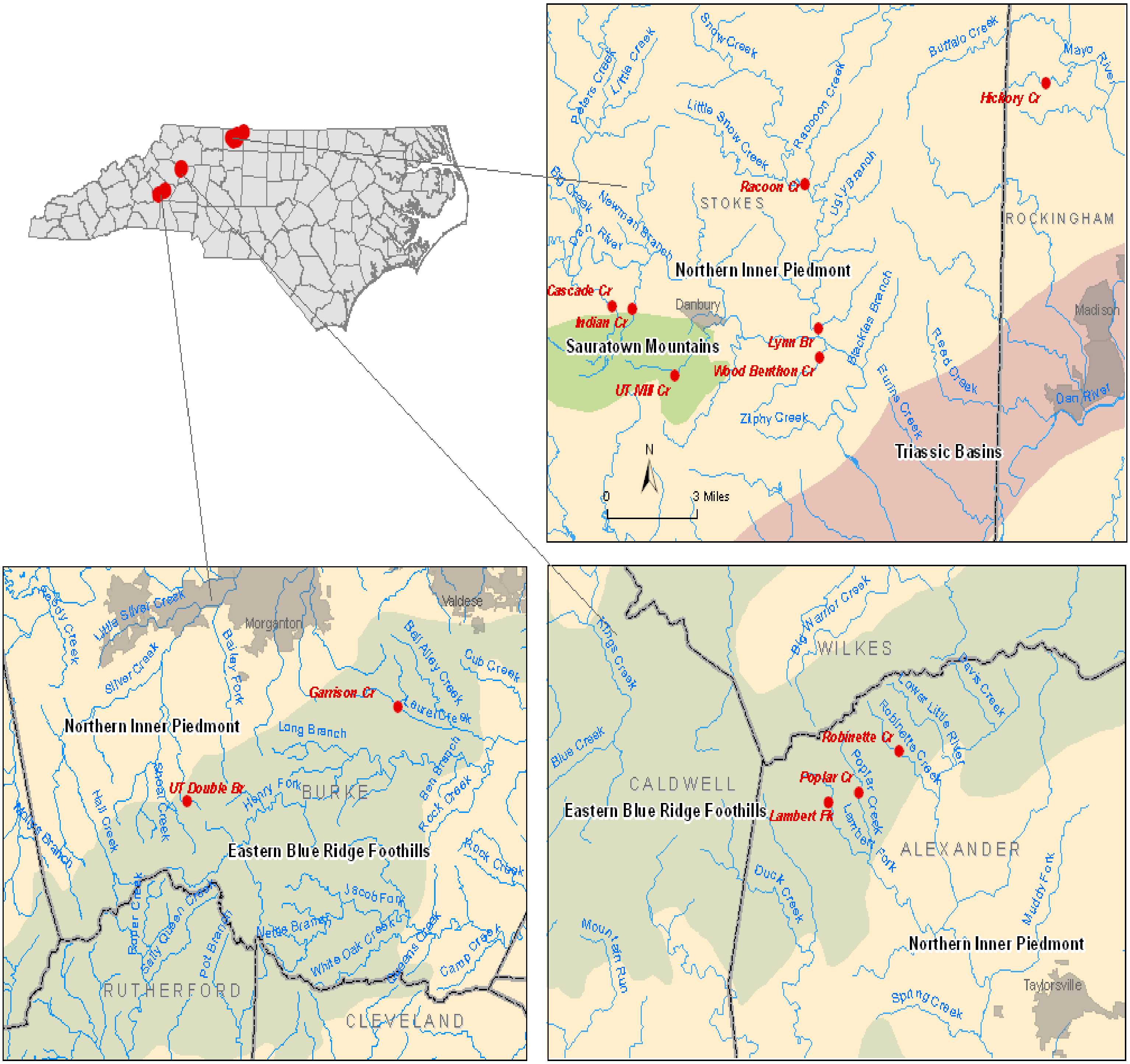



**Figure 39. Level IV Ecoregions and "Foothills" Small Stream Study Sites.**

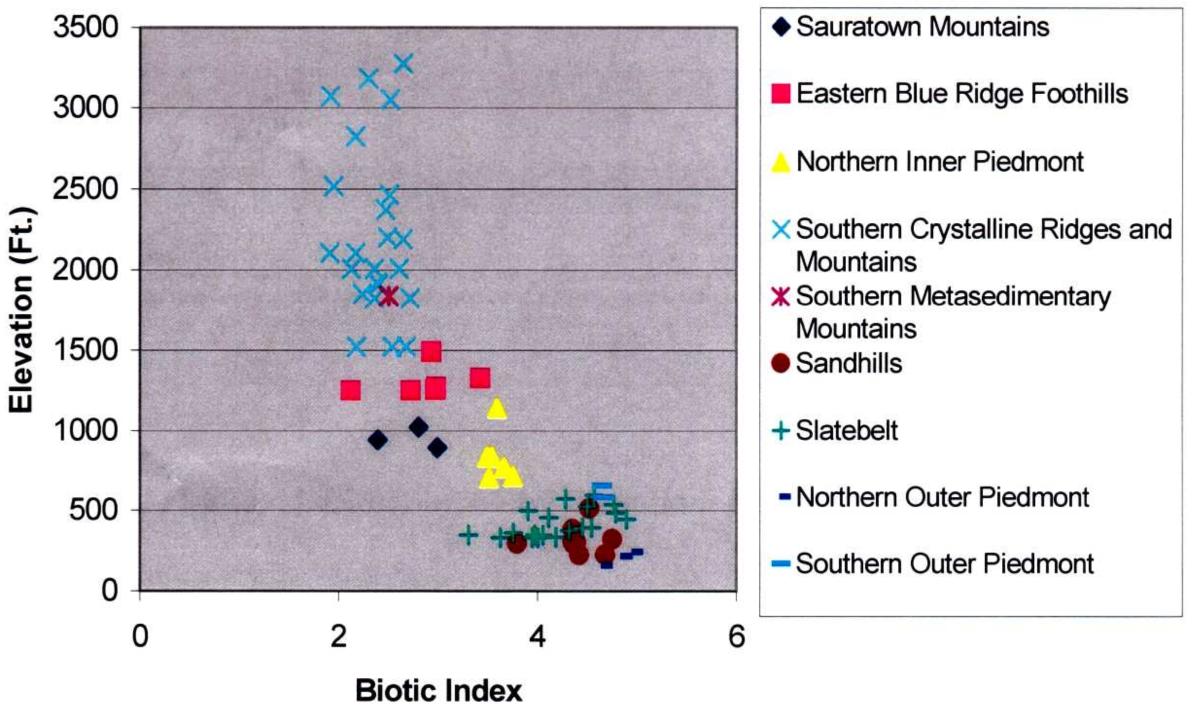
Table 12. Level IV and Level III Reference Elevation and Biotic Index Data.

