

**Developing An Index of Biotic Integrity (IBI) for Warmwater
Wadeable Streams in Virginia**

by

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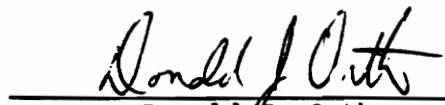
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DEVELOPING AN INDEX OF BIOTIC INTEGRITY (IBI) FOR
WARMWATER WADEABLE STREAMS IN VIRGINIA

by

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(ABSTRACT)

The index of biotic integrity (IBI) comprises several fish-assemblage attributes, called metrics, that reflect how a site differs from least-disturbed (by anthropogenic influences) conditions. Understanding how metrics at least-disturbed sites vary across landscape classes (e.g., physiographies, ecoregions) and stream sizes helps one determine appropriate regions and stream-size ranges in which to develop and use the IBI. The IBI's utility depends on how accurately and reliably each metric reflects disturbance. I make recommendations for developing the IBI for use in Virginia.

I examined metric variation across landscape classes: physiographies, ecoregions, and drainage groups; and across stream sizes. I examined intra-region relations between metrics and disturbance measures and whether relations met conventional IBI assumptions.

Taxonomic metrics (e.g., number of native minnow species) and reproductive metrics (e.g., proportion of individuals as lithophils) varied more across physiographies than across ecoregions or drainages. Trophic metrics (e.g., proportion as invertivores) varied least across landscape classes and most with stream size. For Virginia, I recommend three regions: Coastal Plain, Piedmont, and Mountain, in which to develop and use distinct versions of the IBI.

In Coastal Plain, disturbance-vs-metric relations were mostly contrary to IBI assumptions. In Piedmont, trophic and tolerance metrics best reflected disturbance and met IBI assumptions; in Mountain, reproductive metrics did so. Disturbance measures accounted for about 20% of the variance in metrics, suggesting that my data incompletely represented disturbance effects on fish. Until further validation, I recommend that each regional IBI retain at least two taxonomic, two trophic, two reproductive, and one tolerance metric.

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"I could wade away the hours,
conversing with the darters,
from here to the Coastal Plain.
I'd fulfill my greatest wish:
to learn more about the fishes, if
I only had a seine."

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TABLE OF CONTENTS

CHAPTER ONE: REGIONAL AND STREAM-SIZE EFFECTS ON POTENTIAL IBI FISH METRICS

Abstract	1
Introduction	3
Methods	8
<i>Habitat variables</i>	8
<i>Ranking sites from least- to most-disturbed</i>	11
<i>Fish sampling</i>	12
<i>Sampling considerations</i>	12
<i>Fish variables</i>	13
<i>Testing for statistical effects of</i> <i>landscape class or year</i>	15
<i>Testing for statistical effects of stream size or link</i>	16
<i>Statistical considerations</i>	17
Results	
<i>Effects of physiography</i>	20
<i>Effects of stream size and of link</i>	22
<i>Effects of drainage</i>	24
<i>Effects of year</i>	25
Discussion	
<i>IBI reference regions for Virginia</i>	26
<i>Regional variation in taxonomic variables</i>	28
<i>Regional variation in reproductive variables</i>	29
<i>Regional variation in trophic variables</i>	31
<i>Stream size and IBI</i>	31
<i>Summary and conclusions</i>	33
Literature cited	35
Tables 1 - 14	42
Figures 1 - 5	64

CHAPTER TWO: RELATIONS BETWEEN IBI FISH METRICS AND MEASURES OF ANTHROPOGENIC DISTURBANCE

Abstract	69
Introduction	71
Methods	73
<i>Habitat variables</i>	74
<i>Fish sampling and sampling considerations</i>	75
<i>Fish metrics</i>	76

Statistical tests and considerations 80

Questions 1 and 2 of CCA 82

Question 3 of CCA 84

Results

Relations in Coastal Plain 85

Relations in Piedmont 86

Relations in Mountain 88

Interrelations among habitat variables 89

Discussion 90

Conclusions 96

Literature cited 98

Tables 1 - 8 104

Figures 1 - 4 117

Appendix A: Taxonomic, trophic, and reproductive
classifications of fish species 121

Vita 126

LIST OF TABLES

Chapter One

- Table 1. Fish variables used to determine reference regions within which to set IBI-metric criteria and habitat variables used to determine least-disturbed sites in Virginia. Fish variables are arranged in three classes: taxonomic, trophic, and reproductive. Habitat variables are arranged in three watershed-scale classes: mining, urban, and forest; and one on-site class. For habitat variables, ranges of raw-data values and ranks are given. "N" is number of statewide reference sites on which habitat-variable ranges were based. "Proportion" refers to proportion of individuals. Subsequent tables refer to fish variables by their codes. 42
- Table 2. Number of reference sites in each physiography, ecoregion, river drainage, year, or stream-order-link group, at two spatial scales: statewide (VA) and within physiography (CP=Coastal Plain, PD=Piedmont, MT=mountain). See text for additional explanations 44
- Table 3. Spearman correlations (i.e., r_s) between taxonomic, trophic, and reproductive variables, for 63 reference sites statewide. Only correlations with absolute value of $r_s > 0.40$ ($P < 0.002$) are shown. See Table 1 for codes of fish variables 45
- Table 4. Results of one-way MANOVAs of taxonomic, trophic, or reproductive variables for sites among physiographies (PHYS), among ecoregions (ECOR), or among years (YEAR). For PHYS, sample size (N)=9 for CP, N=29 for PD, N=11 for BR, N=14 for RV. For ECOR, N=37 for SEP, N=4 for BRM, N=16 for CARV, N=5 for NP, N=1 for MACP (excluded from MANOVAs). For YEAR, N=10 for 1987, N=13 for 1988, N=16 for 1989, N=24 for 1990 (see Table 2). A multivariate eta-square (η_m^2) is shown and equals the ratio (of determinants) of the among-group sum-of-squares-and-cross-products matrix (SSCP) to the total SSCP, or simply, $1 - \text{Wilks's } \lambda$. Also shown are F-ratio approximations (F) and corresponding significance values (P) of each MANOVA test. "DF" are the degrees of freedom for the numerator (i.e., the among-group SSCP) and for the denominator (the within-group SSCP), respectively 46
- Table 5. Results of Hotelling's T^2 tests of taxonomic, trophic, or reproductive variables for sites between pairs of physiographies. Coastal Plain=CP (N=9 sites), Piedmont=PD, Blue Ridge=BR, Ridge and Valley=RV, and Mountain=MT=BR+RV. Multivariate eta square (η_m^2), F-ratio approximation (F), and significance value (P) of each test is shown. "DF" are the degrees of freedom for the numerator (between-group SSCP) and for the denominator (within-group SSCP), respectively 47
- Table 6. Summary of canonical analysis of taxonomic, trophic, or reproductive variables for sites among physiographies: CP=Coastal Plain, PD=Piedmont, MT=mountain. CAN1 and CAN2 are the first and second canonical functions. Squared (adjusted) canonical correlations (r^2) represent the relative amount of among-physiography separation explained by CAN1 or CAN2 (see

"Group means" and Figure 3). Multivariate eta square (η_m^2), F-ratio approximation, and significance value (P) of each canonical function are shown. "DF" are the degrees of freedom for the numerator (among-group SSCP) and for the denominator (within-group SSCP), respectively. Within-group structure coefficients are the within-physiography Pearson correlations between each fish variable and the scores of each canonical function. Within-group discriminant-function coefficients are the standardized partial regression coefficients of a within-physiography multiple linear regression of CAN1 or CAN2 scores as a function of taxonomic, trophic, or reproductive variables. Discriminant ratio coefficients (Thomas 1992) are the product of the within-group structure and function coefficients. See text (*Statistical Considerations*) for further explanation and discussion. See Table 1 for codes of variables 48

Table 7. Summary of canonical analysis of taxonomic, trophic, or reproductive variables for sites between pairs of physiographies (e.g., CPvPD=Coastal Plain versus Piedmont, PDvMT=Piedmont versus Mountain). For each pair tested, squared (adjusted) canonical correlations (r_c^2) represent the relative amount of between-physiography separation explained by the first (and only) canonical function. Multivariate eta square (η_m^2), F-ratio approximation, and significance value (P) of the canonical function are shown. "DF" are the degrees of freedom for the numerator (between-group SSCP) and for the denominator (within-group SSCP), respectively. Within-group structure coefficients are the within-physiography Pearson correlations between each fish variable and the canonical-function scores. Within-group discriminant-function coefficients are the standardized partial regression coefficients of a within-physiography multiple linear regression of the canonical-function scores as a function of taxonomic, trophic, or reproductive variables. Discriminant ratio coefficients (Thomas 1992) are the product of the within-group structure and function coefficients. See text (*Statistical Considerations*) for further explanation and discussion. See Table 1 for codes of variables 50

Table 8. Within-physiography Spearman correlations (r_s) between taxonomic, trophic, and reproductive variables, for 63 reference sites. Variables are arranged horizontally in same order as vertically, but are abbreviated (i.e., NON=NONNATSP, etc.). Only correlations with absolute value of r_s ($|r_s|$) > 0.40 are shown. See Table 1 for codes of variables 52

Table 9. Values of taxonomic, trophic, and reproductive variables at sites within each physiography. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Superscripts after some fish variables show significance (only $P < 0.10$) of the omnibus Kruskal-Wallis test of the variable among three physiographies. Lower-case letters after some medians show comparisons of the variable between pairs of physiographies; different letters denote a statistical difference, with $a < b < c$. Superscripts of lowercase letters denote statistical significance of each paired comparison (Wilcoxon two-sample test). For example, for VMANPRP, $a^{2,3}$, $b^{4,3}$, and $c^{4,2}$ show that VMANPRP in MT= $a < c$ =VMANPRP in CP ($0.0001 < P < 0.001$); VMANPRP in PD= $b < c$ =VMANPRP in

MT ($0.01 < P < 0.1$); and VMANPRP in MT=a <b=VMANPRP in PD ($0.001 < P < 0.01$). Number of sites = N. See Table 1 for codes of variables 55

Table 10. Results of one-way MANCOVAs for taxonomic, trophic, or reproductive variables for sites between pairs of physiographies (PHYS: CP=Coastal Plain, PD=Piedmont, MT=Mountain), or among stream-order link categories, statewide (N=24 for LINK=0, N=27 for LINK=1, and N=12 for LINK=2). Covariate is watershed area (WAREA) for both sets of tests. Multivariate eta square (η^2_m), F-ratio approximation (F), and significance value (P) of each MANCOVA are shown. "DF" is the degrees of freedom for the numerator (between-group SSCP) and for the denominator (within-group SSCP), respectively. Number of sites = N. Interaction terms are abbreviated with first letter of each single-effect name, e.g., PHYS x WAREA interaction denoted as "P x W" 57

Table 11. Spearman correlations (r_s) between each taxonomic, trophic, or reproductive variable and watershed area (WAREA in km^2), for reference sites within each physiography (CP=Coastal Plain; PD=Piedmont; MT=mountain). Only correlations with absolute value of $r_s > 0.40$ are shown. Number of sites= N. Range of watershed areas is shown below N. The second columns for PD and for MT are for sites with WAREA < 300 km^2 . See Table 1 for codes of variables 58

Table 12. Values of fish variables at reference sites within stream-order link categories, within each physiography. Link categories are: SLINK 0= downstream reach is same order as site; SLINK 1= downreach is 1 order larger; SLINK >1= downreach is > 1 order larger. Medians, means (X), and standard errors (SE) are shown. Superscripts after some fish variables show significance (only $P < 0.10$) of the omnibus Kruskal-Wallis test for the variable among physiographies. Lower-case letters after some medians show comparisons of the variable between pairs of physiographies; different letters denote a statistical difference, with a < b < c (Wilcoxon two-sample test, only $P < 0.10$ shown). Number of sites = N. See Table 1 for codes of variables . . 59

Table 13. Values of fish variables at Mountain sites within selected river-drainage groups. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Superscripts after some variables show significance (only $P < 0.05$ shown) of a Wilcoxon two-sample test of the variable between drainage groups. Number of sites = N. See Table 1 for codes of variables . . 62

Table 14. Values of taxonomic, trophic, and reproductive variables at reference sites within drainage groups, in Coastal Plain. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Superscripts after some variables show significance (only $P < 0.05$ shown) of Wilcoxon two-sample tests of the variable between drainage groups. Number of sites = N. See Table 1 for codes of variables 63

Chapter Two

Table 1.	Assumptions of metrics most commonly used in versions of the IBI adapted for freshwater stream-fish assemblages. Assumptions describe how metrics are expected to change with increasing degradative anthropogenic disturbance to stream systems (modified from Fausch et al. 1990)	104
Table 2.	Values of habitat variables at sites within each physiography. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Categories of discrete variables and ranges of continuous variables are also shown. Number of sites (N) is followed by the range of stream sizes (shown as watershed area) sampled in each physiography. See Methods for further explanation of habitat variables	105
Table 3.	Fish metrics used in analyses of fish-vs-disturbance relations. Metrics are arranged in three classes: taxonomic, trophic, and reproductive. "Proportion" refers to proportion of individuals. Subsequent tables refer to fish metrics by their codes. See Methods for further explanation of metrics	107
Table 4.	Spearman (r_s , for raw values in CP) and Pearson (r , for residual values in PD and MT) correlations between taxonomic, trophic, or reproductive metrics and continuously distributed habitat variables, for sites within each physiography. Number of sites = N. Only absolute values (e.g., $ r $) of r_s or $r > 0.30$ are shown. Dashes denote that a fish metric was not included in analyses for the particular physiography. See Table 3 for fish-metric codes. For Piedmont correlations, two sites with extremely outlying fish-metric values were excluded: one for MINSP and one for GENPRP and TOLPRP. Similarly, two Mountain sites with extreme DARSCLSP were excluded. See Methods for further explanation	108
Table 5.	Spearman (r_s , for raw values, Coastal Plain only) and Pearson (r , for residual values, Piedmont and Mountain) correlations between taxonomic, trophic, and reproductive metrics for sites within each physiography. Number of sites = N. Only absolute values (e.g., $ r $) of r_s or $r > 0.30$ are shown. Dashes denote that a fish metric was not included in analyses for the particular physiography. See Table 3 for metric codes. For column headings, metric codes are limited to the first 3-5 letters (e.g., NON=NONNATSP, DARSC=DARSCLSP, etc.). For Piedmont correlations, two sites with extremely outlying fish-metric values were excluded: one site for MINSP and one site for GENPRP and TOLPRP. Similarly, two mountain sites with extreme DARSCLSP were excluded. See Methods for further explanation	109
Table 6.	Summary of canonical correlations (CC) of selected fish metrics, for Piedmont or for mountain sites. "CAN1" to "CAN3" are the first through third CCs; "FSH1" to "FSH3" and "HAB1" to "HAB3" are the fish and habitat variates, respectively, of each CC. For each CC, "RED" (=redundancy) is the variance in fish metrics accounted for by the habitat variate; total redundancy (TOTAL) is the variance in fish metrics accounted for by all (=4) of the habitat variates. Wilks's lambda (W), its F-test approximation (F), significance (P), degrees of freedom (DF=degrees for	

numerator, degrees for denominator), and canonical correlation coefficient (r_c) are shown for successive CCs that accounted for up to > 0.90 of TOTAL. For individual fish metrics or habitat variables of each CC, "FC1" to "FC3" are the standardized function coefficients; numbers under variate names are the standardized intraset and interset (for fish metrics only) structure coefficients for each CC. For fish metrics, squares of the interset structure coefficients were summed to yield "COM1" to "COM3" (=communality), which is the accumulated amount of each fish metric's variance accounted for by each successive habitat variate. Number of sites is "N". Two Piedmont sites with extremely outlying fish-metric values were excluded: one site for GENPRP and TOLPRP, and one for MINSP. Similarly, two mountain sites with extreme DARSCLSP were excluded. See Table 3 for fish-metric codes and **Methods** for further explanation of metrics and habitat variables. "Wtrshd." = Watershed and "w." = woody . 112

Table 7. Summaries of (1) a rerun canonical correlation analysis (CCA) of and (2) a descriptive discriminant analysis of selected fish metrics, for 48 or 47 Piedmont sites, respectively. For the CCA, see Table 6 for explanations and definitions of terms; the analysis depicted here is identical to that in Table 6 except "MINSP" and "AGE3SP" were omitted, and no sites were excluded. The discriminant analysis is analogous to a CCA in which one set of variables is a set of dummy variables representing group membership of each site (i.e., least-, most-, or moderately-disturbed). For the discriminant analysis, "FISH1" and "FISH2" are canonical composites (variates), of the fish metrics, that define the space in which 26 least-, 13 most-, and 8 moderately-disturbed sites are maximally separated (also see Figure 4). "FC1" and "FC2" are the standardized function coefficients as in CCA. Numbers in columns labelled "FISH1" and "FISH2" are the intraset structure coefficients, which equal the Pearson correlations between each fish metric and each canonical composite (variate). Means of "FISH1" and "FISH2" for each group of sites are also shown under "Least-", "Moderately-", and "Most-". As in the original CCA, two sites with extremely outlying fish-metric values were excluded from the discriminant analysis: one site for GENPRP and TOLPRP, and one for MINSP. For the rerun CCA only one site with extreme GENPRP and TOLPRP was excluded 114

Table 8. Fish metrics judged especially useful for Piedmont or Mountain versions of the IBI for Virginia warmwater Wadeable streams. "Proportion" refers to proportion of individuals 116

LIST OF FIGURES

Chapter One

Figure 1. Maps of Virginia showing location of 63 reference sites with respect to physiography (top: Cp=Coastal Plain, PD=Piedmont, BR=Blue Ridge, RV=Ridge and Valley, AP=Appalachian Plateau), major river drainage (middle: SHE=Shenandoah, POT=Potomac--no sites, RAP=Rappahannock and adjacent Chesapeake Bay drainages, YOR=York, JAM=James, ROA=Roanoke, CHO=Nottoway + Blackwater + Meherrin, NEW=New, HOL=Holston), and ecoregion (bottom: MACP=Mid-Atlantic Coastal Plain, SEP=Southeastern Plain, BRM=Blue Ridge Mountains, CARV=Central Appalachian Ridge and Valley, NP=Northern Piedmont, CA=Central Appalachians) 64

Figure 2. Plot of mean width vs. total length sampled for 66 reference sites. Solid line is fitted linear regression line. Dashed line is a line parallel to regression line, below which sampling effort was judged inadequate; the three sites below this line were deleted from analyses 65

Figure 3. Plots of 63 statewide reference sites, grouped by physiography, in the spaces defined by the first (CAN1) and second (CAN2) canonical functions of taxonomic (TAX), trophic (TRO), or reproductive (REP) variables. Circles =Coastal Plain sites, squares=Piedmont sites, and triangles=Mountain sites. Triangles with dark centers are 11 Blue Ridge sites, 9 above and 2 below the dashed line (one site above the line is almost entirely hidden). See Table 6 for corresponding statistical summary 66

Figure 4. Plots of taxonomic variables versus watershed area for 29 Piedmont reference sites. See Table 11 for corresponding statistics 67

Figure 5. Plots of taxonomic variables versus watershed area for 25 Mountain reference sites. See Table 11 for corresponding statistics 68

Chapter Two

Figure 1. Map of Virginia showing locations of 108 sample sites in three physiographic regions: Coastal Plain (CP), Piedmont (PD), and Mountain (MT)= Blue Ridge (BR) + Ridge and Valley (RV). For CP, number of sites (N) =12 (circles); for PD, N=49 (squares); and for MT, N=47 (triangles) 117

Figure 2. Plots of 47 (upper panels) or 48 (lower panels) Piedmont sites in the canonical spaces defined by the first (left) and second (right) canonical correlations between fish metrics and four habitat variables. Least- (R), most- (D), and moderately- (O) disturbed sites are shown. Lower two panels are for an analysis that excluded number of native minnow species and number of late-maturing species, two metrics shown to be non-linearly related to disturbance. Intraset structure coefficients are shown after each metric or habitat-variable name. Vertical- and horizontal-axis scales are identical among plots. 118

Figure 3. Plots of 45 Mountain sites in the canonical spaces defined by the first (left) and second (right) canonical correlations between fish metrics and four habitat variables. Least- (R), most- (D), and moderately- (O) disturbed sites are shown. Intraset structure coefficients are shown after each metric or habitat variable name. Vertical- and horizontal-axis scales are identical among plots

119

Figure 4. Plot of a canonical discriminant analysis of 47 Piedmont sites grouped by disturbance. Least- (R), most- (D), and moderately- (O) disturbed sites are shown in the space defined by the first (FISH1) and second (FISH2) canonical composites of the fish metrics. Intraset structure coefficients are shown after each metric or habitat variable name. See Table 7 for summary of analysis. Vertical and horizontal axes span equal canonical distances . . .

120

**CHAPTER ONE: REGIONAL AND STREAM-SIZE EFFECTS ON POTENTIAL IBI
FISH METRICS**

Abstract

I used fish-survey and habitat data from warmwater, wadeable streams in Virginia to assess variation in fish-assemblage attributes (potential IBI metrics) across physiographies, ecoregions, drainage groups, and stream sizes. I needed to investigate this variation in order to define appropriate regions within which to develop and use an IBI in Virginia; metric criteria should reflect expectations in specific regions and at particular stream sizes. I used land-use, riparian, and instream variables to rank site quality. I identified least-disturbed sites and used uni-, bi-, and multivariate analyses to examine variation in taxonomic (e.g., number of native species), trophic (e.g., proportion of individuals as generalist feeders), and reproductive (e.g., proportion as simple, mineral-substrate spawners) metrics.

Metrics differed more among physiographies than they did among ecoregions or drainage groups; largest differences were between Coastal Plain and mountain sites. Taxonomic metrics, especially number of native minnow and number of native darter species, differed more among physiographies than did other metrics. This largely reflected the zoogeographic history of Virginia's freshwater fishes. Some reproductive metrics varied moderately across physiographies, although not fully independently of stream-size effects. Differences in reproductive metrics between Coastal Plain and mountain sites presumably reflected reproductive adaptations of species to lowland vs. to upland stream habitats. Trophic metrics varied more with stream size than they did across regions or drainages. I judged physiographies to be the most reasonable regional units in which to develop and use the IBI in Virginia.

Within physiographies, some taxonomic, trophic, and reproductive metrics varied across stream sizes or drainages; the particular metrics involved and the extent of the variation differed among physiographies. For example, typically expected relations between taxonomic metrics and stream size were not evident at Coastal Plain sites. Also, some trophic and reproductive metrics were related with stream size at Coastal Plain or Piedmont sites, but similar relations at mountain sites were as likely due to drainage effects. These results suggest that setting realistic IBI-metric criteria requires accounting for region-specific

variation (in metrics) with stream size and perhaps drainage. Moreover, for a Virginia IBI, some trophic and reproductive metrics would require region-specific adjustments for stream size, a result contrary to prevailing IBI emphases. Further information from a larger and more evenly distributed (with respect to regions, drainages, and stream sizes) sample of streams is needed before reliable and definitive, region-specific metric criteria can be determined for Virginia.

Introduction

The Index of Biotic Integrity (IBI; Karr 1981; Fausch et al. 1984; Karr et al. 1986) is a bioassessment index often used to compare, among stream sites, the effects of human actions on stream-fish assemblages. The IBI comprises several metrics (i.e., attributes of the assemblage) that describe some of the structure and function of the stream-fish assemblage at a site. Typical structural metrics are total number of fish species or number of species in particular taxa (e.g., Catostomidae=suckers, Etheostomatinae=darters). Typical functional metrics are proportions of individuals in particular functional groups (e.g., omnivores, piscivores, or simple, lithophilous spawners). Each metric is assumed to vary predictably with typical anthropogenic effects such as excessive siltation, chemical pollution, channelization, instream habitat degradation or restoration, or land-use changes. For example, total number of native fish species is expected to be greatest at sites least-disturbed by anthropogenic effects and to decrease with increasing levels of disturbance. (Here and hereafter I use the terms "disturbance" and "least-" or "most-disturbed" to refer to exclusively anthropogenic disturbance). To assign an IBI score to a given test site, one typically compares observed metric values for the site with values expected at least-disturbed sites (= reference sites) that are "located in a similar geographic region" (Karr et al. 1986); a high IBI score represents high similarity between test-site metrics and their reference-site expectations (= reference criteria), thus reflecting high biotic integrity. For the IBI to accurately and reliably reflect the effects (on fish assemblages) of anthropogenic disturbance, one must consider carefully which metrics to include, which reference criteria to use, and which spatial scales and regions to consider for comparing sites. Determining metrics and their reference criteria first requires defining the spatial scales and regions in which IBI assessments will be made. Second, one must know the fish-assemblage attributes at least-disturbed sites within each defined region and how these attributes vary within the range of stream sizes (or other pertinent environmental variables) considered.

For the IBI and similar biotic indices, a tradeoff exists between the widespread utility of the index and the ability of the index to detect variation in the biota that is due solely to effects of anthropogenic actions (Hughes et al. 1990; Karr 1991). Theoretically, one could fine-

tune an index to account for site-specific natural variation in the biota: each site would have its own set of reference criteria. Although such an index presumably would have great ability to detect human-caused disturbance, it would have limited utility for among-site comparisons because each site's score would be based on unique reference criteria. Moreover, the time, cost, and information demands of determining site-specific reference criteria likely would preclude any practical use of such an index. To account for the aforementioned tradeoff between capability and widespread utility of the IBI, one must first understand how fish-assemblage attributes vary spatially and temporally among least-disturbed sites. Then, one must weigh this knowledge with the time and cost constraints and spatiotemporal scales of interest for using the IBI.

In general, the composition of stream-fish assemblages can vary across spatial scales from habitat type (e.g., riffles vs. pools) to reach (e.g., upstream vs. downstream) to landscape class (e.g., physiographies, major drainages, ecoregions) to larger regions. The IBI has been used mostly at the landscape scale. At this scale, the number and types of stream-fish species are known to differ among physiographic regions (Pflieger 1971; Pflieger et al. 1981; Hawkes et al. 1986), major drainages (Hocutt and Wiley 1986), and ecoregions (Larsen et al. 1986; Rohm et al. 1987; Hughes et al. 1987; Whittier et al. 1988). Much less is known of how functional attributes of stream-fish assemblages vary across these landscape classes. For Ohio stream fishes, Larsen et al. (1986) showed that the number of species as intolerant piscivores or intolerant insectivores differed distinctly among four ecoregions. However, comparing presence/absence of species classified into trophic or tolerance groups may be too general an approach to determine how functional attributes vary across regions. Karr (1981) and Karr et al. (1986) suggested using relative abundances of individuals in various trophic groups because abundances likely reflect functional dynamics of energy flow better than do simple presence/absence data. Because fish species differ in trophic and reproductive (= functional) roles, and because species' relative abundances vary across regions (Matthews 1986; Angermeier and Smogor 1995), one can expect functional attributes to vary among physiography, drainage, or ecoregion.

In addition to varying across landscape classes, some fish-assemblage attributes are known to vary with stream size. Specifically, species

richness typically increases with stream size (Shelford 1911; Kuehne 1962; Sheldon 1968; Whiteside and McNatt 1972; Lotrich 1973; Horwitz 1978; Evans and Noble 1979; Platts 1979). Later studies indicated that this relation could be confounded by stream location in a drainage, i.e., downstream link (Osborne and Wiley 1992; Osborne et al. 1992). Users of a fish IBI typically "adjust" richness-metric criteria (e.g., total number of species, number of intolerant species, number of darter species) to account for this stream-size "effect" (see Fausch et al. 1984; Karr et al. 1986; Ohio EPA 1988; Lyons 1992a); however, automatically incorporating these adjustments into an IBI protocol is unwarranted. Most of the earlier studies (cited above) examined only total species richness across a limited range of stream sizes (e.g., 1st- through 5th-order) in a single region. Moreover, many did not account for other sources of variation influencing the richness-vs-stream size relation (e.g., differing levels of anthropogenic degradation at sample sites). Results of several recent studies, some comprising inter-regional comparisons, suggest that landscape-scale differences in geomorphological, hydrologic, or local habitat (biotic and abiotic) can obfuscate or even preclude the expression of stream size-vs-richness relations evidenced at smaller scales (Matthews 1986; Maurakis et al. 1987; Beecher et al. 1988; Ohio EPA 1988; Morin and Naiman 1990; Lyons 1992a). Moreover, for any given study, the presence and strength of such relations likely depends much on the range of stream sizes being examined. For example, Ohio EPA (1988) evidenced strong stream size-vs-metric relations only across limited stream-size ranges. Therefore, for setting realistic and practicable IBI-metric criteria over a geologically and hydrologically variable area (e.g., the state of Virginia), one should compare how fish metrics vary with stream size (or link) relative to how they vary across all other relevant scales of interest (e.g., physiographies, ecoregions, drainages).

