A TERRAIN-BASED PAIRED-SITE SAMPLING DESIGN TO ASSESS BIODIVERSITY LOSSES FROM EASTERN HEMLOCK DECLINE

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Abstract. Biodiversity surveys are often hampered by the inability to control extraneous sources of variability introduced into comparisons of populations across a heterogenous landscape. If not specifically accounted for *a priori*, this noise can weaken comparisons between sites, and can make it difficult to draw inferences about specific ecological processes. We developed a terrain-based, paired-site sampling design to analyze differences in aquatic biodiversity between streams draining eastern hemlock (*Tsuga canadensis*) forests, and those draining mixed hardwood forests in Delaware Water Gap National Recreation Area (USA). The goal of this design was to minimize variance due to terrain influences on stream communities, while representing the range of hemlock dominated stream environments present in the park. We used geographic information systems (GIS) and cluster analysis to define and partition hemlock dominated streams into terrain types based on topographic variables and stream order. We computed similarity of forest stands within terrain types and used this information to pair hemlock-dominated streams with hardwood counterparts prior to sampling. We evaluated the effectiveness of the design through power analysis and found that power to detect differences in aquatic invertebrate taxa richness was highest when sites were paired and terrain type was included as a factor in the analysis. Precision of the estimated difference in mean richness was nearly doubled using the terrain-based, paired site design in comparison to other evaluated designs. Use of this method allowed us to sample stream communities representative of park-wide forest conditions while effectively controlling for landscape variability.

Keywords: biodiversity, GIS, hemlock, sample design, site pairing, terrain

1. Introduction

Eastern hemlock (*Tsuga canadensis*) forests in the U.S. are threatened due to infestation by an exotic aphid-like insect, the hemlock woolly adelgid (*Adelges tsugae*). Many areas in the eastern U.S. have seen dramatic declines in hemlock forests in the past 10 years due primarily to this forest pest (Souto *et al.*, 1996). Some natural areas, including the Delaware Water Gap National Recreation Area (Delaware Water Gap NRA), are only just beginning to exhibit effects of hemlock woolly adelgid, but may suffer substantial losses due to the ecological and recreational importance of hemlock forests to the park. The National Park Service requested a study in 1996 to assess the uniqueness of biota in hemlock forests in comparison to hardwood forests at Delaware Water Gap NRA. Of particular interest was the role hemlock plays in structuring stream communities (in contrast to hardwood forests) since hemlock currently dominates the riparian forests of many

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streams in Delaware Water Gap NRA. Based on long-term studies of recruitment patterns in affected hemlock stands in other parts of the northeastern United States (Orwig and Foster, 1998), we expect hardwood species to replace hemlock as the dominant riparian vegetation should widespread declines occur due to the hemlock wooly adelgid.

In biodiversity studies it is often difficult to isolate individual species-habitat interactions because of other confounding influences on community structure. For example, to determine effects of forest type on aquatic biodiversity it is necessary to consider how vegetation interacts with other environmental factors to structure stream communities. Terrain and stream size are two important influences on aquatic communities in addition to stream-side (e.g. riparian) and upland vegetation. Terrain plays an important role in structuring aquatic communities by regulating energy and allochthonous inputs through moderation of sunlight, temperature, moisture, and delivery of soil and plant materials to streams (Frissell *et al.*, 1986; Poff, 1997). In addition, stream size (Minshall *et al.*, 1985) and position (Osborne and Wiley, 1992) in the drainage network strongly influence aquatic community structure. Our objectives, therefore, were to evaluate the effect of forest type on aquatic biodiversity, while controlling, to the extent possible, for terrain variability.

Meeting these objectives required a sampling design that 1) represented the range of conditions where hemlock occurs in the park, 2) isolated the relationship between forest type and aquatic biodiversity from potentially confounding effects of terrain and stream order, and 3) achieved high statistical power to detect meaningful variation in biodiversity. Our solution was a paired-site sampling strategy that exploited *a priori* information about terrain characteristics and stream size. We found pairs of hemlock and hardwood stands where stream order was equivalent and where differences between terrain variables were minimized. Thus, the design allowed us to observe differences in stream biota among forest types with 'all else being equal', to the greatest extent possible. We used geographic information systems (GIS) and statistical analysis to incorporate terrain information into our sampling design and to identify and minimize sources of confounding variation.

Other authors have incorporated landscape-scale information into sampling designs in order to account for terrain variation (Bourgeron *et al.*, 1994; Austin and Heyligers, 1989). The 'gradsect' sampling method, for example, uses maps of landscape factors to optimize placement of transects that capture the range of natural variation while minimizing logistical requirements (Haila and Margules, 1996; Gillison and Brewer, 1985). However few authors have used terrain information to define closely matched pairs of sites as a means of controlling for sample site variation on a biological endpoint. This paired-site design can help isolate the effect under consideration, thus reducing sample variance, increasing statistical power, and enabling stronger inferences.