| Stream | Level III Ecoregions | Level IV Ecoregions | County | Elevation (Ft.) | BI |
|---|---|---|---|---|---|
| **"Foothills"** | | | | | |
| Cascade Cr | Piedmont | Sauratown Mountains | Stokes | 1018 | 2.8 |
| UT Mill Cr | Piedmont | Sauratown Mountains | Stokes | 891 | 2.99 |
| Indian Cr | Piedmont | Sauratown Mountains | Stokes | 940 | 2.39 |
| UT Double Br | Mountain | Eastern Blue Ridge Foothills | Burke | 1487 | 2.93 |
| Poplar Cr | Mountain | Eastern Blue Ridge Foothills | Alexander | 1266 | 2.98 |
| Robinette Cr | Mountain | Eastern Blue Ridge Foothills | Alexander | 1,250 | 2.97 |
| Garrison Cr | Mountain | Eastern Blue Ridge Foothills | Burke | 1,244 | 2.72 |
| Garrison Cr | Mountain | Eastern Blue Ridge Foothills | Burke | 1,244 | 2.12 |
| Lambert Fk | Mountain | Eastern Blue Ridge Foothills | Alexander | 1,324 | 3.42 |
| Lynn Br | Piedmont | Northern Inner Piedmont | Stokes | 710 | 3.75 |
| Racoon Cr | Piedmont | Northern Inner Piedmont | Stokes | 769 | 3.66 |
| Hickory Cr | Piedmont | Northern Inner Piedmont | Rockingham | 834 | 3.49 |
| Hickory Cr | Piedmont | Northern Inner Piedmont | Rockingham | 834 | 3.54 |
| Wood Benthon Cr | Piedmont | Northern Inner Piedmont | Stokes | 704 | 3.51 |
| UT Dobbins Cr | Piedmont | Northern Inner Piedmont | Yadkin | 1,139 | 3.59 |
| **Mountains** | | | | | |
| UT Wash Cr | Mountain | Southern Crystalline Ridges and Mountains | Henderson | 2,373 | 2.46 |
| Ledford Br | Mountain | Southern Crystalline Ridges and Mountains | Buncombe | 2,200 | 2.48 |
| Bearwallow Br | Mountain | Southern Crystalline Ridges and Mountains | Transylvania | 3,272 | 2.64 |
| Singe Cat Br | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 2,104 | 1.9 |
| Singe Cat Br | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 2,104 | 2.16 |
| Buchanan Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,910 | 2.39 |
| Log Hollow Br | Mountain | Southern Crystalline Ridges and Mountains | Transylvania | 3,068 | 1.91 |
| Reedy Br | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,520 | 2.17 |
| Reedy Br | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,520 | 2.53 |
| Reedy Br | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,520 | 2.67 |
| Big Bearpen Br | Mountain | Southern Crystalline Ridges and Mountains | Transylvania | 3,047 | 2.51 |
| Boyd Br | Mountain | Southern Crystalline Ridges and Mountains | Buncombe | 2,191 | 2.64 |
| Pepper Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,851 | 2.23 |
| Cow Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,923 | 2.38 |
| Bee Rock Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 2,009 | 2.12 |
| Bee Rock Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 2,009 | 2.6 |
| Bee Rock Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 2,009 | 2.35 |
| Lower Cr | Mountain | Southern Crystalline Ridges and Mountains | Yancey | 3,177 | 2.29 |

**Table 12. Level IV and Level III Reference Elevation and Biotic Index Data.**

| Stream | Level III Ecoregions | Level IV Ecoregions | County | Elevation (ft) | BI |
|---|---|---|---|---|---|
| Roses Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,825 | 2.71 |
| Roses Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,825 | 2.35 |
| Bad Fk | Mountain | Southern Crystalline Ridges and Mountains | Henderson | 2,464 | 2.5 |
| Bartlett Cr | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 2,518 | 1.94 |
| Stone Mountain Cr | Mountain | Southern Crystalline Ridges and Mountains | Watauga | 2,824 | 2.16 |
| Laurel Br | Mountain | Southern Crystalline Ridges and Mountains | McDowell | 1,835 | 2.5 |
| **Piedmont** | | | | | |
| North Prong Anderson Cr | Piedmont | Sandhills | Harnett | 221 | 4.42 |
| North Prong Anderson Cr | Piedmont | Sandhills | Harnett | 221 | 4.68 |
| Big Branch | Piedmont | Sandhills | Richmond | 317 | 4.75 |
| Millstone Cr | Piedmont | Sandhills | Richmond | 379 | 4.35 |
| UT Drowning Cr | Piedmont | Sandhills | Moore | 510 | 4.52 |
| W Pr Juniper Br | Piedmont | Sandhills | Scotland | 300 | 4.39 |
| UT Hitchcock Cr | Piedmont | Sandhills | Richmond | 304 | 4.35 |
| Joes Cr | Piedmont | Sandhills | Richmond | 292 | 3.79 |
| Joes Cr | Piedmont | Sandhills | Richmond | 292 | 4.36 |
| Bones Fk Cr | Piedmont | Sandhills | Richmond | 327 | 4.37 |
| Arnett Branch | Piedmont | Carolina Slate Belt | Montgomery | 520 | 4.5 |
| Hogpen Br | Piedmont | Carolina Slate Belt | Montgomery | 354 | 3.75 |
| UT Drowning Cr | Piedmont | Carolina Slate Belt | Montgomery | 492 | 3.9 |
| Little Cr | Piedmont | Carolina Slate Belt | Montgomery | 480 | 4.78 |
| Poisin Fk | Piedmont | Carolina Slate Belt | Montgomery | 450 | 4.11 |
| Dutchmans Cr | Piedmont | Carolina Slate Belt | Montgomery | 341 | 3.97 |
| Dutchmans Cr | Piedmont | Carolina Slate Belt | Montgomery | 341 | 3.3 |
| Wood Run | Piedmont | Carolina Slate Belt | Montgomery | 326 | 4 |
| Wood Run | Piedmont | Carolina Slate Belt | Montgomery | 326 | 3.94 |
| Wood Run | Piedmont | Carolina Slate Belt | Montgomery | 326 | 3.62 |
| Little Dumas Cr | Piedmont | Carolina Slate Belt | Montgomery | 567 | 4.28 |
| Crooked Fork | Piedmont | Carolina Slate Belt | Person | 531 | 4.76 |
| Dial Creek | Piedmont | Carolina Slate Belt | Durham | 440 | 4.89 |
| Iron Hill Branch | Piedmont | Carolina Slate Belt | Montgomery | 370 | 4.32 |
| Horsepen Branch | Piedmont | Carolina Slate Belt | Montgomery | 328 | 4.18 |
| West Branch Maclean Cr | Piedmont | Carolina Slate Belt | Montgomery | 388 | 4.45 |
| West Branch Maclean Cr | Piedmont | Carolina Slate Belt | Montgomery | 388 | 4.54 |
| Moccasin Cr | Piedmont | Carolina Slate Belt | Montgomery | 342 | 4.05 |
| UT Talbots Cr | Piedmont | Carolina Slate Belt | Randolph | 592 | 4.57 |
| UT Bear Swamp | Piedmont | Northern Outer Piedmont | Halifax | 153 | 4.65 |
| Hubquarter Creek | Piedmont | Northern Outer Piedmont | Warren | 236 | 4.96 |
| Jordan Cr | Piedmont | Northern Outer Piedmont | Warren | 210 | 4.85 |
| Tanyard Creek | Piedmont | Southern Outer Piedmont | Davidson | 652 | 4.64 |
| Negro Creek | Piedmont | Southern Outer Piedmont | Caswell | 577 | 4.66 |