Contrary to taxon-richness patterns, relations between functional attributes (e.g., trophic, reproductive) and stream size (or link) have received scant attention, despite the fact that the River Continuum Concept (Vannote et al. 1980) explicitly predicts changes in fish trophic structure from headwater streams (typically 1st-3rd order) to large rivers (typically \geq 7th order). Similarly, Horwitz (1978) and Schlosser (1982, 1987) hypothesized a trophic progression from predominance of generalized-feeding, small fishes in headwaters (1st-3rd

order) to more specialized-feeding, large fishes in midsize streams (4th-6th) to more detritivorous/herbivorous fishes in large rivers (7-8th). They postulated that increasing environmental stability from upstream to downstream, in turn, effects increasing stability in food availability and biotic interactions, resulting in a trophic progression of fish. For 22 1st- through 4th-order Coastal Plain streams in South Carolina, Paller (1994) found evidence supporting this hypothesis. However, the trophic differences occurred mostly between second-order and larger streams; this trophic-progression hypothesis remains untested for most stream sizes and most spatial scales broader than within a stream. Very few published uses of IBI have addressed explicitly stream-size (or link) effects on trophic-metric criteria (but see Ohio EPA 1988). Karr (1991) stated that "scoring criteria for these functional metrics have been remarkably consistent throughout North America, suggesting a general pattern for stream fishes"; however, there is scant published evidence to support this statement.

Even fewer studies have addressed stream-size (or link) effects on reproductive or life-history attributes of stream-fish assemblages. Mahon (1984) indirectly showed that smaller-stream fishes tended to have smaller adult body size, younger age at maturity, shorter reproductive span, and lower fecundity than did species typical of larger streams; however, his comparisons were between two stream systems (one in Ontario, Canada and one in Poland) with probably very different drainage and anthropogenic-disturbance histories. Schlosser (1990) found that lifespan, maximum body size, and age at maturity were greatest for large-river (7th-12th order) fishes and least for headwater (1st-3rd order) fishes of the Illinois River basin. Currently, no well-supported, conceptual basis exists to predict how fish-assemblage reproductive attributes change with stream size (or link), especially for varying ranges of stream size or across multiple landscape classes. Nevertheless, knowledge of such relations may be critical for setting realistic bioassessment criteria.

In Virginia, fish-species richness and diversity differ among physiographies, major drainages, ecoregions, and stream sizes (Hocutt et al. 1986; Jenkins and Burkhead 1994; see Figure 1). These differences likely are attributable to variation in channel gradient, elevation, stream flow, drainage history, human influence, and local habitat (abiotic and biotic) within and across each of these landscape classes

or gradients (e.g., Maurakis et al. 1987). How functional attributes vary across landscape classes is largely unknown. Understanding this variation at least-disturbed sites in Virginia is essential for establishing a statewide IBI protocol.

For Virginia streams, physiography, ecoregion, and major river drainage are reasonable landscape classes within which to compare variation in fish attributes. The relative advantages of using each of these classes to define IBI regions depends on (1) how well each one stratifies natural fish-attribute variation, and on (2) the time, cost, and information demands of developing and using distinct IBIs for regions in each class. Virginia comprises five physiographic provinces, six ecoregions, and at least nine major river drainages in three major river basins (Figure 1); therefore, statistical comparisons of variation (in fish attributes) and development of a statewide IBI at the landscape scale seem practicable.

Several studies used fish-species presence/absences to assess correspondence between geographic patterns of fish distribution and physiographic provinces, river basins, or ecoregions (e.g., Hawkes et al 1986; Whittier et al. 1987; Hughes et al. 1987; Matthews and Robison 1988; Lyons 1989). For example, in Oregon, Hughes et al. (1987) found higher correspondence among ecoregions than that found among river basins or physiographies. However, in Arkansas, Hawkes et al. (1986) found higher correspondence among physiographies than that found among ecoregions. Moreover, for Arkansas fishes, Matthews and Robison (1988) evidenced that fish distributions varied considerably due to zoogeographic chance, manifested as differences in distributions among major river drainages. Results of these and similar studies suggest that no one regional classification is likely to consistently and universally stratify variation in fish-assemblage attributes. To my knowledge, no studies have compared directly how stream-fish IBI metrics vary across physiographies, across ecoregions, and across drainages. Such direct comparisons are needed to develop a sound IBI for Virginia stream fishes.

In this study I use statewide data from least-disturbed sites to determine how IBI metrics (i.e., selected taxonomic, trophic, and reproductive attributes of stream-fish assemblages) differ among physiographies, ecoregions, major drainages, sampling years, and stream sizes (or links). I use these determinations to address the following

questions about developing a fish IBI for warmwater, wadeable streams in Virginia:

- (1) Which landscape class(es) provide the most reasonable and practicable regional framework for a Virginia IBI?
- (2) Which metrics are likely to be especially useful for an IBI with the chosen regional framework?
- (3) Within each IBI region, how do metrics vary with stream size or link? Will a Virginia IBI require adjustments for this variation?
- (4) What are some major concerns about developing a statewide IBI?

Methods

I used data from a fish survey of Virginia warmwater streams conducted July-October 1987, and May-October 1988-1990 (Angermeier and Smogor 1992). Survey data included catch-per-effort of individual fish species and selected instream- and riparian-habitat measures at each of 189 wadeable sites sampled from June-September 1987-1990. These sites occurred in third- through sixth-order streams across most of the major physiographic regions and drainages of the state (Figure 1). Sites were about 50 to 250 m long and drained areas 7 to 454 km²; sites with larger watersheds received greater sampling effort. Sites were selected to provide uniform coverage statewide and to complement existing statewide fisheries data (Angermeier and Smogor 1992).

Habitat variables

The habitat variables that I used comprised selected land-use, riparian, and instream variables that reflected watershed deforestation, watershed urbanization, watershed mining, on-site disruption of well-vegetated riparian zones, and instream habitat heterogeneity for fish. Watershed land use and degradation of riparian areas and instream habitat structure can alter fish assemblages via effects on flow regime, energy source, water quality, physical habitat, and biotic interactions (*sensu* Karr and Dudley 1981 and Karr 1991; Larimore and Smith 1963; Smith 1971; Karr and Schlosser 1978; Angermeier and Karr 1984; Karr et al. 1985; Berkman and Rabeni 1987; Detenbeck et al. 1992; Weaver and Garman 1994). For example, deforestation and mass removal of riparian woody vegetation increases sunlight, nutrients, and sediments to streams. Increases in temperature, nutrient enrichment, and excessive siltation can alter fish richness and abundance by affecting water quality, flow, food availability, spawning substrate, and cover.

Moreover, because streams occur in drainage hierarchies, localized habitat disturbances upstream can have much broader, cumulative effects downstream.

In Virginia, general effects of watershed land-use and riparian degradation are evident. The Virginia Department of Environmental Quality (1994) documented "habitat alteration," mostly due to agricultural and urban land-use impacts, as the second major cause of streams failing to meet "fishable and swimmable" uses designated by the Clean Water Act (PL 95-217). Specific to stream fish in Virginia, Jenkins and Burkhead (1994) stated that, "siltation and turbidity are the most pervasive deleterious factors to the Virginia ichthyofauna." Although the land-use, riparian, and instream variables that I used did not encompass all possible human-caused effects on fish assemblages throughout Virginia, I believe that they ~~they~~ best depicted the least-disturbed sites, given the available information.

I chose least-disturbed sites by comparing, among sites, watershed-scale and on-site habitat measures. I used variables in three watershed-scale classes (mining, urban, forest; Table 1) and one on-site class (riparian/instream cover). Each class presumably reflected distinct anthropogenic effects on fish-assemblage structure or function at each site; there were no strong correlations between any pair of class ranks (see below; absolute values of Spearman's ρ [r_s] < 0.45).

I used the most recent U.S. Geological Survey (USGS) Land-use/Land-cover maps (1:250,000; 1974-1977) to determine percent barren land use, percent urban land use, and percent forest land use in the entire watershed upstream of each sample site. Watershed boundaries were digitized and superimposed on Land-use/Land-cover maps. For each watershed, a grid with regularly spaced dots was superimposed on the map, and dots were counted three times for each of three land-use types: barren, urban, and forest. The mean proportion of dots in each land-use type yielded percent barren, percent urban, or percent forest (Table 1). The most recent USGS 7.5' topographic maps (1:24,000) were used to count the numbers of pollution point-sources in each site's watershed. Mining point-sources comprised dumps, tailings ponds, or coal tipples; urbanization point-sources were water- or sewage-treatment sites.

At each site, I visually quantified bank erosion as slight (value=1), moderate (3), or heavy (5). I visually estimated: (1) riparian width=the width of riparian vegetation extending away from each

streambank (0 to ≥ 50 m), (2) riparian forest= the percent of riparian zone that comprised mature trees, naturally occurring gaps, and relatively undisturbed understory, and (3) bankside woody cover= the percent of banks containing rooted trees or shrubs. For each habitat unit (see below), I noted presence of woody cover or logs in bank-to-bank, 1 m-wide transects. Transects occurred at regular intervals of 1-15 m that were scaled to habitat-unit size, i.e., larger units had greater intervals between each transect. "Instream woody cover" was the percent of transects (in all habitat units) that contained cover. Watershed area of each site was calculated by using Arc-Info, after digitizing the drainage area upstream of each site on USGS 7.5' topographic maps (P. L. Angermeier, personal communication).

Physiographic region of each site was determined by using Arc-Info coverages (P. L. Angermeier, personal communication). A few sites lay on or very near physiographic boundaries; I classified them based on personal on-site observations of surrounding landforms and vegetation, stream gradient, and types and sizes of predominant instream mineral substrates. I used sites in four physiographic regions: Coastal Plain (CP), Piedmont (PD), Blue Ridge, and Ridge and Valley (collectively, MT; Figure 1). Only 12 of 189 sites lay in the Appalachian Plateau physiographic province in extreme southwestern Virginia (Figure 1). Because of this small sample and the relatively unique history of severe anthropogenic disturbance (mostly related to coal mining) in this province (see Jenkins and Burkhead 1994), I deleted the 12 Appalachian Plateau sites, yielding 177 sites for analyses. These twelve sites occurred in the Big Sandy and Clinch (in part) river drainages.

I determined river drainage from a U.S. Geological Survey (USGS) Hydrologic Unit Map of Virginia (1:500,000 scale). Stream orders (Horton-Strahler method) were determined from USGS topographic quadrangle maps, 1:24,000 scale. Stream-order link (hereafter, link) was the difference between the stream order of each site and that of the nearest downstream confluence that was the same order or greater; I used three link categories: (1) link=0, i.e., nearest downstream confluence was the same order as site, (2) link=1, and (3) link > 1 (see Table 2). I used geographic-information-system data provided by the U.S. Environmental Protection Agency (J. Omernik, unpublished data) to determine the ecoregion for each site: Middle Atlantic Coastal Plain (MACP), Southeastern Plain (SEP), Northern Piedmont (NPD), Blue Ridge

Mountains (BRM), and Central Appalachian Ridges and Valleys (CARV; Figure 1).

Ranking sites from least- to most-disturbed

Within each class of habitat variables (i.e., Mining, Urban, Forest, Riparian/instream cover), I ranked sites according to each variable's value. Higher ranks indicated less-disturbed conditions. For each site, I then summed the ranks of each variable. For example, suppose site A ranked 6th (of 177 sites) for percent barren, 15th for number of mining-related point sources, 20th for percent urban, 17th for number of urban-related point sources, 10th for percent forest, 3rd for bank erosion, 8th for riparian width, etc. (see Table 1). Then, site A would receive sum-rank scores of $15+6=21$ for Mining, $17+10=27$ for Urban, 10 for Forest, and $3+8+$ etc. for Riparian/Instream cover classes.

Next, I standardized the sum-rank scores among classes so that each class had equal weight in representing a site's overall amount of anthropogenic disturbance. I did this because I had no prior justification for assigning different weights to each class: I could not quantify the relative potential effects (on fish-assemblage structure and function) of mining vs. urbanization vs. deforestation vs. riparian degradation, across Virginia. For each class, I divided the range of each site's sum-rank scores into thirds. Then I gave each site a standard score of 1, 2, or 3 for each class of variables. This class score represented the site's rank from least-disturbed (1) to most-disturbed (3).

Finally, for each site, I summed the standard class-scores to get a total score, which ranged potentially from 4-12. I ranked all 177 sites according to their total scores and chose sites with scores of 11 or 12 (69 sites) to be the "least disturbed" (hereafter, "reference") sites.

Note that I standardized ranks of variables among but not within each class: I did not account for some variables having greater weight within a class. However, within all but the Riparian/instream cover class, site ranks for individual variables ranged similarly (e.g., for Mining: 1-16 for percent barren and 1-11 for number of point-sources; Table 1). Within the Riparian/instream cover class only one variable's (bank erosion) ranks ranged considerably less (1-5) than did the others' ranks, and only 13 of 177 sites ranked less than 5. Therefore, I do not think that lack of intra-class standardization significantly affected the overall determination of least-disturbed sites.

Individual habitat variables were not redundant. Among all ten variables across all reference sites, absolute values of only three bivariate correlations were > 0.30 , and only one exceeded 0.50: riparian width and riparian forest ($r_s=0.83$, $P=0.0001$). Similarly, site sum-rank scores for each class (Mining, Urban, Forest, Riparian/instream cover) were intercorrelated weakly, except for Forest and Riparian/instream cover ($r_s=-0.43$; $P=0.002$). These results showed that no single class of habitat measures dominated the ranking scheme.

Fish Sampling

At each site we (I and 3 or 4 co-workers) used an electric seine (Angermeier et al. 1991) to collect fish in a series of habitat units (each a single riffle, riffle/run, run, run/pool, or pool) that represented all meso-habitat types in the vicinity. We visually classified habitat units based on water depth, surface turbulence, and obvious changes in channel morphology. For most sites, each habitat unit was blocked bank-to-bank with upstream and downstream nets (0.64 cm mesh). Workers made two seine passes in an upstream direction in each blocked habitat unit. We used dipnets (0.64 cm mesh) to capture all stunned fish, and we identified each fish to species. Individuals > 100 mm (total length) usually were returned to the stream; smaller individuals and those difficult to identify on-site were preserved and identified in the laboratory. Total length of every individual was measured to the nearest mm.

Some habitat units were sampled with upstream blocknets only, downstream blocknets only, or no blocknets due to stream-condition or time constraints. In such cases, whenever possible, we used natural obstructions (to fish) and breaks in stream habitat (e.g., rock ledges, debris dams, heads of riffles, sediment bars) as habitat-unit endpoints. We used a downstream-only blocknet in riffles or runs and an upstream-only blocknet in pools. In faster-flow, shallower habitat units, stunned fish were more likely to drift into downstream nets if missed by hand-netters. In slower-flow, deeper habitat units, stunned fish were netted relatively easily and not flushed downstream; moreover, an upstream blocknet prevented fish from escaping ahead of the electric seine.

Sampling considerations

I screened the reference sites for gross differences in fish-sampling efficiency or effort. First, I eliminated three sites where sampling

efficiency was relatively low due to equipment failure, excessive stream flow, or excessive turbidity. I eliminated three more sites where sampling effort was much less than that at other sites: these three sites lay farthest below a regression line fitted to a plot of mean site width versus site length sampled (Figure 2). Screening yielded 63 reference sites with similar ratios of sample length to stream width.

Adequate estimates of fish species richness and relative abundances are essential for the IBI. The amount of sampling effort (often measured in stream length or number of stream-widths) needed to yield such estimates is not readily known. Angermeier and Smogor (1995) suggested that, given the variation in fish densities, species composition, and habitat among streams, determining a definitive amount of sampling effort is probably impossible, especially when considering streams across large spatial scales (e.g., physiographies). Lyons (1992b) reported that 5 to 49 stream-widths needed to be sampled to yield 95% of the species present in each of nine Wisconsin streams. Only four of our sites were sampled with less than 10 stream-widths (least was 8.0) of effort.

Fish variables

I chose fish variables that potentially could be used as IBI metrics, i.e., variables that presumably reflect effects of typical anthropogenic disturbance on fish communities and are relatively easy to determine from field data (Karr et al. 1986; Miller et al. 1988; Fausch et al. 1990). I grouped fish variables into three classes: taxonomic, trophic, and reproductive (Table 1) for separate analyses. For each class, I chose from an initial list of fish variables that have been used widely in IBI analyses or that I judged potentially useful, but remained unexamined. Due to statistical constraints (see below), I limited variables to those that varied adequately across reference sites, were nearly symmetrically distributed and without extreme outliers (after transformation), and were not highly intercorrelated with many others, i.e., showing many pairwise Pearson correlations > 0.70. For example, I did not include in analyses the "number of intolerant species" (see below), a commonly used IBI metric, because only 4 reference sites had intolerant species, each with only 1 intolerant species.

I based taxonomic, trophic, reproductive, and tolerance classifications of species (Appendix A) on various regional texts (e.g.,

Jenkins and Burkhead 1994, Pflieger 1975), on personal communication with P. L. Angermeier, and on 8 years of personal experience sampling fishes throughout Virginia. I based native versus non-native status (by major river drainage) on Jenkins and Burkhead (1994). Number of native minnows, suckers, and sunfishes each comprised all native species in the families Cyprinidae, Catostomidae, and Centrarchidae, respectively. Number of native darter species comprised all native *Percina* spp. or *Etheostoma* spp.

I classified "intolerant" species as those whose ranges or abundances have decreased presumably due to anthropogenic influences. I classified "tolerant" species as those that are affected least detrimentally by typical anthropogenic disturbances to streams and watersheds (e.g., common carp, *Cyprinus carpio*; gizzard shad, *Dorosoma cepedianum*; green sunfish, *Lepomis cyanellus*). I determined each species' "tolerance" and "intolerance" classifications before ranking sites from least- to most-disturbed; therefore, I avoided any circularity or temptation to "recalibrate" these classifications based on species' occurrences relative to anthropogenic disturbance. Karr et al. (1986) recommended that less than 10% of the species in an IBI region be classified as "intolerant." This limit ensures that an "intolerance" metric contributes exclusively to the highest IBI scores, i.e., only reflects sites at the least-disturbed end of the biotic integrity continuum. My classifying 5% (7 of 142 species; Appendix A) as intolerant seems reasonable. Similarly, I suggest that any IBI "tolerance" classification be limited to a small percentage of the included species; this ensures that the "tolerance" metric reflects exclusively the lowest end of the biotic-integrity continuum, i.e., only those severely disturbed sites dominated by tolerant species or individuals. I classified 12% (17 of 142; Appendix A) of species as tolerant.

For trophic variables I considered three classification factors: number of food types typically eaten, feeding behavior, and feeding group. I designated four food-type categories: (1) detritus, (2) algae or vascular plants, (3) invertebrates, and (4) fish (including fish blood) or crayfish. "Generalist feeders" were species in which adults eat from more than two food-type categories; "specialists" eat from two or fewer categories (Table 1). I designated two mutually exclusive feeding behaviors, benthic and non-benthic. Benthic feeders feed, as adults, mostly along the stream bottom and require foods that are

associated strongly with the stream substratum (e.g., many types of aquatic insects). I assigned fish species to one of five feeding groups based on the primary food type(s) of subadults and adults. Groups represented a continuum from (1) detritivore/algivore/herbivore to (2) algivore/herbivore/invertivore to (3) invertivore to (4) invertivore/piscivore to (5) piscivore or fish parasite. Group 4 comprised species in which subadults eat primarily invertebrates, but adults eat primarily fish or crayfish (e.g., American eel, *Anguilla rostrata*; yellow bullhead, *Ameiurus melas*; redbreast sunfish, *Lepomis auritus*; crappies, *Pomoxis* spp.; yellow perch, *Perca flavescens*). "Carnivores" were species in groups 4 or 5 (Table 1).

For reproductive variables, I classified species as non-obligate versus obligate mineral-substrate (unsilted sand to boulder) spawners, which I refer to as lithophils. Also I designated "manipulative" versus "simple" (non-manipulative) spawners (see Table 1). Manipulative spawners build nests, depressions, or cavities or actively guard eggs or young (e.g., lampreys, Petromyzontidae; trouts, Salmonidae; central stoneroller, *Camptostoma anomalum*; catfishes, Ictaluridae; sunfishes, Centrarchidae; some darters, *Etheostoma* spp.). Simple spawners exhibit relatively little nest preparation or parental care. I defined "nest-associate spawners" (e.g., some Cyprinidae) as members of species known to spawn in or over the nests of other species, namely minnows or sunfishes. I defined "late-maturing species" as those whose females typically do not spawn before 3 years of age.

Testing for statistical effects of landscape class or year

Considering all 63 reference sites, I used one-way MANOVAs to test for differences in fish variables (in each of three classes) among physiographies, ecoregions, or sample years. I subsequently refer to these differences as (statistical) "effects" of physiography, ecoregion, or year on fish variables. Uneven distributions of sites among physiographies, major drainages, ecoregions, and years largely precluded using multiway MANOVAs (see Table 2). For example, I could not test simultaneously for physiography and drainage effects because most drainages do not span all physiographies, or sites in a drainage did not occur across all physiographies. However, I did test for physiography and year effects simultaneously by using two-way MANOVAs. Distributions of sites limited this test to comparisons (of each class of fish variables) between two physiographies (PD and MT) only (see Table 2).

Given that drainage and physiography historically have influenced fish-assemblage structure throughout Virginia, I sought to determine which had the strongest effect (on fish variables), in the context of determining useful IBI regions. To partially test the relative strengths of physiography versus drainage effects, I compared fish variables among particular drainage groups (grouped according to their past and present basin similarities; see Jenkins and Burkhead 1994) within each physiography (see Figure 1). For comparisons within MT, I used SHE (Shenandoah; N=8) sites versus RAP (Rappahannock)+ JAM (James; N=4+6=10). For comparisons within PD I used YOR (York; N=5) versus JAM (N=6) versus ROA (Roanoke; N=10) versus CHO (Chowan; N=8) sites. Finally, for comparisons within CP I used a Chesapeake Bay drainage group (CHE= 3 RAP + 1 YOR + 1 JAM sites) versus an Albemarle Sound drainage group (ALB= 4 CHO sites). Because of smaller sample sizes at these intra-physiography scales, I could not rely on MANOVA or MANCOVA results; therefore, I used Kruskal-Wallis and Wilcoxon two-sample tests (Sokal and Rohlf 1981) to test for differences in fish variables among drainage groups within each physiography.

I used descriptive discriminant analysis (i.e., canonical analysis; Williams 1983; Gittins 1985; Huberty 1986; Huberty and Morris 1989) to show how fish variables contributed to the statistical separation (if any) among physiographies, ecoregions, or years. Specifically, the canonical analysis had three main objectives: (1) For fish variables in each class, define linear combinations (hereafter, canonical functions) that best separate sites by group (e.g., physiographies, ecoregions, drainage groups, or years), in canonical space. (2) Identify the canonical function(s) that contribute most to explaining group separation. (3) For selected canonical function(s), identify fish variables that contribute most to group separation.

Testing for statistical effects of stream size or link

At the statewide scale, I tested for potential stream-size (via one-way MANCOVA with watershed area as covariate) or link (via two-way MANOVA) effects on fish variables while simultaneously accounting for landscape effects (physiography, ecoregion) that were judged most important based on previous one-way MANOVAs. Sample sizes were too small to allow tests of simultaneous effects of stream size, link, and landscape class; however, I first determined that watershed area and link were not related at the statewide scale (Kruskal-Wallis test;

P=0.73). For each fish-variable class, to test for stream-size or link effects sub-statewide, i.e., within potential IBI reference regions (physiographies, ecoregions, drainages), I used one-way MANCOVA with link as the categorical variable and watershed area as the covariate. These MANCOVAs simultaneously accounted for relations between stream size and link at this intra-landscape class level. Because of possible problems with multivariate tests (see *Statistical considerations*), I supplemented them with univariate and bivariate comparisons by using Kruskal-Wallis tests and Spearman correlations of relations between each fish variable and stream size or link.

Statistical considerations

I used an F-ratio approximation of Wilks's lambda statistic to judge the statistical significance of MANOVA and MANCOVA. Wilks's lambda is a scalar that equals the ratio (of determinants) of the within-group sum-of-squares-and-cross-products matrix (SSCP) to the total SSCP. I interpreted P-values as the probability of getting differences (in fish-variable vectors of means) as large or larger than those found, assuming that those vectors of means came from the same population (Carver 1978; Gold 1969). Carver (1978) argued that because one can rarely test the validity of this assumption, using statistical significance to judge the importance or "scientific significance" of findings is inappropriate. Similarly, Thompson (1989) cautioned against sole reliance on statistical-significance measures and recommended comparing magnitudes of statistical effects (e.g., ratios of sums of squares) to achieve meaningful interpretation of results. Given the aforementioned arguments and that the validity of my inferences based on MANOVA or MANCOVA may be in question (see below), I chose to stress more the relative strengths of multivariate effects than the statistical reliability of results.

I used a multivariate eta-square (η_m^2) to assess multivariate differences among and between groups (Barker and Barker 1984). Univariate η^2 is the ratio of the between-group sum of squares to the total sum of squares (Thorndike 1978), which conveniently depicts the variance due to group membership. Analogously, η_m^2 is the ratio (of determinants) of the between-group SSCP to the total SSCP or, simply, $1 - \text{Wilks's lambda}$ (Barker and Barker 1984).

Proper use of multivariate tests and the stability of results of such tests require that the number of variables be limited and that variables

have few high intercorrelations; moreover, inferences based on MANOVA and MANCOVA variance-covariance structure require that variables meet assumptions of multivariate normality and homogeneity of variance-covariance structure (Williams 1983; Huberty 1986; Smith et al. 1988). For MANOVA, Huberty (1986) suggested, as a "rough guide", that sample size of the smallest statistical class (= cell) be at least three times the number of outcome variables (e.g., fish variables). Others (Tabachnick and Fidell 1983) have recommended merely that the number of variables be greater than the number of samples within each cell. Though I limited the number of variables by running separate analyses for each fish-variable class, my cell sizes still fell short of 1 site:1 variable for two MANOVAs of ecoregion effects (e.g., see Table 2). Within each fish-variable class, no pairwise correlations between variables exceeded 0.61, and most were less than 0.50 (Table 3); therefore, "high" multicollinearity apparently was not a problem. I did not assess higher-order (i.e., simultaneous relations among 3 or more variables) multicollinearity.

I did not test directly for multivariate normality because few conclusive tests are readily available. Although not ensuring multivariate normality and homogeneity of variance-covariance structure, I increased their likelihood by transforming variables to best meet analogous univariate assumptions (Tabachnick and Fidell 1983). For fish variables that were counts of species (e.g., number of native darters; see Table 1), I used a square-root transformation, $(X+0.5)^{0.5}$. For fish variables that were proportions, I used an arcsine transformation, $\arcsin(X^{0.5})$; Sokal and Rohlf 1981). I observed only two extreme uni- or multivariate outliers. Bernards Creek, a lower Piedmont site, had the fewest (i.e., none) number of native minnow species and the third fewest number of native species among PD sites; therefore, it resembled CP sites more than it did other PD sites. David Creek, an upper Piedmont site, had the second highest number of native minnow species and highest number of non-native species among PD sites; therefore, it resembled MT sites more than it did other PD sites. I deleted these sites and reran analyses; overall results changed little, and original patterns became even better defined. However, I had few compelling reasons to characterize these sites as overall anomalies; each was very near or in the transition zone between physiographies so it was not unreasonable for each to resemble sites in the bordering physiography.

Therefore, I included both sites in the reported analyses.

Given that sample sizes were small and that some moderate outliers, asymmetry in distribution, and non-homogeneity of variances-covariances were evident among the transformed fish variables and their residuals, I also ran univariate, nonparametric analyses (Kruskal-Wallis and Wilcoxon two-sample tests) to test for differences in fish variables among physiographies, ecoregions, drainages, or years, at the statewide scale. A severe limitation of these univariate tests is that they fail to account for interdependence of fish variables (multicollinearity). Moreover, one cannot use the combined results of multiple univariate tests to validly assess multivariate effects (Huberty and Morris 1989; Share 1984). Despite the potential shortcomings of small sample sizes, my conclusions based on multivariate results reflect a best-possible realistic representation of the multiple relations among fish variables. Alternatively, because some assumptions of multivariate tests were violated, I did not rely solely on multivariate-test results; I also examined data uni- or bivariate to facilitate multivariate-based interpretations. The combined information from multivariate and univariate tests provides the best analysis, given the somewhat "sloppy" data.

No fixed rules exist for how to interpret the relative contributions of individual variables to explaining overall group separation in canonical analysis. Two commonly used approaches are: (1) using within-group canonical-structure coefficients or (2) using within-group standardized canonical-function coefficients. Structure coefficients are the Pearson correlations between each variable and the scores of each canonical function (i.e., directly analogous to factor loadings in factor analysis). Function coefficients are the standardized partial linear-regression coefficients of each canonical function's scores as a function of the variables (e.g., taxonomic, trophic, or reproductive variables). Because structure coefficients do not account for intercorrelations of the variables, interpretations based solely on structure coefficients partly nullify the initially intended multivariate analysis (Share 1984; Huberty and Wisenbaker 1992; Rencher 1992; Thomas 1992). Alternatively, sole use of function coefficients subjects interpretations to problems associated with high multicollinearity or sample-specific covariation, namely, suppressor effects and instability (Meredith 1964; Thorndike and Weiss 1973; Levine

1977; Williams 1983; Thompson and Borrello 1985). Given that the relative utility of structure versus function coefficients varies inconsistently among specific applications, Thomas (1992) proposed using a combined measure, the discriminant ratio coefficient (DRC). This coefficient is simply the product of the two. Herein, I use the DRC to judge the relative contributions of fish variables to "explaining" potential multivariate differences among landscape groups or years. Although this coefficient has limitations (see Thomas 1992), I believe that it provides a reasonable interpretation. However, given that structure and function coefficients each contribute unique information, I reported them also.