For site pairing to be effective, however, landscape-level measures must be derived that are ecologically relevant to the community under study. Terrain information can be extracted from a GIS in various ways to produce ecologically relevant variables that can be analyzed for relationships to species occurrence, species distribution, or wildlife-habitat interactions (Bailey, 1996; Davis *et al.*, 1990; Band, 1989; Davis and Dozier, 1988). Terrain characteristics relevant to stream habitats are surface shape, steepness, shading, and drainage area; all of these measures are easily derived from digital elevation models using a GIS (Blaszczynski, 1997; Davis and Goetz, 1990; Skidmore, 1990; Jenson and Dominque, 1988). While stream size (e.g. width) of small streams is difficult to represent in a GIS, surrogate measures such as stream order, or hierarchical position in the drainage network, can be easily assigned to stream segments in a GIS using the Strahler (1964) or similar methods.

In this paper we describe a terrain-based, paired site sampling design derived *a priori* to field investigations from a digital landscape-scale information base. We discuss its application to a study of aquatic biodiversity in Delaware Water Gap NRA, and we evaluate statistical power of this design in contrast to less structured random sampling designs.

2. Study Area

Delaware Water Gap NRA is located in northeastern Pennsylvania and northwestern New Jersey (Figure 1). The park encompasses 27,742 ha of hills, ravines, and bottom lands straddling the Delaware River. Two physiographic provinces occur in the park. The Southern Appalachian Plateau Province occurs in the western (Pennsylvania) portion and consists of nearly flat lying sandstone and shale. On the eastern margin of this province lies the Pocono Escarpment where softer rock has been eroded and streams draining to the Delaware River drop off the plateau in a series of waterfalls. To the east (New Jersey) lies the Appalachian Ridge and Valley Province characterized by a series of northeast to southwest sandstone-capped ridges. Of the 21,885 ha of Delaware Water Gap NRA that is forested, 18,575 ha (85%) is deciduous forest, 1,295 ha (6%) is evergreen forest, and 2,015 ha (9%) is mixed evergreen-deciduous forest (Myers and Irish, 1981). The dominant hardwood species are red oak (*Quercus rubra*), followed by sugar maple (*Acer saccharum*), chestnut oak (*Quercus prinus*), red maple (*Acer rubrum*), and sweet birch (*Betula lenta*). Dominant evergreens are white pine (*Pinus strobus*), eastern hemlock (*Tsuga canadensis*) and red cedar (*Juniperus virginiana*). Eastern hemlock occurs in nearly pure stands, primarily as a riparian species along stream corridors. However, it also occurs as a significant understory species due to its shade tolerance. Altogether, eastern hemlock occurs as a primary, secondary, or tertiary forest component in approximately 1,130 forested hectares within Delaware Water Gap NRA (Myers and Irish, 1981).

The topographic setting of Delaware Water Gap NRA is varied with terraced benches and ravines to the west, significant river bottom habitats surrounding the