**Figure 40. Biotic Index by Elevation: Level IV Ecoregions of the Mountains and Piedmont.**




**(10.0) Ecoregions Effects**

Ecoregions generally denote areas of similarity in ecosystems as well as the type, quality, and quantity of environmental resources (Griffith et al. 2002). Ecoregions are designed to function as a spatial framework for research, assessment, management, and monitoring of ecosystems and components of ecosystems (Griffith et al. 2002). By classifying the spatial differences in the capacities and potential of ecosystems, ecoregions stratify the environment by its probable response to disturbance (Bryce et al. 1999). These general purpose regions are critical for structuring and implementing ecosystem management strategies across federal agencies, state agencies, and nongovernmental organizations that are responsible for different types of resources within the same geographical areas (Omernik et al. 2000). A Roman numeral hierarchical system has been developed for different levels of ecological regions. Level I is the coarsest level dividing North America into 15 ecological regions, level II divides the continent into 52 regions, while level III further divides the continent into 104 ecoregions (Griffith et al. 2002). The level IV ecoregions division is the finest scale and in North Carolina there are 27 level IV ecoregions (Griffith et al. 2002). Previous work in larger North Carolina streams (generally drainage areas greater than 3.0mi$^2$) demonstrated a significant difference in bioclassification thresholds between the Mountain level III, Piedmont level III, and Coastal Plain level III Ecoregions (Lenat 1993). For example, minimum Excellent bioclassification thresholds (based on the BI) in the Mountain level III Ecoregions must be less than 4.00, while minimum Excellent thresholds in the Piedmont level III must be less than 5.14, and in the Coastal Plain level III the minimum is 5.42 (Lenat 1993, NCDWQ, 2006). Relative to the BI scores from larger streams, minimum Excellent BI thresholds in the Mountain level III Ecoregions for small streams were lower (less than or equal to 3.75), and this was also the case for the Piedmont study sites (less than or equal to 4.36). As previous investigations in North Carolina and the southeast have demonstrated, the BI (Lenat 1993) and invertebrate assemblages in general (Feminella 2000) vary substantially by level III Ecoregion. Underlying drivers of this pattern are likely a combination of temperature and elevation (Lenat 1993, Poff 1997, Vinson and Hawkins 1998, Cuffney et al. 2000, Feminella 2000, Hawkins et al. 2000, Carlisle et al. 2008). These findings generally support the physical conditions observed at most of the Mountain and Piedmont reference streams sampled for this study. In general, and particularly in the Mountains, these small headwater streams were 1-2 meters in width with an enclosed (or largely so) riparian canopy. This intense shading, coupled with the naturally close proximity of these headwater streams to their (cooler in temperature) groundwater sources (Alexander et al. 2007) would help explain the lower minimum BI score thresholds established for small Piedmont and Mountain streams relative to thresholds for larger streams in these ecoregions.

## (11.0) Seasonal Effects

### Piedmont

All of the Piedmont sites were taken from the reference dataset. Wood Run was sampled on three occasions: once in early April, once in early May, and once again in early June. As Figures 53-54 depict, there were no substantial changes in the invertebrate community and there were no changes in the bioclassification between sampling events at this location. West Branch Maclean Creek (Figure 49-50), and Dutchmans Creek (Figure 47-48) were each sampled in early May and then again in early June and there were no changes in bioclassification noted and little variation among the BMI metrics from these months. Hickory Creek (Figure 51-52), Garrison Creek (Figure 45-46), and North Prong Anderson Creek (Figure 43-44) were sampled in early April and then again in early May with no change in bioclassification measured between sampling events and little change among the BMI metrics through time. Jordan Creek was sampled in late April and then again in early June. As was the case with all other Piedmont sites sampled for seasonal effects, there were no changes in bioclassification and little overall change in the BMI metrics temporally (Figure 41-42).