Results

Effects of physiography

Taxonomic, trophic, and reproductive variables differed slightly more among physiographies (e.g., $\eta^2_m = 0.83$) than they did among ecoregions and much more among physiographies or ecoregions than they did among years (Table 4); the magnitude of these effects was lessened by stream-size effects (see *Effects of stream size...*), especially for trophic variables. For each fish-variable group, CP sites differed most from MT (=BR + RV) sites (Table 5). Taxonomic variables differed more among physiographies than did trophic or reproductive variables (Tables 4 and 5; Figure 3).

For taxonomic variables, the first canonical function (CAN1) accounted for 64% (adjusted) of the variance among physiographies; the second canonical function (CAN2) accounted for 34% (Table 6). For three physiographic regions, the greatest multivariate taxonomic differences were between CP and MT and smallest differences were between CP and PD (Table 5 and Figure 3). Number of minnow species (discriminant ratio coefficient [DRC] for CAN1 = 0.67) contributed highly and number of darter species (DRC for CAN2 = 0.76) contributed secondarily to the overall multivariate differences among physiographies (Table 6); however, the relative contributions of taxonomic variables varied, depending on which two physiographies were compared. Expectedly, number of native species was positively and strongly correlated with numbers of minnow and of darter species (Table 3); therefore, function coefficients for number of native species were negative, which reflected a suppressing effect.

For taxonomic variables, number of minnow species mostly

discriminated CP from MT sites (DRC=0.85), whereas numbers of darter (DRC=0.50) and of minnow (DRC=0.64) species co-contributed to discriminating CP from PD (Table 7). For discriminating PD from MT sites, numbers of darter (DRC=0.28) and sunfish (0.23) species contributed similarly and weakly (Table 7). The contribution of number of native species (DRC=0.18) largely suppressed that of number of minnow species (DRC=0.18 but function coefficient=-0.75) due to their high intercorrelation in PD and in MT (Table 8). Univariate comparisons corroborated multivariate results: CP sites had fewer minnow species than did PD or MT sites; PD sites had more species, including more darter species, than did CP or MT sites; and CP and PD sites had more sunfish species than did MT sites (Table 9).

Univariate tests (Table 9) and even some multivariate measures showed that number of non-native species differed distinctly among physiographies; however, when all variables and their inter-physiographic differences were considered together, these relations were less important than were those mentioned previously. High negative function coefficients (-0.87 for CAN1 in Table 6; -0.97 for CPvPD and -1.44 for CPvMT in Table 7) for number of native species suggested that this variable's influence was suppressed (except for PDvMT) due to correlations (multicollinearity) with other influential variables (e.g., number of minnow species). Given the consistently detectable influence of number of minnow species and the secondary influences of numbers of darter and sunfish species and given that all three contributed, as expected, to number of native species (see Table 8), the information gained from this analysis probably would change little if number of native species were omitted.

Reproductive variables differed most between MT and CP (Figure 3; Tables 6 and 7). CP sites had more various-substrate, manipulative spawners (DRC for CAN1 = 0.77) and fewer simple lithophils (DRC for CAN1 = 0.39) than did MT sites; values of these fish variables were intermediate in PD. Although initial tests showed strong differences in trophic variables among physiographies (Table 4), subsequent tests showed that trophic variables differed little among physiographies, when stream-size effects were accounted for (Table 10).

Multivariate analyses only accounted for relations among fish variables within a given class: taxonomic, trophic, or reproductive. At the statewide scale, interclass relations among fish variables were

few and easily interpreted (Table 3); therefore, they likely did not confound my statewide interpretations. Overall, fish variables differed more among physiographies than they did among ecoregions across Virginia; taxonomic variation, especially in numbers of minnow and darter species, was more pronounced than was trophic or reproductive variation.

Effects of stream size and of link

Statewide, for each class of fish variables, stream-size effects accounted for much of the original-MANOVA physiographic effects (see Table 4), although a relatively strong physiographic effect on taxonomic variables was still evident between CP and MT sites ($\eta^2_m=0.54$; Table 10). For each class of fish variables, stream-size and interaction effects were greater than were ecoregion effects for all but one possible pairwise MANCOVA comparison (for taxonomic variables between CARV and NPD, $\eta^2_m = 0.46$ for ecoregion effect, 0.44 for interaction effect, and 0.37 for stream-size effect), again suggesting that differences across physiographies were more distinct than were those across ecoregions. For taxonomic and reproductive variables, accounting for stream size did not fully negate earlier results: taxonomic and reproductive variables still differed across physiographic extremes (CP vs. MT; Table 10) more than they did across stream sizes; however, between adjacent physiographies (e.g., PD vs. MT), stream-size effects or interaction effects were as strong or stronger than were physiographic ones. Interaction effects likely were due, in part, to MT sites (median watershed area= 91 km²) being larger than PD (52 km²) or CP sites (57 km²; Kruskal-Wallis and Wilcoxon two-sample tests; all $P < 0.10$). Unlike for taxonomic or reproductive variables, initial, apparently large physiographic effects on trophic variables became largely attributable to stream-size effects. For example, the original MANOVA physiographic effect of $\eta^2_m=0.75$ (CP vs. MT; Table 5) decreased to 0.12 in MANCOVA (Table 10), even less than the singular stream-size effect ($\eta^2_m=0.26$).

Overall at the statewide scale, at least for taxonomic and reproductive variables, physiography remained most relevant with respect to determining IBI reference regions throughout Virginia. However, for some comparisons at less than the statewide physiographic extreme (e.g., PD vs. MT; Table 10), some taxonomic and trophic variables varied equally or more with stream-size than they did across physiographies.

Accounting for stream-order link did not change these results; statewide, fish variables in each class differed less among link categories than they did across stream sizes (Table 10).

Within physiographies, small sample sizes precluded reliable multivariate tests of stream-size or link effects: within each physiography at least one cell had a sample size less than the number of fish variables (Table 2). Therefore, I examined stream-size and link effects, independently, for each fish variable and within each physiography.

Bivariate tests showed that, within physiographies, taxonomic variables were related to stream size more consistently than were other variables (Table 11, Figures 4 and 5). For PD sites, number of native species increased moderately with increasing stream size, mostly due to increasing numbers of minnow and darter species. Similarly, for MT sites, number of native species increased with stream size, however, mostly due to increasing numbers of minnow and sunfish species. The increase in number of native species with stream size seemed to level or drop off at stream sizes $> 250 \text{ km}^2$, although only one PD and two MT sites were this large (Figures 4 and 5). Within each physiography, stream-order link had little effect on fish variables, and no result suggested that potentially large species richness downstream inflated sample-site species richness (Table 12).

For PD and for MT, some trophic or reproductive variables appeared related as much to stream size as were taxonomic variables (Table 11). However, for MT sites, apparent relations between three functional variables and stream size were not independent of possible drainage effects or intercorrelations of the variables (see Table 8). In MT, sites in SHE had greater watershed areas than did sites in RAP, in JAM, or in NEW+HOL (Kruskal-Wallis test; $P=0.03$); therefore, increasing proportion as specialist carnivores and number of non-native specialist carnivore species and decreasing proportion as nest-associate spawners could be attributed as much to a drainage (see Table 13) as to a stream-size effect (see Table 11). In PD, increased proportion as late-maturing species and decreased proportion as tolerants with increasing stream size could be attributed almost solely to stream-size rather than to link or to drainage effects. Similarly, in CP, decreased proportion as generalist feeders could be attributed almost solely to increased stream size (Table 11). In PD or CP, watershed area was not related

strongly with link (Kruskal-Wallis tests; all $P > 0.28$) or with drainage (Kruskal-Wallis test for CP: ALB vs. CHE, $P=0.22$; for PD: RAP vs. JAM vs. ROA vs. CHO, $P=0.56$).

Overall, accounting for stream-size effects dampened the initial, apparently strong physiographic effects on fish variables; nonetheless, physiography still explained the most variation in taxonomic and reproductive variables across the state. For comparisons between adjacent physiographies, stream size effects either equalled, obfuscated (i.e., interaction), or exceeded physiographic effects, depending on which fish variables and physiographies were considered. Within PD and within MT, especially at sites $< 250 \text{ km}^2$, number of native species increased with stream size mostly due to increases in numbers of minnow species and either sunfish or darter species. This richness-vs-stream size effect was not evident in CP. Within each physiography, some trophic and reproductive variables were related to stream size at least as strongly as were taxonomic variables, although, in MT, these relations were not distinguishable from those possibly due to drainage effects.

Effects of drainage

As mentioned previously (see **Methods**), the uneven distribution of reference sites did not allow simultaneous tests of drainage and physiography effects on fish variables, statewide. Moreover, within physiographies, small sample sizes precluded multivariate tests of drainage effects (see Table 2); therefore, I examined possible univariate drainage effects within each physiography.

Within physiographies, some fish variables differed among drainage groups, but few differences could be attributed solely to drainage effects. In MT, SHE sites had fewer darter species, greater proportion as specialist carnivores, slightly more non-native specialist carnivore species, smaller proportion as nest associates, and greater proportion as tolerants than did RAP+JAM (Table 13); however, as previously mentioned, these differences could be attributed as well to stream-size effects or fish-variable intercorrelations: SHE sites were larger than were RAP+JAM sites (Wilcoxon two-sample test; $P= 0.006$), and these variables were correlated with stream size (Table 11, e.g., CARNPRP) or with variables that were correlated with stream size (Table 8, e.g., DARSP). Only one variable, number of late-maturing species, differed between SHE and RAP+JAM while being unrelated to stream size or other

fish variables.

Fewer late-maturing species in SHE than were in RAP+JAM may have reflected a physiographic effect between BR and RV (recall that I grouped these two physiographies as MT). Seven of 8 SHE sites occurred in RV, whereas 8 of 10 RAP+JAM sites occurred in BR; therefore, SHE vs. RAP+JAM comparisons represented, largely, RV vs. BR. Canonical analyses showed that BR and RV sites segregated well along CAN2 (Figure 3), and number of late-maturing species contributed mostly to this separation (DRC=0.69 in Table 6). The two BR sites least like others with respect to CAN2 (i.e., below dotted line in Figure 3) were also the two sites closest to the physiographic boundary of RV and BR (Figure 1).

In CP, few fish variables differed between drainage groups (Table 14). Specifically, CHE sites had more late-maturing species, greater proportion as benthic, specialist invertivores, and more minnow and tolerant species than did ALB; all four variables were positively intercorrelated in CP (Table 8). These differences between CHE and ALB were not attributable to stream size or link effects: differences (in fish variables) between drainages were not consistent across stream sizes or links (see Tables 11 and 12), stream size differed little between the two drainage groups (median watershed area= 42.0 km² for CHE and 59.0 for ALB; Wilcoxon two-sample test, $P=0.22$), and watershed area was not related strongly with link in CP (Kruskal-Wallis test; $P=0.29$).

In PD, no fish variable differed greatly between CHE (5 YOR + 6 JAM = 11 sites) and ALB (10 ROA + 8 CHO = 18 sites) drainage groups (Wilcoxon two-sample tests; all $P > 0.10$). I alternatively divided PD sites into individual river drainages (YOR vs. JAM vs. ROA vs. CHO; see Table 2) and again found no large differences in fish variables among these finer-scale drainage divisions (Kruskal-Wallis tests; all $P > 0.05$). Overall, within physiographies, variation in only a few fish variables could be attributed exclusively to drainage effects.

Effects of year

Statewide, multivariate differences in fish variables among sample years were small relative to those among physiographies (Table 4). Because uneven distribution of sites (see Table 2) precluded multivariate tests of simultaneous physiography and year effects, I used univariate tests to assess differences among years, within each physiography. Of all 17 fish variables tested within each physiography, only number of native sucker species and proportion as generalist

feeders differed among years--but only in PD. There were fewer native sucker species (median = 1.0) and a smaller proportion as generalist feeders (median = 0.03) in 1989 than there were in the other three years (medians = 2.5 and 0.10, respectively; Kruskal-Wallis and Wilcoxon two-sample tests; $0.05 > P > 0.01$). Both variables were positively correlated in PD ($r_s = 0.64$; Table 8). Overall, variation, in fish variables, across four years of sampling seemed too small to negate or confound any aforementioned results.

Discussion

IBI reference regions for Virginia

In Virginia, choosing an appropriate regional framework for using the IBI is complicated because structural and functional attributes of stream-fish assemblages (i.e., potential IBI metrics) did not vary similarly across potential regions: physiographies, ecoregions, or drainages. Furthermore, because the relative abilities of potential IBI metrics to detect anthropogenic effects are yet unexamined (but see Chapter 2) for Virginia streams, I can only assume that at least some taxonomic, some trophic, and some reproductive attributes will be useful for a Virginia IBI (see Chapter 2, **Methods** for rationales of metrics).

Results of this study suggest that a Virginia IBI should have separate reference criteria for each of three physiographic regions: MT, PD, and CP. Both structural (i.e., taxonomic) and functional (i.e., reproductive) metrics varied distinctly enough to merit reference criteria specific to physiography. Trophic metrics varied less among physiographies, especially when accounting for statewide stream-size differences; therefore, trophic criteria specific to physiography are not needed.

Larsen et al. (1986), Rohm et al. (1987), Whittier et al. (1988) and Hughes et al. (1986 and 1990) have recommended using ecoregions as regions within which to set bioassessment (including a stream-fish IBI) criteria. Ecoregion designations are based on land-surface form, soil types, potential natural vegetation, and land use (Omernik 1987), but not on geographic distributions of specific taxa. For some biotic indices, ecoregions may be inadequate because ecoregion boundaries do not coincide well with spatial patterns of the biota being assessed. For example, in Virginia, ecoregion delineations do not recognize the Fall Line, the narrow zone of relatively steep gradient that separates the Piedmont from the Coastal Plain and has "major effects on fish

distribution" (Jenkins and Burkhead 1994; see Figure 1).

For Virginia streams, physiography provides as good a regional framework for the IBI as does ecoregion. Results of studies in other states support this conclusion, although most of these studies primarily used species presence/absences rather than potential IBI metrics. In Ohio, Larsen et al. (1986) consistently found the most distinct differences in fish-assemblage measures between the Huron/Erie Lake Plain and the Western Allegheny Plateau, which represented the physiographic extremes (in elevation and gradient) across the state. Similarly, for streams in Arkansas (Rohm et al. 1987), in Oregon (Whittier et al. 1988), and in Wisconsin (Lyons 1989) fish assemblages differed more across elevational or gradient extremes than among ecoregions. For streams in Kansas, Hawkes et al. (1986) found that fish-assemblage patterns corresponded much better with physiography than they did with ecoregion. One study of streams in Oregon did find that fish-species presence/absence patterns corresponded more with ecoregion and drainage basin than they did with physiography (Hughes et al. 1987). Apparently, no one regional classification (physiography, ecoregion, major drainage) will always be best for using IBI. For IBI, the relative utility of various regional classifications depends on the correspondence between regional delineations and geographic patterns in pertinent fish-assemblage attributes. In Virginia, I found that physiography accounted more consistently for the variation in metrics (especially for taxonomic variables) than did ecoregion.

As mentioned previously, time and cost constraints limit using the IBI at increasingly finer or more variable spatial scales. For Virginia, physiographic boundaries are slightly broader, better defined, and less subject to change over time than are ecoregion boundaries, which are based partly on human land use. Therefore, ecoregion boundaries and their corresponding biocriteria would require periodic reevaluation more frequently than would physiographic boundaries and biocriteria. The costs of such reevaluations may not be justifiable, given the lack of information gained relative to a physiography-based approach. Given these arguments, I recommend using physiographies (CP, PD, MT) as regions for IBI in Virginia.

For Virginia, some IBI reference criteria developed at the physiographic scale may require adjustment based on further study of intra-physiographic variation in fish assemblages. This may be true

especially for MT sites; the zoogeographic, land-use, and fish-sampling histories of Virginia's MT drainages (or portions thereof) differ from each other more than do the histories of drainages in PD or CP (Jenkins and Burkhead 1994). No doubt, some of what I call "intra-MT" variation may be due to differences between BR and RV, which I have combined as MT. However, even within RV, notable differences in fish-assemblage attributes occur between drainages.

For example, the Clinch River (CLI) and Holston River (HOL) drainages, in southwestern Virginia (Figure 1), likely would require a set of reference criteria quite different from that of the adjacent New River drainage. Being part of the extremely diverse Tennessee River basin, the Clinch and Holston drainages in Virginia contain relatively high numbers of native and endemic fish species. In contrast, the New River drainage has relatively few native fish taxa and ranks lowest of eastern U.S. drainages in number of taxa relative to drainage area (Sheldon 1988; Jenkins and Burkhead 1994). Similar to differences among MT drainages, a few potential taxonomic, trophic, and reproductive metrics differed between CHE and ALB drainage groups in the CP. For some cases (e.g., Clinch vs. New), setting drainage-specific IBI reference criteria for (at least) taxonomic variables seems justifiable. However, in other cases (e.g., in MT for SHE vs. RAP+JAM; or in CP) studies with larger sample sizes than mine are needed to distinguish possible finer-scale effects due to physiography (e.g., BR vs. RV) from effects due solely to drainage.

Regional variation in taxonomic variables

In Virginia's least-disturbed, warmwater Wadeable streams, taxonomic variables differed more among physiographies than did trophic and reproductive variables. This result reflects the pervasive association between physiography and stream-fish distribution in Virginia (Gilbert 1980; Maurakis et al. 1987; Jenkins and Burkhead 1994). Differences in stream-fish taxonomic attributes among physiographies were due mostly to differences in the numbers of native minnow and darter species. Both taxa have relatively many small-bodied, localized or ecologically specialized species; therefore, it is not surprising that they contributed most of the taxon-richness variation across a large spatial scale (Hocutt et al. 1986; Jenkins and Burkhead 1994). The fact that CP sites harbored fewer minnow and darter species than did more upland PD sites is consistent with the belief that Virginia members of these two

taxa radiated principally from upland centers of evolution (Gilbert 1980; Hocutt et al. 1986).

My finding very few (median= 1.0) native darter species at MT reference sites was unexpected, although interpretable. Eight of the twenty-five MT reference sites occurred in the Shenandoah River drainage (SHE). This drainage contains only two native darter species, which is atypical of Virginia MT drainages (Jenkins and Burkhead 1994). Darters and other obligately benthic species tend to be susceptible to excessive siltation (Muncy et al. 1979; Berkman and Rabeni 1987). Perhaps for historical, geomorphological, and hydrological reasons, SHE has been more susceptible to siltation effects than have other drainages in Virginia. Jenkins and Burkhead (1994) speculated that the low number of darter species in SHE could be due to a longer history of anthropogenic disturbance there than that in other Virginia drainages. Recently, of all major river drainages in Virginia, only in the Shenandoah/Potomac drainage was siltation the primary cause of failure to support the federal Clean Water Act's designated uses (VDEQ 1994). The relatively low number of native darter species expected in SHE and the predominant representation of this drainage among MT reference sites explains the unexpectedly low average number of native darters for MT sites. Other warmwater, wadeable MT streams of Virginia drainages (RAP, JAM, ROA, NEW, HOL, CLI; see Figure 1) typically contain four or more native darter species (personal observation; Jenkins and Burkhead 1994).

For a Virginia IBI, number of native minnow species and of native darter species may prove more useful as metrics for PD or MT (depending on drainage) sites than they would for CP sites. Too few members of a taxon limit the observable range of metric values based on that taxon. For example, number of native darter species--expected to be highest at least-disturbed sites--is unlikely to be a useful measure of anthropogenic disturbance in Virginia CP streams because, on average, the least-disturbed sites harbor only 1 darter species (Table 9).

Regional variation in reproductive variables

Stream-fish reproductive attributes differed among physiographies, but less than did taxonomic attributes. Moreover, reproductive differences among physiography were more dependent on differences with stream size, possibly due, in part, to MT sites being larger than were PD or CP sites. Differences in some reproductive variables (e.g., number of late-maturing species) among drainages within MT or within CP

further complicated the interpretation. Despite these complications, some patterns of reproductive variables were evident and interpretable.

Among physiographies, reproductive attributes differed mostly due to CP sites having the largest proportion as reproductive generalists (various-substrate, manipulative spawners; DRC=0.77 for CAN1 in Table 6; Figure 3), the smallest proportion as reproductive specialists (mineral-substrate, simple spawners, DRC=0.39 for CAN1), and the fewest late-maturing species (DRC=0.69 for CAN2; also see Table 9). These results reflect the broad habitat differences between CP and more upland regions. Low-gradient, fine-substrate streams of the CP harbor biota whose reproductive requirements and behaviors are less constrained to stream-bottom and flow characteristics than are those of the biota of steeper, hard-bottomed upland streams (Smock and Gilinsky 1992; Jenkins and Burkhead 1994). Furthermore, characteristically greater proportions of specialist carnivores in CP than in more upland regions (see Table 9; e.g., Huish and Pardue 1978) may cause stronger selection for nest guarding or parental care among CP fish species than that among upland-adapted species.

Intra-physiographic differences in reproductive attributes were most evident and interpretable in MT. For MT sites, fewer late-maturing species in SHE than in RAP+JAM possibly reflected finer-scale physiographic differences between BR and RV (see Figure 3). Also for MT sites, the larger proportion as tolerants in SHE than in RAP+JAM could have reflected true drainage differences; as discussed previously, due to drainage history or character, least-disturbed SHE sites naturally may harbor greater proportions as tolerants than do least-disturbed sites in other Virginia drainages.

For a Virginia IBI, as for taxonomic metrics, the utility of particular reproductive metrics varies with physiography. For example, proportion as simple, mineral-substrate spawners--expected to be highest at least-disturbed sites--was so low at least-disturbed CP sites that, for all practical purposes, it would be insensitive to anthropogenic disturbance. This contrasts with the use of this metric in other statewide versions of IBI (e.g., Ohio EPA 1988; Lyons 1992a). One may need to consider alternative metrics, perhaps diametric to original ones (e.g., proportion as generalist spawners), which vary enough to allow measurable effects of anthropogenic disturbance. Development of an IBI should include examination of natural intra-regional variation in

potential metrics; such examination will aid in streamlining IBI by region, by identifying metrics that vary so little as to preclude their utility.

Regional variation in trophic variables

Statewide, variation in stream-fish trophic attributes was less attributable to physiographic effects than was variation in taxonomic or reproductive attributes. Apparent trophic differences among physiographies (Table 5) could be attributed to overriding stream-size effects (Table 10). Even within physiographies, apparent drainage effects on trophic attributes could be attributed to stream-size effects. For example, in MT, proportion as specialist carnivores and number of non-native specialist carnivore species were greater in the larger SHE streams than they were in the smaller RAP+JAM streams (Table 13).

Similar to my results, Schlosser (1982), Lotrich (1973), and Horwitz (1978), each examining stream size-vs-trophic relations at different spatial scales, found that more specialized, invertivorous/piscivorous fish species predominated (in density) in larger streams or reaches; whereas, more generalized, invertivorous fish species predominated in smaller reaches. Mechanistically, if proportion of piscivores increases with temporal stability of local habitats (Horwitz 1978; Schlosser 1987) or availability of pool habitats (Schlosser 1987), then typically, more piscivores will occur in larger streams, because larger streams provide more local stability

(Horowitz 1978) and pool development (Schlosser 1987). Although my data from MT sites are consistent with these predictions, unequivocal tests of the simultaneous effects (on fish trophic attributes) of stream size and drainage, within physiographies, must await a larger and more evenly distributed sample than was available for this study.

For an IBI in Virginia, trophic metrics do not require reference criteria specific to physiography. However, in contrast to previous IBI emphases, any comparisons of trophic metrics across fairly large spatial scales (inter-physiographic or inter-basin) should consider potentially confounding variation due to possible stream-size effects.

Stream size and IBI

For a Virginia IBI, reference criteria for some potential taxonomic metrics likely would need adjustment for stream size, some specific to physiography. For MT and for PD sites, numbers of native species and of

native minnow species increased with increasing stream size; therefore, expected values of these metrics would be higher for larger streams. However, similar adjustments would be required for number of native darter species only in PD and for number of native sunfish species only in MT. These results are consistent with the prevailing emphasis on adjusting IBI taxonomic-metric criteria for stream size (e.g., Fausch et al. 1984; Karr et al. 1986; Ohio EPA 1988; Steedman 1988; Lyons 1992a). Only one of these studies (Lyons 1992a) accounted explicitly for potential differences in the richness-vs-stream size relation among IBI regions. Similar to my results, Lyons (1992a) found that richness-vs-stream size relations differed among three large geographic regions comprising the state of Wisconsin. Also similar to my results, he found that, for several richness metrics, the slope and levelling-off point of the relation differed among regions.

In Virginia, for CP sites, the typically observed positive relation between species richness and stream size was lacking. Similarly, Paller (1994) found only slight differences, in number of fish species, between 3rd (21.4 species on average) and 4th-order (22.0 species) CP streams, the sizes most similar to CP sites examined herein. However, he did find that 1st and 2nd-order CP streams had fewer species (12.7 and 17.5 species, respectively) than did the larger streams. These results and those of Lyons (1992a), of Ohio EPA (1988), and for the three largest sites in this study (see Figures 4 and 5) showed that increasing species richness tended to level off at larger stream sizes. The stream size at which this levelling off occurs can vary by metric among geographic regions (e.g., Lyons 1992a; Paller 1994), emphasizing the need to account for region-specific variation in richness-vs-stream size relations, in order to set realistic reference criteria.

When adjusting reference criteria for stream size, one should be careful to include data from the entire range of stream sizes likely to be assessed. From personal experience I judge that Virginia warmwater streams that range from about 10-450 km² could be assessed adequately by an IBI based on wade-sampling data. The sites used herein ranged from 13-363 km², with only three sites >250 km². I recommend examining more data from large (> 250 km²) streams in PD and MT and from small (1st and 2nd order) streams in CP before any definitive taxonomic-metric criteria are adopted for a Virginia IBI.

Unlike for taxonomic-metric reference criteria, very few published

uses of a stream-fish IBI have adjusted functional (trophic and reproductive) criteria for variation with stream size, despite conceptual expectations and prior evidence that such variation exists (Horwitz 1978; Vannote et al. 1980; Schlosser 1982; Schlosser 1987; Schlosser 1990). Only one study (Ohio EPA 1988) provided specific evidence for or against adjusting functional metrics for stream size: Ohio EPA (1988) adjusted two trophic metrics and a tolerance metric to account for statewide variation with stream size, but they did not account for potential (eco)region-specific variation in relations. Lyons (1992a) chose not to adjust functional metrics for stream size, but provided no explicit justification. His data did not account for geographic variation in functional metric-vs-stream size relations. I found only weak statewide relations between individual functional metrics and stream size (absolute value of all $r_s < 0.42$), but stronger relations within physiographies (Table 11). Because the IBI is regionally based, accounting for possible region-specific variation in metric-vs-stream size relations is necessary to develop realistic scoring criteria for IBI metrics.

For Virginia sites within each physiography, some trophic and reproductive variables varied with stream size, but which metrics varied and the shapes of such relations differed among physiographies, similar to results for taxonomic variables. For MT sites, apparent relations between functional variables and stream size could be attributed as likely to drainage as to stream-size effects. For non-MT sites, particular trophic (in CP) and reproductive (in PD) variables varied with stream size (see Table 11), mostly independent of other effects. Before definitive functional-metric criteria--some adjusted for stream size--are adopted for a Virginia IBI, I suggest obtaining information from a broader range of stream sizes, especially one including large wadeable streams with watersheds of about 250-450 km².

Summary and conclusions

In Virginia, the fish assemblages of least-disturbed warmwater, wadeable streams are more distinctive among physiographies than they are among ecoregions or among major river drainages. Physiographies are fairly well defined and delineated and provide reasonable and practicable regions within which to develop and use the IBI. Physiographic distinctness is most pronounced for taxonomic metrics and least for trophic metrics; therefore, fish IBIs for Virginia streams

should include taxonomic- and reproductive-metric reference criteria that are specific to physiography. Preliminary evidence suggests that a different set of metrics may be required in each physiography; however, further study is needed to determine which metrics would be most useful in assessing relative anthropogenic disturbance at sites within each physiography (see Chapter 2).

Within each physiography, particular metric reference criteria will require adjustment for stream size or perhaps for drainage. For example, for PD sites, a few taxonomic metrics (numbers of native species, minnow species, and darter species) and reproductive metrics (number of late-maturing species and proportion as tolerants) would require adjustment for stream size. Whereas, for MT sites number of sunfish rather than darter species and perhaps a few trophic and reproductive metrics would require adjustment for stream size or for drainage. Given the limited sample sizes of this study, I can not reliably recommend specific criteria at this time. Within physiographies, more data from sites with watersheds $>250 \text{ km}^2$ and more evenly distributed with respect to major river drainage would allow definitive recommendations.

