

## ABSTRACT

Title of Thesis: STREAM MACROINVERTEBRATE COMMUNITIES IN  
URBANIZING WATERSHEDS IN MARYLAND, USA

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The conversion of agricultural landscapes into residential developments is a prominent form of land use change in Maryland. Macroinvertebrates were sampled in Maryland headwater streams, and used to test for differences in diversity and abundance between agricultural and developed sites. Macroinvertebrate richness was highest in the agricultural streams, possibly due to a negative relationship between richness and impervious surface cover. The nature of farming operations and their proximity to the stream may contribute to increased richness values.

In Chapter 2, I compare macroinvertebrate communities collected with single-habitat and multi-habitat sampling methods. Communities were compared using IBI scores, and the community variables comprising these IBI's. Several community variables differed between methods, however the IBI scores calculated using the two methods were strongly correlated. Single-habitat samples had a slightly stronger relationship with development, indicating that they may be better for monitoring important focal sites.

STREAM MACROINVERTEBRATE COMMUNITIES IN URBANIZING  
WATERSHEDS IN MARYLAND, USA

by

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## DEDICATION

This work is dedicated to Genevieve Elizabeth, life's next great adventure.

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**Chapter 1:** Changes in macroinvertebrate communities resulting from the development of agricultural landscapes

ABSTRACT

The conversion of agricultural lands into residential developments is currently one of the dominant patterns of land use change in the United States. Both agriculture and development have been shown to alter stream macroinvertebrate communities, but current research suggests that the impacts of urbanization may be more detrimental to stream health. I examined macroinvertebrate assemblages in Maryland, USA headwater streams to determine whether agricultural watersheds support more diverse communities than developed watersheds, and to identify specific factors that may be driving community changes. Of 124 macroinvertebrate taxa collected, 25 were found significantly more often at agricultural sites than developed sites, while no taxa were more tolerant of conditions in urbanizing watersheds. Macroinvertebrate richness (including total, EPT and feeding group variables) responded to land use characteristics, with agricultural streams sustaining the highest community diversity. Decreasing richness in developed sites may be a result of a strong negative relationship with impervious surface cover. Positive relationships between richness and riparian forest cover at highly developed sites suggest that this factor may help offset the harmful effects of urbanization. When compared to other published estimates of macroinvertebrate richness in agriculturally impacted streams, the agricultural sites in this study appear to be considerably more diverse. Possible reasons for the higher richness values in this study may be due to a greater proportion of riparian forest adjacent to our sampling reaches, and the absence of acute stressors caused by cultivation that affect many

agricultural areas. These overall findings indicate that the preservation of agricultural land from development may help conserve biodiversity, and that the nature of farming practices may contribute to relatively high diversity in these areas.

## INTRODUCTION

The expansion of human settlements both globally and in the United States has caused large portions of natural forested areas to be cleared for increasingly large and intensive agricultural operations (Richards 1990; Dale et al. 2000). This has been followed by the rapid expansion of urban developments into agricultural areas (Douglas 1994; Dale et al. 2000; Moglen 2000). It has recently been estimated that as much as 50% of global lands have now been transformed and/or impacted by human activities (Vitousek et al. 1997).

The ecological consequences of land use change can be severe, and are especially prevalent in lotic ecosystems, which integrate environmental impacts over large spatial scales (Karr and Chu 1999; Palmer et al. 2000; Nilsson et al. in press). Tilling practices in crop fields can lead to soil erosion (Kang et al. 2001), and runoff from these agricultural areas can cause large amounts of fine sediment deposition in nearby streams and rivers (Richter et al. 1997). While sediment deposition has been described as the most extensive type of agricultural stream pollution (Cooper 1993), drastically altered hydrology is a dominant problem affecting water quality in urbanizing watersheds (Paul and Meyer 2001; Palmer et al. 2002). Specifically, increased impervious surface cover associated with development causes greater peak flood discharge volumes, leading to changes in stream geomorphology and channel erosion (Booth and Jackson 1997).

Physical impacts resulting from land use change are often compounded by excessive inputs of chemical pollutants, such as nutrients and pesticides in agricultural systems (Schulz and Leiss 1999; Cuffney et al. 2000), or metals, oils and road salts from developed catchments (Whipple and Hunter 1979; Wilber and Hunter 1979; Howard and Haynes 1993).

These anthropogenic disturbances in stream habitats can cause dramatic ecological transformations, including changes in ecosystem processes (e.g. Young and Huryn 1999; Buffagni and Comin 2000; Gessner and Chauvet 2002) and community structure (e.g. Kerans and Karr 1994; Weaver and Garman 1994; Barbour et al. 1996). Alterations to macroinvertebrate assemblages have been the most extensively studied ecological response to human impacts in lotic environments (Paul and Meyer 2001). Land use change has been associated with decreases in macroinvertebrate diversity (Whiting and Clifford 1983; Thorne et al. 2000), decreases in overall abundance (Lenat and Crawford 1994), increases in the relative abundance of pollution tolerant taxa (Hall et al. 2001; Walsh et al. 2001), and changes in the distribution of invertebrate feeding groups (Lamberti and Berg 1995). These types of changes in macroinvertebrate community structure have been documented with the conversion of natural landscapes for both agricultural uses (Barton 1996; Rothrock et al. 1998; Genito et al. 2002), as well as residential development (Lenat and Crawford 1994; Morley and Karr 2002; Stepenuck et al. 2002).

Stream ecosystem impairment in response to land use change is often evaluated by comparing degraded streams with undisturbed reference sites in the same region (Reynoldson et al. 1997). However, finding streams within a limited geographical area

that have not been influenced by human activities can be difficult. In many regions of the United States, large tracts of land have been dominated by agriculture for much of the last two centuries (Dale et al. 2000), and the prevailing form of land use change is now the development of these agricultural areas (e.g. Riebsame et al. 1996). For example, forested land in most counties in the state of Maryland is significantly less than the combined area of agriculture and urban development, while urban development has increased at the expense of agriculture throughout Maryland during the 1990's (Moglen 2000).

This general pattern of land use change has caused agricultural areas to be the primary form of remaining undeveloped land in many regions of the country. This in turn has led to the establishment of agricultural land preservation programs to prevent urban sprawl and promote conservation in nearly every state of the U. S.

([www.farmlandinfo.org/fic](http://www.farmlandinfo.org/fic)). For instance, many state governments purchase conservation easements from private citizens for the permanent protection of large tracts of agricultural land. Three of the states contributing to the Chesapeake Bay watershed that have had agricultural easement programs for more than a decade (i.e. Pennsylvania, Maryland and Delaware) have combined to permanently preserve over 530,000 acres of agricultural land from more than 4000 privately owned farms

([www.agriculture.state.pa.us/farmland](http://www.agriculture.state.pa.us/farmland), [www.mda.state.md.us/geninfo/genera3.htm](http://www.mda.state.md.us/geninfo/genera3.htm), [www.state.de.us/deptagri/aglands/Indpres.htm](http://www.state.de.us/deptagri/aglands/Indpres.htm)). Many of these programs advocate the conservation value of agricultural preservation, yet ecological research in many areas has historically shown that farming practices can be detrimental to stream health (e.g. Barton 1996; Rothrock et al. 1998; Genito et al. 2002). It has only recently been suggested that

streams in urbanizing areas have compositionally different and less diverse communities when compared to assemblages in adjacent agricultural lands (Lenat and Crawford 1994; Wang et al. 2000; Stepenuck et al. 2002).

Determining whether agricultural land preservation has a conservation value for lotic ecosystems requires an explicit examination of the ecological differences between stream communities in landscapes dominated by agriculture and development. Recognizing changes in macroinvertebrate communities at urbanizing sites along this gradient could help establish management priorities for the conservation of biological diversity. Furthermore, changes in the diversity of particular invertebrate taxa (i.e. sensitive populations and functional feeding groups) could indicate changes in consumer resources and identify which types of organisms are particularly susceptible to land use change. Understanding community patterns across this land use gradient is necessary so that we can begin to investigate specific factors within agricultural or developed areas that are affecting stream ecosystems. For example, while it has been suggested that the influences of impervious surface (Schueler 1994) and riparian forest (Weigel et al. 1999; Stewart et al. 2001) contribute to ecosystem health, their relative importance at streams along this gradient is unknown. Identifying whether these types of factors are affecting community changes can further allow for the establishment of appropriate restoration goals for mediating the harmful effects of land use change.

In this paper, I use data from watersheds in the Chesapeake Bay drainage area to examine differences in macroinvertebrate communities from headwater streams across a region dominated by a gradient of agriculture and residential development. I use this information to ask what the ecological impacts of the development of agricultural land



are for stream ecosystems, and to look at the effects of specific abiotic land use factors on community variables. To address this question, I: (1) use groups of agricultural and developed stream sites to compare community tolerance, diversity and abundance between land use types, (2) investigate the strength and shape of the relationships between specific land use factors (i.e. impervious surface and riparian forest cover) and taxa richness, including the ability of these factors to explain community variation within the most impaired stream sites, and (3) use a literature survey to compare agricultural streams from this study to other agricultural systems for the purpose of identifying how different farming operations may influence diversity. From these analyses, I hope to determine the relative effects of residential development on stream communities in agricultural landscapes, and to suggest specific factors within these landscapes that may help exacerbate or remediate the negative effects of different land use practices. This will help evaluate the importance of protecting agricultural land from development, and help establish appropriate research priorities for managing impaired streams in developed watersheds.

## METHODS

### *Study Sites & Land Use*

This study took place in the Piedmont region of Maryland, USA, on the outskirts of the Washington, DC metropolitan area (Fig. 1). All sampling sites were located within four watersheds (29-68 km<sup>2</sup>) that ultimately drain into the Chesapeake Bay. These four watersheds were historically undeveloped (all  $\leq$  10% residential) and dominated by agriculture (all  $>$  61%) as recently as 1951. The southern area of this study region

experienced a dramatic increase in residential development (and a corresponding loss of agricultural land) as a result of urban sprawl during the 1960's and 1970's. Currently, Northwest Branch and Paint Branch (tributaries of the Potomac River) have watersheds that are characterized by large amounts of residential development (Fig. 1; 53% and 64%, respectively). Nearly all of the development in these watersheds is low (0.2–2.0 dwellings/acre) or medium (2–8 dwellings/acre) density residential, with only small amounts of high density residential, industrial or commercial land use. Cattail Creek and Hawling's River are both tributaries of the Patuxent River. The watershed of the former is dominated by agricultural land use (56%), while the latter is a mix of agriculture and residential development (36% and 25%, respectively).

Approximately two thirds of the agricultural land in this region is used for pasture, while the remainder is actively cultivated for corn, soybean and winter wheat crops. All four watersheds have relatively similar proportions of deciduous forest (21-32%), with much of this wooded area found along the riparian corridor of the stream network as a result of proactive conservation strategies. The 30 m buffer surrounding the stream network of these four watersheds is 47-68% forested, with the maximum area of riparian forest in Paint Branch, the most developed watershed. The relatively low overall percentage of forest in these watersheds results in a primary gradient of agriculture to low and medium density residential development across the entire region. For comparative purposes, I included a small (3.24 km<sup>2</sup>) headwater stream in northwestern Montgomery County that has a drainage area dominated by deciduous (59%) and coniferous (28%) forest. This watershed was historically used for agriculture (similar to all other sites used

in this study), but was reclaimed for public use and has naturally reforested over the last 30 years.

Sixty-nine sites distributed throughout these four main watersheds were sampled for macroinvertebrate communities. Sampling sites were located near the confluence of each tributary joining the stream network that had a drainage area  $\geq 2.6 \text{ km}^2$ , as well as immediately above and below all confluences along the downstream network. The small headwater streams on the outlying tributaries ( $n = 30$ ) were often dominated by single form of land use, and these sites were separated for my subsequent analyses of invertebrate diversity and abundance. The length of the reach sampled at each site was determined by the distance necessary to include the three riffle habitats necessary for macroinvertebrate sampling, but was kept at a minimum of 75m.

Land use information for this region (historical and current) was obtained from Maryland Office of Planning (MOP) GIS coverages available in the ArcView supplement program GISHydro2000 2<sup>nd</sup> ed. ([www.gishydro.umd.edu](http://www.gishydro.umd.edu)). These MOP coverages use level II Anderson land use classifications, with 30 m resolution. For most subsequent analyses, land use classifications were grouped into 3 broader categories; agricultural (crops+pasture), developed (low, medium and high density residential+commercial+industrial), and forest (deciduous+ coniferous). Streams and drainage areas for each of the sampling sites (i.e. subwatersheds) were delineated using digital elevation maps (Fig. 1). Land use percentages were determined for each subwatershed, and riparian land use was calculated by examining the land use within 30 m of the stream along the entire upstream network of each sampling site.

### *Macroinvertebrate Communities*

Macroinvertebrate communities were sampled from March 15-April 15 in both 2001 and 2002. All sites were sampled in both years, except three sites that were removed from the project following the 2001 sampling season due to site access problems. Samples were collected throughout each sampling reach using a 0.04 m<sup>2</sup> Surber sampler (0.25 mm mesh). Two Surber samples were collected at random locations within each of three riffle habitats (0.24 m<sup>2</sup> total area) at each site. Consecutive riffles at each site were sampled, except where consecutive riffles occurred within a distance less than 75 m. In these cases, alternating riffles were sampled to ensure the required minimum reach length. The six individual samples were pooled together, and one third of this composite material was subsampled. Composite samples were preserved in the lab using a 10% formalin solution.

Samples were rinsed from preservative using a 0.5 mm sieve, and all invertebrates were removed from detrital organic matter and sediment debris. All macroinvertebrates were identified to the lowest possible taxonomic level under a dissecting microscope (100X magnification) and assigned functional feeding group (FFG) designations using Merritt & Cummins (1996) and Thorp & Covich (2001). Most insect taxa were identified to the genus level, while most non-insect taxa were identified to class or order. Fifty organisms from the family Chironomidae from each sample were slide-mounted and identified to genus in 2001 under a compound microscope (400x magnification) using Merritt & Cummins (1996) and Epler (2001) to obtain estimated values of richness and density for these taxa. Chironomid taxa were left at the family level in 2002.

Variables related to diversity and abundance were used to examine community changes across study sites. Total taxa richness, density, diversity, and evenness were determined for both years. Diversity and evenness were computed using the Shannon-Wiener indices:

$$(1) \text{ Diversity } (H') = - \sum P_i \log P_i$$

$$(2) \text{ Evenness } (J) = H' / \log S$$

where  $P_i$  is the proportion of the total number of individuals occurring in species  $i$ , and  $S$  is the total taxa number. Richness of Ephemeroptera, Plecoptera and Trichoptera taxa (EPT; a conventional group of indicator taxa) was also determined across both years, while FFG richness required genus level identification of Chironomidae, and was calculated for 2001 only.

#### *Data Analysis*

The relative sensitivity of invertebrates to land use was tested using the presence/absence of each population in subwatersheds dominated by either agriculture ( $\geq 50\%$ ;  $n = 22$ ) or residential development ( $\geq 50\%$ ;  $n = 22$ ). This was done with a technique developed by McCoy and Mushinsky (2002) that uses a chi-square contingency table to compare the distribution of each population across these two land use groups to the 1:1 distribution expected if an organism does not favor a certain land use type. As an example, taxa "A" was found at 20 sites; 17 agricultural sites, and only 3 developed sites. If taxa A did not favor either land use, we would expect its distribution at 20 sites to be split evenly between 10 agricultural sites and 10 developed sites. The chi-square test indicates that the 17:3 ratio is significantly different from the 10:10 null ratio ( $p = 0.02$ ). Significant chi-square values ( $p < 0.10$ ) were used to classify a set of

“sensitive” taxa that occurred significantly more often at either agricultural or developed sites, a “tolerant” group showing no differences in distribution across these two land use types, and “rare” taxa that were found at too few sites for significance testing. This analysis was performed for 2001 only, as genus level information was used for the family Chironomidae.

Thirty headwater streams (2.7–9.2 km<sup>2</sup>) were used to test for differences in macroinvertebrate diversity and density between land use types. These streams were divided into distinct categorical land use groups based on the percentages of agriculture, forest, and development in their subwatersheds using cluster analysis with Ward’s minimum variance method (Proc Cluster, SAS v8.2). Differences in total community diversity, richness, evenness and density between these land use groups were examined across both 2001 and 2002 using a 2x2 repeated-measures analysis of variance (ANOVA), with autoregressive covariance structure to account for temporal autocorrelation across sampling years (Proc Mixed, SAS v8.2). Chironomid taxa were grouped at the family level in these analyses, for the purpose of standardizing taxonomic resolution across both years. Differences in EPT richness between land use groups were similarly tested across sampling years using repeated-measures ANOVA, while differences in FFG richness were tested in 2001 only using simple one-way ANOVA’s. Tukey’s test for pairwise comparisons was used to test for differences between individual land use groups when a significant overall main effect of land use was found.

Simple linear regression models were used to examine the strength of the relationships between subwatershed land use (i.e. agriculture, development and impervious surface cover) and total macroinvertebrate richness throughout all sampling

sites (Proc Reg, SAS v8.2). Linear models were also used to examine the relationship between riparian forest cover and richness at the most developed sites. Riparian forest models for developed sites were tested separately using groups with 15-20% and >25% impervious surface to separate the confounding influences of this variable on macroinvertebrate richness.