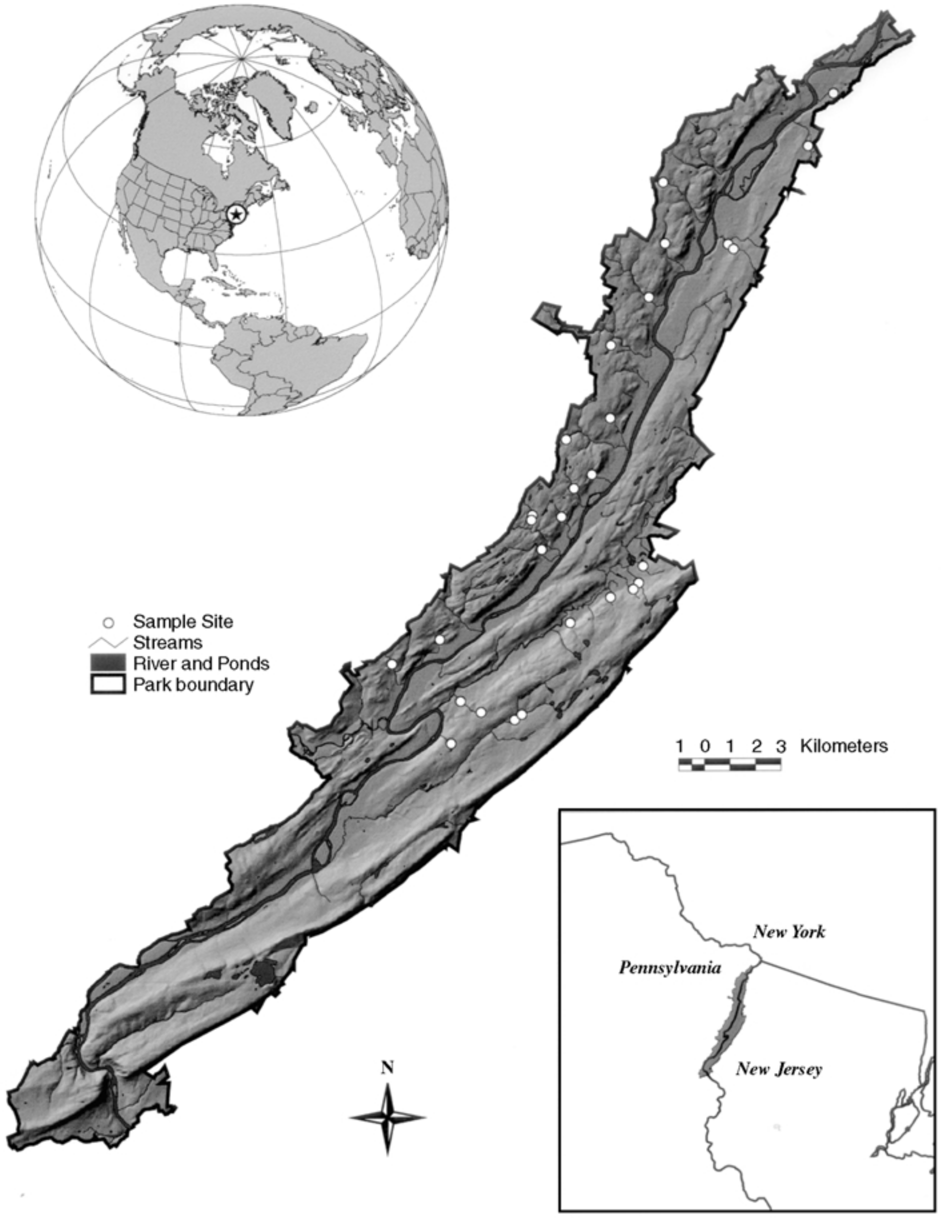


Figure 1. Map of Delaware Water Gap National Recreation Area (USA) showing terrain and selected sample site locations.

Delaware River, and steeply sloping ridge habitats to the east. Minimum elevation is approximately 84 m and maximum elevation is approximately 490 m. Approximately 60 km of the Delaware River flow through the park. Additionally the park has 87 km of 1st order streams, 32 km of 2nd order streams, and 60 km of 3rd or higher order streams, many of which originate outside the park.

3. Methods

We used a GIS to manage landscape-scale data and to assess the range of terrain characteristics in the park for the paired-site design. We examined environmental factors (vegetation, terrain, and stream size) that have known importance in structuring aquatic communities and that were available as GIS maps at the time of the study. Geology is also an important influence on stream communities, but we were unable to incorporate it into our analysis due to lack of available data at the time of our study. However, our general procedures could easily incorporate geologic data. Landscape analysis consisted of six main steps: 1) classifying a digital vegetation map into two forest types (hemlock and hardwood), 2) characterizing topography into ecologically relevant units, 3) using cluster analysis to identify distinct terrain types supporting hemlock forests, 4) characterizing stream order (size) and length by forest stand, 5) defining landscape treatments based on forest type, terrain type, and stream order, and 6) pairing hemlock dominated streams with similarly structured hardwood dominated streams within each treatment.

National Park Service personnel provided digital GIS maps of vegetation, roads, streams, and boundaries for Delaware Water Gap NRA. Vegetation was mapped from 1:12,000 aerial photographs in the early 1980’s (Myers and Irish, 1981). Vegetation was grouped into ‘stands’ or polygons of similarly structured plant composition on this map; each stand was coded with cover type, species composition, and crown closure percentage. The vegetation map contained non-forest vegetation components (e.g. grasses, herbaceous plants, agriculture, etc.) as well as forest components; therefore, we created a new map containing only forest polygons to use in subsequent analyses. The vegetation map defined primary, secondary, and tertiary vegetation composition for each stand, reflecting the dominance by canopy area in each species. Because effect of hemlock forests on biodiversity was of particular interest, we re-selected stands with hemlock defined as either the primary, secondary, or tertiary component into a hemlock forest stand map (N=141 stands), and other forest stands were placed into a separate hardwood forest stand map (N=2145).

After initial polygon selection, we converted all GIS maps to a grid representation where geographic space is divided into a matrix of equal size cells of a given ground distance. In this grid or ‘raster’ representation, each cell is tagged with an attribute (e.g. elevation, forest type, etc.), and a stand is represented as a collection of adjacent cells with identical attributes. We used a cell size of 900 m^2

TABLE I

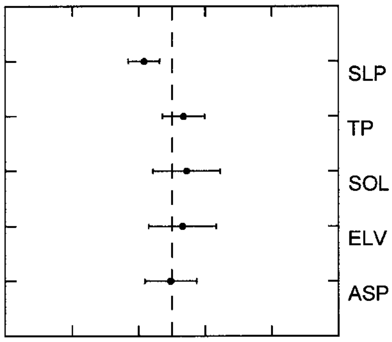
Terrain variables calculated using GIS and used for sample stratification and pairing

Terrain variable	Definition, and calculation method	Reference
Elevation	Elevation in meters, recorded directly from DEM	USGS (1993)
Slope	Slope (percent) of a plane fit to a 9 pixel window	ESRI (1994), Burrough (1986)
Aspect (Northness)	Cosine of aspect (direction of slope), measures degree to which slope is facing north (1) or south (-1)	ESRI (1994), Roberts (1986)
Terrain Shape	Local convexity (value > 0) or concavity (value < 0) of surface, eg. the difference in elevation at a pixel from the mean elevation in a 5 pixel circular neighborhood	McNab (1991)
Solar illumination	Relative amount of sunlight striking surface, computed using 'hillshade' function based on sun altitude and azimuth at summer and winter solstices	Marsh (1983), ESRI (1994)