Until general mechanistic theories emerge concerning fish-assemblage structural and functional attributes, setting adequate biocriteria will require empirical examination of landscape-level (e.g., physiography, ecoregion, drainage) and stream-size effects (on potential metrics) that are relevant to the spatial scale and stream sizes of sites to be assessed. Examining how natural variation in fish-assemblage structural and functional attributes is related to landscape classes and stream sizes may help elucidate specific mechanisms that influence particular attributes. In turn, more of the natural variation in attributes can be accounted for relative to that due to anthropogenic influence. Distinguishing between these two types of variation is essential for setting realistic bioassessment criteria. As more mechanistic explanations for the variation observed in stream-fish attributes become available, the better equipped one will be to prevent anthropogenic actions from upsetting natural patterns and processes of freshwater streams and their watersheds.

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Table 1. Fish variables used to determine reference regions in which to set IBI-metric criteria and habitat variables used to determine least-disturbed sites in Virginia. Fish variables are arranged in three classes: taxonomic, trophic, and reproductive. Habitat variables are arranged in three watershed-scale classes: mining, urban, and forest; and one on-site class. For habitat variables, ranges of raw-data values and ranks are given. "N" is number of statewide sites ranked and number on which habitat-variable ranges are based. "Proportion" refers to proportion of individuals. Subsequent tables refer to fish variables by their codes.

Taxonomic variables	Code
Number of native species	NATSP
Number of non-native species	NONNATSP
Number of native minnow species	MINSP
Number of native sucker species	SUCSP
Number of native sunfish species	SUNSP
Number of native darter species	DARSP
Number of tolerant species	TOLSP
Trophic variables	
Proportion as generalist feeders	GENPRP
Proportion as invertivores	INVPRP
Proportion as benthic, specialist invertivores	BINVPRP
Proportion as specialist carnivores	CARNPRP
Number of non-native specialist carnivore species	CARNSP
Reproductive variables	
Proportion as mineral-substrate, simple spawners	MINSIMPRP
Proportion as various-substrate, manipulative spawners	VMANPRP
Proportion as nest-associate spawners	ASSOPRP
Number of late-maturing (> 2 yr) species	AGEESP
Proportion as tolerant species	TOLPRP

Mining variables	Range of values	Range of ranks	N
Percent barren	0-28	1-16	177
Number of mining point-sources	0-13	1-11	177
Urban variables			
Percent urban	0-49	1-28	177
Number of urban point-sources	0-6	1-22	177
Forest variables			
Percent forest	0-100	1-73	177
On-site variables			
Bank erosion	1-5	1-5	177
Width (in meters) of riparian zone	0-50	1-23	177
Percent riparian as forested	0-100	1-17	177
Percent bank cover as trees or shrubs	0-100	1-17	177
Percent instream cover as woody or log debris	0-92	1-19	177

Table 2. Number of reference sites in each physiography, ecoregion, river drainage, year, or stream-order-link group, at two spatial scales: statewide (VA) and within physiography (CP=Coastal Plain, PD=Piedmont, MT=mountain). See text for additional explanations.

Physiography	VA	CP	PD	MT
Coastal Plain	9	9	0	0
Piedmont	29	0	29	0
Blue Ridge	11	0	0	11
Ridge and Valley	14	0	0	14
Ecoregion				
Mid-Atlantic Coastal Plain ¹	1	1	0	0
Southeastern Plain	37	8	26	3
Blue Ridge Mountains	6	0	0	6
Central Appalachian Ridge and Valley	14	0	0	14
Northern Piedmont	5	0	3	2
River drainage				
Shenandoah	8	0	0	8
Rappahannock	6	3	0	3
York	6	1	5	0
James	14	1	6	7
Roanoke	11	0	10	1
Chowan	12	4	8	0
New	4	0	0	4
Holston	2	0	0	2
Year				
1987	10	2	2	6
1988	13	4	6	3
1989	16	0	7	9
1990	24	3	14	7
Stream-order link				
0	24	4	10	10
1	27	3	13	11
> 1	12	2	6	4

¹ excluded from one-way MANOVA with ECOR (see Table 4)

Table 3. Spearman correlations (r_s) between taxonomic, trophic, and reproductive variables, for 63 reference sites statewide. Only correlations with absolute value of $r_s > 0.40$ ($P < 0.002$) are shown. See Table 1 for codes of fish variables.

Taxonomic	Taxonomic				Trophic				Reproductive			
	MINSP	SUCSP	SUNSP	DARSP	GENPR P	CARNPR P	CARNSP P	MINSIMPRP	VMANRP	ASSOPR P	AGE3SP	TOLPRP
NATSP	0.58		0.45	0.60							0.63	
NONNATSP	0.45						0.90					
TOLSP	0.52	0.41										
MINSP		0.47			0.41			0.53	-0.45		0.49	
SUCSP											0.51	
SUNSP					-0.46	0.47			0.49			
DARSP												
Trophic												
GENPRP									-0.43			
INVPRP									0.42			
BINVPRP								0.54				
CARNPRP										-0.53		
CARNSP												
Reproductive												
MINSIMPRP									-0.47			
VMANPRP												0.61
ASSOPRP												
AGE3SP												

Table 4. Results of one-way MANOVAs of taxonomic, trophic, or reproductive variables for sites among physiographies (PHYS), among ecoregions (ECOR), or among years (YEAR). For PHYS, sample size (N)=9 for CP, N=29 for PD, N=11 for BR, N=14 for RV. For ECOR, N=37 for SEP, N=4 for BRM, N=16 for CARV, N=5 for NP, N=1 for MACP (excluded from MANOVAs). For YEAR, N=10 for 1987, N=13 for 1988, N=16 for 1989, N=24 for 1990 (see Table 2). A multivariate eta-square (η^2) is shown and equals the ratio (of determinants) of the among-group sum-of-squares-and-cross-products matrix (SSCP) to the total SSCP, or simply, $1 - \text{Wilks' } \lambda$. Also shown are F-ratio approximations (F) and corresponding significance values (P) of each MANOVA test. "DF" are the degrees of freedom for the numerator (i.e., the among-group SSCP) and for the denominator (the within-group SSCP), respectively.

Source	DF	η^2	F	P
Taxonomic variables				
PHYS	21,153	0.83	6.30	0.0001
ECOR	21,150	0.67	3.38	0.0001
YEAR	21,153	0.42	1.54	0.0720
Trophic variables				
PHYS	15,152	0.69	5.32	0.0001
ECOR	15,149	0.64	4.41	0.0001
YEAR	15,152	0.45	2.46	0.0030
Reproductive variables				
PHYS	15,152	0.63	4.35	0.0001
ECOR	15,149	0.55	3.35	0.0001
YEAR	15,152	0.20	0.86	0.6067

Table 5. Results of Hotelling's T^2 tests of taxonomic, trophic, or reproductive variables for sites between pairs of physiographies. Coastal Plain=CP (N=9 sites), Piedmont=PD, Blue Ridge=BR, Ridge and Valley=RV, and Mountain=MT=BR+RV. Multivariate eta square (η^2), F-ratio approximation (F), and significance value (P) of each test is shown. "Df" are the degrees of freedom for the numerator (between-group SSCP) and for the denominator (within-group SSCP), respectively.

	Taxonomic variables				Trophic variables				Reproductive variables			
	PD (N=29)	BR (N=11)	RV (N=14)	MT (N=25)	PD (N=29)	BR (N=11)	RV (N=14)	MT (N=25)	PD (N=29)	BR (N=11)	RV (N=14)	MT (N=25)
Coastal Plain												
DF	7,30	7,12	7,15	7,26	5,32	5,14	5,17	5,28	5,32	5,14	5,17	5,28
η^2	0.56	0.96	0.93	0.91	0.41	0.77	0.80	0.75	0.33	0.73	0.58	0.59
F	5.55	36.69	28.87	38.26	4.39	9.28	14.02	16.61	3.17	7.68	4.68	7.93
P	0.0004	0.0001	0.0001	0.0001	0.0037	0.0005	0.0001	0.0001	0.0195	0.0012	0.0072	0.0001
Piedmont												
DF	7,32	7,35	7,46	7,46	5,34	5,37	5,48	5,48	5,34	5,37	5,48	5,48
η^2	0.48	0.64	0.60	0.60	0.38	0.54	0.49	0.49	0.32	0.53	0.38	0.38
F	4.16	8.97	9.85	9.85	4.21	8.59	9.23	9.23	3.18	8.22	5.97	5.97
P	0.0024	0.0001	0.0001	0.0001	0.0044	0.0001	0.0001	0.0001	0.0184	0.0001	0.0002	0.0002
Blue Ridge												
DF		7,17				5,19				5,19		
η^2		0.47				0.36				0.43		
F		2.14				2.15				2.85		
P		0.0954				0.1035				0.0440		

Table 6. Summary of canonical analysis of taxonomic, trophic, or reproductive variables for sites among physiographies: CP=Coastal Plain, PD=Piedmont, MT=mountain. CAN1 and CAN2 are the first and second canonical functions. Squared (adjusted) canonical correlations (\hat{r}_c^2) represent the relative amount of among-physiography separation explained by CAN1 or CAN2 (see "Group means" and Figure 3). Multivariate eta square (η^2), F-ratio approximation, and significance value (P) of each canonical function are shown. "DF" are the degrees of freedom for the numerator (among-group SSCP) and for the denominator (within-group SSCP), respectively. Within-group structure coefficients are the within-physiography Pearson correlations between each fish variable and the scores of each canonical function. Within-group discriminant-function coefficients are the standardized partial regression coefficients of a within-physiography multiple linear regression of CAN1 or CAN2 scores as a function of taxonomic, trophic, or reproductive variables. Discriminant ratio coefficients (Thomas 1992) are the product of the within-group structure and function coefficients. See text (*Statistical Considerations*) for further explanation and discussion. See Table 1 for codes of variables.

	Within-group structure coefficients		Within-group function coefficients		Discriminant ratio coefficients		Group means	
	CAN1	CAN2	CAN1	CAN2	CAN1	CAN2	CAN1	CAN2
	Taxonomic variables							
\hat{r}_c^2	CAN1	CAN2						
P	0.64	0.34					CP	-2.53
η^2	0.0001	0.0002					PD	-0.55
	0.68	0.38					MT	1.55
DF	14,108	6,55						-0.43
NATSP	-0.10	0.65	-0.87	-0.31	0.09	-0.20		
NONNATSP	0.46	0.02	0.41	0.15	0.19	0.00		
TOLSP	0.13	0.50	-0.24	0.05	-0.03	0.03		
MINSP	0.57	0.55	1.17	0.33	0.67	0.18		
SUCSP	0.15	0.43	0.11	0.28	0.02	0.12		
SUNSP	-0.47	0.28	-0.14	0.42	0.07	0.12		
DARSP	-0.16	0.87	0.02	0.87	0.00	0.76		

Trophic variables								
r^2_c	0.57	0.07			CP	-2.21	0.54	
P	0.0001	0.1606			PD	-0.42	-0.35	
χ^2_{df}	0.64	0.11			MT	1.28	0.21	
DF	10,112	4,57						
GENTRP			0.50	0.57	0.43	0.70	0.22	0.40
INVPRP			-0.22	-0.23	-0.48	0.18	0.11	-0.04
BIVPRP			0.26	-0.44	0.59	-0.41	0.15	0.18
CARNRP			-0.23	0.60	-0.42	0.78	0.10	0.47
CARNSP			0.61	0.22	0.69	-0.04	0.42	-0.01
Reproductive variables								
r^2_c	0.37	0.16			CP	1.41	-0.83	
P	0.0001	0.0185			PD	0.37	0.46	
χ^2_{df}	0.53	0.18			MT	-0.94	-0.23	
DF	10,112	4,57						
MINSIMPRP			-0.70	0.36	-0.56	0.13	0.39	0.05
VMANPRP			0.79	-0.31	0.97	0.45	0.77	-0.14
ASSOPRP			-0.16	0.59	0.17	0.58	-0.03	0.34
AGE3SP			-0.13	0.83	0.39	0.83	-0.05	0.69
TOLPRP			0.32	-0.31	-0.23	-0.18	-0.07	0.06

Table 7. Summary of canonical analysis of taxonomic, trophic, or reproductive variables for sites between pairs of physiographies (e.g., CPvPD=Coastal Plain versus Piedmont, PDvMT=Piedmont versus Mountain). For each pair tested, squared (adjusted) canonical correlations (r_c^2) represent the relative amount of between-physiography separation explained by the first (and only) canonical function. Multivariate eta square (η^2_u), F-ratio approximation, and significance value (P) of the canonical function are shown. "DF" are the degrees of freedom for the numerator (between-group SSCP) and for the denominator (within-group SSCP), respectively. Within-group structure coefficients are the within-physiography Pearson correlations between each fish variable and the canonical-function scores. Within-group discriminant-function coefficients are the standardized partial regression coefficients of a within-physiography multiple linear regression of the canonical-function scores as a function of taxonomic, trophic, or reproductive variables. Discriminant ratio coefficients (Thomas 1992) are the product of the within-group structure and function coefficients. See text (*Statistical Considerations*) for further explanation and discussion. See Table 1 for codes of variables.

	Within-group structure coefficients				Within-group function coefficients				Discriminant ratio coefficients			
	CPvPD		CPvMT		PDvMT		CPvPD		CPvMT		PDvMT	
Taxonomic variables												
r_c^2	0.50		0.90		0.56							
P	.0004		.0001		.0001							
η^2_u	0.56		0.91		0.60							
DF	7,30		7,26		7,46							
NATSP				0.41	0.02	0.43		-0.97	-1.44	0.43	-0.40	0.18
NONNATSP				0.41	0.25	-0.39		0.53	0.26	-0.24	0.22	0.09
TOLSP				0.52	0.13	0.13		-0.10	-0.48	0.17	-0.05	0.02
MINSP				0.75	0.46	-0.2		0.85	1.84	-0.75	0.64	0.18
SUCSP				0.40	0.20	0.08		0.34	0.58	0.10	0.14	0.01
SUNSP				-0.13	-0.24	0.60		0.27	-0.26	0.39	-0.04	0.23
DARSP				0.57	0.01	0.59		0.87	0.31	0.48	0.50	0.28

Table 8. Within-physiography Spearman correlations (r_s) between taxonomic, trophic, and reproductive variables, for 63 reference sites. Variables are arranged horizontally in same order as vertically, but are abbreviated (i.e., NON=NONNATSP, etc.). Only correlations with absolute value of r_s ($|r_s|$) > 0.40 are shown. See Table 1 for codes of variables.

Coastal Plain ¹ (N=9)																
	NON	TOL	MIN	SUC	SUN	DAR	GEN	INV	BINV	CARN	CRNS	MNSM	VMAN	ASSO	AGE3	TOLPR
NATSP	.44	.60	.87						.61			.83		.49	.71	
NON-NATSP		.78	.52	.56	.42	-.53					.79	.52			.64	
TOLSP			.76	.49			.45		.58		.68				.69	
MINSP								-.63	.67	.47		.82	-.56	.59	.83	
SUCSP					.60	-.56	.76				.57					
SUNSP									-.43		.46					
DARSP							-.44	.41								-.63
GENPRP																
INVPRP										-.82		-.52	.85		-.50	
BINV-PRP												.55			.78	
CARN-PRP													-.73			
CARN-SP																
MIN-SIMP														.51	.87	
VMAN-PRP															-.52	
ASSO-PRP																.49
AGE3SP																

¹ If $|r_1| > 0.79$ then $P < 0.01$; if $0.79 > |r_1| > 0.66$ then $0.05 > P > 0.01$; if $0.66 > |r_1| > 0.59$ then $0.10 > P > 0.05$; if $0.59 > |r_1| > 0.48$ then $0.20 > P > 0.10$; if $0.48 > |r_1| > 0.40$ then $0.30 > P > 0.20$

¹ If $|r_s| > 0.79$ then $P < 0.01$; if $0.79 > |r_s| > 0.66$ then $0.05 > P > 0.01$; if $0.66 > |r_s| > 0.59$ then $0.10 > P > 0.05$; if $0.59 > |r_s| > 0.48$ then $0.20 > P > 0.10$; if $0.48 > |r_s| > 0.40$ then $0.30 > P > 0.20$

Piedmont² (N = 29)

	NON	TOL	MIN	SUC	SUN	DAR	GEN	INV	BINV	CARN	CRNS	MNSM	VMAN	ASSO	AGE3	TOLPR
NATSP			.70	.47		.78									.71	
NON-NATSP											.92					
TOLSP			.45					-.44		-.43						
MINSP				.48		.59	.49			-.42		.47	-.65	.41	.67	-.51
SUCSP						.44	.64			-.46		.52	-.61	.51	.47	
SUNSP										.51		-.56	.49			
DARSP													-.42		.56	
GENPRP											.49		-.42	.55		
INVPRP																
BINV-PRP												.62				
CARN-PRP													.52	-.45		
CARN-SP																
MIN-SIMP																
VMAN-PRP														-.67	-.56	.77
ASSO-PRP																-.54
AGE3SP																-.48

² If $|r_c| > 0.59$ then $P < 0.001$; if $0.59 > |r_c| > 0.46$ then $0.01 > P > 0.001$; if $0.46 \geq |r_c| > 0.40$ then $0.05 > P > 0.01$

Mountain^a (N = 25)

	NON	TOL	MIN	SUC	SUN	DAR	GEN	INV	BINV	CARN	CRNS	MNSM	VMAN	ASSO	AGE3	TOLPR
NATSP		.43	.85		.81											
NON-NATSP											.80					
TOLSP			.60													
MINSP				.63									.42			
SUCSP										-.41					.59	-.50
SUNSP										.56						
DARSP										-.44	-.47					-.47
GENPRP														.45		
INVPRP									.50					.57		
BINV-PRP																
CARN-PRP															-.71	
CARNSP																
MIN-SIMPRP																
VMAN-PRP																.44
ASSO-PRP																
AGE3SP																

^a If $|r_s| > 0.70$ then $P < 0.0001$; if $0.70 > |r_s| > 0.62$ then $0.001 > P > 0.0001$; if $0.62 > |r_s| > 0.55$ then $0.01 > P > 0.001$; if $0.55 > |r_s| > 0.40$ then $0.05 > P > 0.01$

Table 9. Values of taxonomic, trophic, and reproductive variables at sites within each physiography. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Superscripts after some fish variables show significance (only $P < 0.10$) of the omnibus Kruskal-Wallis test of the variable among three physiographies. Lower-case letters after some medians show comparisons of the variable between pairs of physiographies; different letters denote a statistical difference, with $a < b < c$. Superscripts of lowercase letters denote statistical significance of each paired comparison (Wilcoxon two-sample test). For example, for VMANPRP, $a^{2,3}$, $b^{4,5}$, and $c^{4,5}$ show that VMANPRP in MT= $a < c$ =VMANPRP in CP ($0.0001 < P < 0.001$); VMANPRP in PD= $b < c$ =VMANPRP in MT ($0.01 < P < 0.1$); and VMANPRP in MT= $a < b$ =VMANPRP in PD ($0.001 < P < 0.01$). Number of sites = N. See Table 1 for codes of variables.

Coastal Plain (N=9)					
	MD		X	SE	CV
NATSP ^a	15.0	a ⁴	14.4	1.53	31.7
NONNATSP ¹	1.00	a ^{3,2}	1.11	0.261	70.36
TOLSP ^a	3.0	a ^{3,4}	2.9	0.31	32.1
MINSP ¹	2.0	a ^{2,1}	2.2	0.68	92.2
SUCSP ^a	1.00	a ^{3,2}	0.89	0.200	67.60
SUNSP ¹	3.0	b ³	3.1	0.59	56.7
DARSP ¹	1.0	a ³	1.4	0.34	70.2
GENPRP ¹	0.062	a ³	0.087	0.0277	95.068
INVPRP	0.53		0.60	0.076	38.42
BINVPRP ⁴	0.07	a ⁴	0.11	0.043	121.14
CARNPRP ⁴	0.18	b ⁴	0.27	0.070	76.92
CARNSP ¹	0.00	a ^{4,1}	0.44	0.176	118.59
MINSIMPRP ^a	0.000	a ^{3,2}	0.011	0.0080	222.663
VMANPRP ^a	0.41	c ^{4,2}	0.44	0.085	57.41
ASSOPRP ⁴	0.09	a ⁴	0.10	0.037	112.27
AGE3SP ⁴	1.0	a ⁴	2.8	0.83	89.6
TOLPRP	0.39		0.30	0.064	63.67
Piedmont (N=29)					
	MD		X	SE	CV
NATSP	18.0	b ^{4,3}	18.9	0.82	23.3
NONNATSP	2.00	b ³	2.31	0.228	53.14
TOLSP	5.00	b ³	4.55	0.251	29.70
MINSP	7.0	b ^{2,4}	6.5	0.43	35.7
SUCSP	2.0	b ³	2.4	0.31	69.6
SUNSP	2.00	b ¹	2.48	0.251	54.49

DARSP	3.00	b ^{3,1}	2.86	0.197	37.03
GENPRP	0.100	a ¹	0.125	0.0225	97.244
INVPRP	0.588		0.576	0.0193	18.032
BINVPRP	0.166	b ⁴	0.181	0.0209	62.202
CARNPRP	0.114	a ⁴	0.134	0.0164	65.963
CARNSP	1.00	b ^{4,2}	1.17	0.165	75.84
MINSIMPRP	0.047	b ^{3,4}	0.059	0.0102	93.437
VMANPRP	0.15	b ^{4,3}	0.23	0.034	79.68
ASSOPRP	0.241	b ⁴	0.227	0.0254	60.083
AGE3SP	5.0	bc ⁴	5.0	0.33	35.9
TOLPRP	0.143		0.191	0.0228	64.150

Mountain (N=25)

	MD		X	SE	CV
NATSP	15.0	a ³	14.8	0.70	23.5
NONNATSP	4.0	c ^{2,3}	3.9	0.41	52.8
TOLSP	4.00	b ⁴	4.12	0.291	35.26
MINSP	8.0	c ^{1,4}	7.7	0.38	24.4
SUCSP	2.00	b ²	2.00	0.183	45.64
SUNSP	1.00	a ^{3,1}	0.92	0.152	82.54
DARSP	1.00	a ¹	1.48	0.184	62.05
GENPRP	0.255	b ^{3,1}	0.268	0.0290	54.065
INVPRP	0.51		0.49	0.040	40.71
BINVPRP	0.221	b ⁴	0.209	0.0257	61.387
CARNPRP	0.08	a ⁴	0.14	0.030	105.62
CARNSP	2.00	c ^{1,2}	2.28	0.204	44.80
MINSIMPRP	0.078	c ^{2,4}	0.105	0.0183	86.553
VMANPRP	0.074	a ^{2,3}	0.105	0.0169	80.773
ASSOPRP	0.13	b ⁴	0.21	0.038	90.88
AGE3SP	4.0	ab	4.2	0.31	36.8
TOLPRP	0.129		0.166	0.0233	70.012

¹ P < 0.0001

³ 0.001 < P < 0.01

² 0.0001 < P < 0.001

⁴ 0.01 < P < 0.1

Table 12. Values of fish variables at reference sites within stream-order link categories, within each physiography. Link categories are: SLINK 0= downstream reach is same order as site; SLINK 1 = downstream is 1 order larger; SLINK > 1 = downstream is > 1 order larger. Medians, means (X), and standard errors (SE) are shown. Superscripts after some fish variables show significance (only $P < 0.10$) of the omnibus Kruskal-Wallis test for the variable among physiographies. Lower-case letters after some medians show comparisons of the variable between pairs of physiographies; different letters denote a statistical difference, with $a < b < c$ (Wilcoxon two-sample test, only $P < 0.10$ shown). Number of sites = N. See Table 1 for codes of variables.

	Coastal Plain									
	SLINK 0 (N=4)			SLINK 1 (N=3)			SLINK > 1 (N=2)			
	Median	X	SE	Median	X	SE	Median	X	SE	
NATSP ^a	17.0	b	17.8	1.70	9.3	0.88	15.5	b	15.5	1.50
NONNATSP	1.0		1.0	0.41	1.0	0.7	2		2	0
TOLSP	3.00		2.75	0.250	2.0	2.3	4.0		4.0	1.00
MINSP	2.5		3.3	1.31	1.0	1.0	2.0		2.0	1.00
SUCSP	1.00		0.75	0.250	1.0	0.7	1.5		1.5	0.50
SUNSP	3.0		3.0	0.82	3.0	2.0	5		5	0
DARSP	2.0		2.0	0.58	1.0	1.0	1		1	0
GENPRP	0.054		0.060	0.0229	0.08	0.07	0.16		0.16	0.110
INVPRP	0.61		0.59	0.119	0.44	0.56	0.68		0.68	0.144
BINVPRP	0.15		0.18	0.083	0.022	0.030	0.07		0.07	0.052
CARNPRP	0.28		0.31	0.103	0.20	0.31	0.14		0.14	0.033
CARNSP	0.00		0.25	0.250	0.0	0.3	1		1	0
MINSIMPRP	0.009		0.023	0.0172	0	0	0.003		0.003	0.0033
VMANPRP	0.42		0.45	0.151	0.36	0.34	0.58		0.58	0.178
ASSOPRP	0.094		0.094	0.0148	0.02	0.13	0.06		0.06	0.055
AGE3SP	4.5		4.0	1.47	1	1	3.0		3.0	2.00
TOLPRP	0.15		0.21	0.095	0.39	0.33	0.44		0.44	0.047

Piedmont

	SLINK 0 (N=10)			SLINK 1 (N=13)			SLINK > 1 (N=6)		
	Median	X	SE	Median	X	SE	Median	X	SE
NATSP	19.5	19.2	1.48	17.0	18.1	0.96	18.0	20.0	2.48
✓ NONNATSP ¹	2.0	1.9	0.35	2.00	2.08	0.265	3.0	3.5	0.56
TOLSP	5.0	4.5	0.40	5.0	4.5	0.39	4.5	4.7	0.67
MINSP	7.0	6.9	0.60	6.0	6.2	0.37	8.0	6.7	1.76
SUCSP	2.5	3.0	0.58	2.0	1.7	0.33	3.0	3.0	0.82
SUNSP	2.0	2.3	0.40	2.0	2.5	0.39	3.0	2.8	0.65
DARSP	3.0	2.9	0.35	3.00	2.85	0.249	2.5	2.8	0.60
GENPRP	0.10	0.12	0.040	0.085	0.099	0.0175	0.11	0.18	0.079
INVPRP	0.60	0.60	0.032	0.59	0.56	0.031	0.57	0.57	0.044
BINVPRP	0.180	0.183	0.0269	0.23	0.21	0.039	0.132	0.122	0.0280
CARNPRP	0.106	0.095	0.0152	0.159	0.153	0.0209	0.08	0.15	0.060
CARNSP	1.00	0.90	0.277	1.00	1.08	0.239	2.0	1.8	0.31
MINSIMPRP	0.066	0.061	0.0144	0.028	0.044	0.0150	0.094	0.088	0.0274
VMANPRP	0.12	0.19	0.051	0.25	0.27	0.045	0.11	0.21	0.107
ASSOPRP	0.30	0.26	0.043	0.20	0.19	0.033	0.26	0.24	0.074
AGE3SP	5.0	4.9	0.62	4.0	4.6	0.40	6.5	5.8	0.87
TOLPRP	0.11	0.15	0.036	0.18	0.22	0.035	0.20	0.20	0.055

Mountain

	SLINK 0 (N=10)			SLINK 1 (N=11)			SLINK > 1 (N=4)		
	Median	X	SE	Median	X	SE	Median	X	SE
NATSP	14.0	14.0	1.24	15.0	15.3	1.05	16.0	15.5	1.32
NONNATSP	4.5	4.4	0.54	2.0	3.5	0.77	4.0	3.8	0.63
TOLSP	4.5	4.3	0.52	4.0	4.1	0.41	4.0	3.8	0.75
MINSP	7.5	7.9	0.74	6.0	7.5	0.51	8.5	8.0	0.71
SUCSP	2.00	1.60	0.221	2.0	2.3	0.33	2.00	2.25	0.250
SUNSP	1.00	0.90	0.233	1.00	0.91	0.285	1	1	0
DAKSP	1.00	1.10	0.233	2.00	1.82	0.296	1.0	1.5	0.50
GENPRP	0.26	0.29	0.047	0.22	0.22	0.036	0.40	0.35	0.094
INVPRP	0.50	0.45	0.057	0.51	0.51	0.071	0.49	0.50	0.074
BINVPRP	0.14	0.17	0.038	0.26	0.25	0.041	0.19	0.19	0.054
CARNPRP	0.19	0.18	0.044	0.04	0.10	0.050	0.16	0.17	0.071
CARNSP	3.0	2.7	0.34	2.0	1.9	0.31	2.00	2.25	0.250
MINSIMPRP ¹	0.040	0.067	0.0223	0.116	0.132	0.0257	0.09	0.13	0.070
VMANPRP	0.072	0.087	0.0213	0.080	0.112	0.0231	0.09	0.13	0.074
ASSOPRP	0.14	0.22	0.069	0.20	0.22	0.052	0.08	0.14	0.093
AGESSP	4.00	3.89	0.291	5.0	4.5	0.59	4.0	4.5	0.87
TOLPRP ²	0.18	0.22	0.043	0.099	0.114	0.0230	0.18	0.18	0.062

¹ P=0.0424

² 0.05 < P < 0.10

Table 13. Values of fish variables at Mountain reference sites within selected river-drainage groups. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Superscripts after some variables show significance (only $P < 0.05$ shown) of a Wilcoxon two-sample test of the variable between drainage groups. Number of sites = N. See Table 1 for codes of variables.