To compile estimates of macroinvertebrate richness (total and EPT) in other agricultural systems, I used the ISI Web of Science literature database (<http://isi5.newisiknowledge.com>) to search for articles containing the keywords “agriculture” or “agricultural” and “macroinvertebrate”. This search returned 88 articles that were subsequently considered. All relevant articles (i.e. empirical studies investigating macroinvertebrates in agriculturally impacted streams) were examined, and richness values were estimated from graphical, tabular or text values.

## RESULTS

A total of 124 macroinvertebrate taxa were collected throughout the four watersheds examined in this study, including 42 EPT taxa. The chi-square contingency analysis revealed 25 “sensitive” taxa in 2001 that were found significantly more often at agricultural sites than at developed sites (Table 1;  $p < 0.10$ ). In contrast, no macroinvertebrate taxa were found significantly more often at developed sites than agricultural sites (all  $p > 0.10$ ). A total of 49 taxa showed no significant difference in their presence at agricultural and developed sites (“tolerant” taxa), while 50 “rare” taxa were found at too few sites for significance testing. I further examined the presence/absence of each of the sensitive taxa throughout all study sites, and recorded the

maximum amount of agriculture or development occurring among the subwatersheds that each taxa was found in (Figure 2). These comparisons indicate that while all invertebrate taxa are found in areas dominated by agriculture, many can only tolerate moderate amounts of development.

The cluster analysis for the 30 headwater streams revealed five distinct underlying groups (Fig. 3). The first of these land use groups was limited to our single forested subwatershed. A second group was represented by high percentages of agricultural land use, with relatively low amounts of residential development ( $n = 11$ ). The “developed” cluster included sites that have large proportions of residential development, and a complete absence of agriculture ( $n = 6$ ). The final two groups have sites with relatively mixed land use, the first with low percentages of development and moderate amounts of agriculture ( $n = 6$ ), and the second with low percentages of agriculture and moderate amounts of development ( $n = 6$ ). An examination of historical land use patterns within these groups indicates that all sites were similarly agricultural and undeveloped until approximately 1960 (Fig. 4). The large amount of variability in development and agriculture within the land use groups around 1963 indicates a period of localized and rapid land use change at this time. In 1970 there is a distinct separation in mean residential development between the two more urbanized groups, followed by the complete separation of development patterns for all four groups around 1990 (Fig. 4).

These categorical groups (excluding the single forested site) were used to test for differences in macroinvertebrate communities resulting from land use. There was a significant main effect of land use on the diversity and richness of the entire community (Table 2;  $p < 0.0001$ ), with developed sites having lower richness and diversity than all



other land use groups across both sampling years (Fig. 5). In both cases there was no effect of sampling year, but there was a significant interaction between year and land use group (Table 2;  $p < 0.05$ ). This was due to a change in the magnitude of differences in richness and diversity values between land use groups during the 2002 sampling season. There was no significant effect of land use when we examined changes in community evenness or community density (Table 2;  $p > 0.05$ ).

Very similar community patterns were found when examining differences in richness between specific macroinvertebrate groups. Mean EPT richness varied between land use groups (Table 2;  $p < 0.0001$ ), with agricultural sites having significantly more taxa than developed sites across both sampling years (Fig. 5;  $p < 0.0001$ ). The two mixed land use groups had similar mean EPT richness values, intermediate between the agricultural and developed sites. There was no difference in EPT richness between sampling years, nor was there an interaction between year and land use group ( $p > 0.05$ ). There was a significant effect of land use on the richness of all functional feeding groups, with the exception of shredders (Table 2). Collector, filterer, predator and scraper richness were all significantly higher in the agricultural streams compared to the developed sites (Fig. 5). The single forested site had total and EPT richness values that were intermediate to the agricultural and developed groups. This stream also had intermediate values of collector, predator and scraper richness, with relatively high shredder richness and relatively low filterer richness compared to all other land use groups.

The amount of development in each subwatershed explained 52% of the variation in total taxa richness in 2001 (Fig. 6a;  $p < 0.0001$ ). The strong negative linear

relationship between impervious surface cover and richness may help explain decreasing diversity in developed subwatersheds (Fig. 6b;  $p < 0.0001$ ). It has been previously suggested that thresholds may exist in this relationship, whereby richness declines only above 10-15% imperviousness (Schueler 1994), or that decreases in diversity will level off above 10-15% impervious cover (Stepenuck et al. 2002). Separate regression models on sites with  $<15\%$  imperviousness ( $r^2 = .30$ ,  $p = 0.02$ ) and  $>15\%$  imperviousness ( $r^2 = 0.55$ ,  $p = 0.009$ ) suggest an absence of thresholds, and linear declines in richness throughout the entire range of impervious surface cover.

Despite the strong relationships between richness and land use throughout all sites, there appeared to be considerable variation in richness within land use groups affected by development (Fig. 6). A great deal of this variation is explained by the amount of forest within the 30 m riparian zone of the upstream network of each site (Fig. 7). Richness in developed sites was positively related to riparian forest when examined separately for groups of moderate (15-20%;  $r^2 = 0.82$ ,  $p = 0.03$ ) and high ( $>25\%$ ;  $r^2 = 0.60$ ,  $p = 0.04$ ) impervious surface cover. These groups were tested separately to remove the confounding effect of impervious surface on diversity, and the subsequent relationships between richness and imperviousness within each group were non-significant.

The literature survey produced 31 journal articles from which I was able to estimate total and/or EPT richness values in agricultural stream systems (Table 3). Mean richness estimates from these studies were separated by geographical region and level of taxonomic resolution, and compared to richness values from agricultural streams in the current study (Fig. 8). It is evident that the richness values from this study (both total and

EPT) are within the upper range of values found both globally and within the United States, regardless of taxonomic resolution. Mean richness values from this study also appear to be higher than the combined means of other published studies (Fig. 8), although these differences were not statistically tested.

## DISCUSSION

Agricultural areas represent the last remaining undeveloped land in many regions of the United States (Riebsame et al. 1996; Moglen 2000; Dale et al. 2000), and this has led to the establishment of agricultural land preservation programs throughout the country. The goals of many of these programs include biological conservation, yet the benefits of agricultural preservation for lotic ecosystems have yet to be explored. Only recently has there been research to suggest that community health in agricultural streams may be better than that in adjacent urbanizing areas (e.g. Wang et al. 2000; Stepenuck et al. 2002). Understanding how macroinvertebrate communities change across this land use gradient is essential for establishing management priorities and stream restoration strategies. My findings suggest that agricultural streams in Maryland have more diverse communities when compared to streams in developed watersheds. I show that impervious surface cover may be causing the decreases in diversity at developed sites, although there is evidence to suggest that the presence of riparian forest may mitigate the impacts of development. My data, combined with the results of a literature survey, suggest that the relative health of agricultural streams may depend on the nature and management of agricultural operations.

Macroinvertebrate abundance (e.g. Hachmoller et al. 1991; Hall et al. 2001) and the dominance of certain taxa (Karr and Chu 1999) can be used as indicators of stream health. However, total macroinvertebrate density and evenness throughout these sites did not show any significant differences between land use groups (Table 2). This is likely due to a relatively high density of chironomid and oligochaete taxa in both agricultural and residential areas. This explanation is supported by other studies that have found an increased relative abundance of these groups in disturbed streams (Whiting and Clifford 1983; Hall et al. 2001).

Of the 124 taxa found throughout the study area, 25 were found significantly more often at agricultural sites than at developed sites, and not a single population was found more often in urbanizing watersheds. This indicates that many taxa are sensitive to the development of farmlands, and that no organisms survive better in residential areas when compared to agricultural streams. The relative sensitivity of invertebrate populations to development is supported by the diversity patterns found along this land use gradient. Diversity and richness are strong and effective measures that are often used to assess anthropogenic impacts on macroinvertebrate communities (eg. Kerans and Karr 1994; Stribling et al. 1998; Barbour et al. 1996). Invertebrate diversity and richness were significantly lower at developed sites, suggesting the localized disappearance of several invertebrate populations in these streams (Fig. 5). For both total community and EPT taxa groups, agricultural streams sustained approximately twice the richness of invertebrate taxa compared to developed sites. The pattern of decreased richness among these developed stream sites was also evident within all of the feeding groups except for the shredder taxa, indicating that most FFG's were negatively affected by development.

Decreased functional group richness could have important consequences if there is a top-down relationship between macroinvertebrate consumer diversity and stream ecosystem processes. Invertebrate consumers do influence periphyton growth and decomposition rates (e.g. Lamberti et al. 1995; Jonsson and Malmqvist 2000), and relationships between diversity and function have previously been demonstrated in stream systems (Cardinale et al. 2002). This overall decrease in richness (including EPT and FFG taxa) with development in agricultural landscapes indicates that agricultural land preservation may be an important practice for maintaining stream biodiversity.

Interestingly, macroinvertebrate richness values in the single forested stream were similar to or less than the mean richness values at the agricultural sites, with the exception of shredder taxa richness. This may be partially due sampling design, with macroinvertebrate samples taken from riffle habitats only. Coarse riffle substrates sustain a large abundance of invertebrate groups (i.e. scrapers and collectors) that feed on the algae and bacteria that grow primarily in this habitat. Closed tree canopies in forested headwater streams should lead to relatively low algal productivity in riffles, and cause assemblages to be dominated by invertebrates that feed on detrital leaf inputs (Vannote et al. 1980). Thus, macroinvertebrate diversity in forested streams could be greater than agricultural streams when all stream habitats (i.e. leaf packs, woody debris) are sampled. This is supported by Lenat and Crawford (1994), who found comparable macroinvertebrate richness in riffle samples of agricultural and forested sites, but significantly greater richness in forested areas when multiple habitats were sampled.

The finding that agricultural streams sustain relatively diverse communities compared to those in residential landscapes has been recently supported by other

empirical research. Wang et al. (2000) and Stepenuck et al. (2002) examined stream communities across a similar land use gradient in Wisconsin, and found that macroinvertebrate and fish diversity, respectively, decreased with the amount of residential and commercial development in a watershed. These authors suggested that one of the principal factors contributing to decreased diversity in these landscapes was the quantity of impervious surface cover. I also found a highly significant negative relationship between macroinvertebrate richness and imperviousness in Maryland streams (Fig. 7a). Impervious surface cover in the highly developed subwatersheds examined in this study (all >25%) significantly exceeds levels that were implicated in community degradation in the Wisconsin stream sites. Schueler (1994) suggests that macroinvertebrate diversity will decline drastically once a threshold of 10-15% impervious surface cover is reached. In contrast, Stepenuck et al. (2002) demonstrated that decreases in macroinvertebrate richness leveled off above 10-15% impervious surface cover. My data indicates a distinct linear relationship, even when I only considered sites with low levels of imperviousness (<15%). Furthermore, richness continues to decrease dramatically even at highly developed sites. This pattern suggests that there may be some level of imperviousness in developed watersheds that will cause water quality to be completely inhospitable for macroinvertebrate survival. The absence of the previously suggested thresholds shows that the negative effects of imperviousness must be considered even at the early stages of development, and that sustaining aquatic life in highly developed watersheds will require active management of stormwater flow.

While impervious surface cover in subwatersheds may be an important factor causing decreases in richness, it appears that the amount of riparian forest along the

stream channels of these subwatersheds may mitigate the impacts of development (Fig. 7b). Positive relationships between the amount of riparian buffer and richness were apparent within both groups of developed sites having similar levels of imperviousness. These riparian forests may alleviate the impacts of development in several ways, including decreasing erosion due to bank stabilization by the vegetation, and increased consumer resources from detrital leaf inputs. Recognizing the relationships between richness and both imperviousness and riparian forest can have important implications for stream management. Restoration strategies that take these factors into account, such as stormwater management ponds or riparian reforestation, may be extremely useful in alleviating the harmful affects of development.

Maryland agricultural streams do have more diverse communities than those in adjacent urbanizing areas. Nevertheless, agricultural watersheds represent human-altered ecosystems that can receive large inputs of both chemical (Schulz and Leiss 1999; Anderson et al. 2003) and sediment (Davis et al. 2003) pollution. Several previous studies have found that macroinvertebrate communities in agricultural streams are degraded compared to natural streams (Lenat and Crawford 1994; Roth et al. 1996; Rothrock et al. 1998; Genito et al. 2003). Understanding how communities in Maryland agricultural streams compare to those from other agricultural systems is essential for pinpointing specific factors in farming landscapes that may contribute to elevated diversity. Maryland agricultural streams were found to have higher richness than most other agricultural streams, suggesting comparatively healthy invertebrate communities in the current study.

Watzin and McIntosh (1999) suggest that the impact of agriculture on macroinvertebrate community health will vary depending on the type of agriculture and its proximity to the stream. The agricultural subwatersheds examined in this study were dominated by pasture that was typically separated from the stream network with riparian buffers, thus avoiding many of the harmful impacts that have been associated with cultivation. Ten of the eleven agricultural sites had uninterrupted riparian forests throughout the sampling reaches, and the average agricultural site had 44% forest within the riparian zone of the entire upstream network. Prior research has shown the usefulness of forested riparian buffers for removing chemical (Lee et al. 2003) and sediment (Muscott et al. 1993; Osborne and Lovacic 1993) pollution in agricultural areas. Furthermore, Weigel et al. (1999) and Stewart et al. (2001) have specifically indicated the importance of forested buffers in agricultural areas for maintaining macroinvertebrate communities. Although there was not a significant linear relationship between richness and forested buffer within the agricultural streams (as was found within the developed sites), this may be a result of less variability in riparian forest among the agricultural sites.

Of the agricultural systems examined in the literature survey, only four had total or EPT richness higher than the mean richness values from this study (i.e. Lenat and Crawford 1994; Richards and Host 1994; Clenaghan et al. 1998; Delong and Brusven 1998). A closer examination of these systems reveals similar qualities to those present in the Maryland agricultural streams. While specific information on riparian forest cover at these sites was generally unavailable, the watersheds themselves were 31-77% forested. All of these sites had less than 50% crop cultivation, in contrast to many of the other



studies considered (Table 3). The combination of relatively low amounts of crop cover and high proportions of forest in these four systems and the Maryland agricultural sites could prevent harmful impacts from specific agricultural stressors that have been causally implicated in decreased community health, such as pesticides (Barton and Metcalfe-Smith 1992; Liess and Schulz 1999), irrigation (Armitage and Petts 1992; Koetsier 2002), and salinization (Williams et al. 1991).

The results of the field study presented here show that agricultural streams in Maryland support healthy communities compared to adjacent developed sites, and indicate that agricultural land preservation may be helpful for maintaining biodiversity. Furthermore, the literature survey suggests that these Maryland agricultural streams are comparatively diverse relative to other agricultural systems. This suggests that the relative health of macroinvertebrate communities in agricultural streams may depend on the nature and management of farming operations. The low macroinvertebrate richness in developed subwatersheds may be a consequence of high levels of impervious surface cover, though it appears that riparian forest at these sites may alleviate some of these negative impacts. Focusing on restoration strategies in developed areas that diminish the effects of impervious surface cover and riparian deforestation may be particularly successful in maintaining community diversity. Establishing a direct causal link between these variables and invertebrate community health will require more research, but the strength of the relationships presented here justifies the further exploration of these factors.

Table 1. Macroinvertebrate taxa found throughout all 69 sampling sites, and their frequency of occurrence during 2001 (2002 Chironomidae were only identified to the family level). Taxa were considered “sensitive” if they were found significantly more often at 22 agricultural sites when compared to 22 developed sites ( $p < 0.10$ ). “Tolerant” taxa were not found significantly more often at either agricultural or developed sites. Rare taxa (with frequency of occurrence) were not represented at enough sites in 2001 to determine significant differences between 22 agricultural and 22 developed sites. Rare taxa with zero frequency of occurrence were found only in the 2002 sampling season. Invertebrate order (ORD) classifications are; co = Coleoptera, di = Diptera, ep = Ephemeroptera, me = Megaloptera, ot = other (non-insect), od = odonata, pl = plecoptera, tr = trichoptera. Functional feeding group (FFG) designations are; c = collector, f = filterer, p = predator, sc = scraper, sh = shredder.