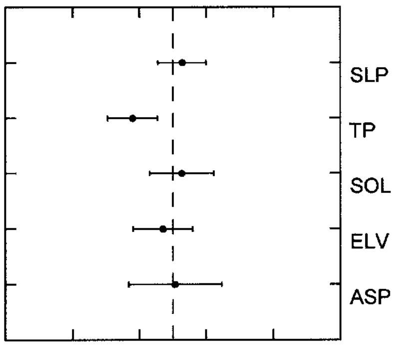
for compatibility with existing digital elevation maps used for terrain modeling. We conducted subsequent analyses using both the grid and polygon representations of forest stands (and other GIS files); the map representation used depended on the requirements of a particular analysis task and the tools available in Arc/Info GIS software (ESRI, Inc., 1994).

We classified terrain into ecologically relevant units by deriving measures of elevation, slope, aspect (as 'northness'), terrain shape, and solar illumination from a digital elevation model (Table I). We computed summaries of these five terrain variables for all forest stands. This was accomplished by using map overlay techniques where we summarized the mean, variance, and range of pixel values for each stand. We standardized the terrain variables to mean zero and unit variance (e.g. Z-scores) to eliminate effects of differing measurement units.

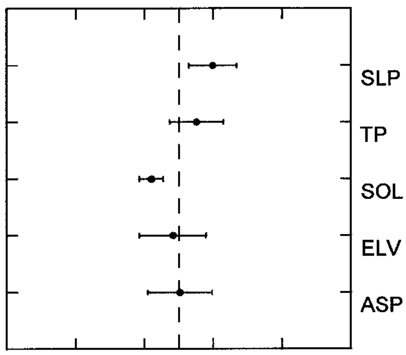
We used Euclidean distance-based k-means clustering (Wilkinson 1998) to identify distinct terrain types among 141 hemlock stands using five terrain variables. Upon examination of resulting profile plots and cluster means of terrain variables, we determined that hemlock stands could be represented as occurring in three major terrain types in Delaware Water Gap NRA; bench, ravine, and mid-slope (Figure 2). The 'bench' type represented gently sloping, topographically flat to slightly convex areas at moderately high elevations. The 'ravine' type represented large, generally northwest trending, concave-shaped drainages. The 'mid-slope' type represented low incident light, steeply sloped, topographically convex areas that generally occur in the mid-slope regions of hillsides in the park. This grouping captured the main terrain types where hemlock occurs in Delaware Water Gap NRA, and formed the basis of treatment groups used to examine the interaction of forest type and terrain type in structuring aquatic communities in the park. We used



Bench sites - Low gradient, topographically flat to slightly convex areas with moderatly high elevation



Ravine sites - Moderate to high gradient, topographically concave areas at lower elevations.



Mid-slope sites - Very steep, topographically convex areas with low light.

Figure 2. Distribution by terrain type of five topographic variables used in cluster analysis of hemlock stands (SLP = slope, TP = terrain shape, SOL = solar radiance, ELV = elevation, and ASP = aspect or northness), summarized as Z-scores. Dotted lines in center of plots indicate the grand mean for each variable, circles indicate the within-cluster mean, and horizontal lines indicate one standard deviation above and below the mean.

discriminant analysis (Davis, 1986) to test the separation of hemlock stands used in stand pairing into ‘landscape types’ defined by terrain characteristics.

We characterized stream size (order) and length using a digital stream map. Streams were initially mapped by the US Geological Survey at 1:24,000 map scale and are from the ‘Digital Line Graph’ file series (USGS, 1990). We augmented the stream map by calculating stream order for major tributary stream systems flowing into Delaware Water Gap NRA using the Strahler method (Strahler, 1964). We then selected only those hemlock (N=56) and hardwood (N=297) forest stands from the vegetation map that were drained by 1st or 2nd order streams by intersecting the stream and vegetation maps in GIS using map overlay. We limited our design to 1st and 2nd order streams because larger streams were drained by significant land areas containing both hemlock and hardwood forests, thus compromising the ability to determine the influence of a particular forest type on stream communities.

We defined five terrain-type/stream order combinations based on the three terrain types (bench, ravine, and mid-slope) and two stream order types (stands with 1st order streams, stands with 2nd order streams). One of the terrain types (‘mid-slope’) did not contain streams of greater than 1st order. Terrain type/stream order combinations (hereafter termed simply ‘terrain types’) were assigned designations as follows: bench, stream order 1; bench, stream order 2; ravine, stream order 1; ravine, stream order 2; mid-slope, stream order 1.