	Shenandoah drainage (N=8)				Rappahannock + James drainages (N=10)			
	MD	X	SE	CV	MD	X	SE	CV
NATSP	16.0	15.4	1.21	22.2	14.0	14.6	0.87	18.9
NONNATSP	3.0	4.1	0.58	39.8	2.0	3.1	0.48	49.2
TOLSP	4.0	4.3	0.65	43.1	3.0	3.7	0.40	33.8
MINSIP	7.0	8.1	0.88	30.5	6.0	7.3	0.54	23.3
SUCSP	2.00	1.75	0.164	26.45	2.00	2.20	0.200	28.75
SUNSP	1.00	1.13	0.227	56.97	1.00	0.70	0.153	69.01
DARSP ²	1.00	0.75	0.164	61.72	2.00	1.80	0.249	43.82
GENPRP	0.22	0.29	0.056	54.37	0.29	0.31	0.043	44.70
INVPRP	0.39	0.42	0.052	34.89	0.47	0.51	0.081	50.22
BINVPRP	0.15	0.19	0.042	62.64	0.16	0.20	0.044	67.91
CARNPRP ²	0.25	0.25	0.043	48.98	0.05	0.11	0.056	158.11
CARNSP ²	3.0	2.9	0.35	34.5	2.00	1.80	0.249	43.82
MINSIMPRP	0.086	0.086	0.0264	86.877	0.08	0.14	0.036	81.79
VMANPRP	0.07	0.11	0.036	93.09	0.053	0.083	0.0253	96.277
ASSOPRP ²	0.03	0.10	0.044	126.49	0.20	0.26	0.066	80.08
AGE3SP ¹	4.0	3.6	0.32	25.3	5.0	5.3	0.52	30.9
TOLPRP ²	0.19	0.25	0.044	48.51	0.088	0.108	0.0258	75.754

¹ $P = 0.006$

² $0.01 < P < 0.05$

Table 14. Values of taxonomic, trophic, and reproductive variables at reference sites within drainage groups, in Coastal Plain. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Superscripts after some variables show significance (only $P < 0.05$ shown) of Wilcoxon two-sample tests of the variable between drainage groups. Number of sites = N. See Table 1 for codes of variables.

	Chesapeake group (N=5)				Albemarle group (N=4)			
	MD	X	SE	CV	MD	X	SE	CV
NATSP	17.0	16.8	1.85	24.7	11.5	11.5	1.76	30.5
NONNATSP	1.00	1.40	0.245	39.12	0.5	0.8	0.48	127.7
TOLSP ¹	3.0	3.4	0.40	26.3	2.00	2.25	0.250	22.22
MINS ¹	3.0	3.4	0.93	61.0	1.00	0.75	0.250	66.67
SUCSP	1.0	1.0	0.32	70.7	1.00	0.75	0.250	66.67
SUNSP	3.0	3.0	0.63	47.1	4.0	3.3	1.18	72.7
DARSP	1.0	1.4	0.40	63.9	1.5	1.5	0.65	86.1
GENPRP	0.12	0.12	0.045	85.65	0.057	0.049	0.0175	71.354
INVPRP	0.46	0.48	0.082	38.30	0.81	0.75	0.103	27.74
BINVPRP ¹	0.13	0.17	0.065	84.52	0.018	0.026	0.0146	112.764
CARNPRP	0.41	0.37	0.108	64.85	0.154	0.149	0.0236	31.832
CARNSP	1.00	0.60	0.245	91.29	0.00	0.25	0.250	200.00
MINSIMPRP	0.007	0.019	0.0137	158.268	0.00	0.00	0.0000	—
VMANPRP	0.22	0.30	0.096	70.22	0.66	0.62	0.099	32.04
ASSOPRP	0.100	0.091	0.0186	45.862	0.03	0.11	0.086	162.01
AGESP ¹	5.0	4.4	0.98	49.8	1.00	0.75	0.250	66.67
TOLPRP	0.15	0.25	0.081	72.53	0.44	0.37	0.105	57.58

¹ $0.01 < P < 0.05$

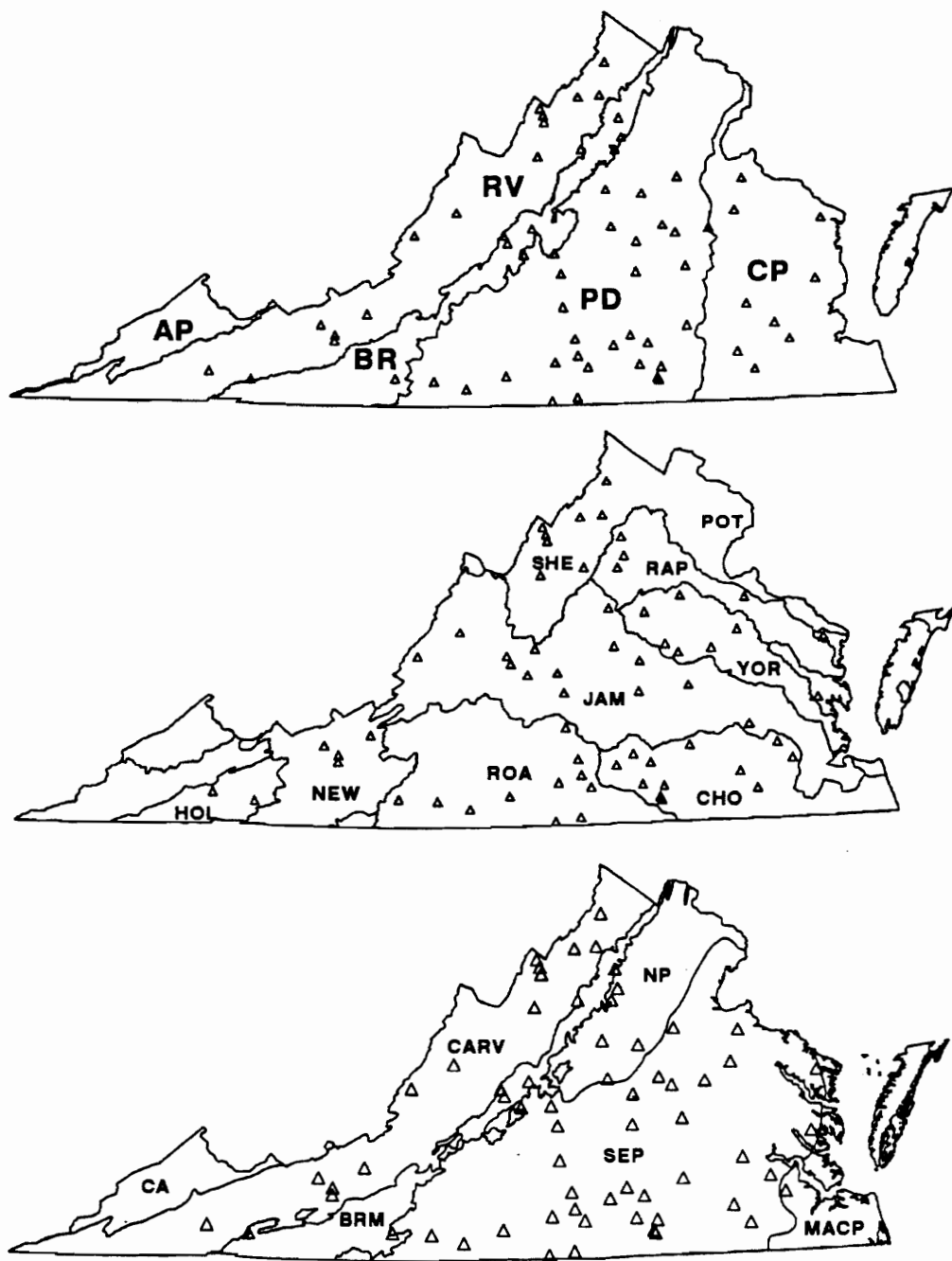


Figure 1. Maps of Virginia showing location of 63 reference sites with respect to physiography (top: CP=Coastal Plain, PD=Piedmont, BR=Blue Ridge, RV=Ridge and Valley, AP=Appalachian Plateau), major river drainage (middle: SHE/POT=Shenandoah/Potomac, RAP=Rappahannock and adjacent Chesapeake Bay drainages, YOR=York, JAM=James, ROA=Roanoke, CHO=Nottoway+Blackwater+Meherren, NEW=New, HOL=Holston), and ecoregion (bottom: MACP=Mid-Atlantic Coastal Plain, SEP=Southeastern Plain, BRM=Blue Ridge Mountains, CARV=Central Appalachian Ridge and Valley, NP=Northern Piedmont, CA=Central Appalachians).

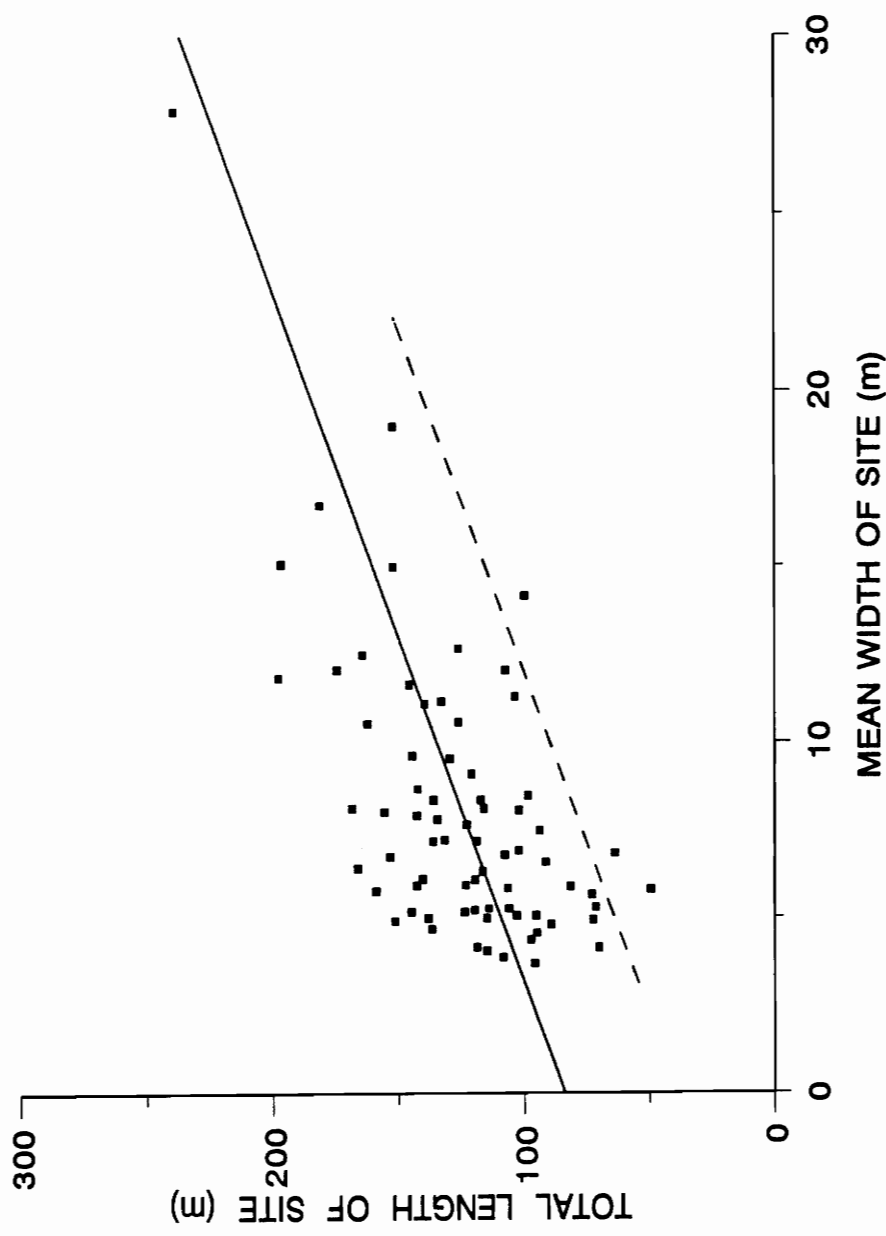


Figure 2. Plot of mean width vs. total sampled length for 66 reference sites. Solid line is fitted regression line. Dashed line is a line parallel to regression line, below which sampling effort was judged inadequate; the three sites below this line were deleted from analyses.

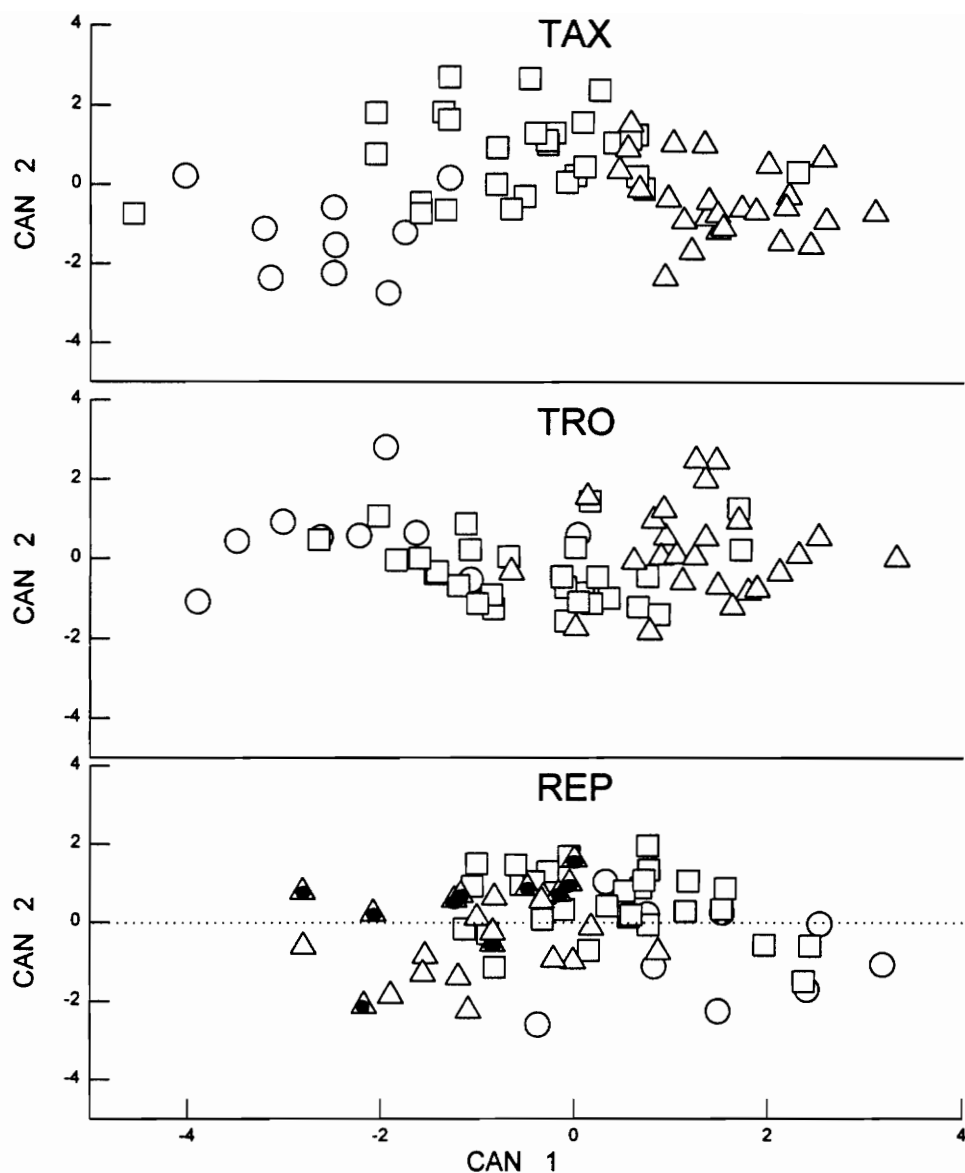


Figure 3. Plots of 63 statewide reference sites, by physiography, in the spaces defined by the first (CAN1) and second (CAN2) canonical functions of taxonomic (TAX), trophic (TRO), or reproductive (REP) variables. Circles=Coastal Plain sites, squares=Piedmont sites, and triangles=mountain sites. Triangles with dark centers are 11 Blue Ridge sites, 9 above and 2 below the dashed line (one site above the line is almost entirely hidden). See Table 6 for corresponding statistical summary.

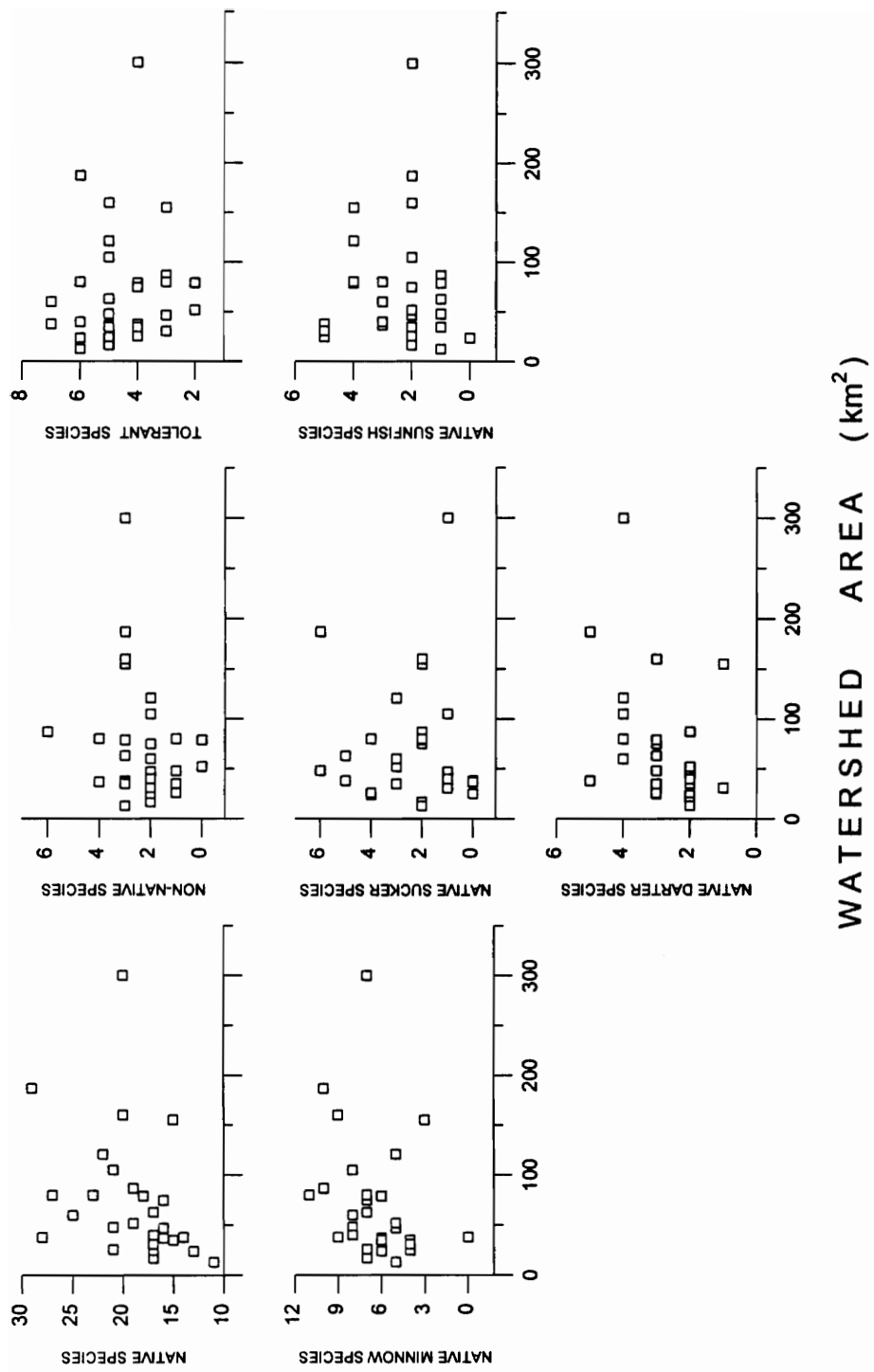


Figure 4. Plots of taxonomic variables versus watershed area for 29 Piedmont reference sites. See Table 11 for corresponding statistics.

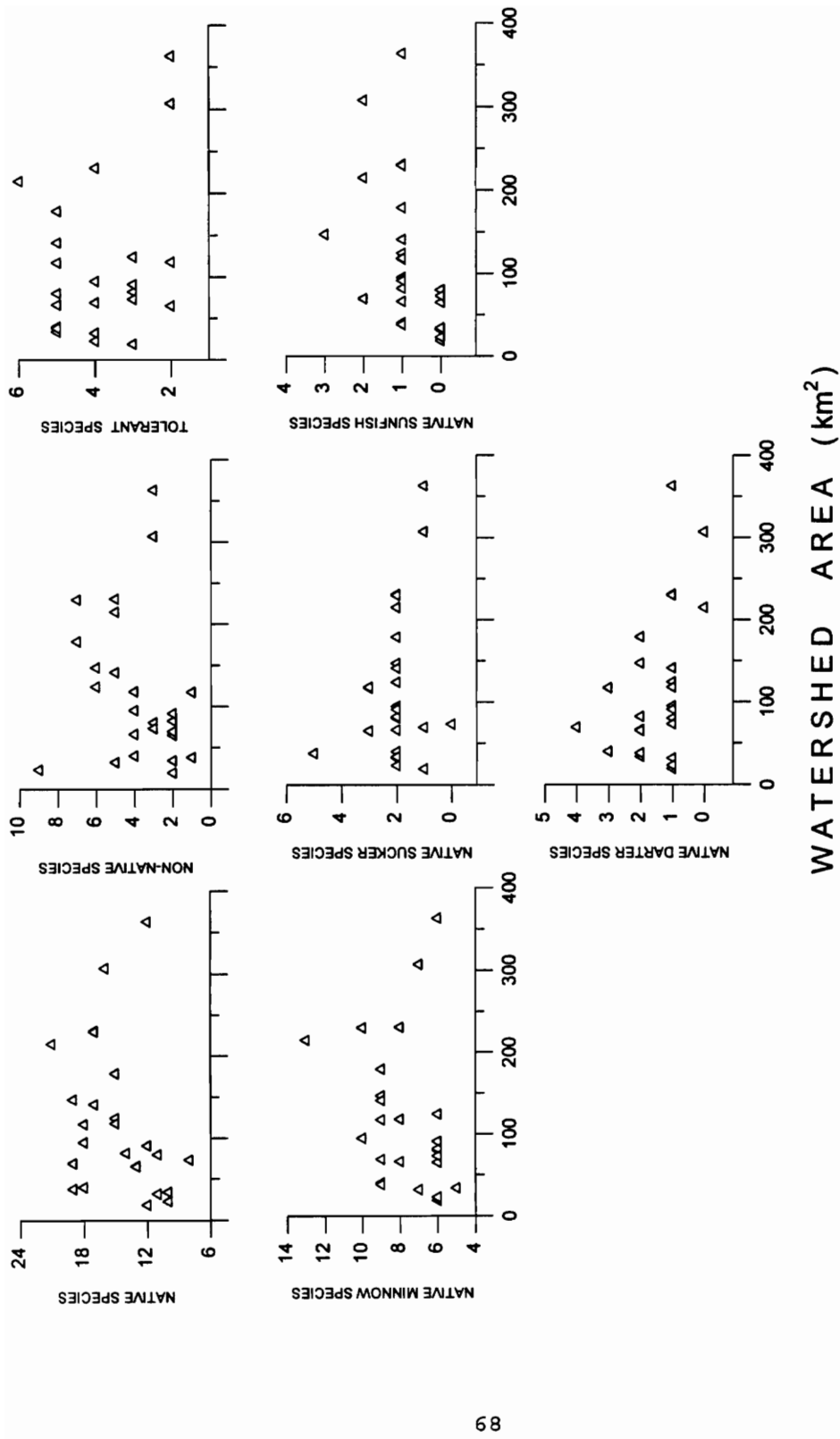


Figure 5. Plots of taxonomic variables versus watershed area for 25 MT reference sites. See Table 11 for corresponding statistics.

CHAPTER TWO: RELATIONS BETWEEN IBI FISH METRICS AND MEASURES OF ANTHROPOGENIC DISTURBANCE

Abstract

The ability of the index of biotic integrity (IBI) to reflect anthropogenic disturbance depends directly on how its individual metrics do so. By convention, each metric is assumed to vary predictably with anthropogenic degradation; for example, in warmwater streams, native fish-species richness typically is expected to decrease monotonically with increasing degradation. For sites in each of three physiographic regions across Virginia, I examined relations between potential metrics and habitat variables to determine if metrics met conventional IBI assumptions.

Relations at Coastal Plain sites were least consistent with IBI assumptions. The metrics that I used, most of which were developed for upland streams, may be inappropriate for the Coastal Plain, where flowages are geologically, hydrologically, and biologically distinct from more-upland streams. Developing an IBI for such systems will require a more distinctive understanding of their fish-vs-habitat relations than is now available. Relations at Piedmont and at mountain sites were more consistent with IBI assumptions. Trophic metrics best reflected anthropogenic disturbance at Piedmont sites; whereas, reproductive metrics were most diagnostic at mountain sites. Overall, habitat variables accounted for up to about 20% of the variance in fish metrics, suggesting that the data incompletely represented anthropogenic effects on fish assemblages.

Multivariate methods, such as canonical correlation analysis, can provide comprehensive and realistic depictions of the relations between fish-assemblage attributes and measures of anthropogenic disturbance. However, judging the reliability of these relations requires validation. Until further validation of my results, I recommend that IBIs for each region in Virginia retain two or three metrics from each class: taxonomic, trophic, reproductive, and tolerance. I also recommend including the following metrics because they best reflected disturbance, consistent with prior IBI assumptions. An IBI for PD should include proportion as generalist feeders, proportion as specialist carnivores, and proportion as tolerants. An IBI for MT should include proportion as lithophils, proportion as tolerants, and numbers of native minnows and

of late-maturing species.

Introduction

The index of biotic integrity (IBI) can be used to assess the ecologic health of freshwater streams in a pre-defined region (Karr 1981; Karr et al. 1986). Various versions of the IBI for stream fish have been used in more than thirty U.S. states and Canadian provinces, and several agencies now use it to regulate and monitor freshwater biotic resources (Karr 1991). The IBI reflects comparisons of attributes (=metrics) of a stream-fish assemblage at a sample site with attributes expected at sites least disturbed by anthropogenic actions. (Hereafter, I use the terms "disturbance" and "least- " or "most-disturbed" to refer exclusively to anthropogenic disturbance). Metrics typically include numbers of species in selected taxa or proportions of individuals in selected trophic or reproductive functional groups, e.g., omnivores or top carnivores. For each sample site, each metric receives a score based on comparisons with expectations (e.g., score of 5 = sample site most resembles least-disturbed conditions; 1 = sample site differs most from least-disturbed conditions), and the total IBI score for a sample site (hereafter called "total IBI") is the sum of metric scores. Therefore, a high total IBI should reflect conditions of relatively low disturbance. A resource manager can use the IBI to help determine management priorities among sites.

The utility of any bioassessment index depends on its (1) practicality and (2) ability to adequately reflect disturbance (e.g., Pratt and Bowers 1992). For the IBI, practical issues include choosing the number and types of metrics and the spatiotemporal framework (e.g., physiographies, ecoregions, drainages, and ranges of stream size and disturbance) within which to use the index. These issues may be subject to non-biological factors such as data or cost constraints, or politically rather than ecologically relevant spatial boundaries. Most recent and extensive investigations of the IBI for stream fish have been bound, for practical reasons, to intra-state spatial scales (Ohio EPA 1988, for Ohio; Lyons 1992, for Wisconsin).

The ability of the IBI to accurately and reliably reflect disturbance depends directly on the validity of the assumptions used to score each metric (Table 1). Despite their ultimate importance, these assumptions have received little critical examination, and as late as nine years after the IBI was first proposed, Fausch et al. (1990) still gave

"highest priority" to the need to validate them. Investigating IBI-metric assumptions is especially critical given that how metrics vary with disturbance may depend on the IBI region, the range of stream sizes, or the types and range of disturbance considered. The natural variation in fish-assemblage attributes across regions and stream sizes (e.g., Chapter 1) strongly implies that the utility of individual metrics will also vary spatially. For example, metrics most diagnostic of disturbance in small upland streams will not necessarily be as useful in large lowland streams, and vice-versa.