TAXA	Total # of Sites (/70)	# of Ag Sites (/22)	# of Dev Sites (/22)	ORD	FFG	TAXA	Total # of Sites (/70)	# of Ag Sites (/22)	# of Dev Sites (/22)	ORD	FFG
<b>SENSITIVE TAXA</b>						<b>TOLERANT TAXA (CONT)</b>					
Parametricnemus sp.	53	22	8	di	c	Stenonema sp.	52	21	10	ep	sc
Tvetenia sp.	50	22	8	di	c	Optioservus sp.	51	21	11	co	sc
Neophylax sp.	47	22	6	tr	sc	Polypedilum sp.	49	19	14	di	sh
Stenelmis sp.	41	20	6	co	sc	Hemerodromia sp.	47	18	13	di	p
Amphinemura sp.	38	21	2	pl	sh	Clinocera sp.	45	13	16	di	p
Paraleptophlebia sp.	34	19	2	ep	c	Chelifera sp.	43	18	14	di	p
Shipsa sp.	31	14	2	pl	sh	Tanytarsus sp.	43	19	11	di	f
Baetis sp.	30	19	1	ep	c	Tanypodinae	42	15	9	di	p
Chimarra sp.	30	16	2	tr	f	Eukiefferiella sp.	41	15	7	di	c
Glossosoma sp.	30	18	4	tr	sc	Tipula sp.	37	12	13	di	sh
Helichus sp.	30	16	1	co	sc	Ceratopsyche sp.	36	19	8	tr	f
Hydrachnida	29	14	4	ot	p	Hydrobaenus sp.	35	8	10	di	sc
Serratella sp.	25	17	0	ep	c	Diplectrona sp.	31	10	7	tr	f
Acerpenna sp.	22	10	1	ep	c	Ferrissia sp.	31	8	10	ot	sc
Isoperla sp.	22	13	1	pl	p	Turbellaria	31	13	8	ot	p
Psephenus sp.	22	11	2	co	sc	Nigronia sp.	29	11	8	me	p

Leuctra sp.	21	11	2	pl	sh	Stegopterna sp.	27	10	3	di	f
Rhyacophila sp.	20	11	0	tr	p	Eurylophella sp.	25	5	9	ep	c
Strophopteryx sp.	15	7	0	pl	sh	Bezzia sp.	23	11	3	di	p
Leucrocuta sp.	11	9	0	ep	sc	Corbicula sp.	23	8	4	ot	f
Nixe sp.	11	8	0	ep	sc	Copepoda sp.	20	2	9	ot	c
Acentrella sp.	9	5	0	ep	c	Microtendipes sp.	20	10	4	di	f
Hexatoma sp.	7	5	0	di	p	Theinmanniella sp.	20	8	4	di	c
Limnophila sp.	6	5	0	di	p	Corynoneura sp.	18	6	2	di	c
Epeorus sp.	5	5	0	ep	sc	Diamesa sp.	18	4	5	di	c
<b>TOLERANT</b>						Arigomphus sp.	16	5	3	od	p
Oligochaeta	68	22	21	ot	c	Parakiefferiella sp.	15	7	3	di	c
Cheumatopsyche	67	22	21	tr	f	Culicoides sp.	12	5	5	di	p
sp.	67	22	21	tr	f	Pilaria sp.	12	5	1	di	p
Antocha sp.	65	22	20	di	c	Rheocricotopus sp.	12	2	8	di	c
Hydropsyche sp.	65	21	20	tr	f	Chrysops sp.	11	7	2	di	c
Orthocladius sp.	64	21	20	di	c	Probezzia sp.	11	7	2	di	p
Nematoda	62	22	18	ot		Ceratopogon sp.	9	7	1	di	p
Simulium sp.	62	22	17	di	f	Dolophilodes sp.	9	1	5	tr	f
Rheotanytarsus	60	21	18	di	f	Amphipoda	8	2	3	ot	sh
sp.	60	21	18	di	f	Isopoda	8	3	2	ot	c
Oulimnius sp.	59	22	15	co	sc	Sialis sp.	6	4	1	me	p
Cricotopus sp.	57	16	21	di	sh						
Ephemerella sp.	55	22	11	ep	c						
Prosimulium sp.	52	19	11	di	f						

**Rare taxa:** Pycnopsyche sp. (10), Ameletus sp. (9), Dicranota sp. (8), Psychomyia sp. (7), Acroneuria sp. (6), Dubiraphia sp. (6), Hirudinea (6), Macronychus sp. (6), Cambaridae (5), Ephemera sp. (5), Polycentropus sp. (5), Clioperla sp. (4), Collembola (4), Cryptochironomus sp. (4), Potthastia sp. (4), Psilometriocnemus sp. (4), Brillia sp. (3), Diploperla sp. (3), Ectopectera sp. (3), Isonychia sp. (3), Neozavrelia sp. (3), Promoresia sp. (3), Sphaeromias sp. (3), Sublettea sp. (3), Caenis sp. (2), Diplocladius sp. (2), Ectopria sp. (2), Endochironomus sp. (2), Physidae (2), Stempellina sp. (2), Stempellinella sp. (2), Stenochironomus sp. (2), Cardiocladius sp. (1), Chaoborus sp. (1), Cladotanytarsus sp. (1), Corydalus sp. (1), Curculionidae (1), Habrophlebia sp. (1), Hydrophilus sp. (1), Paratanytarsus sp. (1), Planorbidae (1), Stenacron sp. (1), Stratiomys sp. (1), Ancronyx sp. (0), Cymellus sp. (0), Drunella sp. (0), Habrophlebiodes sp. (0), Lepidoptera (0), Leptophlebia sp. (0), Psilotreta sp. (0)

Table 2. Results of ANOVA mixed models on macroinvertebrate variables using land use groups at 29 headwater streams (agricultural, n = 11; mixed-agricultural, n = 6; mixed-developed, n = 6; developed, n = 6), with the forested site excluded. Models using all taxa and EPT taxa were tested using repeated-measures ANOVA across both the 2001 and 2002 sampling seasons, with chironomid taxa identified to the family level. Functional feeding group models include genera level chironomid identification, and use the 2001 data only. For land use main effects: numerator degrees of freedom (ndf) = 3, and denominator degrees of freedom (ddf) = 25. For main effects of year: ndf = 1, ddf = 23. For year x land use interaction terms: ndf = 3, ddf = 23. Italicized F-ratios are significant at  $p < 0.05$ .

Response	Model		Model	
	F-ratio	p	F-ratio	p
	(Total Density)		(Total Evenness)	
Land Use	0.45	0.72	2.68	0.07
Year	<b>19.00</b>	0.0002	0.04	0.85
LU*Year	0.27	0.84	0.63	0.6
	(Total Richness)		(Total Diversity)	
Land Use	<b>18.51</b>	<0.0001	<b>10.74</b>	0.0001
Year	0.62	0.44	0.63	0.44
LU*Year	<b>3.99</b>	0.02	<b>3.19</b>	0.04
	(EPT Richness)		(Collector Richness)	
Land Use	<b>13.85</b>	<0.0001	<b>8.18</b>	0.0006
Year	0.07	0.8	-	-
LU*Year	2.67	0.07	-	-
	(Filterer Richness)		(Predator Richness)	
Land Use	<b>9.73</b>	0.0002	<b>8.34</b>	0.0005
	(Scraper Richness)		(Shredder Richness)	
Land Use	<b>19.44</b>	<0.0001	1.97	0.14

Table 3. Results of a literature search for published articles providing macroinvertebrate richness values in agriculturally impacted streams. Articles were included if: (1) the studies were in lotic systems, (2) were empirical in nature, (3) did not have significant land use impacts other than agriculture (e.g. urban, mining), and (4) macroinvertebrate richness could be estimated from text, graphical or tabular values. In many cases, only a subset of sites were used from a study according to the author's indication of streams under agricultural influences.

Study	Geographical Location	Land Use Description and Extent	Taxonomic Resolution	Estimated Mean Total Richness	Estimated Mean EPT Richness	Determination of Mean Richness
Anderson et al. (2003)	California	Row crops throughout watershed, heavy agricultural impacts from local tributaries	Genus or Species	16.9	-	Mean of tabular values for composite samples at stations 2, 3, and 4
Armitage and Petts (1992)	United Kingdom	Agriculture influenced sites subject to spray irrigation and runoff from fertilized land	"Genus" (family and species mean)	21	-	Mean of graphical values (estimated from mean of species and family richness) for WP, EP and CD sites
Barton and Metcalfe-Smith (1992)	Quebec	Grain crops dominate watersheds of sampling sites; influences of pesticides, fertilizers and tile drainage	Unknown (lowest possible)	40	-	Mean of estimated graphical values for July Surber samples at sites 31 and 32
Bis et al. (2000)	Poland	50-86% Agriculture, 6-36% forest throughout study watersheds	Unknown (lowest possible)	16.4	-	Mean of estimated graphical values for all sites
Brewin et al. (2000)	Nepal	39-98% Terraced agriculture (mostly rice and maize), 2-60% forest throughout watersheds	Family	22.7	-	Mean of tabular values of winter and pre-monsoon samples at Likhu Khola sites
Brown and May (2000)	California	Sites in agricultural drainages or streams affected by agriculture	Family	17	6	Mean richness given for Twinspan group "drain 1"; may exclude some rare taxa
Caruso (2002)	New Zealand	One site (of 12 sites sampled) described as an "agricultural" catchment	Unknown	35.5	-	Mean of endpoints of the range of richness values (34-37) given at agricultural site
Clenaghan et al. (1998)	Ireland	25% Agriculture; 40% forest (conifer plantation); 35% moorland (sheep grazing) throughout watershed	Species	50	-	Estimated mean from graphical values for all sites
Corkum (1996)	Ontario	Dominated by agricultural land	Family	20	8	Richness values obtained from graphical density plots at 3 agricultural sites using natural rocks

Crane et al. (1995)	United Kingdom	Cereal crops, orchards, grazed pastures throughout watershed; some sewage influences	Family	13.9	2.6	Mean of richness values obtained from taxa density tables for all sampling stations
de Billy et al. (2000)	France	Upper portion of catchment dominated by meadows used for cattle grazing	Genus	24.4	-	Mean of tabular values for Spring and Winter samples for all sites
Delong and Brusven (1998)	Idaho	43% Crop land (wheat/pea/barley rotation), 13% grazing pasture, 44% woodland throughout watershed	Genus	50.8	24.8	Mean of tabular values for all sites (after richness standardized to genus level)
Dovciak and Perry (2002)	Minnesota	Corn-soy crop rotation and hog-cattle production throughout watersheds	Genus	-	7	Estimated mean from scatterplot values throughout all 68 study sites
Genito et al. (2002)	Pennsylvania	Overall study area land use is 57% cropland, 35% forest, and 8% permanent pasture	Genus	10	-	Estimated mean from scatterplot values of all sites with >50% agriculture
Kay et al. (2002)	Australia	Most sites located in wheat and wool growing agricultural areas	Family	13.8	-	Mean of average richness values given for 7 site groups (weighted by site number in group)
Koetsier (2002)	Idaho	Irrigated agricultural land in watershed, streams receive return water from fields	Species	8	-	Mean of estimated graphical values for sites MC and ICI in winter samples
Lenat and Crawford (1994)	North Carolina	48% Row crops, 5% grasslands and pasture, 31% forest throughout watershed	Unknown (probably species)	60	11.3	Mean richness values given for kick-net samples in agricultural catchment
Liess and Schulz (1999)	Germany	Beet, barley and wheat crops dominate region	Species	11	-	Mean of estimated graphical values for 2 months prior to insecticide contamination at site
Matagi (1996)	Uganda	Region influenced by fallow agriculture with some bush land	Genus	10	2	Mean is number of taxa found at B1 sampling site across entire year
Monaghan et al. (2000)	Ecuador	Mixed forest and pasture, some crop land throughout study area	Family	23	-	Mean of graphical values for agriculturally impacted "modified" sites
Neumann and Dudgeon (2002)	China	Vegetable and flower crop lands near sampling stations, secondary forest upstream	Species	8.8	-	Mean of estimated graphical values for 3 downstream sites in March and April
O'Connor and Lake (1994)	Australia	Sheep and cattle grazing, broad-acre cereal cropping dominate watershed	Species	20	-	Estimated mean from graphical values for all sites across all sampling dates
Richards and Host (1994)	Minnesota	23% Agriculture, 77% forest in watershed (but agriculture heaviest near watershed outlet)	Genus	25	18	Richness values given for East Branch Knife River (agricultural) site

Rothrock et al. (1998)	Montana	Region subject to irrigation of hay and alfalfa crops; Livestock production at various intensities	Genus or Species	30.8	12.3	Mean of tabular values for Cottonwood, Nevada, Rock and Union tributaries
Shieh et al. (1999)	Colorado	Irrigated agriculture (corn, hay, wheat, beans, barley) dominate region, some urban influence	Genus	13	-	Mean of estimated graphical values for sites 3, 4, and 6 (agriculturally impacted sites)
Sorace et al. (1999)	Italy	Tributary characterized as an agricultural zone, with some meadows and uncultivated areas	Unknown	7	1	Richness value given at Paglia River (agricultural) site
Stewart et al. (2001)	Wisconsin	20-90% agricultural land use throughout 38 watersheds used in study	Species	18	5.2	Median total richness given; EPT richness back-calculated from median "% EPT species" value
Walsh et al. (2001)	Australia	Hinterlands; dominated by a mixture of agriculture and forest, with some small urban centers	Unknown (lowest possible)	10	5	Estimated means from scatterplot values for hinterland sites in Spring riffle samples
Weigel et al. (2000)	Wisconsin	Sites in valleys dominated by agriculture; riparian land use varying from wooded to continuous grazing	Genus	15.6	-	Mean of tabular values for generic richness for all sites
Williams et al. (1991)	Australia	Agriculture (and vegetation clearing) throughout river catchments; influenced by agricultural salinization	Genus or Species	23.3	3.5	Mean of tabular presence/absence values for all sites (except estuarine stations)
Wohl and Carline (1996)	Pennsylvania	50-85% Agricultural land throughout watersheds of all sites (mostly pasture)	Family	12.8	-	Mean of 3 median richness values for each site during May sampling period

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## FIGURE LEGENDS

Figure 1. Location of the four watersheds containing the 69 macroinvertebrate sampling sites. Map shows the position of the study region within the Chesapeake Bay drainage basin, as well as the general patterns of agriculture, forest and development in the four study watersheds. Locations of the sampling sites are shown with open shapes, with stars representing the 30 headwater sites. An example of subwatershed delineation used to estimate land use percentages (designated by heavy black lines) is given at the 8 headwater streams in Northwest Branch.

Figure 2. The maximum tolerance of sensitive taxa for agricultural land use and development, ranked according to their frequency of occurrence during the 2001 sampling season. Bars represent the maximum amount of each land use in the most agricultural or developed site that a particular taxa was found in (land use quantities expressed as the proportion of the most agricultural/developed subwatershed throughout all 70 sampling sites).

Figure 3. Results of a cluster analysis performed on headwater stream sites ( $n = 30$ ), based on the percentages of forest, agriculture and development within each subwatershed. Clusters (ellipses surrounding individual sites) are plotted against percentages of agriculture and development to demonstrate their distinct separation along these two variables.



Figure 4. Historical changes in subwatershed development and agriculture since 1938 for the four land use groups (agriculture = 11; mixed-ag = 6; mixed-dev = 6; developed = 6) used to compare macroinvertebrate communities. Symbols represent mean values of development (+/- SE).

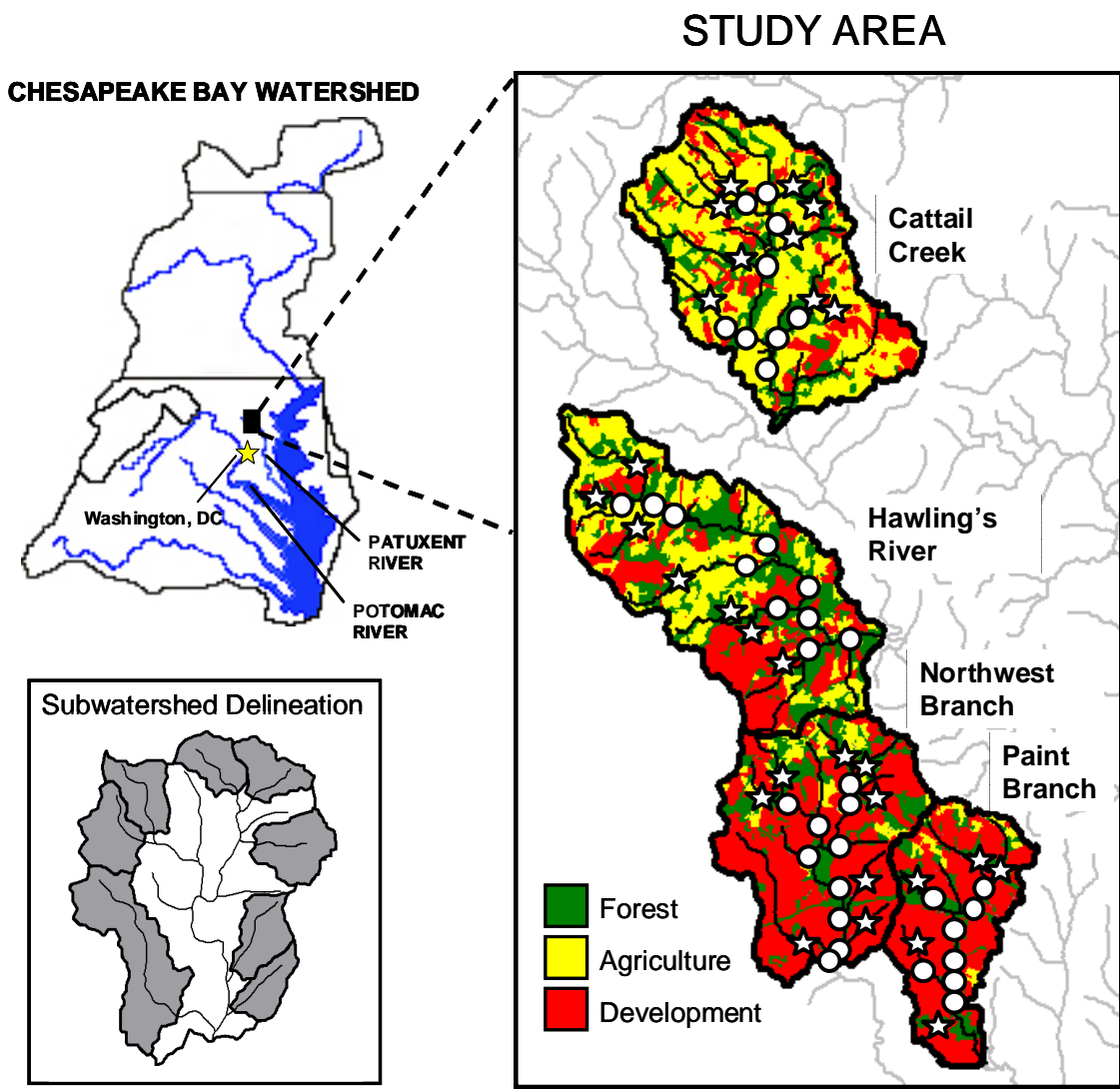
Figure 5. Mean invertebrate total, EPT and FFG richness (+/- SE) for land use groups. A significant main effect of land use was found for all richness variables (all  $p < 0.001$ ) except shredder richness. Bars connected by a line indicate no significant differences between land use groups (Tukey's  $p > 0.05$ ). Richness values for the single forested site were not included in the ANOVA models, and are shown here strictly for comparative purposes.