Within each of the resulting terrain types, we paired hemlock stands to ‘terrain equivalent’ hardwood stands containing streams of the same order. Stand pairing was accomplished by computing a multivariate Euclidean distance between each hemlock forest stand and all hardwood forest stands using standardized terrain variables (elevation, slope, northness, terrain shape, and relative solar radiance). We selected the 10 closest hardwood matches for each input hemlock stand. We then examined each hemlock-hardwood matched pair for viability as sampling sites based on several considerations: access, minimum stand size, influence of human disturbance, beaver activity, other forest types upstream of the stand, and length of stream within the stand available for aquatic sampling. Field inspection confirmed the strength of site similarity based on terrain, and led to selection of 15 pairs (3 pairs in each of 5 stream types) for sampling. One pair was discarded (bench, stream order 2) during Spring 1997 sampling due to dramatic differences in stream discharge.

We conducted statistical power analyses to evaluate the effectiveness of site pairing. The null hypothesis underlying the power analysis was that forest type does not affect mean species richness, and we used the t-test to test this hypothesis. To compute power, we set values for difference in mean species richness (i.e., difference in aquatic invertebrate taxa richness), sample variance, sample size, and Type I error rate based on field studies we conducted at Delaware Water Gap NRA in 1997. We considered 4 cases to contrast sampling designs; each case resulted in different variances.

For Cases I, II, and III, we included aquatic species counts from 4 terrain types (bench/stream order 1, bench/stream order 2, ravine/stream order 1, ravine/stream order 2). Mid-slope terrain types were excluded from this analysis because we found that in this terrain type, forest did not affect richness in the same way as the other terrain types. Thus, by removing mid-slope sites the forest effect could be tested in the absence of a terrain/forest interaction. We also considered a Case IV where a random sample is taken from stands that included mid-slope terrain types.

Case I represented the paired-site design with terrain type included as a design factor. Error variance resulted from weighting the within terrain type variances by the respective degrees of freedom. This pooled variance was equal to the mean square error from a general linear model with terrain type included as an explanatory variable. The response was the difference in aquatic invertebrate taxa richness for each site pair; thus the test was a paired t-test.

For Case II we removed the pairing but retained terrain type as a design factor. This resulted in 2 error variances, 1 for each forest type. The variances, as in Case I, resulted from weighting across terrain type. We used a 2 sample t-test with Satterthwaite's approximation (Zar, 1984) for degrees of freedom because variances were unequal (the variance for hemlock was less than half that for hardwood).

In Case III and IV, neither pairing nor terrain type were retained. Again there were 2 error variances, 1 for each forest type. The relevant variances were those that would have resulted from a random sample of stands without regard to terrain type. Cochran (1977:136) presents a general procedure to compute a sample variance when the sample design was stratified. We sampled roughly equal number of sites within each terrain type. However, the frequency of forest stands potentially occurring in each terrain type was not uniform across the landscape (e.g. among hardwood stands, 80% occurred in the 'bench' terrain type). Cochran's procedure takes this disparity into account. Again the test was a 2 sample t-test with Satterthwaite's approximation for degrees of freedom.

In all cases sample size was 22 stands (11 hemlock and 11 hardwood), and Type I error rate was 0.10. We varied treatment effect from a difference of 0 to 20 species, roughly corresponding to the observed differences in species richness (from field studies). We used functions in SAS to compute probabilities under a non-central t-distribution (SAS Institute, Inc. 1990).

In addition to (or perhaps instead of) hypothesis testing, a thorough analysis would include estimating the difference in mean richness. The precision of the estimated difference is design-dependent. We calculated relative efficiency as a ratio of variances; that is, the variance of the difference from an alternative design divided by the variance from the paired-site design (Case I).

TABLE II

Distribution of terrain variables among vegetation stands within Delaware Water Gap National Recreation Area as measured on GIS maps. Terrain attributes in all hemlock stands (n=141) and all hardwood stands (n=2145) occurring in the park are contrasted with terrain conditions in hemlock stands (n=14), and hardwood stands (n=14) selected for sampling

Forest type	Terrain variable	Minimum	Maximum	Range	Mean	Std. dev.
All hemlock	Elevation (m)	88.00	422.00	334.00	203.08	55.37
All hardwood	Elevation (m)	85.00	490.00	405.00	203.07	125.02
Selected hemlock	Elevation (m)	120.00	375.00	255.00	220.03	45.83
Selected hardwood	Elevation (m)	108.00	379.00	271.00	248.70	65.79
All hemlock	Slope (%)	0.00	99.39	99.39	25.82	16.99
All hardwood	Slope (%)	0.00	147.58	147.58	15.70	14.96
Selected hemlock	Slope (%)	0.59	87.90	87.31	26.13	15.69
Selected hardwood	Slope (%)	0.00	77.25	77.25	18.51	11.78
All hemlock	Northness (aspect)	–1.00	1.00	2.00	0.33	0.64
All hardwood	Northness (aspect)	–1.00	1.00	2.00	0.08	0.66
Selected hemlock	Northness (aspect)	–1.00	1.00	2.00	0.26	0.62
Selected hardwood	Northness (aspect)	–1.00	1.00	2.00	0.21	0.68
All hemlock	Solar illumination	58.00	241.50	183.50	129.89	44.64
All hardwood	Solar illumination	60.50	241.50	181.00	128.23	69.65
Selected hemlock	Solar illumination	73.00	241.00	168.00	132.85	41.77
Selected hardwood	Solar illumination	79.00	241.50	162.50	141.77	46.11
All hemlock	Terrain shape	–42.93	28.43	71.36	–2.24	8.36
All hardwood	Terrain shape	–39.09	44.41	83.49	0.42	5.18
Selected hemlock	Terrain shape	–34.30	21.72	56.01	–3.12	8.71
Selected hardwood	Terrain shape	–28.99	16.93	45.91	–0.56	5.28