Various researchers reported and discussed how individual metrics were related with the total IBI (Angermeier and Karr 1986; Karr et al. 1987; Steedman 1988). Several others examined how the total IBI related with measures of water or physical-habitat quality (e.g., Berkman et al. 1986; Leonard and Orth 1986; Angermeier and Schlosser 1987; Hughes and Gammon 1987; Ohio EPA 1988; Steedman 1988; Lyons 1992; Shields et al. 1995). Despite the emphasis on disturbance as reflected by the total IBI, few of these studies provided explicit evidence of the validity of individual IBI-metric assumptions. Moreover, the studies that used the same sample-site data to determine IBI-metric scoring criteria as to calculate total IBIs (e.g., Leonard and Orth 1986; Hughes and Gammon 1987; Steedman 1988; Shields et al. 1995) merely showed that a single-value index, the total IBI, could reflect how its individual metrics were assumed to vary with disturbance. This approach does not represent a valid investigation of those assumptions. A better approach would be (1) to define the spatial regions relevant to the IBI assessment, (2) to define and assess disturbance in non-IBI terms, from least- to most-disturbed, at sites in each region, and (3) to determine how each IBI metric is related to disturbance in each region. Ideally, the data used for such an investigation should be independent of that used for any future IBI assessment. Only two studies approximately met these criteria: Ohio EPA (1988) for Ohio streams, and Lyons (1992) for Wisconsin streams. Although both studies accounted for points (1) and (2) above, neither examined how individual metrics reflected least- to most-disturbed conditions in each region.

In Virginia, anthropogenic effects on stream habitat and fish assemblages are likely to differ across the state's major physiographic regions: Coastal Plain, Piedmont, Blue Ridge, and Ridge and Valley. First, disregarding effects due to disturbance, the types and extents of

processes that link watershed, riparian, and instream features differ naturally between upland, higher-gradient, coarse-substrate streams (e.g., those in mountain physiographies) and lowland, lower-gradient, fine-substrate streams (e.g., those in lower Piedmont or Coastal Plain; Smock and Gilinsky 1992). Perhaps not surprisingly, fish metrics of potential use for the IBI also differ "naturally" (i.e., at least-disturbed sites) across physiographic regions in Virginia (Chapter 1). Second, region-specific relations between fish metrics and measures of disturbance could further complicate attempts to determine how metrics reflect that disturbance throughout the state. Therefore, a region-specific, multivariate-based investigation of these relations provides a biologically realistic and comprehensive first step toward developing IBIs for streams in Virginia.

In this study, I examine uni-, bi-, and multivariate relations between measures of disturbance and potential IBI fish metrics, for wadeable, warmwater streams in each of three physiographic regions in Virginia (Figure 1). Specifically, I investigate (1) if fish metrics relate with selected habitat variables in ways consistent with IBI assumptions, (2) how these relations differ across IBI regions, and (3) which fish metrics may be most useful for stream-fish IBIs in Virginia.

Methods

I used a subset of data from a fish survey of Virginia warmwater streams conducted July-October 1987 and May-October 1988-1990 (Angermeier and Smogor 1992). Sites were selected to provide uniform coverage statewide and to complement existing statewide fisheries data. The data subset included catch-per-effort and presence of fish species, and selected instream- and riparian-habitat measures at each of 108 wadeable sites sampled from June-September 1988-1990 (Figure 1). These sites occurred in third- through sixth-order streams in three major physiographic regions that represent potential IBI reference regions for Virginia (Chapter 1). Sites were chosen at least a few km upstream of any larger-order downstream tributary to limit possible influence of the larger stream on fish-assemblage attributes. For a subset of the streams, no downstream effect was evident (Chapter 1). Sites were distributed among physiographies (see **Methods**, Chapter 1) as follows: for Coastal Plain (CP) N=12, for Piedmont (PD) N=49, and for Mountain (MT) N=47. The Mountain region comprised the Blue Ridge and Ridge and Valley physiographic provinces (Figure 1). Sites were about 50 to 250 m

long and drained areas 7 to 454 km²; sites with larger drainage areas had longer sample lengths.

Habitat variables

I used variables that presumably reflected anthropogenic disturbance. These variables were riparian-condition and instream-cover variables that were measured at each site coincident with fish sampling. I also used three watershed-scale measures: proportion of urban land use, proportion of forest, and number of pollution point-sources (P. L. Angermeier, unpublished data). I refer to on-site and watershed variables, collectively, as "habitat" variables.

Habitat variables comprised measures that reflected watershed deforestation, watershed urbanization, watershed mining, and on-site condition of riparian zones, stream banks, and instream habitat structure for fish. Watershed land use and disruption of riparian areas and instream structure can alter fish assemblages via effects on flow regime, food (energy) source, water chemistry and temperature, physical habitat, and biotic interactions (*sensu* Karr and Dudley 1981 and Karr 1991; Larimore and Smith 1963; Smith 1971; Gorman and Karr 1978; Karr and Schlosser 1978; Muncy et al. 1979; Angermeier and Karr 1984; Neves and Angermeier 1990; Detenbeck et al. 1992; Weaver and Garman 1994; Rabeni and Smale 1995). For example, deforestation and mass removal of riparian woody vegetation can effect increased sunlight, nutrients, and sediments to streams. Temperature increases, nutrient enrichment, and excessive siltation can alter fish richness and abundance via changes in water chemistry and temperature, altered flows, food availability, spawning substrate, and cover. Moreover, because streams belong to drainage hierarchies, localized habitat disturbances upstream can have much broader, cumulative effects downstream.

In Virginia, general effects of watershed land use and riparian degradation are evident. The Virginia Department of Environmental Quality (1994) documented "habitat alteration", mostly due to agricultural and urban land-use impacts, as the second major cause of streams failing to meet "fishable and swimmable" uses designated by the Clean Water Act (PL 95-217). Specifically for stream fish in Virginia, Jenkins and Burkhead (1994) stated that, "siltation and turbidity are the most pervasive deleterious factors to the Virginia ichthyofauna." Although the habitat variables that I used did not encompass all possible anthropogenic effects on fish assemblages throughout Virginia,

they did include measures that reflected the most-documented and pervasive anthropogenic effects on stream systems in Virginia and elsewhere. Moreover, the habitat variables represented a set of relatively easy-to-measure parameters that could be incorporated in long-term monitoring programs and further tests of the relations examined herein.

For each site, three watershed variables were estimated: (proportion) watershed as urban, (proportion) watershed as forest, and number of pollution point sources (Table 2; see **Methods** in Chapter 1 for further details). Also for each site, I or P. Angermeier (senior investigator) visually estimated the following on-site variables (Table 2; see **Methods**, Chapter 1 for further explanation): riparian width, riparian forest, bankside woody cover, maximum depth, and instream woody cover (PD or CP only) or instream cover (MT only). Instream woody cover or instream cover was the proportion of 1 m-wide, bank-to-bank transects that contained woody debris or logs (for CP and PD) or woody debris, logs, or rock crevices/ledges (for MT). Maximum depth was the maximum of water-depth measurements (in cm) that were taken approximately along the thalweg. Depth and instream-cover variables presumably reflected amounts of structural cover (for fish) and pool development at sites. Bank-to-bank transects and depth measurements occurred at regular intervals of 1-15 m that were scaled to habitat-unit size, i.e., larger units had greater intervals between each transect or measurement.

In addition to the previously mentioned habitat variables, I used site rankings of overall disturbance to describe fish metric-vs-habitat relations. I ranked sites based on class sums of ranks of individual habitat variables in four disturbance classes: mining, urban, forest, and riparian/instream cover (see **Methods**, Chapter 1). These rankings used some of the habitat variables used herein and a few additional ones. The site ranks represented a continuum from least- to most-disturbed, and I defined the approximate upper and lower thirds of all ranked sites as least- and most-disturbed, respectively, for comparisons herein.

Fish sampling and sampling considerations

At each site we (I and 3 or 4 co-workers) used an electric seine (Angermeier et al. 1991) to collect fish in a series of habitat units (each a single riffle, riffle/run, run, run/pool, or pool) that represented all meso-habitat types in the vicinity. Though our sampling

probably did not capture every species present at each site, I believe that our techniques (see Angermeier et al. 1991) yielded information adequate enough to allow valid tests of association between fish metrics and habitat variables across sites within each physiography. See **Methods**, Chapter 1 for further explanation of sampling methods and considerations.

Fish metrics

I chose fish variables that potentially could be used as IBI metrics, i.e., variables that presumably reflect effects of typical anthropogenic disturbance on fish communities and are relatively easy to determine from field data (Karr et al. 1986; Miller et al. 1988; Fausch et al. 1990). I grouped fish variables (hereafter called fish metrics or metrics) into three classes: taxonomic, trophic, and reproductive (Table 3). For each class, I chose from an initial list of fish metrics that have been used widely in IBI analyses or that I judged potentially useful, but remained unexamined. Due to statistical constraints (see below), I limited metrics to those that showed adequate variation across sites within each physiography, were nearly symmetrically distributed and without extreme outliers (after transformation), and were not highly intercorrelated with many others (i.e., showing many pairwise Pearson correlations > 0.80). For example, I excluded from all analyses the number of native sucker species because: too few such species occur in Virginia's CP; and PD and MT distributions of suckers were asymmetric with extreme outliers, a reflection of the highly variable distribution of sucker species across Virginia drainages (Jenkins and Burkhead 1994).

I used 14 fish metrics in analyses: 5 taxonomic, 4 trophic, 4 reproductive, and 1 tolerance metrics. The following 5 metrics have been used widely and their rationales discussed elsewhere (see Karr 1981; Karr et al. 1986; Miller et al. 1988; Fausch et al. 1990; Karr 1991): number of native species, number of non-native species, number of native sunfish species, number of native darter or sculpin species, proportion (of individuals) as members of tolerant species. Therefore, I elaborate only on those metrics that have not been widely justified or used previously.

I based taxonomic, trophic, reproductive, and tolerance classifications of species (Appendix A) on various regional texts (e.g., Jenkins and Burkhead 1994, Pflieger 1975), on personal communication with P. Angermeier, and on eight years of personal experience sampling

fishes throughout Virginia. I based native versus non-native status (by major river drainage) on Jenkins and Burkhead (1994). Number of native minnows and number of native sunfish comprised all native species in the families Cyprinidae and Centrarchidae, respectively. Number of native darter or sculpin species comprised all native *Percina* spp., *Etheostoma* spp., or species of Cottidae. Because 20 sites in MT had no native sunfish species, I included non-native species in this metric, for analyses of MT sites.

Number of native minnow species has been used as an IBI metric in western streams (Hughes and Gammon 1987), Great Plains streams (Bramblett and Fausch 1991), and midwestern headwater streams (Ohio EPA 1988). For Maryland CP streams, Hall et al. (1994) used a similar metric, number of shiner species excluding golden shiner (*Notemigonus crysoleucas*). Native species of Cyprinidae are numerous and widely distributed throughout Virginia (Jenkins and Burkhead 1994). These species comprise an ecologically diverse group whose local richness may positively reflect local habitat heterogeneity (e.g., Hughes and Gammon 1987; Hall et al. 1994); therefore, I expect this metric to decrease with increased disturbance in Virginia streams. The 48 native Virginian cyprinids represented by my data include five tolerant and two intolerant species (Appendix A).

I classified "intolerant" species as those whose ranges or abundances have decreased, presumably due to anthropogenic effects. I classified "tolerant" species as those that have been affected least detrimentally by typical anthropogenic disturbances to streams and watersheds (e.g., common carp, *Cyprinus carpio*; gizzard shad, *Dorosoma cepedianum*; green sunfish, *Lepomis cyanellus*). For using the IBI, Karr et al. (1986) recommended that less than 10% of the species in a region be classified as "intolerant." This limit ensures that an intolerance metric contributes exclusively to the highest IBI scores, i.e., only reflects sites at the highest end of the biotic-integrity continuum. My classifying 5.6 % (8 of 143 species; Appendix A) as intolerant seems reasonable. Similarly, I suggest that classification as "tolerant" be limited to a small percentage of the included species; this ensures that the tolerance metric reflects exclusively the lowest end of the biotic-integrity continuum, i.e., only those severely degraded sites dominated by tolerant species or individuals. I classified 11.9 % (17 of 143; Appendix A) of species as tolerant.

Before any analyses, I determined intolerance/tolerance classifications of species that occurred in my samples statewide. Subsequent results, indicating the importance of physiographies as IBI regions (Chapter 1), suggested that a more proper approach would be to classify species within each physiographic region. Such re-classification would be highly subject to bias due to revelations of the analyses; therefore, I chose not to re-classify. Consequently, because within PD and within CP very few species were classified as intolerant, the metric (i.e., number of intolerant species) varied little among sites. For MT sites, most species classified as intolerant occurred in the Clinch River drainage, and the metric did not equally represent all MT sites. For these reasons I did not use number of intolerants as a metric in my analyses.

For trophic variables I considered three classification factors: number of food types typically eaten, feeding behavior, and feeding group. I designated four food-type categories: (1) detritus, (2) algae or vascular plants, (3) invertebrates, and (4) fish (including fish blood) or crayfish. "Generalist feeders" were species whose adults eat from more than two food-type categories; "specialists" eat from two or fewer categories (see Table 3). I designated two mutually exclusive feeding behaviors, benthic and non-benthic. Benthic feeders feed, as adults, mostly along the stream bottom and require foods that are associated strongly with the stream substratum (e.g., many types of aquatic insects). I assigned fish species to one of five feeding groups based on the primary food type(s) of subadults or adults. Groups represented a continuum from (1) detritivore/algivore/herbivore to (2) algivore/herbivore/invertivore to (3) invertivore to (4) invertivore/piscivore to (5) piscivore or fish parasite. Group 4 comprised species in which subadults eat primarily invertebrates, but adults eat primarily fish or crayfish (e.g., American eel, *Anguilla rostrata*; yellow bullhead, *Ameiurus melas*; redbreast sunfish, *Lepomis auritus*; crappies, *Pomoxis* spp.; yellow perch, *Perca flavescens*). "Carnivores" were species in groups 4 or 5. The four trophic metrics represented roughly a continuum of food and feeding specialization from generalist feeders to specialist carnivores or specialist benthic invertivores; invertivores represented the middle of this continuum. Trophic specialists are expected to be most abundant at least-disturbed sites, and vice-versa (Table 1). Using similar trophic categories,

Angermeier (1995) found that, among Virginia's native stream fishes, benthic species and those that feed on a single major food type were more likely to be extirpated than were more generalized species, thus implying that specialists are more susceptible to anthropogenic disturbance.

For reproductive metrics, I classified species as obligate versus non-obligate mineral-substrate (unsilted sand to boulder) spawners. For consistency with previous IBI work, I refer to obligate mineral-substrate spawners as lithophils. Also I designated "manipulative" versus "simple" (non-manipulative) spawners (Table 3). "Manipulative spawners" build nests, depressions, or cavities or actively guard eggs or young (e.g., lampreys, *Petromyzontidae*; trouts, *Salmonidae*; central stoneroller, *Campostoma anomalum*; catfishes, *Ictaluridae*; sunfishes, *Centrarchidae*; some darters, *Etheostoma spp.*). "Simple spawners" exhibit relatively little nest preparation or parental care or guarding. Because manipulative spawners can alter spawning substrates or provide extended care to eggs or young, I presume that generally they would be more resilient to disturbance than would be simple spawners. Alternatively, simple lithophils represented the reproductive group (and metric) likely to be most sensitive to disturbance, specifically that contributing to excessive siltation of streambeds (e.g., Muncy et al. 1979; Ohio EPA 1988; Rabeni and Smale 1995), which is the second-most pervasive nonpoint impact in Virginia streams (Virginia Department of Environmental Quality 1994). I excluded any species classified as "tolerant" from the "lithophil" classification to minimize contrary information contributed by species originally classified as both, e.g., blacknose dace (*Rhinichthys atratulus*) (see Appendix A).

As a final metric, I designated "late-maturing species" as those in which females typically do not spawn before 3 years of age. Number of late-maturing species may be indicative of chronic disturbance because such species may be slower to recover from disturbance than are those with shorter generation times (Detenbeck et al. 1992).

The four trophic metrics and three of the reproductive metrics examined represent a continuum of trophic or reproductive specialization that simultaneously represents food types and feeding modes or spawning substrates and behaviors selected to presumably reflect species' susceptibilities to anthropogenic disturbance (Table 3). Consistent with IBI tenets, I expect that the relative abundances of individuals,

categorized along either continuum, will reflect disturbance. Specifically, more-disturbed sites will have more generalists and fewer specialists than will less-disturbed ones, and vice-versa.

Consistent with these continuum concepts, in **Results** and **Discussion** I refer generally to particular metrics as "trophic (or reproductive) specialists" or "reproductive (or trophic) generalists". Also in those sections, when I refer to metrics that represent proportions of individuals, I omit "proportions of individuals as" and use a shortened name for brevity. For example, I refer to "proportion of individuals as members of tolerant species" simply as "tolerants". To avoid confusion with proportional metrics, I refer to "number of species" metrics explicitly as such, for example, "Number of native minnow species was greater at sites with ..."

Statistical tests and considerations

I analyzed sites within each of three physiographic regions: N=12 for CP, N=49 for PD, and N=47 for MT. I examined all pairwise correlations (Pearson's r for PD and for MT; Spearman's ρ [r_s] for CP) between fish metrics and the four continuously distributed habitat variables: bankside woody cover, instream (woody) cover, maximum depth, and (proportion) watershed as forest (see Table 2). Due partly to the method, range, or precision of measurement, the following habitat variables had highly skewed or disjunct distributions: riparian width, riparian forest, presence of point sources, and (proportion) watershed as urban (see Table 2); therefore, I treated these as discretely distributed variables. For PD and for MT sites, I used multivariate analysis of covariance (MANCOVA; SAS 1990), with watershed area as covariate, to examine how fish metrics or the four continuously distributed habitat variables varied among discrete habitat categories. For CP sites, small sample size precluded multivariate tests; therefore, I used Wilcoxon two-sample tests to assess differences in metrics or continuous habitat variables among habitat categories.

Also, for PD and for MT sites I used canonical correlation analysis (CCA; Hotelling 1935 and 1936; Thompson 1984; Gittins 1985) to examine multivariate relations between fish metrics and the four continuous habitat variables. CCA accounts for intraset relations among variables in each of two sets (e.g., fish metrics vs. habitat variables) to account more completely for relations between the two sets. Therefore, it represents a more realistic, comprehensive, concise, and

(potentially) interpretable investigation of relations than would multiple uni- or bivariate analyses. Moreover, given that the IBI was originally intended to be an information-redundant index (Angermeier and Karr 1986; Karr et al. 1986; Miller et al. 1988), CCA allowed a direct way of quantifying and elucidating this redundancy.

Because fish metrics were correlated with stream size and because I wanted to assess relations between metrics and habitat, independent of stream-size effects, I used standardized fish-metric residuals in CCAs. I obtained these residuals from general linear regression models (SAS 1990) of (natural log of) watershed area as a function of transformed fish metrics (see below). For three preliminary CCAs of sites within each physiography, I used residuals of a model that included only the fish metrics in a particular class (i.e., taxonomic, trophic, or reproductive). For two final CCAs (one for PD sites, one for MT sites), I used residuals from a model that included a selected, multi-class set of metrics. Using fish-metric residuals removed most of the (linear) statistical effect of stream size on fish metrics, and allowed for clearer interpretation of CCA results. Hereafter, when referring to CCA and its results, I refer to the fish-metric residuals simply as "fish metrics".

Proper use of multivariate tests and the reliability of their results require that the variable-to-sample size ratio (p/n) be small and that variables have few high intercorrelations (Gittins 1985). Thorndike (1978) suggested that p/n be less than 0.10 (but preferably less than 0.02) for a conclusive CCA; however, Gittins (1985) showed that valid interpretation of ecological data was possible given $p/n > 0.50$. Gittins (1985) explained that as p/n approaches 1.0 the value of the first canonical correlation rapidly approaches 1.0, rendering meaningful interpretation impossible (e.g., see Carleton [1984] for an example where this may be a problem). To limit p/n , I first ran a separate CCA for each of the three classes of fish metrics (taxonomic, trophic, reproductive); all p/n were < 0.20 . For each of two final CCAs (one for PD, one for MT), I used the 2 or 3 fish metrics, from each class, for which preliminary CCAs explained the most variance. For each of the two final CCAs, $p/n < 0.27$.

To complement the two final CCAs, I used MANOVA and descriptive discriminant analysis (i.e., canonical analysis; Thorndike 1978; Gittins 1985; Huberty 1986) to examine how selected fish metrics (i.e.,

residuals) varied among least-disturbed, most-disturbed, and moderately-disturbed sites in PD and in MT. These site groupings were based on rankings as described previously. Whereas the MANOVAs and discriminant analyses did not directly relate fish metrics with the individual habitat variables, they did depict relations between metrics and a general measure of disturbance (i.e., site rankings) that was based largely on those habitat variables. I considered that consistencies in results between CCA and the other analyses indicated robustness of CCA results.

For sites in PD or in MT, each final CCA comprised the following questions:

1. What relations exist between the habitat variables and the fish metrics? Are these relations important, i.e., worthy of further interpretation and discussion?
2. How much information in the set of fish metrics is accounted for by that in the set of four habitat variables?
3. Which individual habitat variables and fish metrics contribute most to the important relations between the two sets?

Questions 1 and 2 of CCA

CCA defines sequential pairs of lines, each a linear composite of one set of the variables (similar to factor or components analysis), so that the points on each pair correlate maximally. For example, a canonical correlation (r_c) is equivalent to a Pearson correlation between a fish-metric composite and a habitat composite. Unlike factor analysis, canonical correlation does not choose lines that necessarily define the longest dimension (i.e., largest variance or covariance) in the composite space of each set of variables. Therefore, it is possible for two linear composites (hereafter called canonical variates or variates) to share a large amount of variance (i.e., r_c^2 is high), but to account for relatively little of the variability in either set of variables.

After the first canonical correlation defines and relates a pair of variates, subsequent canonical correlations use the remaining variability to do so for new pairs, which are restricted to being uncorrelated (=orthogonal) with all previous pairs. The total possible number of canonical correlations (i.e., pairs of variates) extracted equals the number of variables in the set with fewest variables, which was four for the study herein. As alluded to previously, for two sets of variables, a CCA disentangles (i.e., all bivariate correlations

minimize to zero) the within-set relations while it emphasizes and elucidates, in the form of canonical variates, the between-set relations (Gittins 1985).

After all possible canonical correlations were extracted, I needed to judge their importance: which ones were worthy of interpretation and discussion? Statistical reliability does not ensure importance and vice-versa (Gold 1969; Carver 1978; Thompson 1989); therefore, I relied on at least three criteria (including statistical reliability) to assess the importance of each canonical correlation, as recommended by Barcikowski and Stevens (1975), Thorndike (1978), Thompson (1984), and Gittins (1985).

For judging statistical reliability, SAS used an *F*-approximation of Wilks's lambda statistic to test the overall null hypotheses that all canonical correlations were zero and that a given canonical correlation and all smaller ones were zero, in the population (SAS 1990). Validity of these statistical-significance tests depends on representativeness of the sample and multivariate normality of the variables (Thompson 1984; Gittins 1985).

Sites were not chosen randomly or to represent the full range of expression of all habitat variables within a physiographic region. However, they were chosen to represent a wide intra-state geographic range, and they did represent ranges of habitat conditions that likely reflected least to moderate anthropogenic disturbance (personal observation; also see Table 2). The practical value of a bioassessment index lies mostly in its ability to indicate slight to moderate disturbance effects, before such effects have rendered pro-action or recovery impossible (*sensu* Angermeier 1995); therefore, the sites used herein adequately represented the "population" of sites that likely would be of most interest to assessors using the IBI in Virginia.

Few conclusive tests are available for judging multivariate normality (Barker and Barker 1984; Thompson 1984; Maxwell 1992). In practice, if sample sizes are nearly equal, statistical tests of significance in CCA and related multivariate techniques (e.g., MANOVA) are considered robust against non-normality (Barker and Barker 1984; Thompson 1984; Maxwell 1992). I transformed each variable to best exhibit univariate normality, which is necessary but not sufficient to maximize the likelihood of multivariate normality (Tabachnick and Fidell 1983). Data represented as proportions were arcsine transformed ($\arcsin[x^{0.5}]$), and

data represented as counts were square-root transformed ($[x + 0.5]^{0.5}$; Sokal and Rohlf 1981). Watershed area and maximum depth were natural-log transformed. Despite transformations, some sites remained extreme outliers for some fish metrics and, therefore, potentially biased CCA results; CCA is based on least-squares procedures that can be overly sensitive to outliers (Gittins 1985). I deleted from CCAs of PD sites one site with no native minnow species and one site with an extremely high proportion as tolerants. For CCAs of MT sites, I deleted one site with an extremely high and another with an extremely low number of native darters or sculpins.

For the second criterion of importance, I used r_c to judge each canonical correlation, although few formal rules exist for deciding how large an r_c is worth interpreting. I judged that $r_c > 0.30$ (i.e., accounting for >9% of variance) indicated importance. This value is commonly accepted as a criterion for choosing meaningful factor loadings in multivariate analyses (e.g., factor analysis, principal components analysis, or discriminant analysis; Thorndike 1978; Tabachnick and Fidell 1983).

For the third and final criterion, I used measures related to redundancy analysis (Stewart and Love 1968; Cooley and Lohnes 1971; SAS 1990) to judge the importance of each canonical correlation. For each canonical correlation, I report the redundancy, which represents the amount of variance in fish metrics explained by the habitat variables. I examined the cumulative sum of redundancies across all canonical correlations to determine, in part, which canonical correlations were important: cumulative redundancy tends to level off after the first few important canonical correlations. No definitive rules exist for deciding how large a redundancy denotes ecological importance. For ecological data, Gittins (1985) suggested that redundancy values ≥ 0.50 should be considered unusually high. He also showed that redundancies < 0.10 could be readily interpretable and ecologically meaningful.

Question 3 of CCA

I determined which fish metrics and habitat variables contributed most to each important variate by comparing among standardized canonical-function coefficients (i.e., analogs of multiple-regression beta weights) and by comparing among intraset structure coefficients, which were the Pearson correlations between each fish metric or habitat variable and their respective canonical-variate scores. Researchers

disagree about the relative utility of these two indices for determining the importance of individual variables comprising canonical variates (Thorndike and Weiss 1973; Barcikowski and Stevens 1975; Levine 1977; Williams 1983; Share 1984; Thompson and Borrello 1985; Huberty and Wisenbaker 1992; Rencher 1992; Thomas 1992). I mostly used intraset structure coefficients to describe the fish-metric and habitat variates in terms of their individual variables. However, I reported both coefficients because each can be informative in particular instances. For example, for a single variable of a variate, discrepancies in the signs (positive vs. negative) of its intraset structure versus function coefficient can indicate influential correlations among variables in a set (e.g., suppressor effects).

Despite potential shortcomings resulting from small p/n , and a few moderate outliers, moderate asymmetry, and unequal dispersions for some fish metrics and habitat variables, I believe that my conclusions based on multivariate results reflect a best-possible and realistic representation of the multiple relations between habitat variables and fish metrics. Nonetheless, general applicability of these multivariate results is unjustifiable because I did not test their validity. Testing for validity is recommended (Thorndike and Weiss 1973; Thorndike 1978; Thompson 1984; Gittins 1985) because sample-specific covariation of results (e.g., canonical correlation coefficients, canonical function and structure coefficients, redundancies) increases as p/n approaches one (Barcikowski and Stevens 1975; Gittins 1985). Because of these potential shortcomings of multivariate results, I did not rely solely on them; I also examined data uni- or bivariate to facilitate multivariate-based interpretations. I believe that this combined approach provided the most comprehensive analysis, given the somewhat "sloppy" data and limited sample sizes.

Results

Relations in Coastal Plain

Fish metrics were related moderately with habitat variables, but most relations were inconsistent with IBI assumptions. For example, sites in more-forested watersheds had fewer native sunfish species ($r_s = -0.40$) and more tolerants ($r_s = 0.67$) than did less-forested sites (Table 4). Also, sites in more urbanized watersheds or with point sources present had more native sunfish species and trophic specialists (as carnivores) than did less disturbed sites (Wilcoxon two-sample tests, $0.20 > P > 0.10$).

The highest correlations among (pairs of) fish metrics revealed two contrasting groups (Table 5). One group comprised number of native species, number of native minnow species, number of late-maturing species, and proportion as benthic invertivores. Again, inconsistent with IBI assumptions, metrics in this group generally were least at least-disturbed sites, i.e., those with more bankside and instream woody cover (Table 4). A second group, comprising proportions as invertivores and as generalist spawners, had greater values at less-disturbed sites. Other bivariate correlations among fish metrics were too numerous to allow clear and concise interpretation (Table 5).

Relations in Piedmont

Fish metrics were related moderately with the four habitat variables. About 20% of the variance in selected fish metrics could be accounted for and reasonably interpreted via these relations (sum of RED=0.229 for PD sites, Table 6). Taxonomic and trophic metrics contributed most of the explained variance, whereas reproductive metrics contributed little: the first two canonical correlations explained from 0.19 (=COM2 for proportion as specialist carnivores) to 0.40 (=COM2 for number of native minnow species) of the variance in non-reproductive metrics (Table 6).