### Mountain

Five Mountain reference sites were examined for seasonal effects: Reedy Branch (Figure 61-62), Singe Cat Branch (Figure 55-56), Bartlett Creek (Figure 57-58), Roses Creek (Figure 59-60), and Bee Rock Creek (Figure 63-64). Singe Cat Branch, Bartlett Creek, and Roses Creek were all sampled in late April and then again two months later in late June and there were no substantial alterations in the invertebrate communities and (with the sole exception of the 6/21/2005 sample at Roses Creek) there were no changes in bioclassification between months at these sites. The 6/21/2005 Roses Creek sample produced a BI of 2.71 and was therefore just 0.01 over the proposed minimum threshold (2.70) for an Excellent bioclassification for Mountain sites. Reedy Branch and Bee Rock

Creek were each sampled on three occasions: once in late April, once in late May, and once again in late June. The results of these samples further indicate a stable invertebrate community and none of these samples changed bioclassification.

Overall, the Mountain and Piedmont sites exhibited very little change in community metrics between the months examined and (with the exception of the Roses Creek sample which only exceeded the minimum Excellent Mountain bioclassification threshold by 0.01) there were no changes in bioclassification. In summary, there were a total of 11 sites from the Mountains and Piedmont sampled for temporal repeatability between the months of April, May, and June. Only one of these sites (Roses Creek, 6/21/2005) resulted in a different bioclassification from a previous sample. As a result, the temporal repeatability rate of this data set is 91.6%. Consistent temporal repeatability is an important requirement for the validation of biocriteria and previous standards of acceptable levels of temporal repeatability has been set at 90% for estuarine waters (Eaton 2001).

**Figures 41-54. Piedmont Seasonality Samples.**

**Figure 41.**

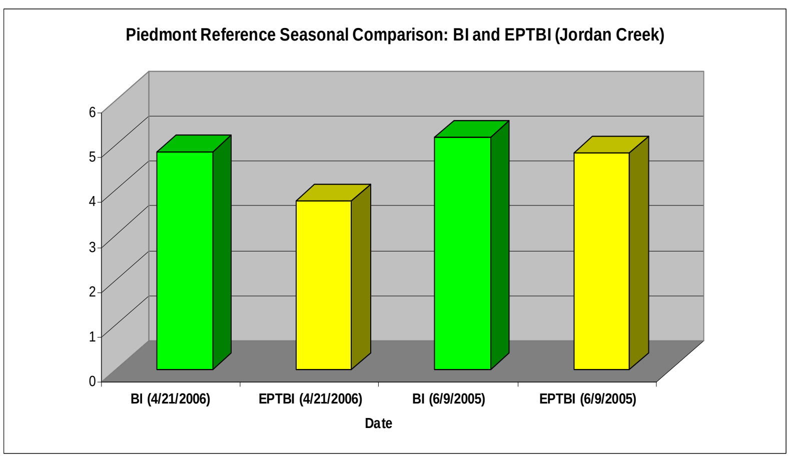
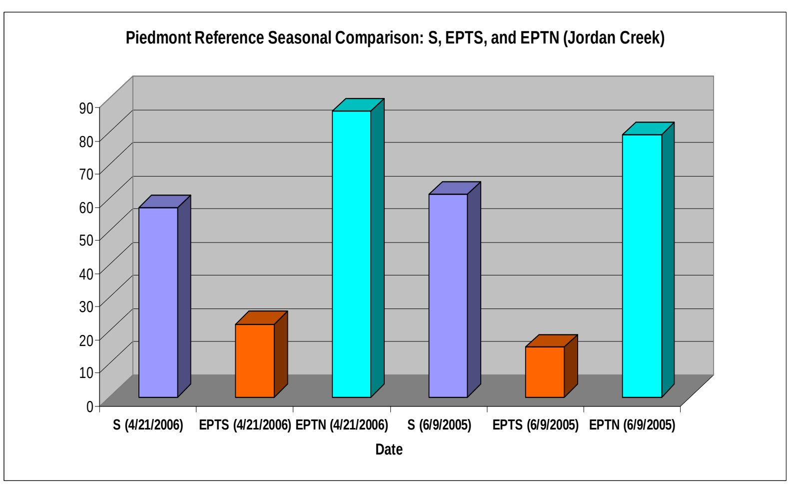