4. Results

Statistical summaries of elevation, slope, 'northness', terrain shape, and solar radiance computed for all forest stands (Table II) show that hemlock stands generally occur in steeper, more northerly-facing slopes, and in more concave terrain shapes than hardwood forests. This finding is in line with expectations that hemlock occurs in more shaded environments and persists in areas that were less accessible to past harvest activities.

We used discriminant analysis to investigate the effectiveness of our *a priori* classification of hemlock stands into three terrain types (before stand pairing and selection). In this case, discriminant analysis was not used as a confirmatory test

TABLE III

Results of discriminant function test on hemlock stands containing 1st or 2nd order streams showing strength of terrain type clustering based on 5 terrain variables. Table shows observations classified from cluster analysis (rows), predicted group membership based on discriminant function test (columns), and between group F statistic in parentheses

	Predicted Group Membership			
Landscape type	1 (bench)	2 (ravine)	3 (mid-slope)	% correct
1 (bench)	22 (0.0)	0 (15.44)	1 (6.237)	96
2 (ravine)	2 (15.44)	23 (0.0)	2 (7.407)	85
3 (mid-slope)	0 (6.237)	0 (7.407)	6 (0.0)	100
Total	24	23	9	91

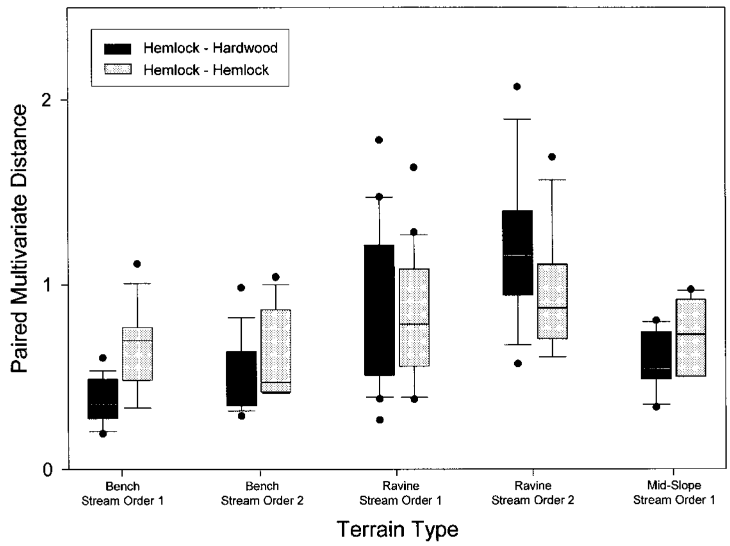


Figure 3. Distribution of mean multivariate distances between hemlock stands and the 10 closest paired hardwood stands (black boxes) compared to multivariate distances between hemlock stands and other hemlock stands of the same stream type (gray boxes). Boundaries of the box mark the 25th and 75th percentile, the line within the box marks the median, and the whiskers mark the 10th and 90th percentiles. Circles represent values lying outside the 10th and 90th percentiles.

but rather to explore the relative power of distinguishing sites among terrain types. Overall, the terrain types were strongly defined with an overall classification accuracy of 91% (Table III). Four out of the five stands mis-classified were in the ravine type, suggesting that some stands may be marginal ravines and may be more similar to either bench or mid-slope sites (Table III).