Figure 6. Relationships between total macroinvertebrate richness and the percentages of (a) development and (b) impervious surface cover in each subwatershed in 2001, based on simple linear regression models ( $n = 30$ ). Similar patterns were found in 2002, and across other invertebrate groups (i.e. EPT and FFG's). The dashed line in (b) represents the proposed threshold at 15% impervious surface. Separate linear regressions for sites with  $<15\%$  imperviousness ( $r^2 = 0.30$ ;  $p = 0.02$ ) and sites with  $>15\%$  imperviousness ( $r^2 = 0.55$ ;  $p = 0.009$ ) suggest an absence of a threshold effect.

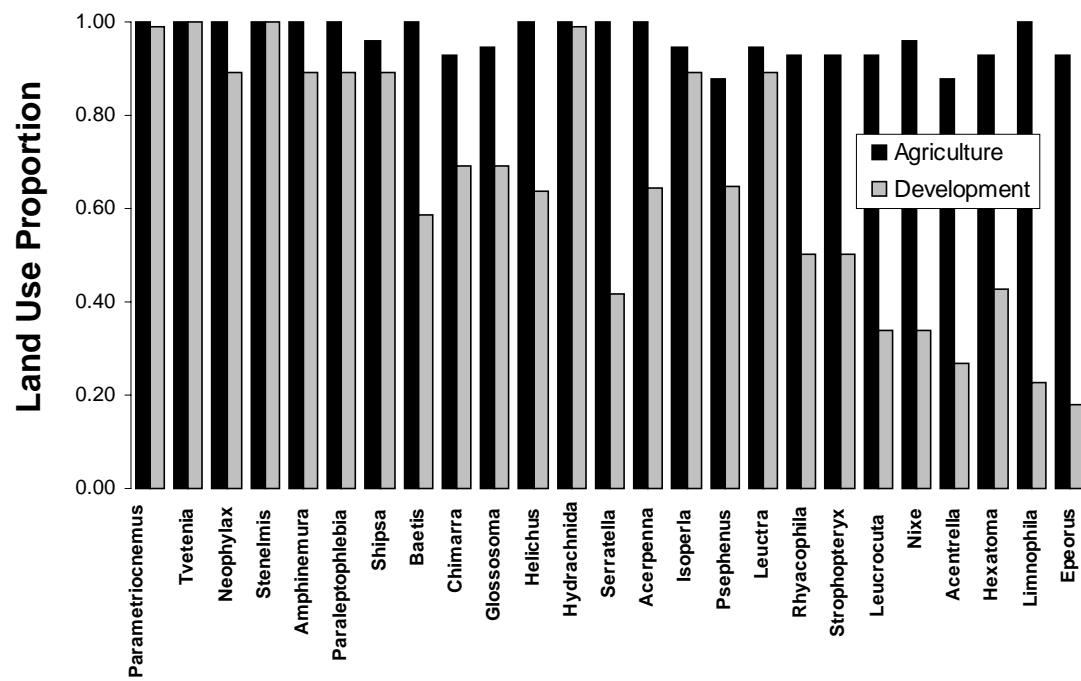
Figure 7. The relationship between taxa richness and upstream riparian forest cover at developed sites. Separate regression models were run for developed sites with 15-20% imperviousness (open circles;  $n = 5$ ) and  $>25\%$  imperviousness (shaded circles;  $n = 7$ ).

Figure 8. Comparison of total and EPT richness values in the current study (Moore 2003) with richness values from published studies in other agricultural systems throughout the world. Symbol shapes indicate level of taxonomic resolution used in each study, while filled shapes indicate values from the current study. “Other” regions include Africa, Asia, Australia and South America for total richness, and includes these continents plus Europe for EPT richness. Lines indicate mean richness values for current study (solid line) and all other studies (dashed line).

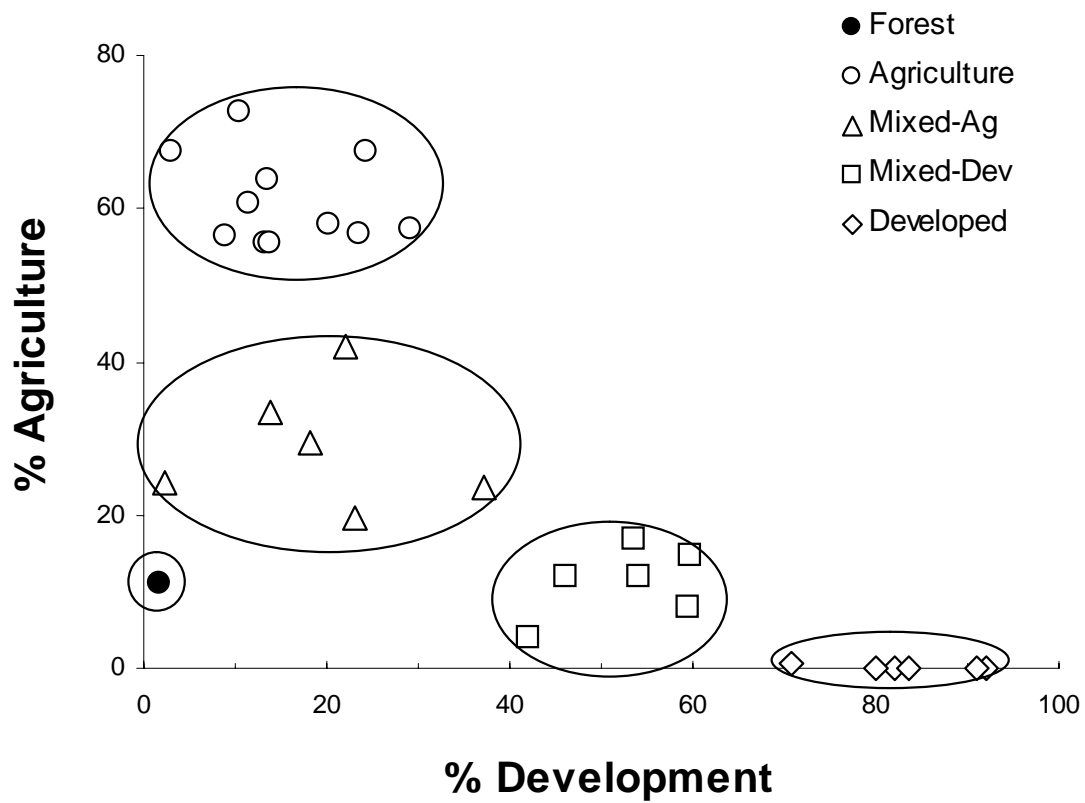
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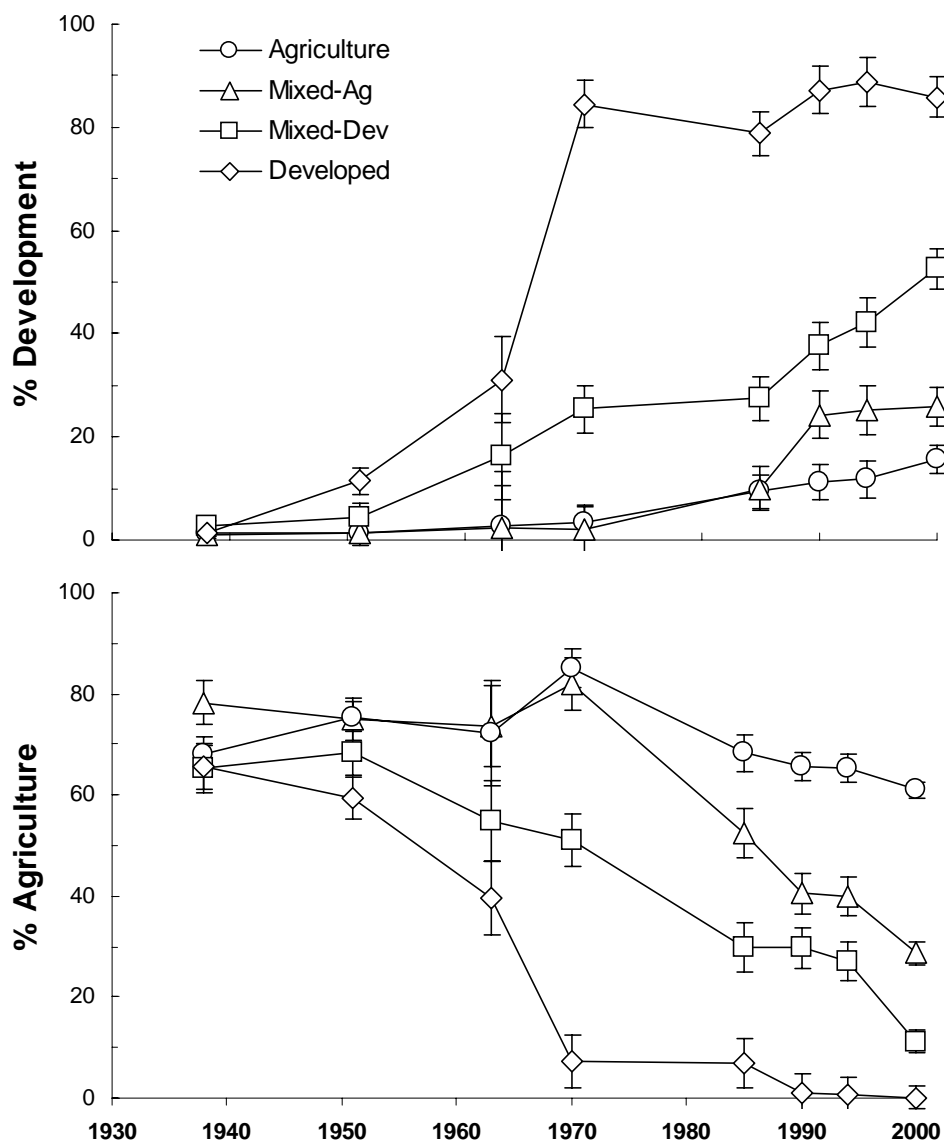
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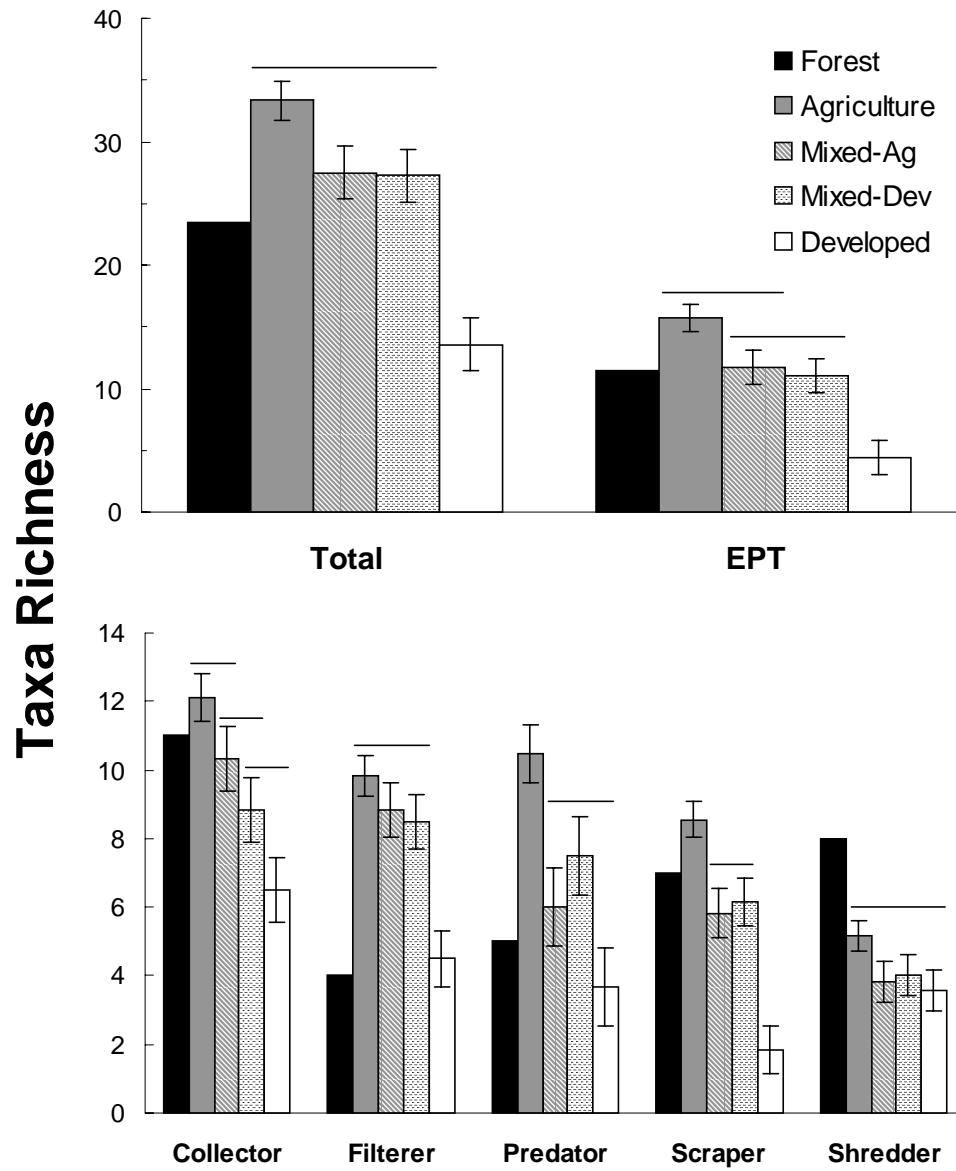
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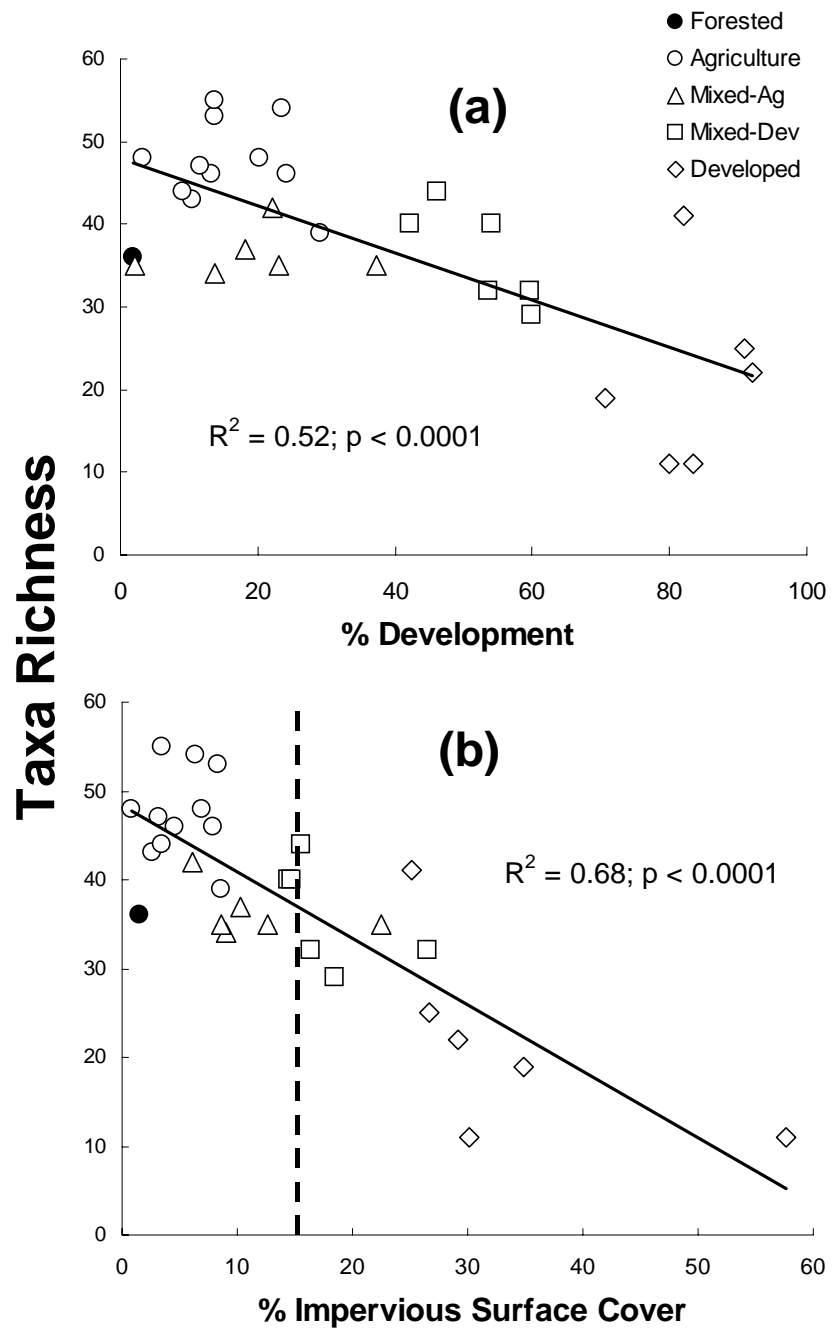
(Figure 4)