**Figure 42.**



## Figures 41-54. Piedmont Seasonality Samples (Continued).

**Figure 43.**

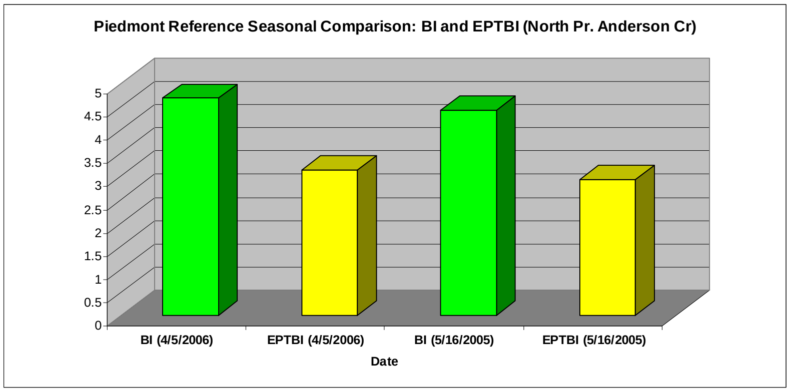



**Figure 44.**

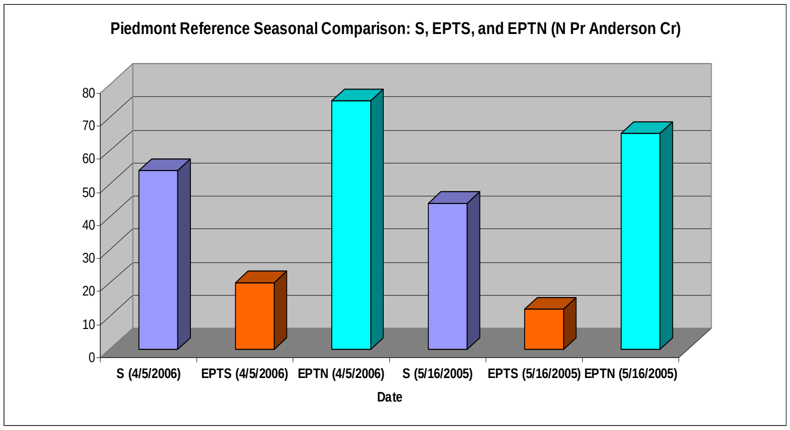



**Figure 45.**

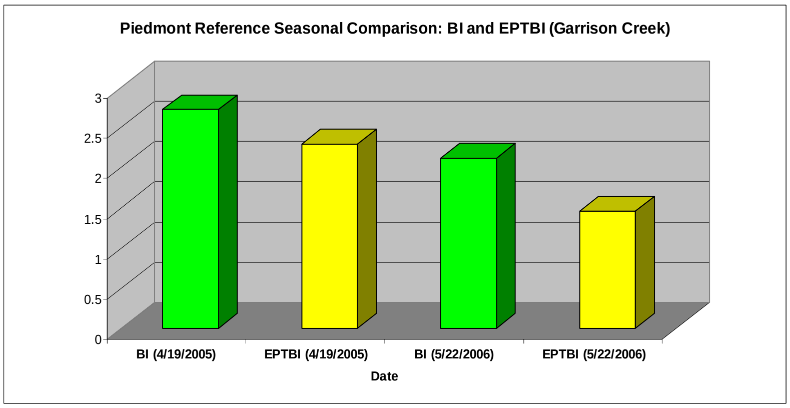
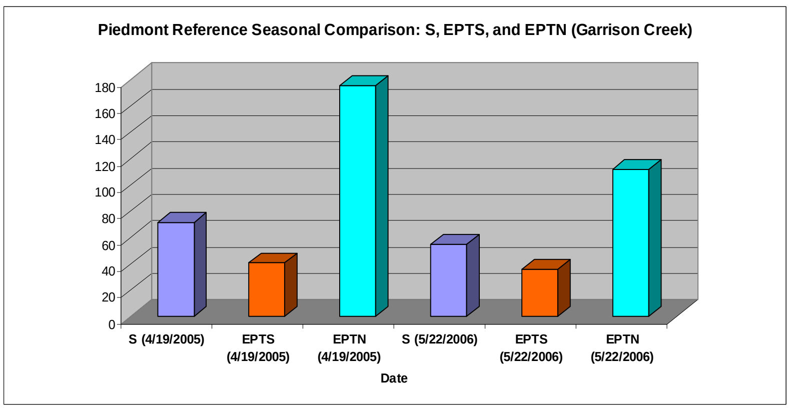



**Figure 46.**



## Figures 41-54. Piedmont Seasonality Samples (Continued).

Figure 47.

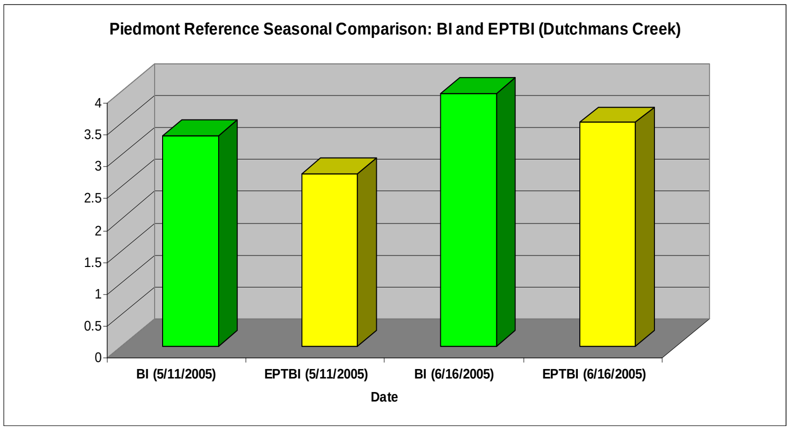



Figure 48.

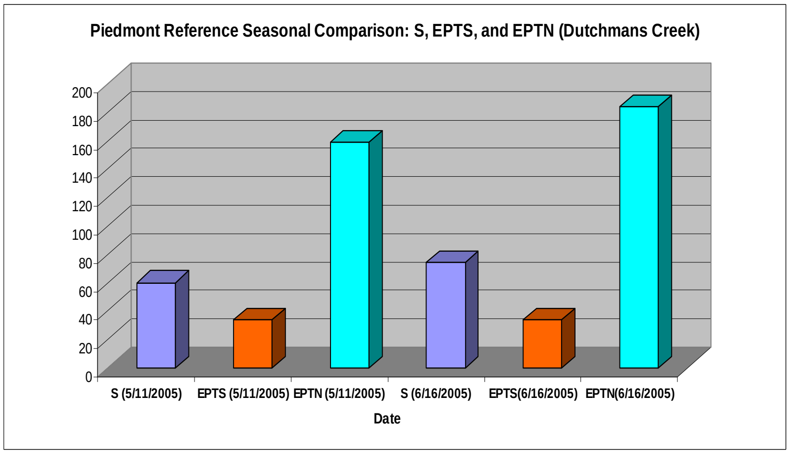



Figure 49.

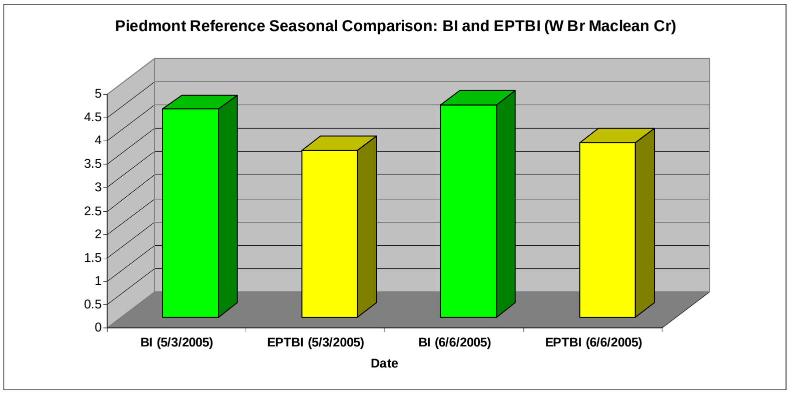
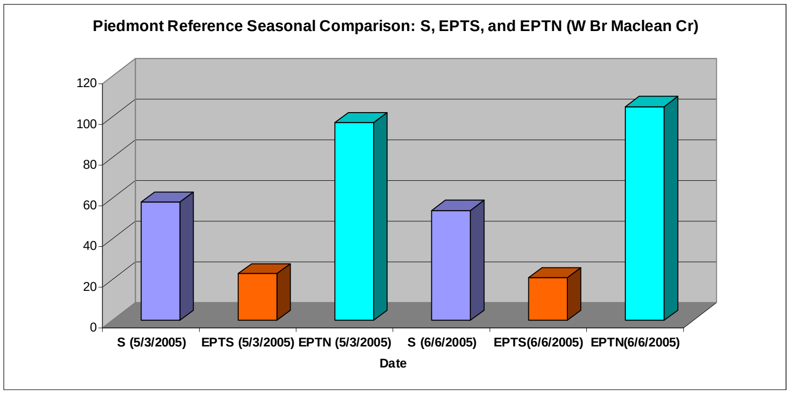



Figure 50.