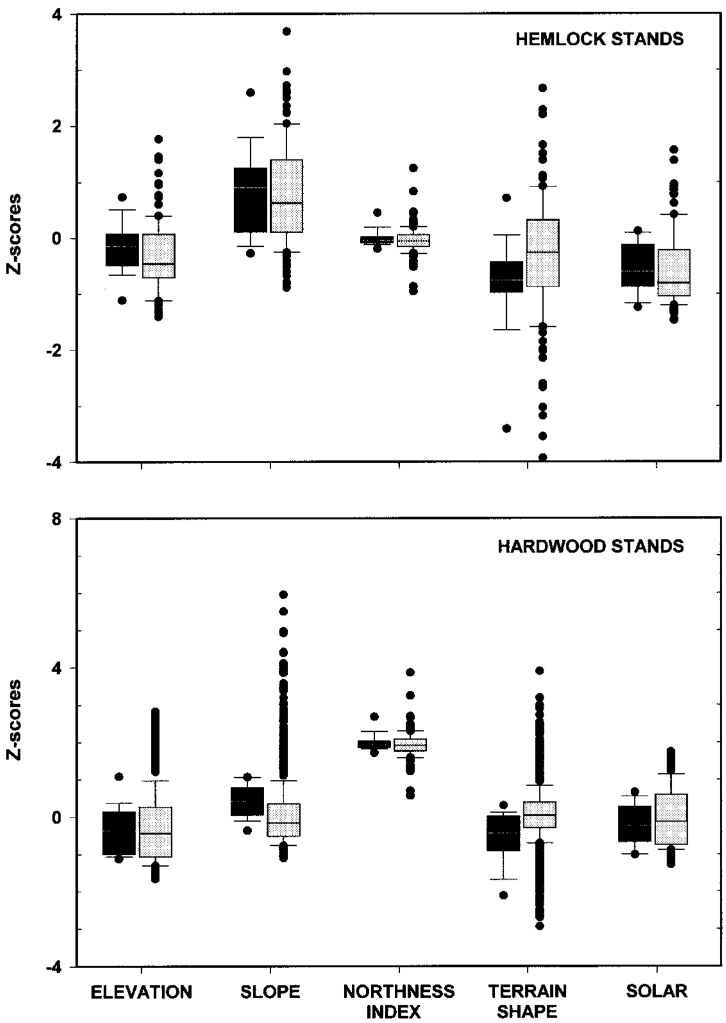


Figure 4. Comparison of the distribution of five terrain variables used for site pairing between stands selected for study (black boxes) and those left unselected (gray boxes). Top panel compares hemlock stands and bottom panel compares hardwood stands. Boundaries of the box mark the 25th and 75th percentile, the line within the box marks the median, and the whiskers mark the 10th and 90th percentiles. Circles represent values lying outside the 10th and 90th percentiles.

An evaluation of the paired hemlock and hardwood stands revealed that in most cases paired hardwood stands were very similar in terrain characteristics to their hemlock counterparts, and in some cases were more similar to the selected hemlock stand than were other hemlock stands in the same terrain type (Figure 3). Multivariate distances between individual hemlock stands and matched hardwood stands were generally smaller (i.e. stands were more similar) than multivariate distances between hemlock stands within the same terrain type.

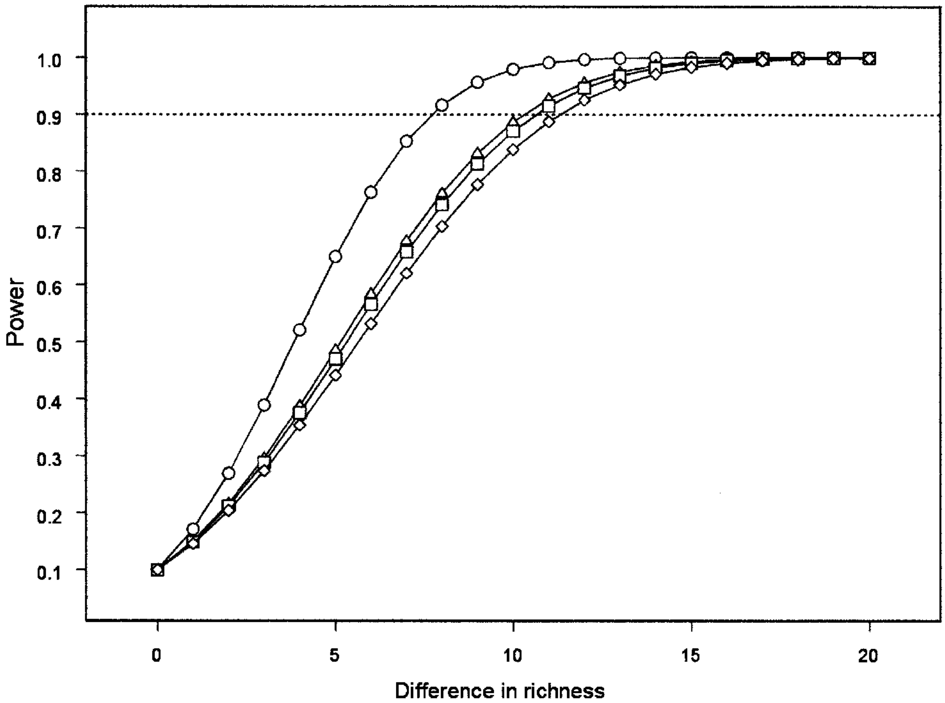


Figure 5. Power to detect differences in species richness between hemlock and hardwood forest types. Case I (circle) is the sampling design that includes paired sites and terrain type as a design variable. This is the terrain-based, paired-site design. Case II (triangle) is the design without pairing, but with terrain types as a design variable. This is a terrain-based stratified design. Case III (square) is the design without pairing and without terrain type as a design variable. This is a random sample of sites without regard to terrain type. Case IV (diamond) is a random sample including mid-slope terrain types.

Mean values of terrain variables for the 14 selected hemlock stands were not markedly different from the 127 non-selected hemlock stands (Figure 4), suggesting that our sample represented the range of hemlock conditions present in the park. Similarly, the 14 selected hardwood stands were representative of the overall terrain conditions found in the 2,131 hardwood forest stands left unselected (Figure 4).