(Figure 5)

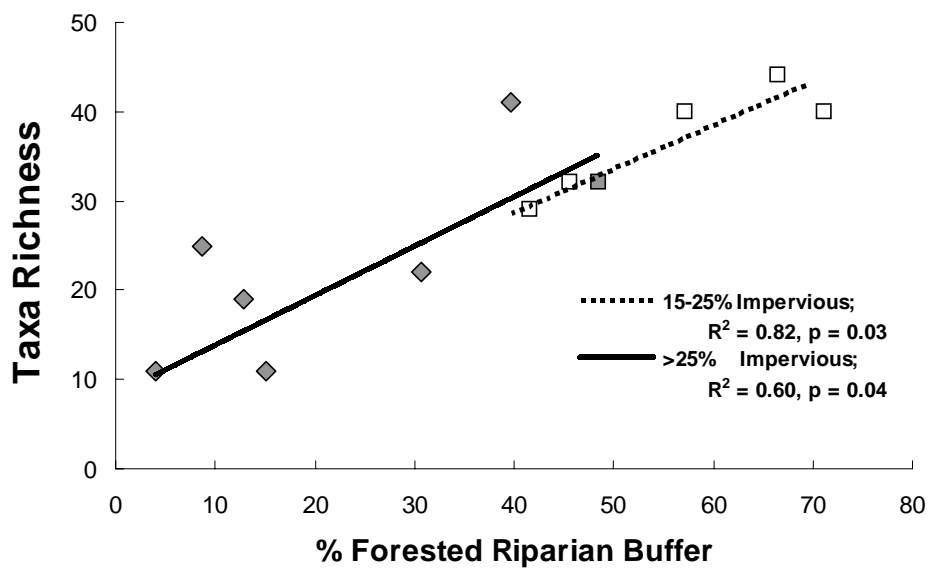


(Figure 6)

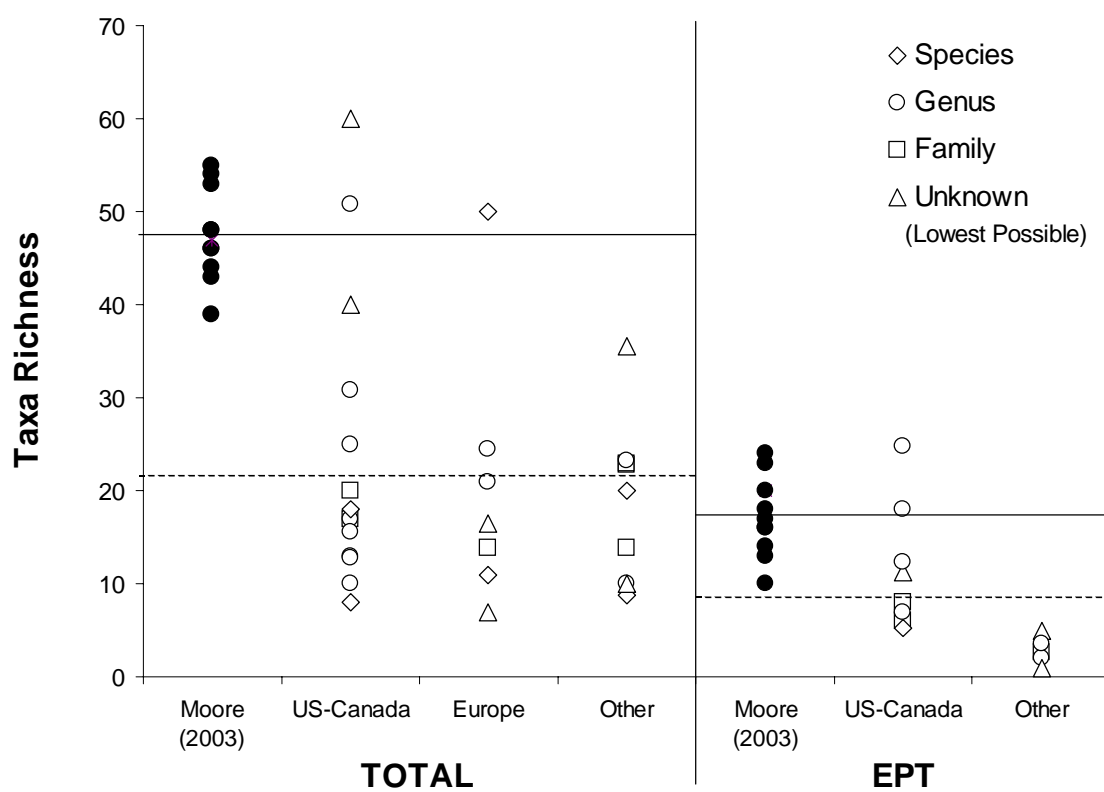




(Figure 7)



(Figure 8)



**Chapter 2:** A comparison of quantitative single-habitat and stratified multi-habitat macroinvertebrate sampling methods for assessing biological integrity

#### ABSTRACT

Stream researchers and biomonitoring agencies often employ different methods for sampling and/or subsampling benthic organisms. Quantitative single-habitat sampling methods may most accurately portray changes in macroinvertebrate populations, but are expensive to implement. Subsequently, stratified multi-habitat subsamples (a commonly used rapid bioassessment protocol) are frequently used to evaluate changes in communities over large numbers of sites. Differences in community data between these methods, and potential dissimilarities in the response to human impacts, are largely unknown. I used single-habitat quantitative (Surber) samples and multi-habitat (d-net) samples to monitor 32 sites along an urbanization gradient in Maryland, USA. Two multi-metric indices of biological integrity (IBI's) were used to compare macroinvertebrate communities and their response to residential development. Several community metrics, and one of the IBI's, showed significant differences between sampling methods. Specifically, multi-habitat d-net samples frequently produced lower richness estimates, due to the exclusion of rare taxa as a result of fixed count subsampling. Despite differences in mean IBI scores between protocols, there were strong correlations in index values between the two methods. Community metric values from both sampling methods were also strongly related to residential development. Richness estimates obtained from single-habitat samples had the highest  $r^2$  values, and thus single-habitat IBI scores were more strongly related to development than were multi-habitat scores (when a lone outlier was removed from the model). Consequently, the

more cost effective multi-habitat method should be sufficient for monitoring large numbers of sites, however quantitative single-habitat samples may be useful for assessing macroinvertebrate community changes at focal sites undergoing rapid land use change.

## INTRODUCTION

The conversion of forested and agricultural areas into landscapes dominated by urban development can cause the rapid deterioration of water resources (Paul and Meyer 2001; Palmer et al. 2002). In turn, this degradation can lead to the extirpation of local populations of aquatic species (e.g. Lenat and Crawford 1994; Moore, Chapter 1). Consequently, structural changes in stream communities are used by both academic researchers and governmental organizations to assess human impacts in disturbed or urbanizing watersheds (e.g. Stribling et al. 1998; Morley and Karr 2002; Stepenuck et al. 2002). The status of benthic macroinvertebrate communities is frequently evaluated through the use of multi-metric indices of biotic integrity (IBI's) that include information on loss of diversity and shifts in the relative abundance of sensitive taxa (Barbour et al. 1999; Karr and Chu 1999).

Researchers and agencies in overlapping geographical areas may have different purposes for assessing biological integrity, and diverse goals can lead to concurrent monitoring programs. For instance, state organizations may need to make assessments of large numbers of sites over short periods of time for the purpose of evaluating water quality or management criteria (Barbour et al. 1999). In contrast, local agencies developing and evaluating stream restoration projects may need to compile extensive long-term data sets at targeted locations that include baseline and post-project monitoring

(Kondolf 1995). There is an excellent potential for agencies to supplement each other's data collection, and thus save time and monetary resources, by collaborating to integrate data (Volstad et al. 2002). However, various monitoring objectives may result in a diversity of methods for collecting and processing benthic samples, making data integration difficult (Diamond et al. 1996). A wide variety of sampling techniques have been proposed for stream monitoring, including electroshocking (Taylor et al. 2001), drift sampling (Pringle and Ramirez 1998), artificial substrates (Barton and Metcalfe-Smith 1992), kick-seine nets (Barbour et al. 1999), Hess and Surber samples (Kerans et al. 1992), and d-frame pond-nets (Stribling et al. 1998).

Many researchers favor the use of "quantitative" single-habitat sampling techniques. The most common single-habitat method is the use of Surber samplers (Surber 1937) to obtain precise estimates of invertebrate richness and density from a specific area of riffle substrate (Hauer and Resh 1996). There are two prominent reasons why quantitative single-habitat Surber samples may be preferred over other methods. First, single-habitat samples may reduce observed variability between sites by concentrating on a distinct, well-defined habitat (Parsons and Norris 1996). Second, accurate estimates of richness and abundance require the identification of all invertebrates collected in a sample, or a substantial "fixed-fraction" of the sample volume (Courtemanch 1996). Consequently, the large number of organisms identified gives a more consistent representation of the community in the given habitat (Doberstein et al. 2000) and accounts for the presence of rare taxa (Vinson and Hawkins 1996). In addition to being valuable for biomonitoring, quantitative samples are used to closely monitor population dynamics and measure invertebrate production (Benke 1996). As a result,

quantitative single-habitat Surber samples may give the most accurate assessment of the response of the local invertebrate community to experimental treatments and anthropogenic disturbances.

While quantitative Surber samples provide excellent community information, they are time-intensive to collect and expensive to process (Storey et al. 1991). As a result, stream monitoring agencies commonly collect invertebrates using “rapid bioassessment protocols” (RBP’s; Barbour et al. 1999). This technique frequently involves the use of a d-frame pond-net (d-net) to sample a variety of habitat types in the relative proportion that they occur within a designated stream reach (e.g. Furse et al. 1981; Stribling et al. 1998). Invertebrates gathered using this method are usually subsampled in the lab, with a fixed-count target of 100-500 organisms removed for identification (Barbour and Gerritsen 1996). There are several advantages of this RBP method, including the suggested importance of collecting invertebrates from several stream habitats (Kerans et al. 1992; Bradley and Omerod 2002). Furthermore, the increased resource efficiency associated with fixed-count subsampling allows for comparisons across a larger number of sites (Barbour and Gerritsen 1996).

Multi-habitat d-net methods and quantitative single-habitat Surber methods collect information on different aspects of community organization, which can make generalization between sampling techniques difficult (Diamond et al. 1996). Recognizing differences in community data, and how these differences alter biological assessments, is essential for monitoring programs that wish to integrate data from multiple sources. Furthermore, quantitative single-habitat Surber samples should fully characterize riffle communities, and thus may show a clearer response of invertebrates to

human impacts. This may indicate that the use of quantitative Surber samples is warranted at sites undergoing restoration or rapid urbanization, but justifying the added expense of this method requires a full understanding of the potential benefits for biological monitoring. Finally, if there are differences in communities and their response to disturbance using these two methods, it is important to identify whether this is due primarily to dissimilarities in field sampling procedures (i.e. sampling tools or habitats sampled) or to laboratory subsampling procedures. Determining the relative contribution of these factors could indicate how to acquire the most useful biological information while maximizing monitoring resources.

In this paper, I compare quantitative macroinvertebrate Surber samples from riffle habitats with stratified multi-habitat d-net samples collected in urbanizing watersheds in Maryland, USA. I use two multi-metric IBI's, and the 15 community variables comprising these indices, to: (1) determine if the two sampling methods provide similar community information, and to test whether IBI scores using these methods can be integrated using linear models; (2) compare the strength of relationships between IBI's and residential development using each collection method; and, (3) test whether differences between methods are due to field sampling tools and/or habitats or due to laboratory subsampling procedures, by analyzing fixed-count subsamples generated from the quantitative data set. From these analyses, I hoped to discern if quantitative single-habitat samples could be used to supplement the data collected by monitoring agencies, and to address the possibility that quantitative samples may provide additional information that may justify their use at important focal sites.

## METHODS

### *Study Sites & Invertebrate Sampling*

This study took place in three Piedmont watersheds in Montgomery County, Maryland, USA. Paint Branch (28.5 km<sup>2</sup>) and Northwest Branch (59.0 km<sup>2</sup>) are tributaries of the Potomac River, while Hawlings River (67.6 km<sup>2</sup>) is a tributary of the Patuxent River (Fig. 9). Each of these watersheds is located within approximately 35 km of Washington, DC, and ultimately drains into the Chesapeake Bay.

These watersheds were similarly dominated by agricultural practices in the mid-1900's (>60%), with lesser amounts of forest (<30%) and residential development (<10%). Currently, Paint Branch and Northwest Branch are dominated by residential development (64% and 53%, respectively), while Hawling's River is characterized by a mixture of residential development (25%) and agriculture (36%). Agricultural land use in this area generally represents a mixture of approximately two-thirds pasture, and one-third crop (i.e. corn, soybean and winter wheat) cultivation. All three watersheds have similar amounts of forested area (21-32%), with much of this forest found in extensive riparian buffers throughout the stream networks.

Macroinvertebrate communities were collected at 32 sites in 2001 and 2002 across these three watersheds. These sites are part of a benthic monitoring program that was implemented by the Montgomery County Department of Environmental Protection (DEP) in 1994 (Roth et al. 2001), and are concurrently being surveyed as a part of a collaborative study addressing the effects of urban sprawl on stream ecosystems (Palmer et al. 2002). Macroinvertebrates were collected from March 15-April 15 within a 75 m reach at each site that has previously been used for benthic monitoring by the DEP.



Macroinvertebrates were simultaneously collected with both a d-net using a stratified multi-habitat technique, and with composite Surber samples taken in several riffle habitats.

The multi-habitat d-net method has been recently adopted by the DEP to correspond to the methodology used by the Maryland Department of Natural Resources (DNR) (Volstad et al. 2002). Twenty d-net “jabs” (approximately 1 ft<sup>2</sup> each) were taken from each reach, and split up according to relative proportions of the “most productive” habitats available (generally riffles, leaf packs, submerged roots and woody debris). All of the benthic material collected was combined, and large pieces of sediment and organic matter were rinsed and removed from the sample. The rest of the material was preserved in the field using 95% ethanol, and returned to the DEP lab for processing. Fixed-count macroinvertebrate subsamples were taken by depositing the collected sample on a partitioned sorting tray, and collecting invertebrates from randomly selected grids until a target of 100 organisms was reached. All invertebrates within the final grid were collected (generally resulting in a final count of 100-200 organisms) and the entire macroinvertebrate sample was identified to the lowest possible taxonomic level, usually genus.

Surber samples were gathered from three consecutive riffles starting at the downstream end of the 75 m reach at each site. These single-habitat samples were collected at the same time as the multi-habitat d-net samples while working in an upstream direction. This simultaneous sampling was done to minimize the influence of substrate disturbance between protocols. In some instances, the length of the reach was extended past 75 m to include the required number of riffles. Surber samples (0.04 m<sup>2</sup>

each) were collected from two random locations in each riffle by disturbing the substrate to a depth of approximately 10 cm for two minutes. A total of six Surber samples were combined into a composite sample in 15 liters of filtered stream water (45  $\mu\text{m}$ ), and a fixed-fraction (33%) of the pooled material was removed while vigorously agitating the sample. This material was stored on ice, and brought back to the lab for preservation in a 10% formalin solution. All organisms collected were identified to the lowest possible taxonomic level, usually genus. Fifty chironomid larvae from each site in 2001 were identified to sub-family, and were then used to calculate relative abundance of these taxa in each sample. Chironomid taxa collected in Surber samples were only sorted to the family level in 2002.

#### *Data Analysis*

Multi-metric IBI's represent the average of several standardized community variables, generally related to diversity and the relative abundance of certain taxa. Differences between sampling methods were analyzed using IBI scores from two indices used throughout this region by the DEP and the DNR (Table 4). In addition to examining overall IBI scores, I also looked at the differences in the 15 individual community variables comprising these two IBI's. Information on tolerance values and functional feeding group designations of individual taxa (Appendix) were taken primarily from Stribling et al. (1998), with missing information supplemented by the Maryland Department of Environment, Montgomery County DEP, Barbour et al. (1999), Lenat (1993), and Bode et al. (1996).