## Figures 41-54. Piedmont Seasonality Samples (Continued).

Figure 51.

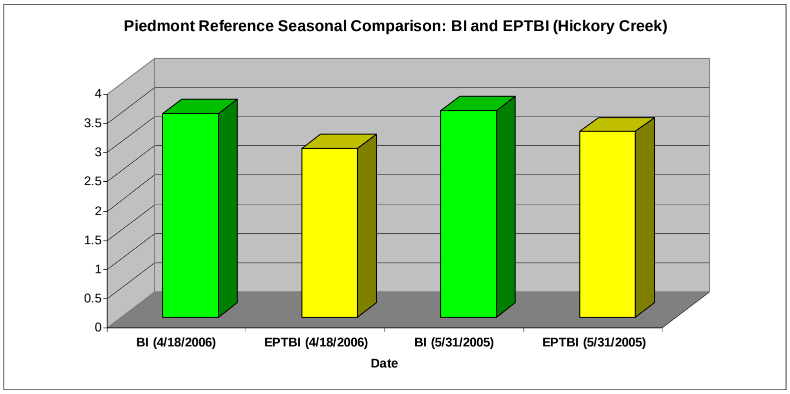



Figure 52.

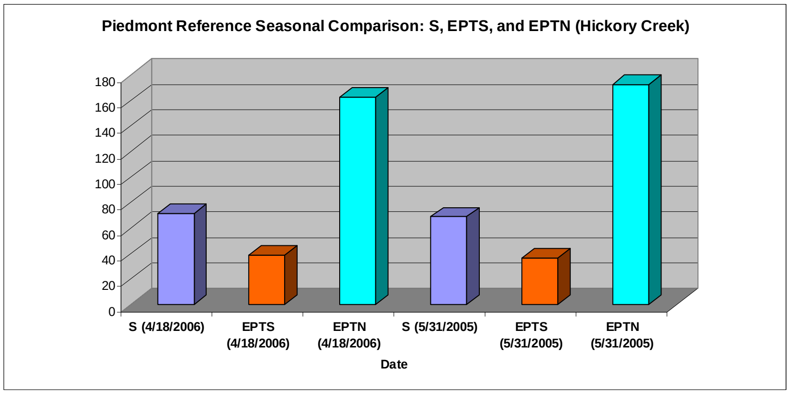



Figure 53.

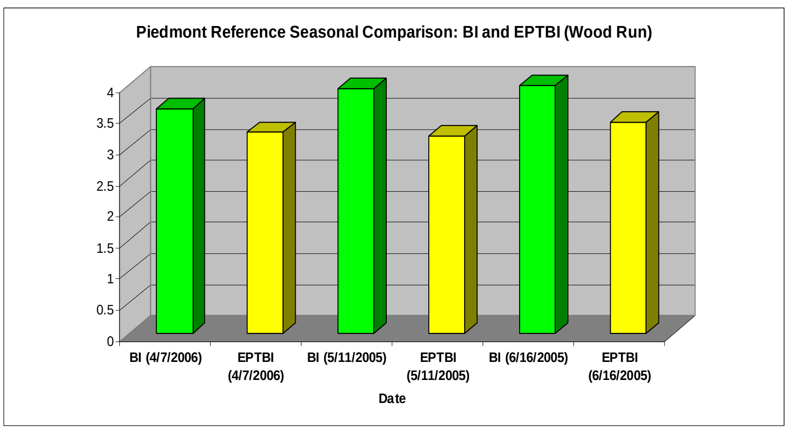
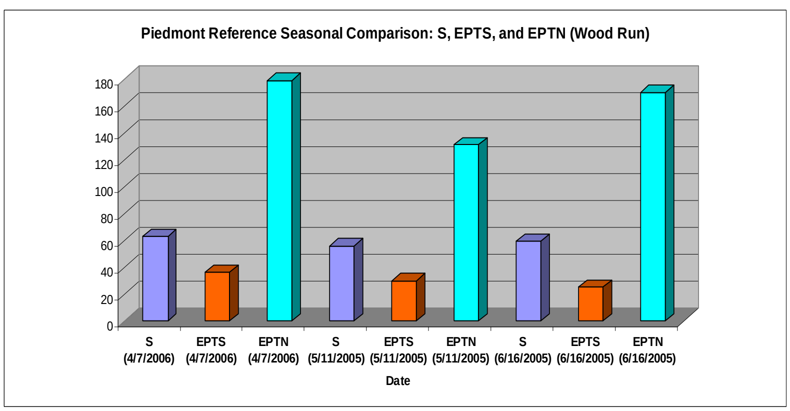



Figure 54.



## Figures 55-64. Mountain Seasonality Samples.

Figure 55.

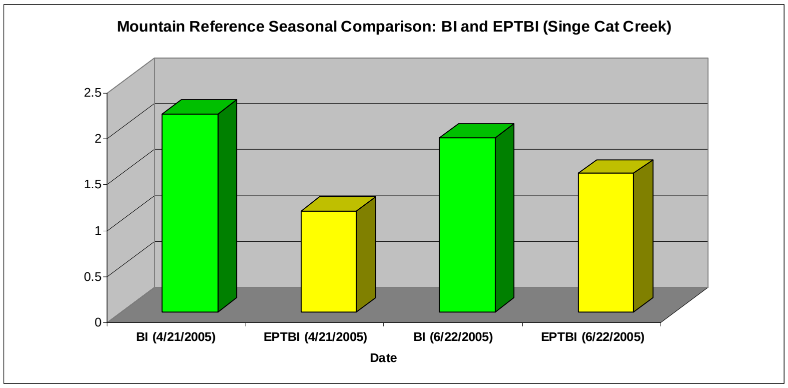



Figure 56.