The power to detect differences in aquatic invertebrate taxa richness was highest when sites were paired and terrain type was included as a factor in the analysis (Case I, Figure 5). Power was lowest when stands were selected at random without regard for terrain type (Cases III and IV). Power was similar for Cases II and III, which differed only by whether terrain type was included as a design factor. That is, Case II was random selection within terrain type and Case III was random selection regardless of terrain type. An effective criteria is to balance Type I and Type II error rates, e.g., both equal to 0.10. For example, to achieve power of 0.90 (Type II error rate of 0.10), for a difference of 8 species, our design resulted in a 31% increase in the power to detect a difference of 8 species in comparison to random sampling. The pairing component of our design (Case I) when compared to Case II resulted in a 21% increase in power to detect a similar difference in richness. In contrast, including terrain type without pairing resulted in only an 8% increase in power when compared to random sampling (Case II versus Case IV).

The variance of the difference in mean richness, useful for computing a confidence interval of the difference, was smallest for Case I. Relative efficiency (i.e. variance of the difference from each case compared to that of Case I) was 2.25, 1.99, and 1.85 for Cases II, III, and IV respectively. Thus, for one of the other designs to result in similar precision as the paired-site design, the number of stands sampled would have to be effectively doubled.

5. Discussion

This sampling scheme successfully blended classical sampling designs (e.g. randomized blocks), with terrain variation measured through use of GIS (*sensu* 'gradsect' sampling: Gillison and Brewer, 1985), and pairing to control for possible confounding variables (Cochran, 1983; Schlesselman, 1982). By using GIS, we took advantage of easily obtainable *a priori* information on terrain and vegetation to characterize hundreds of hemlock and hardwood forest stands within the park. We then capitalized on this information to pair hemlock and hardwood stands with similar terrain types and stream orders prior to field sampling.

We had three goals to meet with our sampling design. The first goal was to represent the range of terrain variation in the landscape and to study how terrain and forest cover interact to affect biodiversity patterns. The second goal was to control the confounding influences of terrain and to test for forest cover effects, all else being equal. The third goal was to achieve a design with adequate power to detect variation in biodiversity. Since the objective of the study was to assess the uniqueness of biota in hemlock forests, and particularly in streams draining hemlock forests, we had to address the potential for stream communities to be influenced by prevailing conditions other than forest cover. We found it possible to characterize components of terrain that have ecological meaning for stream communities (such as elevation, slope, slope position, light conditions, and shape), and to exploit this information through site pairing in a manner that effectively isolated these confounding influences and reduced error variance. The influence of stream order on aquatic communities can be controlled in a similar fashion. The terrain-based, paired-site design met our goals of successfully representing the range of terrain variation in Delaware Water Gap NRA and controlling for the confounding influence of landscape variation by pairing sites of different forest type with similar terrain and stream conditions. Overall terrain variable distributions show greater ranges than the stands selected for sampling, due primarily to stands found on 'extreme sites', such as ridge tops or near river bottoms. Through the use of clustering, we were able to organize structural groupings of hemlock stands, ensuring that we could test for the effects of terrain on aquatic biodiversity across the range of distinctive terrain types where hemlock commonly occurs in the park.

Statistical power in landscape-scale studies is often low due to the difficulty and cost in sampling remote sites. Therefore, any efficiencies that can be gained through the sampling design when small sample sizes are spread across the landscape can be of critical importance. We found that site pairing within terrain type increased the probability of detecting biodiversity differences associated with forest cover. Statistical power decreased in the absence of stand pairing. Including terrain type was important for the control of potentially confounding variation and to understand stream/forest type interactions. For example, we found that aquatic invertebrate communities in mid-slope terrain types responded differently to forest type than those in the other two terrain types. However, incorporating terrain type in the design did not by itself greatly increase power. Rather, it was site pairing that increased the efficiency of the design. Precision of the estimated difference in mean richness was nearly doubled due to pairing in comparison to the design that incorporates terrain type only as a blocking variable. In the random sampling case, assessing interactions between terrain type and forest cover would be difficult because selection of sites would be in proportion to their occurrence in the landscape, regardless of their biological importance. However, we were able to increase power through use of GIS to pair individual sites based on derived topographic variables, minimizing sources of unexplained variance when comparing differences dues to forest type.

In summary, the combination of *a priori* GIS and statistical analysis holds great promise for landscape-scale study designs in ecology. By characterizing terrain conditions into terrain types and controlling for potentially confounding influences, it is possible to capture the range of conditions present, while at the same time providing an opportunity for random selection of sites within each type. Our study provides but one example of the potential of this technique in studies of aquatic communities; the technique could as easily be applied to other aquatic or terrestrial taxa. Indeed, this design is finding continued use in studies of eastern hemlock's importance to other faunal groups including amphibians and birds, and to physical habitat parameters such as water quality at Delaware Water Gap NRA (R. Evans, National Park Service, *pers. comm*). Continued development of methodologies for finding similar terrain morphologies, such as those recently presented by Blaszczynski (2000), and further integration of GIS and statistical designs will increase the applicability of these techniques to ecological studies.

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