Paired t-tests were used to test for differences in individual IBI metrics between sampling methods during the 2001 sampling season (Proc Ttest, SAS v8.2), with type I

error rate controlled using Hommel's adjustment of p-values (Proc Multtest, SAS v8.2). Several of the metrics relating to invertebrate relative abundance required arcsine square root data transformations to meet assumptions of normality and variance homogeneity (Table 5). The difference in mean DNR IBI scores between sampling methods was also tested using a paired t-test. The comparisons above were performed in 2001 only, as one of the metrics used in the DNR IBI (% Tanytarsini) requires sub-family identification of Chironomidae, not available for Surber samples in 2002. A repeated-measures 2 x 2 factorial analysis of variance (ANOVA; Proc Mixed, SAS v8.2) was used to examine the effects of sampling method (single- or multi-habitat) and year (2001 or 2002) on DEP IBI scores, with chironomid taxa identified to the family level. The repeated-measures ANOVA structure was used to account for temporal autocorrelation in macroinvertebrate communities collected at a given site across sampling years. Simple linear models were used to examine the predictive relationships between the two sampling methods using 2001 DEP and DNR IBI scores (Proc Corr, SAS v8.2).

The strength of the relationships between 2001 DEP and DNR IBI scores and residential development were examined using linear regression models (Proc Reg, SAS v8.2). Residential development is defined here as the percentage of land use occurring within the drainage area of each sampling site. Research in this system has shown that macroinvertebrates communities are significantly degraded by development along this land use gradient (Moore, Chapter 1). Percentages of residential development were determined using Maryland Office of Planning GIS land use coverages in the ArcView GIS supplement program GISHydro2000 2<sup>nd</sup> ed. ([www.gishydro.umd.edu](http://www.gishydro.umd.edu)). Land use percentages for each site were calculated by overlaying these coverages on drainage areas

delineated using digitized topographical maps. Linear model regressions were also used to test the strength of the relationships between development and all individual community metrics for each sampling method (Proc Reg, SAS v8.2). Type I error rate for the linear model p-values was not adjusted, as descriptive model comparisons between methods considered only the absolute  $r^2$  values.

Creating subsamples from the quantitative data set, and then reexamining community differences, can help determine the relative contribution of field habitat sampling and laboratory subsampling procedures on observed dissimilarities between methods. Random fixed-count subsamples of 100, 200, 300, 400 and 500 organisms were computer generated from Surber samples, corresponding to the range of subsamples sizes typically used in stream biomonitoring. Community metrics and IBI's were separately computed for each of these single-habitat subsample groups. Differences in IBI scores and metrics between d-net subsamples and each of the Surber subsamples were separately tested using paired t-tests, with error rate controlled using Hommel's adjustment of p-values. Relationships between residential development and community variables for each of the Surber subsamples were examined using simple linear regression models.

## RESULTS

### *Community Comparisons*

Community comparisons between sampling methods were performed separately for the community variables comprising the DEP and DNR IBI's, and for the overall IBI scores. The variables used in the IBI's were separated into metrics representing

invertebrate richness and relative abundance, with the exception of the “biotic index”, which represents the mean tolerance value of all invertebrates in a sample (Table 5). Single-habitat Surber samples had higher values of total, Diptera, and intolerant taxa richness ( $p < 0.01$ ; Fig. 10a). Among abundance metrics, single-habitat Surber samples had higher values for the percentages of Hydropsyche-Cheumatopsyche sp. and tolerant taxa ( $p < 0.01$ ; Fig. 10b). Conversely, there were higher percentages of Ephemeroptera, EPT taxa, and shredder taxa collected in multi-habitat d-net samples. The single-habitat protocol produced higher biotic index values than did the multi-habitat method ( $p = 0.001$ ; Fig. 10a).

The fixed-count subsamples generated from the single-habitat data set did not have any effect on differences between methods for relative abundance metrics (e.g. % Hydropsyche/ Cheumatopsyche; Fig. 11a). However, invertebrate richness in single-habitat samples decreased with subsample size. While total single-habitat Surber samples and large fixed-count subsamples (400 and 500 individuals) had higher richness compared to the multi-habitat d-net method, 100 individual single-habitat subsamples had significantly lower richness than the similar sized multi-habitat subsamples (Fig. 11b).

Differences in community variables between d-net samples and total Surber samples led to overall higher DEP IBI scores for d-net samples when examined across both sampling years ( $F_{1,31} = 7.49$ ,  $p = 0.01$ ; Fig. 12a). There was not a significant effect of year ( $F_{1,31} = 2.82$ ,  $p = 0.10$ ), or an interaction between year and method ( $F_{1,25} = 0.28$ ,  $p = 0.60$ ) in this model. In contrast to the DEP index, there was no overall effect of sampling method in 2001 when comparing DNR IBI scores ( $t_{26} = 0.71$ ,  $p = 0.48$ ; Fig. 12a). Differences between single-habitat Surber and multi-habitat d-net IBI scores were

also dependent on subsampling procedure (Fig. 12b). When p-values were adjusted for multiple comparisons, differences in DEP IBI scores between multi-habitat samples and total or 500 organism single-habitat samples became non-significant. In contrast, all other single-habitat subsamples had significantly lower IBI scores than the multi-habitat subsamples (all  $p_{\text{adj}} < 0.05$ ).

There were highly significant correlations between quantitative single-habitat and multi-habitat IBI scores for both the DEP index ( $r^2 = 0.46$ ,  $p = 0.0001$ ) and the DNR index ( $r^2 = 0.37$ ,  $p = 0.0007$ ; Fig. 13). A distinct outlier was found within the single-habitat scores (site “NW13”), representing a subwatershed with a large percentage of residential development (82%), yet high IBI scores indicative of a healthy invertebrate community. The solitary outlier strongly affected the relationship in IBI scores between methods, and removing this site from the analysis allowed 64% and 61% of the variation in the DEP and DNR index scores to be explained, respectively ( $p < 0.0001$ ).

#### *Community Response to Development*

The response of macroinvertebrate communities to development was examined separately for the 15 IBI community variables, and for the overall IBI scores. Most of the individual community variables demonstrated a significant relationship with residential development for both the single-habitat Surber and multi-habitat d-net methods ( $p < 0.05$ ; Table 5). In general, richness variables seemed to be more strongly related to development (higher  $r^2$ ) than relative abundance ratios. When comparing the strength of the relationships between community variables and development for each method, I found that 11 of the 15 community metrics had higher  $r^2$  values for single-habitat Surber

samples. This suggests that there may be an overall stronger community response to residential development using the quantitative single-habitat method (Table 5).

The linear relationships between residential development and IBI scores using the two sampling methods were remarkably similar for both the DNR and DEP indices, and I give results from only the DEP IBI for the sake of brevity. Percent development explained 43% of the variation in DEP IBI scores using the single-habitat Surber method (Fig. 14;  $p < 0.0001$ ), and 41% of the variation in IBI scores using the multi-habitat d-net methods ( $p = 0.0003$ ). The relationship between IBI scores and development drastically improved when the outlier site NW13 was removed from the analysis ( $r^2 = 0.65$ ). A more detailed discussion of the importance of identifying and/or removing this outlier is provided later in this article.

Fixed count subsample size did not seem to have a notable effect on the relationships between residential development and community variables or overall IBI scores (Table 6). In most cases,  $r^2$  values for subsamples were comparable to  $r^2$  values for the entire sample. This indicates that differences in the observed response of macroinvertebrates to human disturbance are largely due to dissimilarities in field sampling procedures (i.e. sampling tools or habitats sampled).

## DISCUSSION

Single-habitat quantitative collection methods using Surber samplers and stratified multi-habitat sampling using d-nets are two common techniques for gathering macroinvertebrate community information (e.g. Frost et al. 1971; Furse et al. 1981; Mackey et al. 1984; Kerans et al. 1992). While quantitative samples most thoroughly

characterize benthic communities, monitoring programs frequently prefer the use of the more time and cost efficient multi-habitat invertebrate subsamples (e.g. Resh and Jackson 1993; Stribling et al. 1998). In this paper, I examine whether monitoring agencies could potentially increase their ability to manage disturbed watersheds by selectively utilizing quantitative single-habitat Surber samples, and whether these data can be successfully integrated into multi-habitat data sets. To date, few studies have compared differences in macroinvertebrate samples between these two methods (but see Mackey et al. 1984; Storey et al. 1991), or their differential response to human disturbance.

#### *Community comparisons*

Multi-metric benthic IBI scores represent the combination of numerous community variables, and are often used to rate overall site conditions relative to surrounding streams (Kerans and Karr 1994; Resh et al. 1995). Our results suggest that the single-habitat Surber and multi-habitat d-net methods provide somewhat dissimilar community information, evident by several significant differences in community metrics. For instance, there were lower proportions of Ephemeroptera and EPT taxa in single-habitat samples, and higher proportions of tolerant organisms and biotic index values. This likely reflects the proportionately large number of pollution-tolerant Chironomidae and Oligochaeta collected in Surber samples. Palmer (1990) found that these organisms are buried in benthic sediments at relatively high densities. The Surber method thoroughly sampled the top 10 cm of the riffle substrates, and should allow these taxa to be well characterized. In contrast, the d-net method does not sample these habitats as intensively and multi-habitat subsamples contain relatively few riffle taxa, causing this group to be comparatively under-represented. Hydropsychid caddisflies are filtering



organisms that are also found exclusively in areas of turbulent flow (Wallace and Merritt 1980), explaining their greater abundance in single-habitat riffle samples. D-net samples had a significantly higher ratio of shredder taxa, invertebrates that utilize concentrated areas of organic matter (leaf packs) as a primary food resource (Cummins and Klug 1979; Wallace and Webster 1996). Leaf packs are frequently encountered in depositional areas, which are not sampled using the quantitative Surber method.

Interestingly, three of the five richness metrics had significantly higher values for Surber samples, while none of these variables were higher in d-net samples. Story et al. (1991) suggested that the comparatively greater sampling intensity involved in collecting Surber samples favors the collection of more invertebrate taxa, although others have found greater richness in d-net samples (Mackey et al. 1984). A more likely reason for this finding is the fixed-count subsampling used when processing samples collected with the multi-habitat d-net method. Because fixed-count subsampling reduces the number of organisms identified, it should provide lower richness estimates due to the under-representation of rare taxa (Courtemanch 1996). In contrast, the fixed-fraction subsampling employed in the single-habitat sample processing allowed for a much higher abundance of organisms to be identified (mean = 920 individuals). Randomly resampling the single-habitat data set confirmed that differences in richness between methods are due primarily to subsampling. Single-habitat 100 count subsamples actually had fewer taxa than multi-habitat subsamples, probably due to the combination of losing the more rare taxa and having a relatively high abundance of Chironomidae. In contrast, abundance ratio metrics (i.e. percentage values) were not affected by subsampling, indicating that dissimilarities are likely due to taxonomic differences between the habitats sampled.

When considered over both sampling years, the single-habitat Surber samples had lower mean DEP IBI scores. The differences in taxonomic composition discussed above (i.e. smaller proportions of EPT taxa and larger proportions of pollution tolerant organisms in single-habitat samples) likely caused this disparity. In contrast, there was no significant difference in DNR IBI scores between methods. This indicates that there were overall greater differences between methods in the community metrics in the DEP IBI. Interestingly, the differences in DEP IBI scores between sampling methods increased when the index was calculated using fixed-count single-habitat subsamples (Fig. 12). This suggests that IBI scores are particularly susceptible to the exclusion of rare taxa that occurs during fixed-count subsampling. In general, these findings indicate that site assessment (i.e. IBI scores) may be extremely sensitive to field collection methods, the type of subsample processing, and the community metrics used to calculate indices. Consequently, the successful integration of data using different sampling methods will require careful prior evaluation of these differences.

While quantitative single-habitat Surber samples and multi-habitat d-net samples provided different values for several community metrics and one of the IBI's, there was a fairly strong correlation in IBI scores between the two methods when I removed the lone outlier from the model (Fig. 13). This indicates that scores obtained using single-habitat Surber techniques could be integrated into regional multi-habitat data sets with a fair degree of accuracy using simple linear models. Volstad et al. (2002) previously used this correlation technique for integrating data, showing that 78% of the variation in multi-habitat IBI scores could be predicted using single-habitat invertebrate subsamples. However, the difference in absolute IBI values between methods for the DEP index

discussed above suggests that the scoring criteria for determining site impairment may have to be adjusted when integrating data from other sources.

#### *Community response to development*

The strength of linear relationships between community variables and residential development show that richness variables are very important for predicting the effects of urbanization, and that single-habitat Surber samples may be somewhat better at detecting these changes. A potential explanation for this is the inclusion of more rare taxa in the Surber samples. There has been some recent discussion of the importance of including rare taxa in biomonitoring efforts (Cao et al. 1998; Marchant 1999; Cao and Williams 1999). Cao et al. (1998) found that including rare taxa helped discriminate between impaired and reference sites when segregation was based on invertebrate richness. In contrast, Marchant (1999) suggested that the use of rare taxa is largely unnecessary, and removing them from community analyses can result in a nearly identical ordination of sites. Interestingly, the stronger response of the single-habitat community metrics to development seems to be independent of subsampling procedure, suggesting that it is not the presence of rare taxa in single-habitat Surber samples that is driving these relationships. These associations may be due instead to the more thorough community characterization provided by the intensive sampling of the single-habitat method.

Overall IBI scores from both sampling methods are similarly related to subwatershed development (Fig. 14). However, the more intensive single-habitat community sampling revealed that one of the highly developed sites (NW13) had a remarkably healthy invertebrate community. Identifying this type of outlier is important for two reasons. First, removing this point from the single-habitat analysis drastically

increases the relationship between IBI scores and development. This removal would be appropriate if the monitoring goal was to create models predicting the effects of development on stream communities, and may suggest that single-habitat methods may be better in formulating such models. Second, identifying relatively healthy communities in urban areas offers a unique opportunity for investigating the specific factors that are mediating or exacerbating the harmful effects of development (Karr and Chu 1999), and thus has implications for successful stream restoration. Site NW13 is one of four sampling locations having similar (70-85%) development, yet the upstream network draining to this site has a very well protected riparian buffer system (40% forested) relative to the other developed sites (4-15% forested). This riparian forest may be shielding the stream from urbanization effects (Moore, Chapter 1). The fact that the Surber sampler was able to detect a healthy community at this site may be due to either a greater sampling intensity or a lack of subsampling, which is more likely to fully characterize the benthic community (Doberstein et al. 2000).

### *Conclusions*

In general, the results of this study suggest that samples collected using quantitative single-habitat Surber methods and stratified multi-habitat d-net methods may produce significantly different community data. These differences are a result of taxonomic dissimilarities resulting from laboratory subsampling, as well as field collection procedures (including sampling equipment and the habitats sampled), and can lead to overall different IBI scores between sampling methods. Despite these differences in absolute index values, the strong correlations in IBI scores between the two methods suggest that data integration between protocols could be successful. Furthermore, the

communities collected by both sampling methods demonstrated significant degradation in response to residential development. However, the richness variables from the quantitative single-habitat method had the strongest relationships, independent of subsampling procedure. This led to a stronger response of the single-habitat IBI scores to development when a lone outlier was removed from the linear model, and suggests that this method may be extremely useful in monitoring subtle changes in stream health resulting from human impacts. Consequently, if single-habitat Surber data can indeed be successfully incorporated into multi-habitat programs, monitoring agencies may justify the use of this quantitative collection method at sites undergoing rapid urbanization or targeted for restoration, despite additional costs in resources.

Table 4. Community metric variables used in the calculations of Maryland DNR and Montgomery County DEP IBI's, with explanations on how these variables were calculated. Each metric was scored with a 1, 3 or 5, based on the raw values, and the expected direction of change in each metric with increasing urban development is indicated. IBI values for each site are based on the mean scores of all metrics. DEP metrics were scored differently based on stream order, while DNR metric scores are the same for all stream sizes.

Metric	Explanation	Stream Order	Scoring Criteria			Change w/ Development
			1	3	5	
<b>DNR</b>						
Total Richness	Total # of Invertebrate Taxa		<16	16-22	>22	Decrease
EPT Richness	Total # of EPT Taxa		<5	5-12	>12	Decrease
Ephemeroptera Richness	Total # of Ephemeroptera Taxa		<2	2-4	>4	Decrease
Diptera Richness	Total # of Diptera Taxa		<6	6-9	>9	Decrease
Ephemeroptera Percent	# Ephemeroptera/Total Abundance		<5.7%	5.7-20.3%	>20.3%	Decrease
Tanytarsini Percent	# Tanytarsini/Total Abundance		0	0-4.8%	>4.8%	Decrease
Collector Percent	# Collectors/Total Abundance		<13.5	13.5-31%	>31%	Decrease
Intolerant Richness	Total # of Taxa w/ Tolerance 0-3		<3	3-8	>8	Decrease
Tolerant Percent	(# Taxa w/ Tolerance 7-10)/Total Abundance		>48%	11.8-48%	<11.8%	Increase
<b>DEP</b>						
Total Richness	Total # of Invertebrate Taxa	1st-2nd	<12	12-23	>23	Decrease
		3rd-4th	<11	11-22	>22	
EPT Richness	Total # of EPT Taxa	1st-2nd	<6	6-11	>11	Decrease
		3rd-4th	<7	7-12	>12	
EPT Percent	EPT Abundance/Total Abundance	1st-2nd	<28%	28-55%	>55%	Decrease
		3rd-4th	<28%	28-55%	>55%	
Scraper Percent	# Scrapers/(# Scrapers + # Filterers)	1st-2nd	<10%	10-20%	>20%	Decrease
		3rd-4th	<9%	9-18%	>18%	
Shredder Percent	# Shredders/Total Abundance	1st-2nd	<3%	3-5%	>5%	Decrease
		3rd-4th	<3%	3-5%	>5%	
Hydropsyche/Cheumatopsyche Percent	(# Hydropsyche + # Cheumatopsyche)/EPT Abundance	1st-2nd	>57%	15-57%	<15%	Increase
		3rd-4th	>59%	17-59%	<17%	
Dominant Taxa Percent	# of Most Abundant Taxa/Total Abundance	1st-2nd	>67%	33-67%	<33%	Increase
		3rd-4th	>74%	47-74%	<47%	
Biotic Index	(# of Each Taxa*Tolerance of Each Taxa)/Total Abun	1st-2nd	>6.93	3.86-6.93	<3.86	Increase
		3rd-4th	>6.89	3.78-6.89	<3.78	

Table 5. Tests of differences in individual community metrics between sampling methods (paired t-test (Surber - d-net);  $n = 27$ ), using Hommel p-value adjustment for multiple comparisons. Also shown are the results of linear model regressions indicating the strength of the relationship (using  $r^2$  values) between each metric and subwatershed residential development for both Surber ( $n = 31$ ) and d-net ( $n = 27$ ) samples. Eleven of the 15 metrics had higher  $r^2$  values using the single-habitat method, indicated by positive values of  $r^2_{\text{Surber}} - r^2_{\text{d-net}}$ .