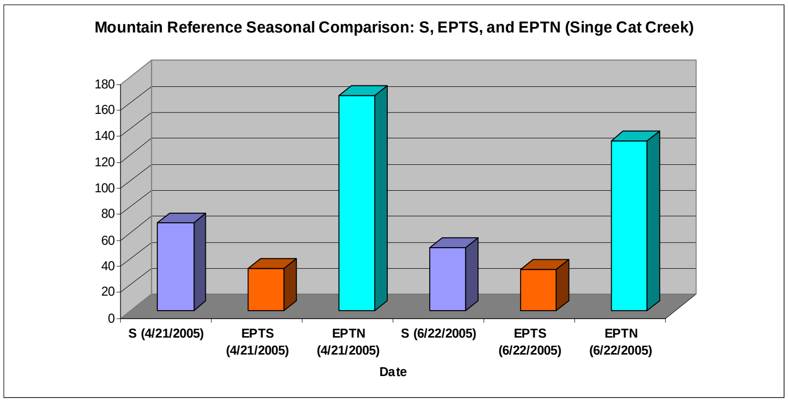



Figure 57.

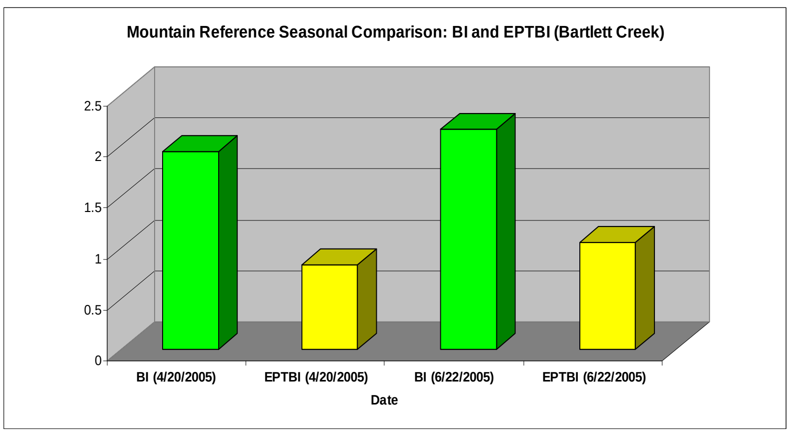
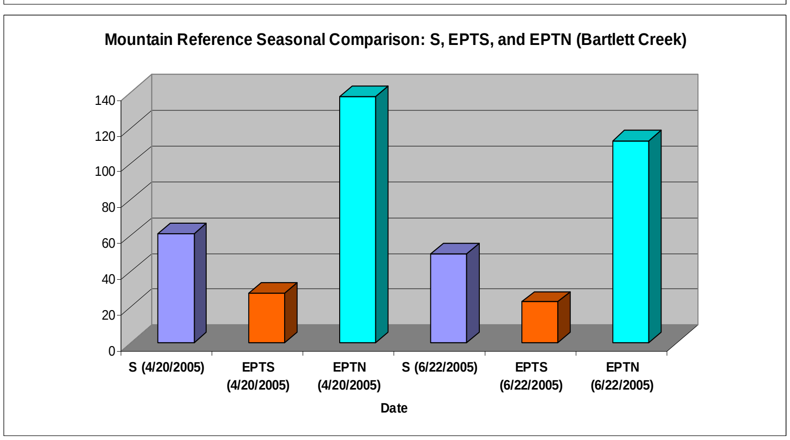



Figure 58.