Relations revealed by the first canonical correlation (CAN1) were mostly inconsistent with IBI assumptions. Apparently, more-disturbed sites, i.e., those with less bankside and instream woody cover, had more native minnow species and fewer individuals as tolerants, as specialist carnivores, and as reproductive generalists (Figure 2; Table 6). Proportion as tolerants and proportion as various-substrate, manipulative spawners loaded similarly on CAN1. A high Pearson correlation ($r=0.75$; Table 5) between the two was due, in part, to a few commonly abundant, PD-dwelling species each being classified as tolerant and as generalist spawners (Appendix A): tessellated darter (*Etheostoma olmstedi*), johnny darter (*E. nigrum*), and bluegill (*Lepomis macrochirus*). A graphical display showed little separation of most- and least-disturbed sites in the space defined by CAN1, except for four leftmost sites (Figure 2, left upper plot).

Relations revealed by CAN2 were much more consistent with IBI assumptions than were those of CAN1; however, they accounted for much less fish-metric variance (RED=0.06 for CAN2 vs. RED= 0.15 for CAN1; Table 6). For CAN2, less-disturbed sites, i.e., those in more forested watersheds and with more instream woody cover, had fewer trophic

generalists, fewer tolerants, and more trophic specialists (as carnivores) than did more-disturbed sites. Most- and least-disturbed sites were largely separate in the space of CAN2, and their location was consistent with how the habitat variate reflected disturbance (Figure 6).

Discriminant-analysis and MANOVA results were similar to those of CCA except emphases of the first two canonical relations were reversed. Sites were separated along the first canonical composite of the discriminant analysis (i.e., FISH1 in Figure 4 and Table 7) similar to their separation depicted by CAN2 of the CCA: most-disturbed sites had more generalist feeders and more tolerants than did least-disturbed sites and tended to group in canonical space accordingly (Figures 2 and 4). Discriminant-analysis, MANOVA, and univariate results showed that number of native minnow species and number of late-maturing species were greatest at moderately-disturbed sites and thus uni-modally, rather than monotonically, related with disturbance (FISH2 in Figure 4; Table 7). A rerun CCA with these two metrics removed accounted for nearly as much fish-metric variance as did the original CCA (sum of RED=0.213; Table 7); and both CAN1 (RED=0.122) and CAN2 (RED=0.074) of the rerun CCA were consistent with IBI assumptions. Specifically, fewer generalists and more specialist carnivores (lower plots in Figure 2; also see FSH1 in Table 7) and more native species and fewer tolerants (see FSH2) occurred at less-disturbed sites, i.e., those with more bankside and instream cover (see HAB1) and more forested watersheds (see HAB1 and HAB2).

I examined plots similar to those in Figure 2, but with PD sites depicted by drainage or by year of sample. Except for CAN1 of the original CCA, I found little separation or clustering of sites by drainage or by year, suggesting that these groupings had little confounding influence on the patterns discussed. For CAN1 of the original CCA (Figure 2, left upper plot), the four leftmost sites that largely defined the overall correlation occurred in the same vicinity in the Roanoke River drainage.

Fish metrics differed little among discrete habitat categories. The most pronounced differences in fish metrics were those for trophic metrics in the presence versus absence of point sources (one-way MANCOVA, Wilks's $\lambda=0.76$, $P=0.05$). This result largely was due to sites with no point sources having the most tolerant individuals, contrary to IBI assumptions. Although this result showed that 0.24

(i.e., 1 - Wilks's lambda) of the variance in trophic metrics could be attributed to presence of point sources, this effect was obscured by a possible simultaneous interaction effect (one-way MANCOVA, Wilks's lambda=0.72, P=0.02; for point source X stream size). Overall, after accounting for fish metric-vs-stream size relations, metrics varied little among discrete habitat categories.

Relations in Mountain

Similar to those at PD sites, fish metrics at MT sites were related moderately with the four continuous habitat variables. Again, about 20% of the variance in selected fish metrics could be attributed to and reasonably interpreted via these relations (sum of RED= 0.204, Table 6). Unlike for PD sites, the total explained variance was more evenly distributed among fish metrics, and reproductive metrics contributed substantially (COM2=0.19-0.28 for three reproductive metrics).

Relations revealed by CAN1 were not readily interpretable as consistent or not with IBI assumptions because the habitat variate did not depict a strong gradient of disturbance. Sites with much instream cover but less bankside woody cover had more trophic specialists (as benthic invertivores and as carnivores), but also had fewer reproductive specialists and more reproductive generalists (Figure 3; Table 6). Graphic display of sites in CAN1 space showed little separation of most- from least-disturbed sites (Figure 3).

Relations revealed by CAN2 were consistent with IBI assumptions. Less-disturbed sites, i.e., those with more bankside woody cover and (to a lesser extent) more forested watersheds, had more native minnow and late-maturing species, more reproductive specialists, and fewer tolerants than did more-disturbed sites. In CAN2 space, least-disturbed sites were distinctly separate from only a subset of most-disturbed sites, three of which largely defined the lower left tail of the canonical correlation (Figure 3). Unlike for analyses of PD sites, MANOVA and discriminant analysis showed few differences in fish metrics among disturbance category (i.e., least- vs. most-disturbed) for MT sites (Wilks's lambda=0.67 and P=0.5125 for omnibus MANOVA). However, some ANOVA results were consistent with CAN2 results (see Figure 3): number of late-maturing species differed more, univariately, among sites than did any other metric and was greatest at least-disturbed and least at most-disturbed sites (F=3.34; P=.0452).

I examined plots similar to those in Figure 3, but with MT sites

depicted by drainage or by year of sample. I found very little separation or clustering of sites by drainage or by year, suggesting that these groupings had little confounding influence on the patterns discussed.

In MT, fish metrics were related slightly more with discrete habitat variables than they were in PD. However, similar to PD results, interpretations were confounded by stream-size effects. Most-pronounced differences were for reproductive metrics at sites in least- versus most-urbanized watersheds and for taxonomic metrics at sites with the narrowest versus widest riparian zones (one-way MANCOVAs: Wilks's $\lambda=0.50$, $P=0.0006$ for urban effect; Wilks's $\lambda=0.51$, $P=0.0051$ for riparian effect). Two metrics dominated these results: proportion as lithophils was greatest at less disturbed (i.e., less urbanized) sites--consistent with CAN2 results--and number of non-native species was greatest at sites with the widest riparian zones, contrary to IBI assumptions. As mentioned above, these relations were dependent, in part, on relations between fish metrics and stream size as well (one-way MANCOVAs: Wilks's $\lambda=0.55$, $P=0.0024$ for urban X stream size; Wilks's $\lambda=0.52$, $P=0.0065$ for riparian X stream size).

Interrelations among habitat variables

My interpretations of fish-vs-habitat relations were not obfuscated by interrelations of habitat variables. Compared to those of fish metrics (see Table 5), interrelations of habitat variables were fewer and easily interpretable. In CP, in PD, and in MT, the four continuously distributed habitat variables mostly were positively and weakly intercorrelated (all Pearson $r > -0.20$ and < 0.31), suggesting that, together, variables neither mis- nor over-represented disturbance. The presence of any strong negative correlations would have diminished the adequacy of these habitat variables as accordant measures of disturbance. Alternatively, the lack of strong positive intercorrelations suggested that each variable reflected possibly unique aspects of disturbance.

The continuously distributed habitat variables were weakly related with stream size (all Pearson $r > -0.18$ and < 0.30) except, sites in larger watersheds in MT tended to be deeper (Pearson $r=0.43$, $P=0.0031$) and, in CP, had more instream woody cover (Spearman's $\rho=0.62$, $P=0.0303$) than did other sites.

Continuous habitat variables differed little among habitat

categories, i.e., Wilks's $\lambda > 0.80$ for all except two one-way MANCOVAs. For these two cases, the differences reflected disturbance in concert with habitat categories. Specifically, in PD and in MT, sites in the most forested watersheds were least urbanized and vice-versa (one-way MANCOVAs: Wilks's $\lambda=0.68$, $P=0.0568$ for urban effect in PD and Wilks's $\lambda=0.62$, $P=0.0198$ for urban effect in MT). Also, least-urbanized PD sites had the most instream woody cover and least-urbanized MT sites had the most bankside woody cover. These relations were not independent of stream-size effects (one-way MANCOVAs, Wilks's $\lambda=0.66$, $P=0.0388$ for urban X stream size in PD; Wilks's $\lambda=0.67$, $P=0.0635$ for urban X stream size in MT). Overall, the few relations among and between continuous and discrete habitat variables were easy to explain and did not confound interpretations of fish-vs-habitat relations.

Discussion

Despite the apparently small to moderate amounts of variance accounted for, my analyses revealed meaningful relations between fish metrics and habitat measures that reflected anthropogenic disturbance. Large amounts of explained variation may be the exception for analyses of assemblage-level ecological relations at large geographic scales (Gauch 1982). For CCA specifically, Gittins (1985) showed that relations explaining < 0.10 of variance nevertheless could be easily interpreted and ecologically meaningful.

Relations between IBI-metrics and measures of disturbance have not been examined systematically or multivariately at large spatial scales; and CCA has not been used properly or widely enough to allow comparison of my results with those of parallel studies. However, a few previous studies provide analyses and results somewhat analogous to a CCA approach.

For a statewide sample of stream sites in Arkansas, Matthews et al. (1991) found a Pearson correlation of 0.39 between a multivariate composite of fish-species abundances and one of water-quality measures, thus accounting for 0.15 of the variance shared by the composites. Based on principal components analysis, the results accounted for a maximum of 0.26 of the variance in any single water-quality variable and a maximum of 0.10 of that in any single species' abundance. Using a similar approach for stream-sites in a single drainage in Oklahoma, Taylor et al. (1993) found a correlation of 0.54 (Mantel test) between a

species-by-sites matrix and a sites-by-habitat (i.e., substrate type, depth, instream woody cover, stream gradient, etc.) matrix, thus explaining 0.29 of shared variance. Based on canonical correspondence analysis, their results accounted maximally for 0.39 of the variance in any single habitat variable. I could not calculate an analogous measure for species' abundances from the reported results. Herein, amounts of explained variance in fish metrics and their canonical variates are similar to those obtained in previous studies. Moreover, the relations revealed were readily interpretable and meaningful with respect to the questions asked of the data.

At CP sites, fish metrics reflected disturbance mostly contrary to typical IBI assumptions. Native minnow species, trophic specialists, and late-maturing species were fewest at least-disturbed sites; whereas, tolerants and reproductive generalists were greatest at least-disturbed sites. Typical IBI metrics and their assumed relations with anthropogenic disturbance are based largely on studies of non-lowland streams and may be inappropriate for CP streams.

For example, for non-lowland, warmwater streams, less-disturbed sites typically have more fish species than do disturbed sites, thus high species richness may directly reflect high biotic integrity (e.g., Karr et al. 1986). However, increased species richness or diversity does not always reflect increased biotic integrity. Prior to anthropogenic disturbance many sites in Atlantic CP flowages probably were dystrophic, low-flow blackwaters (Smock and Gilinsky 1992) with naturally low native species richness compared to river mainstems or more upland streams. Draining and forest-clearing of CP watersheds were common anthropogenic disturbances (Smock and Gilinsky 1992) that likely contributed to localized increases in productivity and species richness.

Currently, at least in Virginia and North Carolina (personal observation), the physical, chemical, and biotic features of CP flowages tend to be variable among sites, yet distinct from those of more upland streams in PD and MT. Therefore, an IBI for CP streams likely would require metrics or metric-score criteria different than those used in most previous versions of IBI. For example, for CP streams, I found little utility for metrics that included proportions as lithophils, a commonly used IBI-metric category (Miller et al. 1988; e.g., Hall et al. 1994). In Virginia, relatively few CP streams contain substantial areas of mineral substrates larger than sand; therefore, fishes requiring such

substrates are extremely localized or rare in CP. Similarly, taxonomic metrics that included numbers of sucker, darter, or sculpin species had limited utility because richness of these taxa is naturally low in CP (Jenkins and Burkhead 1994). Hall et al. (1994), used a fish-IBI to compare physical, chemical, and biotic features of Maryland CP streams. Their version of IBI, which differed little from prior non-CP versions, related weakly or not at all with measures of disturbance. However, the authors provided little evidence to justify their choice of metrics, and the lack of relations could have been due to inappropriateness of individual metrics or metric-score criteria.

For Virginia, the distinctness of CP streams and the relative lack of their study highlight the need to better understand the fish-vs-disturbance relations there--especially before one can choose a definitive set of metrics for an IBI in CP. Bramblett and Fausch (1991) concluded similarly; they found that a traditionally based version of IBI inadequately assessed disturbance in a group of western Great Plains streams. They attributed this result to the inability of their metrics to reflect disturbance in streams that exhibited relatively distinct or less-understood relations between fish-assemblage attributes and habitat.

In PD and in MT in Virginia, fish metrics reflected disturbance more consistently with IBI assumptions than they did in CP. This probably resulted from the relative similarity of many PD and MT streams to those for which IBI has been most fully developed. However, for my study, fish-vs-disturbance relations simply may have been more detectable in MT or PD than they were in CP because variability of the four continuously distributed habitat variables was greatest in MT and least in CP (Table 2). Alternately, similar to the fish metrics, the habitat variables may have represented disturbance less adequately in CP than they did in PD or in MT. The effects of anthropogenic disturbance (e.g., clearing of trees and vegetation, non-point runoff, impoundment) have been studied little for CP compared to more upland streams (Smock and Gilinsky 1992); therefore, upland-based preconceptions of these effects may be misleading when applied to lowland streams.

In addition to overall differences between CP and non-CP sites, fish metrics reflected disturbance at PD sites differently than they did at MT sites. In PD, trophic metrics dominated the relations that were consistent with IBI assumptions; whereas, in MT, reproductive and

taxonomic metrics did so. Despite these consistencies with IBI assumptions, results of the first canonical correlation of each CCA were contrary to or uninformative of such agreement. For PD sites, a supplementary MANOVA and discriminant analysis showed reversed emphasis of the CCA results, whereas supplementary analyses revealed little additional information for MT sites.

For PD sites, the discrepancy between CCA and supplementary analyses may be due to the following. Least-squares procedures, particularly linear correlation, can capitalize heavily on the most extreme observations (Gittins 1985). CAN1 of the original CCA for PD sites (Figure 2, left upper plot) depicted a relation largely dependent on the four leftmost sites representing the lower tail of the canonical correlation. These sites were located in the same drainage and vicinity upstream of a large reservoir (Kerr Reservoir); one site was also about 4 km directly downstream of another impoundment (Mayo Reservoir in North Carolina). The relative paucity of native minnow species and preponderance of tolerants at these sites (see Figure 2) suggested that CAN1 largely represented a reservoir effect expressed by these sites, namely decreased native-species richness and increased abundance of individuals of less specialized, perhaps more tolerant, species (Mahon et al. 1979; Neves and Angermeier 1990). The habitat variables that I used would not necessarily reflect effects (on fish assemblages) of impoundment; therefore, the habitat variate of CAN1 and my groupings of sites by disturbance likely were only artifactually related with the fish variate.

For MT, CAN1 indicated little separation of least- from most-disturbed sites, and the habitat variate did not depict a strong disturbance gradient: sites with more instream cover tended to have less bankside woody cover. CAN1 did reveal a functional gradient: more trophic specialists and reproductive generalists, but fewer reproductive specialists, occurred at sites with more instream cover, and vice-versa. Whereas this relation may be ecologically informative, it contributed little to the focus of the study. CCA is best used as an exploratory technique; it does not guarantee that all important relations will be relevant to particular questions asked.

For PD sites, the first canonical composite of the discriminant analysis, CAN2 of the original CCA, and CAN1 of the rerun CCA represented relations that were most consistent with IBI assumptions:

trophic generalists were fewer and trophic specialists (as carnivores) were greater at less-disturbed sites, but reproductive metrics reflected disturbance weakly (Figures 2 and 4; Table 7). For MT sites, CAN2 and CAN3 of the CCA and MANCOVA results represented relations that were most consistent with IBI assumptions: native minnow species, native darter or sculpin species, lithophils, and late-maturing species were greatest at least-disturbed sites, but trophic metrics reflected disturbance weakly (e.g., Figure 3; Table 6). Only one metric contributed consistently between PD and MT sites: tolerants were most abundant at most-disturbed sites in each physiography.

These differences in fish-vs-disturbance relations across CP, PD, and MT strongly suggest that the utility of particular metrics can vary across IBI regions. Whereas previous researchers suggested the same and adapted versions of IBI to particular regions (see Miller et al. 1988), none explicitly have evidenced the intra-region abilities of individual metrics to reflect disturbance. Although my results suggest that a distinct few metrics may best reflect disturbance in each region, I do not recommend limiting Virginia IBIs to only those metrics because my results remain not validated. Moreover, some of my results suggest that I incompletely accounted for disturbance at sites.

As mentioned previously, in PD, two trophic metrics, proportions as generalist feeders and as specialist carnivores strongly reflected disturbance (Figure 2). However, another trophic metric, proportion as benthic, specialist invertivores, was not included in the final CCA; the preliminary CCA that used only trophic metrics showed that it reflected disturbance least of all trophic metrics. Similarly, in MT, proportion as lithophils strongly reflected disturbance; whereas, the preliminary reproductive-metric CCA found that proportion as *simple* lithophils reflected disturbance least of all reproductive metrics. In each case, the metric representing the most specialized trophic or reproductive category was least related to disturbance. This result and those of CAN1 of the PD CCA (see previously) probably reflect that the general habitat measures used herein encompassed only general or limited effects of overall disturbance to streams. Similarly, Shields et al. (1995) suggested that chemical water quality confounded their relations between total IBI and physical-habitat measures.

Karr (1991 and earlier) proposed five major classes of environmental factors that affect aquatic biota: food (energy) source, chemical

makeup and temperature of water, physical habitat structure, flow regime, and biotic interactions. Disturbance effects can be represented by factors from any or all of these classes; the few habitat variables that I used mostly represented physical-habitat factors, and fish metrics represented, in part, food-source and biotic-interaction factors. Overall, I did not account completely for all possible factors or for all classes. More specific and encompassing measures of disturbance (i.e., physical-habitat variables plus water-chemistry, hydrologic, etc. variables) may relate more closely with functionally specialized groups of fishes than do general physical-habitat or water-quality (e.g., presence/absence of point sources) measures alone.

The ability of fish metrics to reflect disturbance could be improved also by more explicitly defining functional-metric categories and by ensuring that classifications match the species' functional roles in the IBI region of interest. Meeting these two requirements limits the possibility that a metric will contribute superfluous, contrary, or overly redundant information to an IBI assessment. For example, for CP and for PD sites, I classified two common, widespread darter species, tessellated darter and johnny darter, as benthic, specialist invertivores; contrarily, I also classified them as tolerants and as reproductive generalists. Although "benthic, specialist invertivore" apparently represents a specialized functional role, "invertivore" or "insectivore" may be still too general a category to reliably reflect disturbance (e.g., Shields et al. 1995). These two darter species feed largely on midge larvae (Jenkins and Burkhead 1994) and may benefit from moderate anthropogenic disturbance via increased midge abundance, a common indicator of degraded stream conditions (Berkman et al. 1986; Ohio EPA 1988; Plafkin et al. 1989). Even taxonomic categories could be altered to better reflect assumed relations between species richness and disturbance. One obvious way to limit contrary information in taxonomic metrics is to omit tolerants from counts of total species or of species within taxa, for those richness metrics monotonically related with disturbance.

Further efforts should test, within each IBI region, the accuracy and reliability of functional classifications of species, especially for metrics that represent less explicitly defined categories (e.g., tolerant vs. intolerant) and may be more subject to personal bias. For example, I could have run separate CCAs, each with a different number of

species classified as tolerant, to test the sensitivity of results to this metric and its possible manifestations.

Not only should metrics be better defined, but so should the effects of disturbance on possible metrics. Such efforts should choose sites that best reflect the overall range of anthropogenic disturbance in a given region, a recommendation not met by the herein study. Further study in Virginia should try to validate and expand on the metric-vs-disturbance relations revealed in this study, especially with respect to choosing metrics most appropriate for each IBI region. New findings should be used to regularly reassess taxonomic, trophic, reproductive, and tolerance classifications of species and to tune metrics to reflect region-specific disturbance in consistently predictable and reasonable ways. Virginia's CP streams warrant special attention because traditional IBI metrics and their assumptions appear least tenable there.

Conclusions

At least three distinct versions of the IBI are appropriate for assessing the biotic integrity of warmwater, wadeable streams in Virginia: one each for CP, PD, and MT. Relations between fish metrics and measures of anthropogenic disturbance differ across these three physiographic regions; the ability of each IBI to accurately and reliably reflect disturbance will depend on how well its metrics represent these region-specific relations.

In a multivariate context, the ideal IBI would include only those metrics that reasonably could account for maximal differentiation of sites based on overall disturbance. Currently, too little evidence prohibits choosing a complete and distinct set of metrics that would reliably meet this ideal in each of Virginia's physiographic regions. Until further work can validate and expand on the information provided in this study, I recommend that an IBI for each region include at least two metrics from each of a taxonomic, trophic, and reproductive class of metrics; I also recommend including a tolerance metric, at least for PD and for MT IBIs (see Table 8).

For a CP IBI, I do not recommend any particular metrics because my results provided little unequivocal information, given such a small sample of sites. I do recommend less emphasis on traditional IBI-metric expectations that are based on assumed monotonic relations between metrics and disturbance. For PD sites, I recommend more reliance on

trophic metrics--including less-general metrics that may depict more accurately trophic-vs-disturbance relations-- and less emphasis on species- and taxa-richness metrics. As for CP sites, for PD sites high richness may not necessarily reflect high biotic integrity: number of native minnow species and number of native species tended to be highest at moderately-disturbed sites. Metrics that may be especially useful for a PD IBI are: proportion as generalist feeders, proportion as specialist carnivores, and proportion as tolerants (see Table 8). For MT sites I recommend relying most on reproductive and some taxonomic metrics, especially proportion as lithophils, number of late-maturing species, and number of native minnow species (see Table 8). I also encourage study of additional or alternative reproductive metrics, perhaps some that represent how disturbance may affect species' life-history traits (see Balon 1975, 1984), such as temporal or spatial spawning patterns and behaviors.

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Table 1. Assumptions of metrics most commonly used in versions of the IBI adapted for freshwater stream-fish assemblages. Assumptions describe how metrics are expected to change with increasing degradative anthropogenic disturbance to stream systems (modified from Fausch et al. 1990).

Taxonomic and richness metrics
The number of native species decreases
The number of native species in particular taxa or functional groups decreases
The number of intolerant species decreases
The number of non-native species or proportion of non-native individuals increases
Trophic metrics
The proportion of individuals that are trophic specialists (e.g., insectivorous cyprinids, top carnivores) decreases
The proportion of individuals that are trophic generalists (e.g., omnivores) increases
Reproductive metrics
The proportion of individuals that require silt-free, mineral spawning substrates decreases
Tolerance and fish-condition metrics
The proportion of individuals that are tolerant increases
The proportion of individuals that are intolerant decreases
The incidence of externally evident disease, parasites, and morphological anomalies increases

Table 2. Values of habitat variables at sites within each physiography. Medians (MD), means (X), standard errors (SE), and coefficients of variation (CV) are shown. Categories of discrete variables and ranges of continuous variables are also shown. Number of sites (N) is followed by the range of stream sizes (shown as watershed area) sampled in each physiography. See Methods for further explanation of habitat variables.

Variable	Category or range of variable					
	MD	X	SE	CV	Categ.	N
Coastal Plain (N=12; 23-147 km ²)						
Discrete						
Riparian width	50.0	50.0	0.00	0.0	<50 m	0
Riparian forest	1.000	0.975	0.0179	6.375	<1.00	2
Number of point sources	0.00	0.33	0.188	195.40	0	9
Watershed as urban	0.033	0.070	0.0272	135.428	0	4
Continuous						
Bankside woody cover	0.73	0.75	0.043	19.68	0.50-0.95	8
Instream woody cover	0.41	0.48	0.077	54.96	0.08-0.87	8
Maximum depth	84	82	5.8	25	54-112 cm	8
Watershed as forest	0.60	0.61	0.040	22.65	0.41-0.85	8
Piedmont (N=49; 7-454 km ²)						
Discrete						
Riparian width	50.0	40.2	2.02	35.3	<50 m	18
Riparian forest	0.95	0.77	0.042	37.84	<1.00	26
Number of point sources	0.00	0.16	0.061	260.67	0	42
Watershed as urban	0.030	0.051	0.0110	151.345	0.00	15
					0.00<x<0.05	13
					≥0.05	21

Mountain (N=47; 14-363 km ²)									
Continuous									
Bankside woody cover	0.800	0.778	0.0228	20.506				0.30-1.00	
Instream woody cover	0.250	0.293	0.0294	70.149				0.00-0.92	
Maximum depth	80	74	3.2	31				30-120 cm	
Watershed as forest	0.595	0.584	0.0212	25.347				0.24-0.90	
Discrete									
Riparian width	27.5	27.1	2.46	62.2	<20 m	16	20 ≤ x < 50	20	≥ 50 m 11
Riparian forest	0.40	0.46	0.053	79.87	<0.20	11	0.20 ≤ x < 0.80	22	≥ 0.80 14
Number of point sources	0.00	0.13	0.058	310.64	0	42			> 0 5
Watershed as urban	0.014	0.041	0.0098	164.475	0.00	14	0.00 < x < 0.05	21	≥ 0.05 12
Continuous									
Bankside woody cover	0.80	0.72	0.031	29.64			0.05-1.00		
Instream cover	0.080	0.103	0.0140	92.689			0.00-0.35		
Maximum depth	63	69	3.5	35			35-130 cm		
Watershed as forest	0.63	0.59	0.039	45.86			0.00-1.00		

Table 3. Fish metrics used in analyses of fish-vs-disturbance relations. Metrics are arranged in three classes: taxonomic, trophic, and reproductive. "Proportion" refers to proportion of individuals. Subsequent tables refer to fish metrics by their codes. See Methods for further explanation of metrics.

Taxonomic metrics	Code
Number of native species	NATSP
Number of non-native species	NONNATSP
Number of native minnow species	MINSP
Number of native sunfish species ¹	SUNSP
Number of native darter or sculpin species	DARSCLS
Trophic metrics	
Proportion as generalist feeders	GENPRP
Proportion as invertivores	INVPRP
Proportion as benthic, specialist invertivores	BINVPRP
Proportion as specialist carnivores	CARNPRP
Reproductive metrics	
Proportion as mineral-substrate, simple spawners (i.e., simple lithophila) ²	MINSIMPRP
Proportion as mineral-substrate spawners (i.e., lithophila) ²	MINPRP
Proportion as various-substrate, manipulative spawners	VMANPRP
Number of late-maturing (> 2 yr) species	AGE3SP
Proportion as members of tolerant species	TOLPRP

¹For MT sites only, this metric also included non-native sunfish species

²Excluded species also classified as "tolerant"

Table 4. Spearman (r_s), for raw values in CP) and Pearson (r , for residual values in PD and MT) correlations between taxonomic, trophic, or reproductive metrics and continuously distributed habitat variables, for sites within each physiography. Number of sites = N. Only absolute values (e.g., $|r|$) of r or $r > 0.30$ are shown. Dashes denote that a fish metric was not included in analyses for the particular physiography. See Table 3 for fish-metric codes. For Piedmont correlations, two sites with extremely outlying fish-metric values were excluded: one for MINSIP and one for GENPRP and TOLPRP. Similarly, two Mountain sites with extreme DARSCLSP were excluded. See Methods for further explanation.

	Coastal Plain (N=12) ¹				Piedmont (N=47) ²				Mountain (N=45) ³			
	Bank-side woody cover	In-stream woody cover	Maximum depth	Water-shed as forest	Bank-side woody cover	In-stream woody cover	Maximum depth	Water-shed as forest	Bank-side woody cover	In-stream cover	Maximum depth	Water-shed as forest
NATSP			0.39		-0.31	-0.32						
NONNATSP						-0.31		-0.32				
MINSIP		-0.53			-0.54	-0.43			0.38			
SUNSP		0.42	0.40	-0.40								
DARSCLSP						-0.33						
GENPRP				-0.39		-0.43		-0.39				
INVPRP		0.42	-0.34									
BINVPRP	-0.37	-0.36		0.32						0.40		-0.31
CARNPRP			0.38		0.34	0.39	0.31					
TOLPRP				0.67				-0.35				
MINSIMPRP	---	---	---	---								
MINPRP	---	---	---	---	-0.30				0.46			
VMANPRP	0.48	0.45			0.35					0.37		
AGE3SP	-0.40	-0.37							0.41			

¹If $|r_s| = 0.67$, $P = 0.02$
if $0.53 \geq |r_s| > 0.39$, $0.08 \leq P < 0.20$
if $0.39 \geq |r_s| > 0.30$, $0.20 \leq P < 0.33$

²If $0.55 \geq |r| > 0.45$, $0.0001 \leq P < 0.001$
if $0.45 \geq |r| > 0.38$, $0.001 < P < 0.01$
if $0.38 \geq |r| > 0.30$, $0.01 < P < 0.05$

³If $0.46 \geq |r| > 0.37$, $0.001 < P < 0.01$
if $0.37 \geq |r| > 0.30$, $0.01 < P < 0.05$

Table 5. Spearman (r_s , for raw values, Coastal Plain only) and Pearson (r , for residual values, Piedmont and Mountain) correlations between taxonomic, trophic, and reproductive metrics for sites within each physiography. Number of sites = N. Only absolute values (e.g., $|r|$) of r_s or $r > 0.30$ are shown. Dashes denote that a fish metric was not included in analyses for the particular physiography. See Table 3 for metric codes. For column headings, metric codes are limited to the first 3-5 letters (e.g., NON=NONNATSP, DARSC=DARSCCLSP, etc.). For Piedmont correlations, two sites with extremely outlying fish-metric values were excluded: one site for MINSP and one site for GENPRP and TOLPRP. Similarly, two mountain sites with extreme DARSCCLSP were excluded. See Methods for further explanation.