Metric	IBI	Paired t-test		Development (Surber)	Development (d-net)	$r^2_{\text{Surber}} - r^2_{\text{d-net}}$
		t-statistic	p	$r^2$	$r^2$	
<b>Invertebrate Richness</b>						
Total Richness	Both	4.1	0.003	0.53**	0.27*	0.26
EPT Richness	Both	2.04	0.21	0.51**	0.49**	0.02
Ephemeroptera Richness	DNR	0.16	0.88	0.45**	0.49**	-0.04
Diptera Richness	DNR	4.19	0.002	0.22*	0.07	0.16
Intolerant Richness	DNR	2.67	0.09	0.52**	0.34**	0.18
<b>Abundance Ratio</b>						
Ephemeroptera Percent <sup>+</sup>	DNR	-5.64	0.001	0.33**	0.24*	0.09
Tanytarsini Percent <sup>+</sup>	DNR	1.97	0.21	0.13*	0.20*	-0.07
Collector Percent	DNR	1.69	0.31	0.19*	0.13*	0.06
Tolerant Percent <sup>+</sup>	DNR	4.35	0.002	0.05	0.02	0.04
EPT Percent	DEP	-3.95	0.004	0.14*	0.09	0.05
Hydropsyche/Cheumatopsyche %	DEP	5.15	0.001	0.38**	0.19*	0.18
Dominant Taxa Percent <sup>+</sup>	DEP	2.11	0.18	0.14*	0.05	0.09
Scraper Percent <sup>+</sup>	DEP	-1.26	0.44	0.10	0.13*	-0.03
Shredder Percent <sup>+</sup>	DEP	-4.29	0.002	0.18*	0.27*	-0.09
<b>Other</b>						
Biotic Index	DEP	5.43	0.001	0.33**	0.18*	0.15

+ Arcsine transformed ratio metrics

\*  $p < 0.05$ ; \*\*  $p < 0.001$

Table 6. Changes in mean values of IBI scores and community metrics resulting from the subsampling of single-habitat samples. Letters next to mean single-habitat subsample values represent significant (“a”) or non-significant (“b”) differences compared to the multi-habitat subsamples based on paired t-tests ( $p < 0.05$ ). Also shown are the regression coefficients for the strength of the relationship between community variables and residential development for each subsample. All linear relationships shown are significant at  $p < 0.05$ . Only one richness metric and one abundance ratio metric are shown for the sake of brevity, however these general patterns are the same as those found for the other richness and ratio metrics.

Subsample Size	DEP IBI score		Total Richness		% Hydropsyche/ Cheumatopsyche	
	Mean	$r^2_{\text{development}}^*$	Mean	$r^2_{\text{development}}$	Mean	$r^2_{\text{development}}$
Multi-habitat						
100-200	3.08 <sub>a</sub>	0.38	16.93 <sub>a</sub>	0.27	0.26 <sub>a</sub>	0.19
Single-habitat						
100	2.21 <sub>b</sub>	0.56	12.78 <sub>b</sub>	0.39	0.52 <sub>b</sub>	0.25
200	2.38 <sub>b</sub>	0.57	15.75 <sub>a</sub>	0.48	0.53 <sub>b</sub>	0.41
300	2.55 <sub>b</sub>	0.59	18.72 <sub>a</sub>	0.44	0.52 <sub>b</sub>	0.45
400	2.56 <sub>b</sub>	0.57	20.06 <sub>b</sub>	0.47	0.53 <sub>b</sub>	0.35
500	2.62 <sub>a</sub>	0.59	21.38 <sub>b</sub>	0.49	0.52 <sub>b</sub>	0.39
Total	2.68 <sub>a</sub>	0.66	24.72 <sub>b</sub>	0.53	0.53 <sub>b</sub>	0.38

\* single outlier removed from single-habitat linear models



## FIGURE LEGENDS

Figure 9. Regional location of the three study watersheds used for macroinvertebrate collecting in Montgomery County, Maryland, USA. Areas of forest, agriculture, and development, are indicated on the bottom of the figure, with open circles representing sampling locations.

Figure 10. Mean values of invertebrate richness and abundance ratio metrics (+/- SE) for each sampling method. Significant differences in means are noted with an asterisk (Hommel's adjusted  $p < 0.10$ ). Biotic Index values are included in the richness plot, but represent the average tolerance value of all individuals in a sample. Proportions of Ephemeroptera, Tanytasini, collector, tolerant, dominant, scraper and shredder taxa were arcsine square root transformed for analysis and back-transformed for plots.

Figure 11. (a) Mean percentages of Hydropsyche/Cheumatopsyche sp. relative abundance, and (b) mean total taxa richness for multi-habitat (usually 100-200 individual) and single-habitat (100-500 individual) fixed count subsamples. Significant differences between the multi-habitat method and each of the single-habitat subsamples (based on paired t-tests,  $p < 0.05$ ) are indicated by asterisks (\*). Only one richness metric and one abundance ratio metric are shown for the sake of brevity, however these patterns are the same as those found for the other richness and ratio metrics.

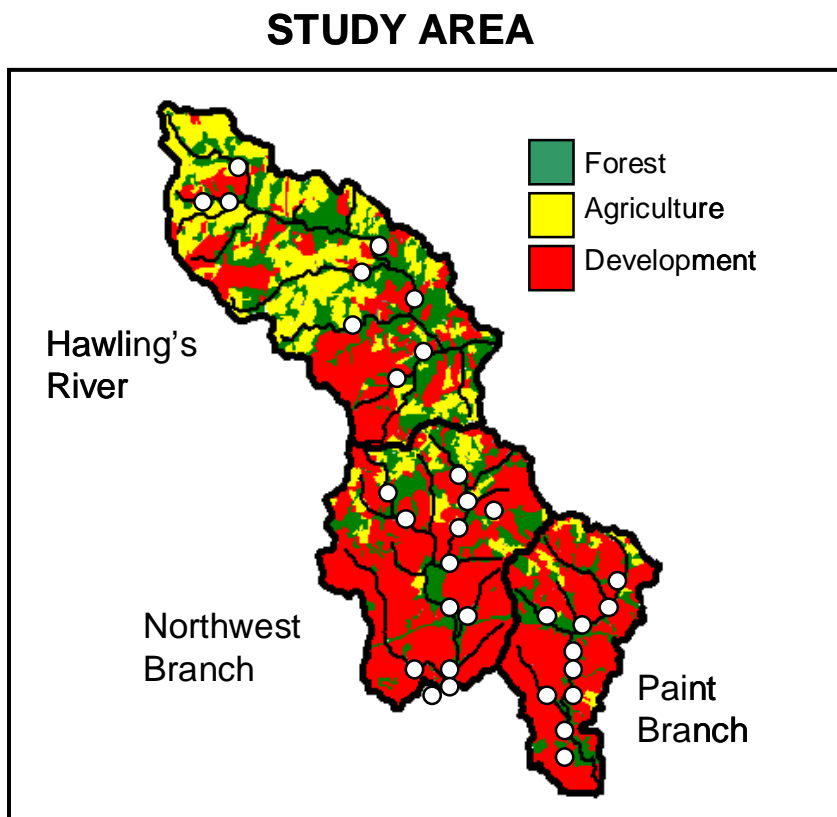
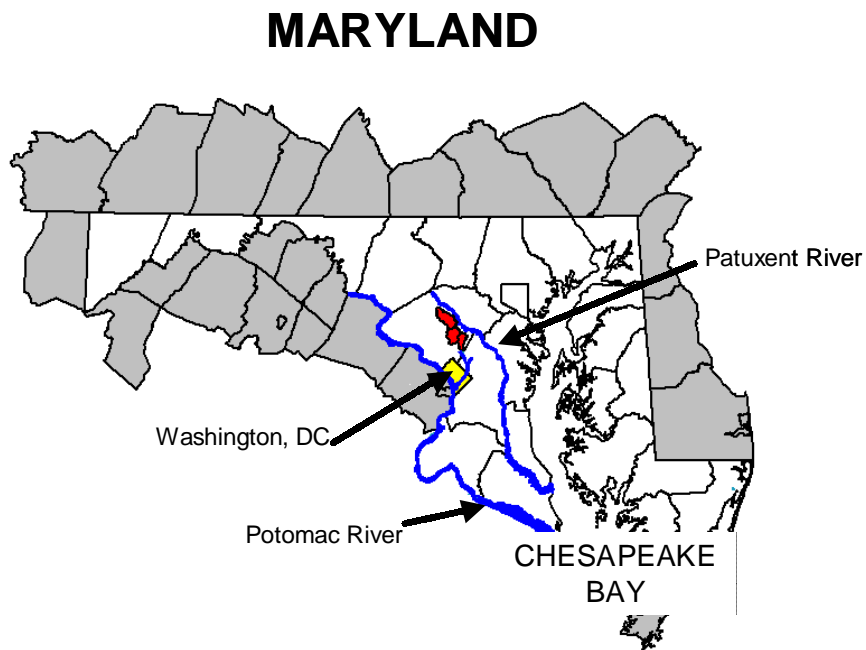
Figure 12. (a) Mean IBI scores (+/- SE) for each sampling method for both DNR and DEP indices ( $n = 32$  for single-habitat Surber samples,  $n = 27$  (2001) or  $31$  (2002) for

multi-habitat d-net samples). Mean DNR values are for 2001 only (required sub-family identification of Chironomidae not available for Surber samples in 2002), while DEP scores represent the average across both sampling years. The line connecting the bars for mean DNR index values indicate no significant difference between sampling methods ( $p > 0.05$ ). (b) Mean DEP IBI scores for multi-habitat (usually 100-200 individual) and single-habitat (100-500 individual) fixed count subsamples. Significant differences between the multi-habitat method and each of the single-habitat subsamples (based on paired t-tests, Hommel's  $p < 0.05$ ) are indicated by asterisks (\*).

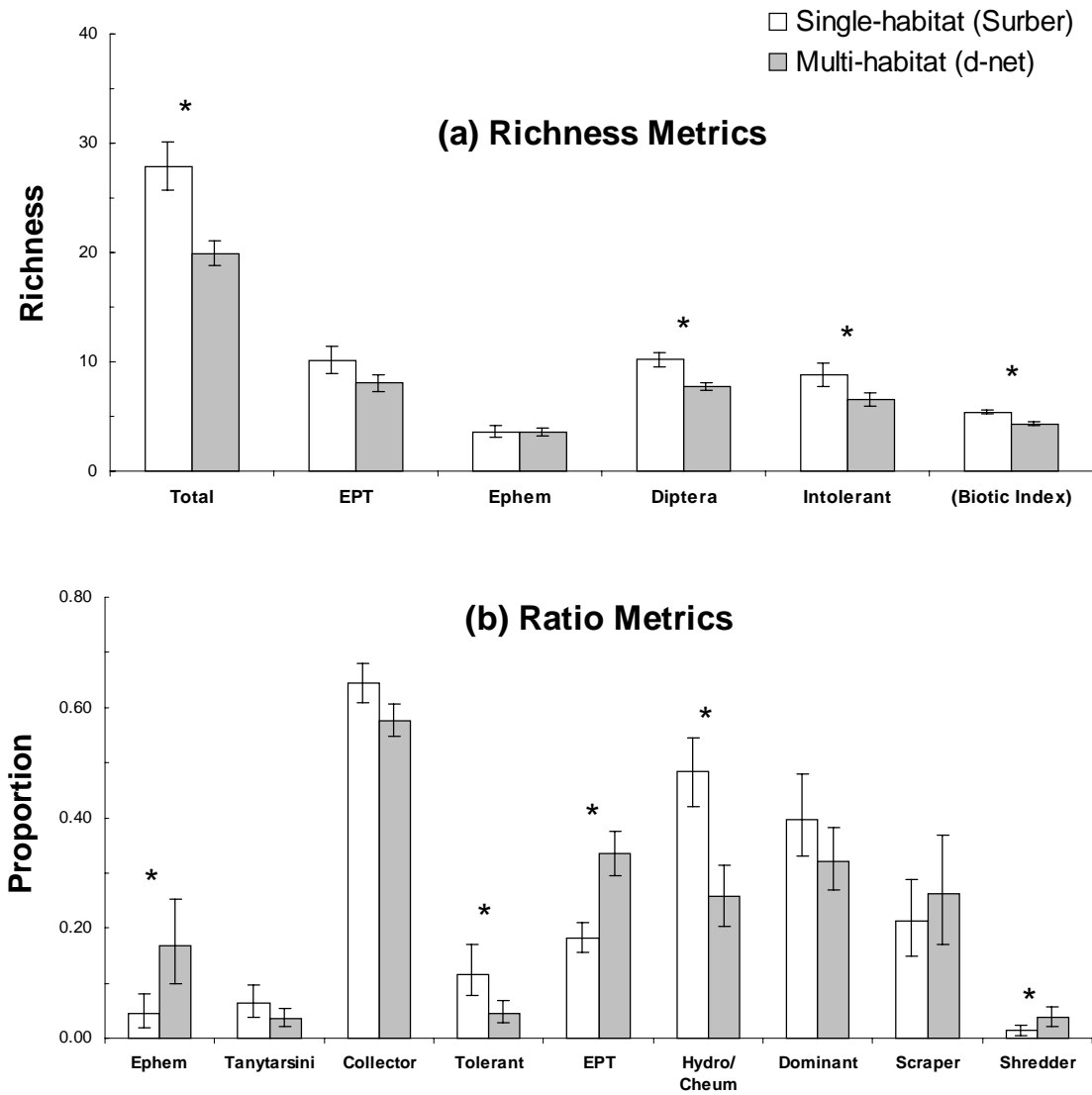
Figure 13. Relationship between 2001 DEP IBI scores using single-habitat and multi-habitat sampling methods ( $n = 27$ ). A considerable outlier site is indicated by the closed diamond, and  $r^2$  values are given with and without this outlier in the analysis. Regression lines indicate the linear relationship in IBI scores between methods with the outlier included, but is nearly identical to the regression line with the outlier removed.

Figure 14. Relationship between subwatershed development and DEP IBI scores in 2001 for both single-habitat ( $n = 32$ ) and multi-habitat ( $n = 27$ ) sampling methods. A considerable outlier in the single-habitat data set is indicated by the closed diamond, and  $r^2$  values are given with and without this outlier in the analysis. Regressions lines indicate the linear relationship between development and IBI scores (with the outlier included in the Surber sample analysis).