## Figures 55-64. Mountain Seasonality Samples (Continued).

**Figure 59.**

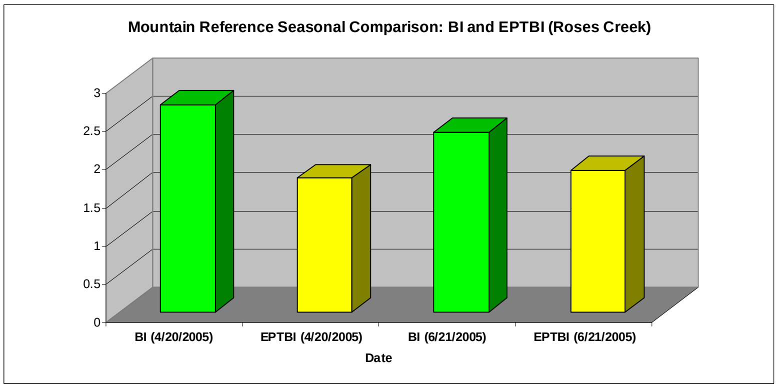



**Figure 60.**

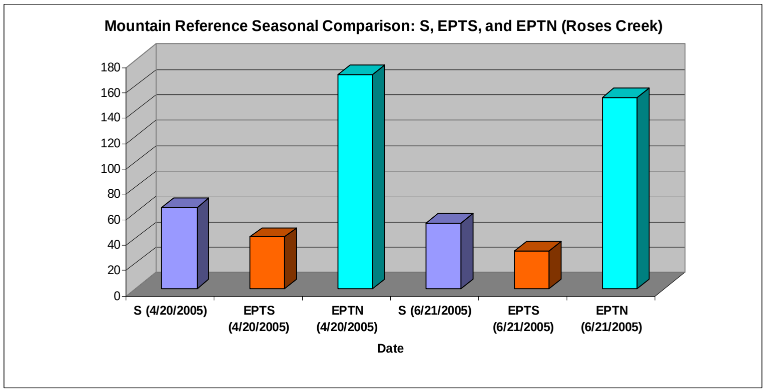



**Figure 61.**

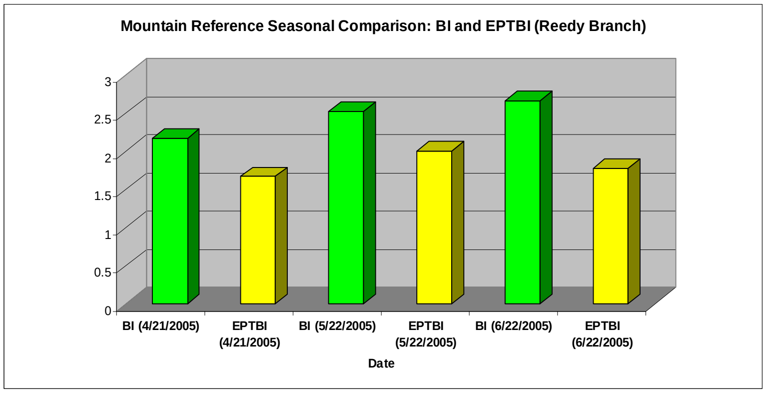
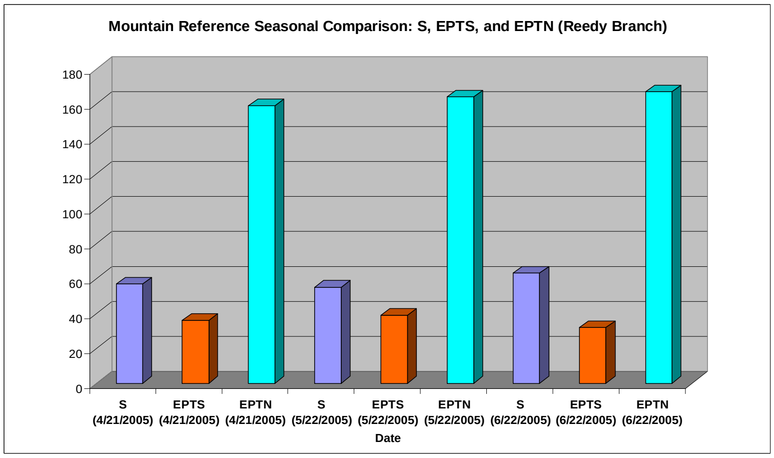



**Figure 62.**



**Figures 55-64. Mountain Seasonality Samples (Continued).**

**Figure 63.**

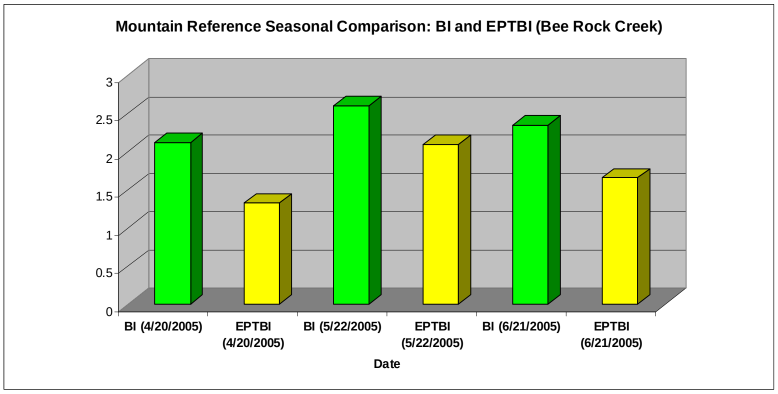
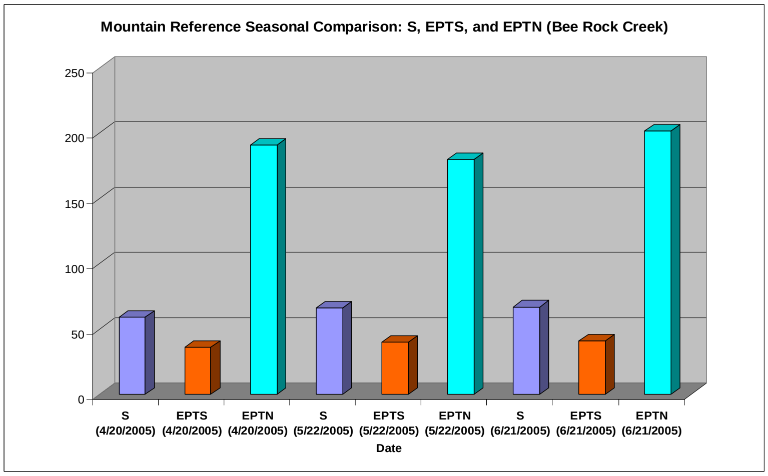



**Figure 64.**




## (12.0) Conclusions

Regardless of ecoregion or disturbance class, the BI had the lowest CV relative to the EPTS, EPTBI, EPTN, and S, and was also the most strongly correlated to two of the most important determinants on water quality and the biological integrity of benthic macroinvertebrate communities: percent forest/wetland and percent developed. In addition, the BI was essentially equivalent in performance to the EPTBI among Mountain sites in response to percent agriculture, percent sand, percent sand+silt, and habitat score, but significantly outperformed the EPTBI in the Mountains when it came to correlation to percent grass/pasture, ag+grass/pasture, and conductivity and in the Piedmont the BI was most strongly correlated to habitat and was comparable with the EPTBI in terms of correlation to conductivity. Moreover, the BI exhibited no overlap among the coarse or fine-scaled disturbance classes in the Mountains or Piedmont. Conversely, in both the Piedmont and Mountains, the S, EPTN, EPTS, and EPTBI demonstrated significant overlap between the landuse derived coarse-scale, and fine-scale disturbance classes. Consequently, of the five BMI metrics examined for this study only the BI was capable of reliably distinguishing between the worst and best sites, as well as between sites with only subtle differences in disturbance gradients.

**(13.0) Future Work**

Although this is a large dataset, it is not comprehensive. To this end, there are some disturbance classes within the level IV and level III ecoregions that are (to varying degrees of severity) somewhat underrepresented here. These would include (in approximate order of priority): 1) severe impact streams in the Mountains , 2) intermediate impact sites in the Mountains, 3)severe impact streams in the Sandhills level IV Ecoregions, and 4) reference Piedmont samples, particularly outside of the Uwharrie National Forest area. In terms of additional seasonal data, repeat sampling from a subset of reference Piedmont and Mountain sites in March and (to a lesser extent) June as well as July and August in an effort to expand the small streams sampling window. In addition, it should be the ultimate goal of this methodology to evaluate how these invertebrate communities vary throughout the calendar year so that these systems could eventually be sampled at anytime. While there was a good overall range of drainage areas sampled in this study, additional effort should be focused on streams that are < $0.3mi^2$. However, accurately finding such sites can be difficult due to poor map resolution and limitations to GIS-accuracy at these very small catchment scales.

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