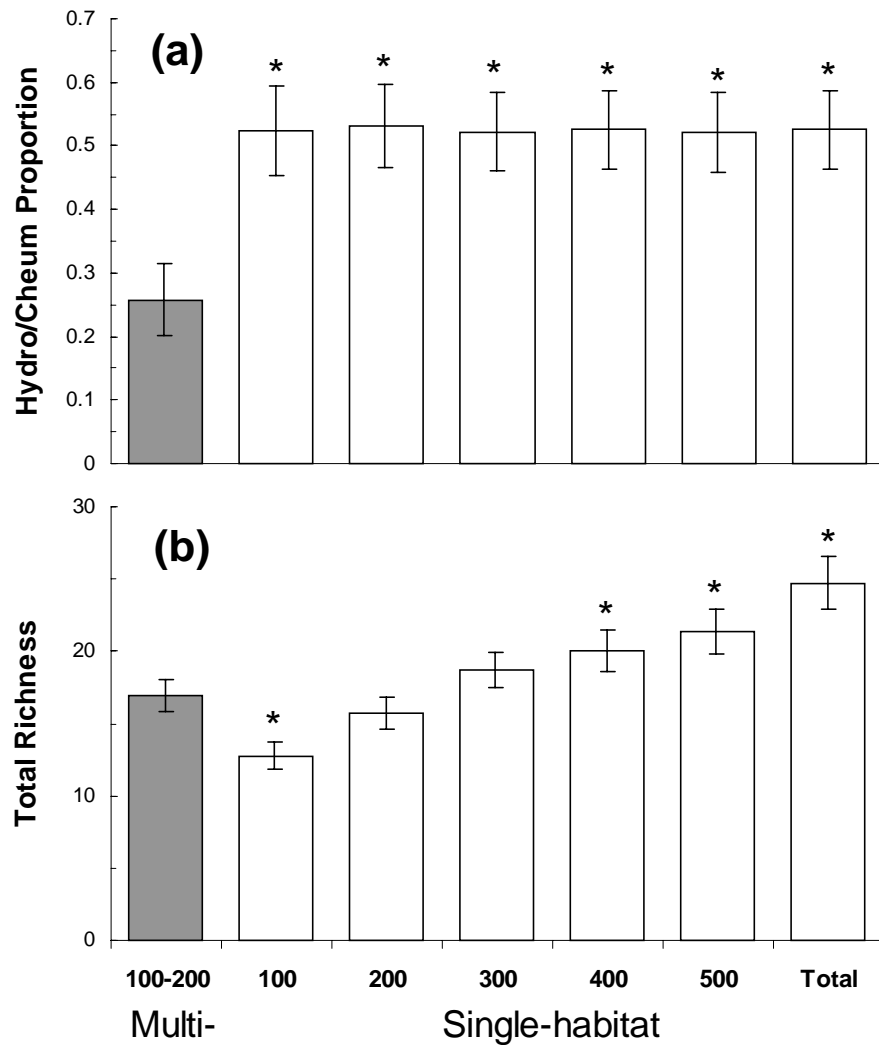
(Figure 9)



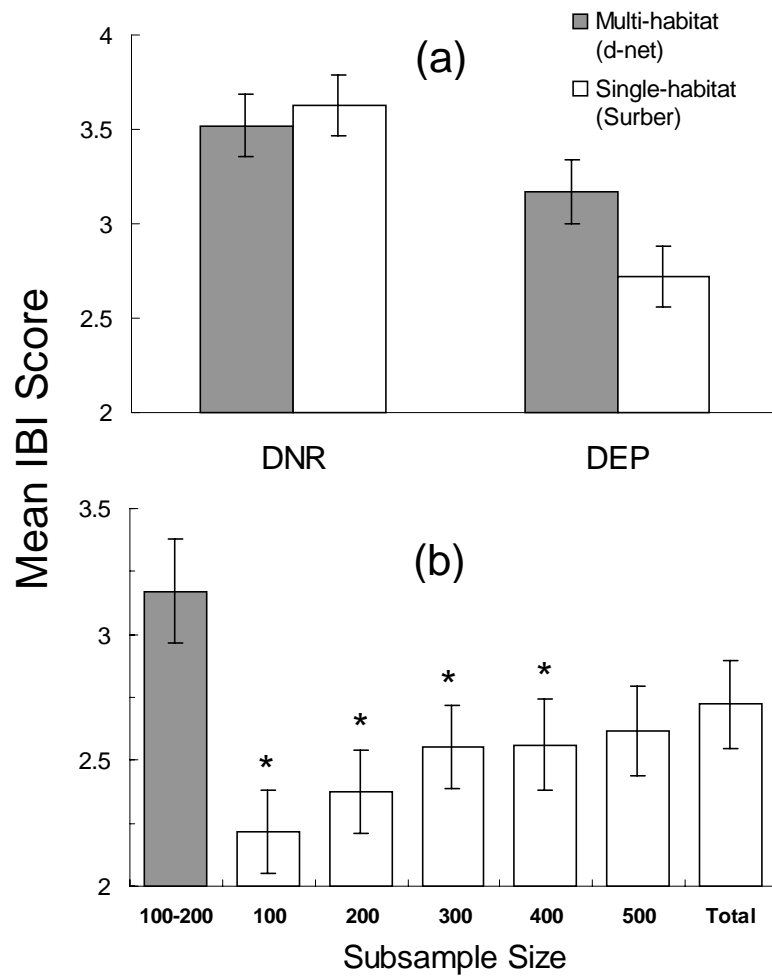
(Figure 10)



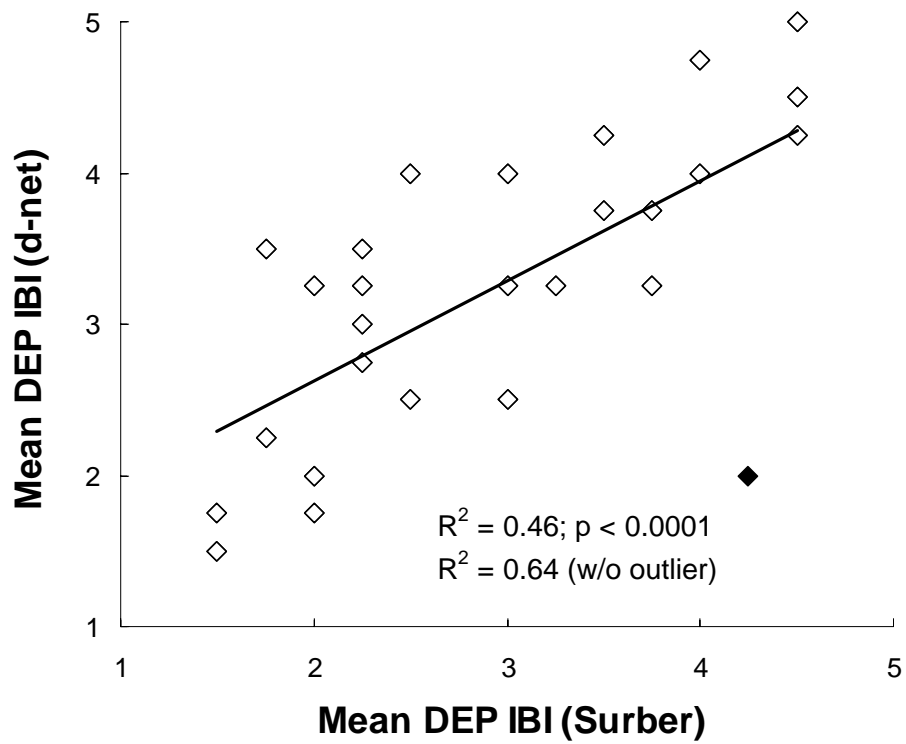
(Figure 11)



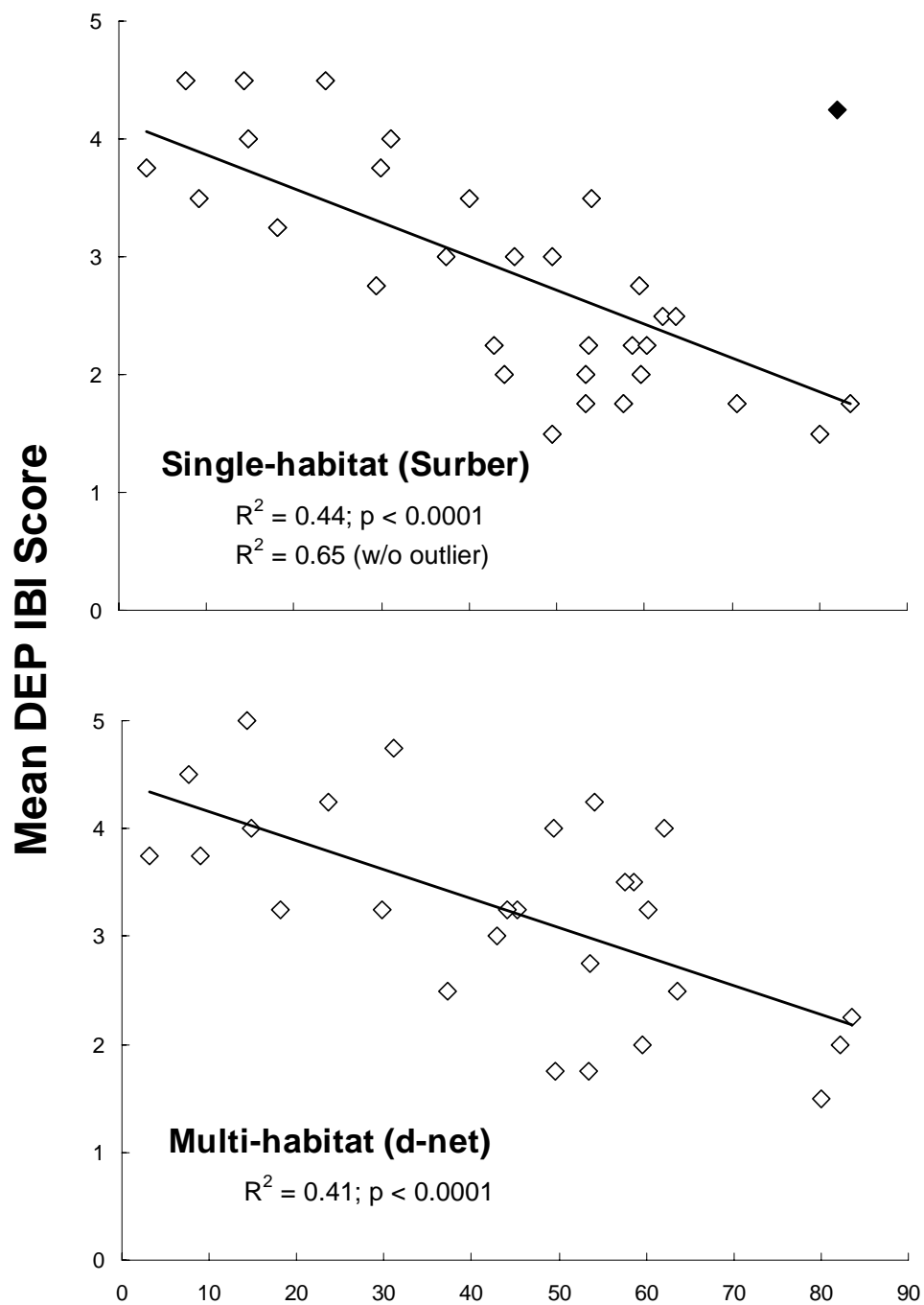
(Figure 12)



(Figure 13)



(Figure 14)





## APPENDIX

List of taxa (divided by order) found throughout the 32 sampling sites in Montgomery County, Maryland, USA. All organisms were identified to genus when possible. The number of sites that each taxon is found at is given for each sampling method for each sampling year (number of sites sampled; Surber 2001/2002 = 32, d-net 2001 = 27, d-net 2002 = 31). Tolerance values are rated 0-10, with low numbers indicating high sensitivity to disturbance. Functional feeding group (FFG) designations are: c = collector; f = filterer; p = predator; sc = scraper; sh = shredder.

Taxa	Surber (2001/ 2002)	d-net (2001/ 2002)	FFG	Toler- ance		Taxa	Surber (2001/ 2002)	d-net (2001/ 2002)	FFG	Toler- ance
<b>Ephemeroptera</b>					-	<b>Diptera (continued)</b>				
Acentrella sp.	3/1	3/1	c	4		Dasyhelea sp.		1/0	c	6
Acerpenna sp.	1/11	2/5	c	4		Dicranota sp.	5/2	0/1	p	3
Ameletus sp.	4/2	1/9	c	0		Dixa sp.		0/2	f	1
Baetis sp.	8/8	7/6	c	6		Dixella sp.		0/2	c	1
Caenis sp.	1/1	1/1	c	7		Hemerodromia sp.	19/26	7/12	p	6
Centroptilum sp.		2/16	c	2		Hexatoma sp.	1/0	0/1	p	2
Dipheter sp.		0/4	c	6		Limnophila sp.	0/1		p	4
Drunella sp.		1/0	sc	0		Limonia sp.		0/1	sh	6
Epeorus sp.	1/0		sc	0		Mallochohelea sp.		0/1	p	
Ephemera sp.	0/1	1/1	c	2		Odontomyia sp.		0/1	c	7
Ephemerella sp.	22/23	19/23	c	1		Ormosia sp.		1/0	c	6
Eurylophella sp.	15/1	2/18	c	2		Pilaria sp.	6/1		p	7
Habrophlebia sp.	1/2		c	4		Probezzia sp.	3/1	3/1	p	6
Habrophlebiodes sp.		1/2	sc	6		Prosimulium sp.	22/14	11/3	f	2
Isonychia sp.	2/1	1/1	c	2		Pseudolimnophila sp.		1/1	p	2
Leptophlebia sp.		7/11	c	4		Simulium sp.	28/28	21/12	f	5
Leucrocuta sp.	4/4		sc	1		Sphaeromias sp.	1/2		p	6
Nixe sp.	2/3		sc	2		Stegopterna sp.	11/2	4/0	f	7
Paraleptophlebia sp.	9/13	1/3	c	1		Stratiomys sp.	1/0		c	4
Serratella sp.	7/8	1/0	c	2		Tipula sp.	18/14	11/16	sh	6
Stenacron sp.	0/2	2/5	sc	4		<b>Coleoptera</b>				
Stenonema sp.	21/24	17/22	sc	3		Agabus sp.		0/3	p	5
<b>Plecoptera</b>						Anchytarsus sp.		3/5	sh	4
Acroneuria sp.	2/0	1/0	p	0		Ancronyx sp.	0/2	1/4	sc	5
Allocapnia sp.		0/1	sh	3		Dineutus sp.		1/0	p	4
Amphinemura sp.	12/2	15/13	sh	3		Dubiraphia sp.	1/1	2/4	sc	6
Chloroperlidae		0/1	p	0		Helichus sp.	9/1	3/3	sc	5
Clioperla sp.	2/3	1/2	p	1		Hydrophilus sp.	1/0		c	5
Cultus sp.		0/2	p	2		Hydroporus sp.		0/1	p	5

Diploperla sp.	1/2	3/4	p	2					
Eccoptura sp.	1/2	1/0	p	3					
Isoperla sp.	8/11	2/1	p	2					
Leuctra sp.	7/15	3/2	sh	0					
Nemoura sp.		1/2	sh	1					
Perlesta sp.		0/1	p	3					
Prostoia sp.		6/4	sh	6					
Shipsa sp.	12/14		sh	2					
Strophopteryx sp.	6/1	2/2	sh	3					
Taenionema sp.		2/0	sh						
<b>Trichoptera</b>									
Ceratopsyche sp.	15/12	6/1	f	3					
Cheumatopsyche sp.	3/29	23/2	f	5					
Chimarra sp.	11/1	5/6	f	4					
Cymellus sp.	0/1		f	8					
Diplectrona sp.	14/14	8/12	f	0					
Dolophilodes sp.	8/4	5/5	f	0					
Glossosoma sp.	8/1	4/3	sc	0					
Hydropsyche sp.	3/26	16/18	f	4					
Ironoquia sp.		3/3	sh	4					
Lype sp.		0/2	sc	2					
Mystacides sp.		0/1	c	4					
Neophylax sp.	18/15	1/9	sc	3					
Polycentropus sp.	2/1		p	6					
Psilotreta sp.	0/1		sc	0					
Psychomyia sp.	2/0		c	2					
Pycnopsyche sp.	3/1	2/8	sh	4					
Rhyacophila sp.	5/8	2/5	p	1					
<b>Diptera</b>									
Antocha sp.	3/25	15/11	c	5					
Bezzia sp.	9/1	4/0	p	6					
Ceratopogon sp.	3/2	0/2	p	6					
Chaoborus sp.	1/0	1/0	p						
Chelifera sp.	17/17	6/4	p	6					
Chironomidae	32/32	27/31	c	8					
Chironomini	23/-	26/28	c	6					
Diamesinae	12/-	9/19	c	7					
Orthoclaadiinae	32/-	27/31	c	5					
Tanypodinae	17/-	22/27	p	7					
Tanytarsini	28/-	24/26	f	6					
Chrysops sp.	5/1	1/2	c	5					
Clinocera sp.	27/25	13/16	p	6					
Culicoides sp.	4/3		p	10					
Macronychus sp.	2/3	2/4	sc	5					
Microcylloepus sp.		2/0	sc	2					
Optioservus sp.	22/24	8/13	sc	4					
Oulimnius sp.	27/27	21/1	sc	3					
Peltodytes sp.		1/0	sh	5					
Promoresia sp.	1/3	1/0	sc	2					
Psephenus sp.	7/8	1/5	sc	3					
Scirtidae		0/1	c	7					
Stenelmis sp.	14/17	9/12	sc	5					
<b>Odonata</b>									
Argia sp.		2/2	p	8					
Arigomphus sp.	6/9		p	4					
Boyeria sp.		1/1	p	2					
Calopteryx sp.		8/1	p	6					
Gomphus sp.		2/0	p	5					
Lanthus sp.		0/2	p	5					
Macromia sp.		1/0	p	3					
Stylogomphus sp.		2/0	p	4					
<b>Megaloptera</b>									
Nigronia sp.	15/12	8/8	p	4					
Sialis sp.	4/1	1/0	p	4					
<b>Other</b>									
Amphipoda	3/3	2/9	sh	6					
Cambaridae	3/3	3/4	c	6					
Collembola	2/3		c	8					
Copepoda	1/15		c	8					
Corbicula sp.	12/9		f	6					
Ferrissia sp.	15/13	0/3	sc	6					
Gerris sp.		1/0	p						
Hirudinea	3/1		p	8					
Hydrachnida	9/14		p						
Isopoda	4/3	3/4	c	8					
Lepidoptera	0/5	0/3	sh	6					
Microvelia sp.		2/0	p	6					
Mooreobdella		1/0	p	8					
Nematoda	26/22	2/0							
Oligochaeta	31/32	11/18	c	10					
Physidae	1/4	2/8	c	8					
Pisidium		0/3	f	6					
Planorbidae		0/6	sc	6					
Sphaeriidae		6/1	f	8					
Turbellaria	13/18		p	4					

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