Coastal Plain (N = 12) ¹													
	NON	MINS	SUN	DARSC	GEN	INV	BINV	CARN	TOLP	MINSI	MINP	VMAN	AGE
NATSP	.60	.71			.42	-.55	.55	.38		-	-	-.70	.68
NONNATSP		.37	.48			-.44	.33	.37		-	-	-.51	.55
MINSP				0.36		-.63	.61	.31		-	-	-.78	.67
SUNSP					.38		-.44	.32	-.31	-	-		
DARSCISP										-	-		
GENPRP								-.34		-	-		
INVPRP								-.84	.38	-	-	.79	-.69
BINVPRP									.55	-	-	-.55	.73
CARNPRP									-.37	-	-	-.48	.49
VMANPRP										-	-		-.80
AGE3SP										-	-		

¹ If $|r_s| = 0.84$, $P = 0.0006$
if $0.83 \geq |r_s| > 0.70$, $0.001 < P < 0.01$;
if $0.70 \geq |r_s| > 0.50$, $0.01 < P < 0.10$;
if $0.50 \geq |r_s| > 0.30$, $0.10 < P < 0.33$

Piedmont (N=47)²

	NON	MINS	SUN	DARSC	GEN	INV	BINV	CARN	TOLP	MINSI	MINP	VMAN	AGE3
NATSP		.60		.64					-.35				.51
NONNATSP		.34			.32								
MINSP				.49	.60			-.49		.43	.44	-.51	.31
SUNSP					-.40			.55		-.40	-.47	.42	
DARSCISP									-.33		.34	-.41	
GENPRP								-.42		.33	.34		
INVPRP							.38				.37		
BINVPRP										.47			
CARNPRP									0.33		-.51	.55	
TOLPRP											-.41	.75	-.38
MINSIMPRP											.52	-.42	
MINPRP												-.73	
VMANPRP													-.52
AGE3SP													

² If $|r| > 0.53$, $P < 0.0001$;
if $0.53 > |r| > 0.47$, $0.0001 < P < 0.001$;
if $0.47 \geq |r| > 0.36$, $0.001 < P < 0.01$;
if $0.36 \geq |r| > 0.30$, $0.01 < P < 0.05$

Mountain (N=45)^a

	NON	MINS	SUN	DARSC	GEN	INV	BINV	CARN	TOLP	MINSI	MINP	VMAN	AGE3
NATSP		.86	.33	.48							.38		.49
NONNATSP			.60									.36	
MINS				.37				-.41			.40		.35
SUNSP								.39				.33	
DARSCISP													
GENPRP								-.54	.59				
INVPRP							.44		.47			.31	
BINVPRP										.35	-.31	.42	
CARNPRP											-.31		
TOLPRP											-.66	.49	
MINSIMP													
MINPRP													-.49
VMANPRP													
AGE3SP													

^a If $|r| > 0.52$, $P < 0.0001$;
if $0.52 \geq |r| > 0.46$, $0.0001 < P < 0.001$;
if $0.46 \geq |r| > 0.37$, $0.001 < P < 0.01$;
if $0.37 \geq |r| > 0.30$, $0.01 < P < 0.05$

Table 6. Summary of canonical correlations (CC) of selected fish metrics, for Piedmont or for mountain sites. "CAN1" to "CAN3" are the first through third CCs; "FSH1" to "FSH3" and "HAB1" to "HAB3" are the fish and habitat variates, respectively, of each CC. For each CC, "RED" (=redundancy) is the variance in fish metrics accounted for by the habitat variate; total redundancy (TOTAL) is the variance in fish metrics accounted for by all (=4) of the habitat variates. Wilks's lambda (W), its F-test approximation (F), significance (P), degrees of freedom (DF=degrees for numerator, degrees for denominator), and canonical correlation coefficient (r_c) are shown for successive CCs that accounted for up to > 0.90 of TOTAL. For individual fish metrics or habitat variables of each CC, "FC1" to "FC3" are the standardized function coefficients; numbers under variate names are the standardized intraset and interest (for fish metrics only) structure coefficients for each CC. For fish metrics, squares of the interest structure coefficients were summed to yield "COM1" to "COM3" (=communality), which is the accumulated amount of each fish metric's variance accounted for by each successive habitat variate. Number of sites is "N". Two Piedmont sites with extremely outlying fish-metric values were excluded: one site for GENPRP and TOLPRP, and one for MINSP. Similarly, two mountain sites with extreme DARSCLSP were excluded. See Table 3 for fish-metric codes and Methods for further explanation of metrics and habitat variables. "Wtrshd." = Waterbed and "w." = woody.

	Piedmont (N=47)									
	DF	W	F	P	r_c			TOTAL		
					FC1	FC2	FC3	FSH1	FSH2	FSH3
CAN1	28,131	0.26	2.11	0.0026	0.66	0.152		0.45	-0.01	-0.02
CAN2	18,105	0.47	1.81	0.0328	0.59	0.061		0.61	-0.17	0.07
CAN3	10,76	0.71	1.42	0.1884	0.48	0.016		0.24	-0.46	0.11
	FC1	FC2	FC3	FSH1	FSH2	FSH3		HAB1	HAB2	HAB3
NATSP	-0.13	-0.36	-0.45	0.67	-0.01	-0.04		0.45	-0.01	-0.02
MINSP	1.13	0.47	0.22	0.92	-0.28	0.14		0.61	-0.17	0.07
GENPRP	-0.19	-0.60	0.31	0.36	-0.78	0.22		0.24	-0.46	0.11
CARNPRP	-0.10	0.53	0.96	-0.50	0.48	0.36		-0.33	0.28	0.18
TOLPRP	-0.48	-0.81	0.53	-0.49	-0.54	0.18		-0.32	-0.32	0.09
VMANPRP	0.50	0.32	-0.79	-0.56	-0.12	-0.21		-0.37	-0.07	-0.10
AGEESP	0.24	0.16	0.62	0.46	0.06	0.44		0.30	0.03	0.21
								0.20	0.20	0.20
								0.37	0.40	0.40
								0.06	0.27	0.28
								0.11	0.19	0.22
								0.10	0.21	0.21
								0.14	0.14	0.15
								0.09	0.09	0.14
Bankside w.cover	-0.82	-0.20	-0.31					-0.89	0.02	-0.05
Instream w.cover	-0.45	0.48	0.30					-0.53	0.71	0.26
Maximum depth	0.29	0.27	0.77					-0.04	0.37	0.79
Wtrshd.as forest	0.28	0.68	-0.60					0.14	0.82	-0.51

Mountain (N=45)

	DF	W	F	P	r _c	RED	TOTAL					
CAN1	32,123	0.17	2.33	0.0005	0.74	0.111	0.216					
CAN2	21,98	0.39	1.82	0.0263	0.65	0.071						
CAN3	12,70	0.68	1.48	0.2597	0.50	0.022						
	FC1	FC2	FC3	FSH1	FSH2	FSH3	HAB1	HAB2	HAB3	COM1	COM2	COM3
MINSP	0.27	0.76	-0.45	-0.19	0.54	-0.01	-0.14	0.35	0.00	0.02	0.14	0.14
DARSCLSP	-0.32	-0.67	0.70	-0.11	-0.23	0.53	-0.09	-0.15	0.27	0.01	0.03	0.10
BINVPRP	0.59	0.23	-0.04	0.73	-0.11	-0.06	0.54	-0.07	0.00	0.30	0.30	0.30
CARNPRP	0.01	0.08	-0.54	0.42	-0.02	-0.14	0.31	-0.01	-0.07	0.10	0.10	0.10
TOLPRP	-0.74	-0.67	-0.85	0.18	-0.55	-0.49	0.14	-0.36	-0.25	0.02	0.15	0.21
MINPRP	-0.49	0.09	-0.66	-0.53	0.54	0.10	-0.40	0.35	0.05	0.16	0.28	0.28
VMANPRP	0.52	0.07	0.24	0.69	-0.11	0.03	0.51	-0.07	0.02	0.26	0.27	0.27
AGE3SP	-0.39	0.08	0.49	-0.23	0.61	0.39	-0.17	0.40	0.20	0.03	0.19	0.23
Bankside w. cover	-0.33	0.92	-0.16				-0.37	0.92	-0.09			
Instream cover	0.86	0.29	-0.48				0.75	0.14	-0.64			
Maximum depth	0.33	0.14	0.48				0.33	0.01	0.61			
Wtrshd. as forest	0.47	0.28	0.56				0.26	0.38	0.68			

Table 7. Summaries of (1) a rerun canonical correlation analysis (CCA) of and (2) a descriptive discriminant analysis of selected fish metrics, for 48 or 47 Piedmont sites, respectively. For the CCA, see Table 6 for explanations and definitions of terms; the analysis depicted here is identical to that in Table 6 except "MINSP" and "AGE3SP" were omitted, and no sites were excluded. The discriminant analysis is analogous to a CCA in which one set of variables is a set of dummy variables representing group membership of each site (i.e., east-, west-, or moderately-disturbed). For the discriminant analysis, "FISH1" and "FISH2" are canonical composites (variables), of the fish metrics, that define the space in which 26 east-, 13 west-, and 8 moderately-disturbed sites are maximally separated (also see Figure 4). "FC1" and "FC2" are the standardized function coefficients as in CCA. Numbers in columns labelled "FISH1" and "FISH2" are the intraset structure coefficients, which equal the Pearson correlations between each fish metric and each canonical composite (variable). Means of "FISH1" and "FISH2" for each group of sites are also shown under "Least-", "Moderately-", and "Most-". As in the original CCA, two sites with extremely outlying fish-metric values were excluded from the discriminant analysis: one site for GENPRP and TOLPRP, and one for MINSP. For the rerun CCA only one site with extreme GENPRP and TOLPRP was excluded.

	Canonical correlation analysis (N=48)									
	DF	W	F	P	r _c			TOTAL		
					FSH1	FSH2	FSH3	HAB1	HAB2	HAB3
CAN1	20,130	0.36	2.37	0.0020	0.62	0.122	0.225			
CAN2	12,106	0.58	1.99	0.0317	0.49	0.074				
CAN3	6,82	0.77	1.90	0.0910	0.44	0.017				
	FC1	FC2	FC3	FSH1	FSH2	FSH3				
NATSP	-0.35	0.56	0.17	-0.45	0.72	0.28	-0.28	0.35	0.12	0.22
GENPRP	-0.48	-0.22	0.26	-0.87	-0.19	0.14	-0.54	-0.10	0.06	0.31
CARNPRP	0.47	-0.22	1.13	0.70	-0.27	0.35	0.44	-0.13	0.15	0.23
TOLPRP	-0.44	-0.85	0.48	-0.07	-0.83	-0.17	-0.04	-0.41	-0.07	0.17
VMANPRP	0.17	0.44	-1.35	0.34	-0.46	-0.44	0.21	-0.23	-0.19	0.13
Bankside w. cover	0.32	-0.56	-0.42				0.53	-0.60	-0.17	
Instream w. cover	0.63	-0.34	0.12				0.87	-0.20	0.21	
Maximum depth	0.02	0.18	0.95				0.29	-0.05	0.89	
Wtrshd. as forest	0.44	0.84	-0.25				0.64	0.72	-0.22	

Discriminant analysis (N=47)

	DF	W	F	P	Least- (N=26)	Moderately- (N=8)	Most- (N=13)
FISH1	14,76	0.44	2.76	0.0024	-0.76	0.56	1.18
FISH2	6,39	0.79	1.70	0.1476	-0.10	1.04	-0.44
	FC1	FC2		FISH1	FISH2		
NATSP	-0.49	-0.51		-0.06	0.50		
MINSF	0.01	1.40		0.22	0.58		
GENPRP	0.79	-0.51		0.68	0.03		
CARNPRP	-0.06	-0.28		-0.21	-0.33		
TOLPRP	0.57	-0.77		0.39	-0.40		
VMANPRP	0.06	1.39		0.03	-0.27		
AGE3SP	0.79	0.81		0.25	0.55		

Table 8. Fish metrics judged especially useful for Piedmont or Mountain versions of the IBI for Virginia warmwater Wadeable streams. "Proportion" refers to proportion of individuals.

IBI version	Metrics
Piedmont	Proportion as generalist feeders
	Proportion as specialist carnivores
	Proportion as members of tolerant species
Mountain	Number of native minnow species
	Proportion as lithophils
	Number of late-maturing (> 2 yr.) species
	Proportion as members of tolerant species

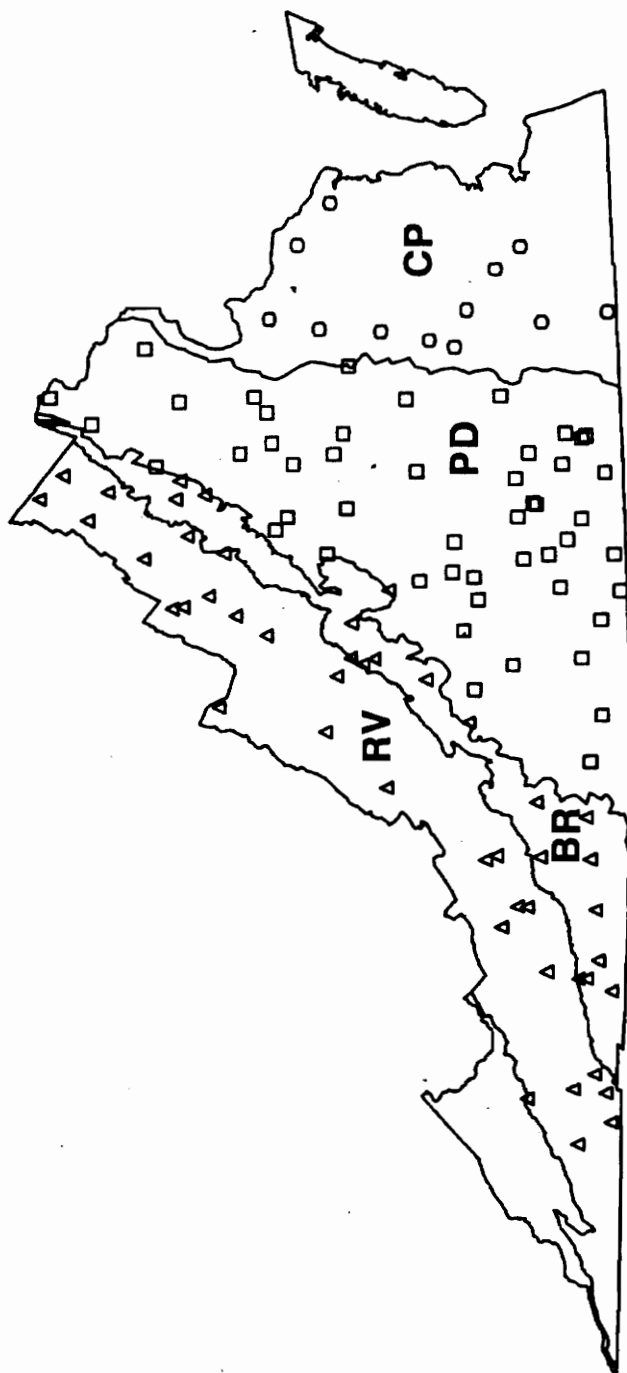


Figure 1. Map of Virginia showing locations of 108 sample sites in three physiographic regions: Coastal Plain (CP), Piedmont (PD), and Mountain = Blue Ridge (BR) + Ridge and Valley (RV). For CP, number of sites (N)=12 (circles); for PD, N=49 (squares); and for MT, N=47 (triangles).

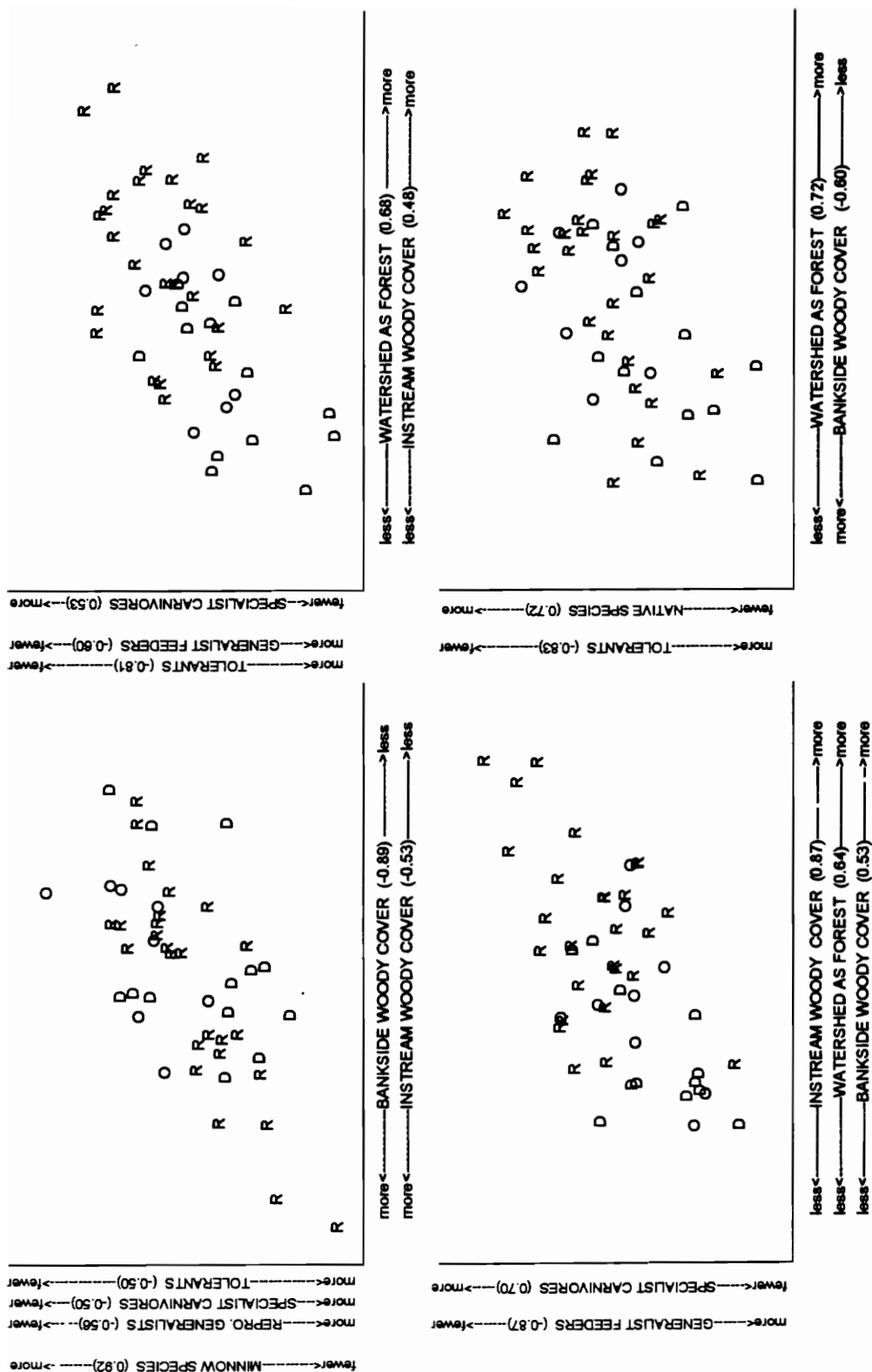


Figure 2. Plots of 47 (upper panels) or 48 (lower panels) Piedmont sites in the canonical spaces defined by the first (left) and second (right) canonical correlations between fish metrics and four habitat variables. Least- (R), most- (D), and moderately- (O) disturbed sites are for an analysis that excluded number of native minnow species and number of late-maturing species, two metrics shown to be non-linearly related to disturbance. Intraspecific coefficients are shown after each metric name. Vertical- and horizontal-axis scales are identical among plots.

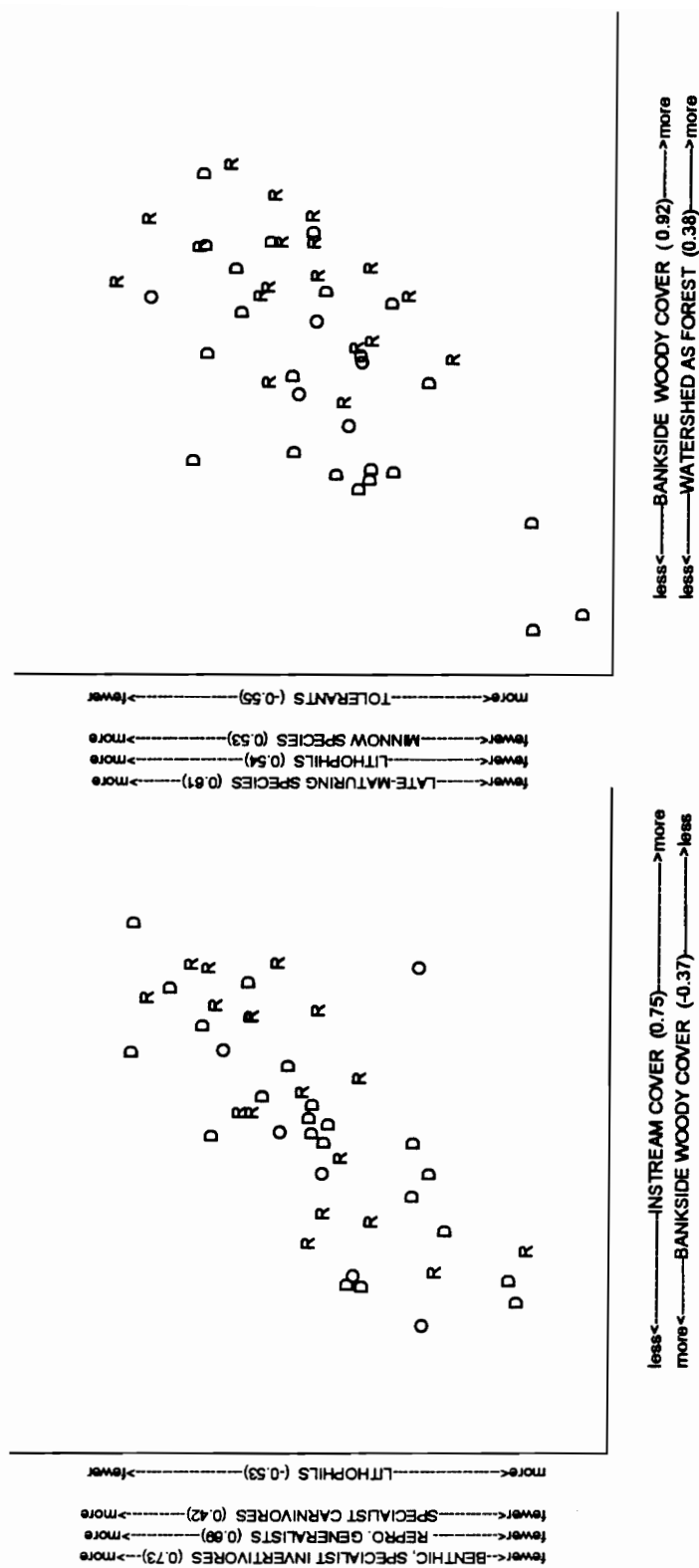
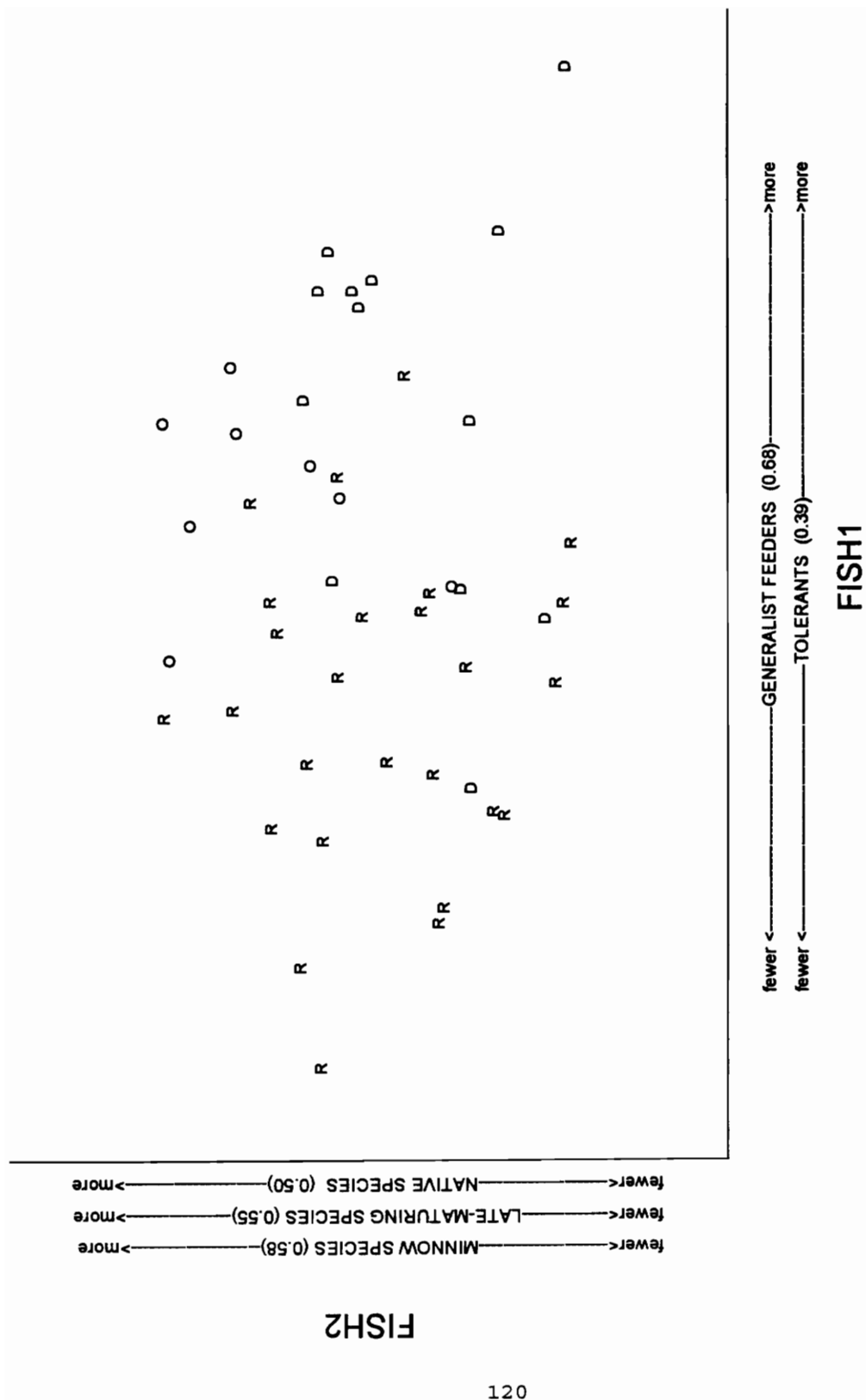


Figure 3. Plots of 45 Mountain sites in the canonical spaces defined by the first (left) and second (right) canonical correlations between fish metrics and four habitat variables. Least- (D), most- (R), and moderately- (O) disturbed sites are shown. Intraset structure coefficients are shown after each variable name. Vertical- and horizontal-axis scales are identical among plots.



Appendix A. Taxonomic, trophic, and reproductive classifications of fish species that were sampled at 143 Wadeable Stream sites in Virginia, 1987-1990. Trophic groups (TRO) are: DAH=detritivore/algivore/herbivore, AHI=algivore/herbivore/invertivore, INV=invertivore, IP=invertivore/piscivore, and PIS=piscivore or parasite. Number of food types (NUM) shows number of following categories from which a species feeds: (a) detritus, (b) algae or vascular plants, (c) invertebrates, or (d) fish or blood. Benthic feeders (BEN=foods strongly associated with stream bottom) and generalist feeders (GEN=feeds on more than 2 food types and foods are not associated strongly with the stream bottom) are shown with a "+". Female age at reproduction (in years) is shown as "AGE". Spawning substrates (SUB) are: NON=none or pelagic, VEG=vegetation or organic debris, VAR=not restricted to particular substrates, and MIN=unsilted mineral substrates from sand to boulder. Nest preparers or parental-care givers (CAR), mineral-substrate, simple spawners (LIT), and nest associates (ASS) are shown with a "+". Simple spawners are species that exhibit no nest preparation or parental guarding/care (i.e., CAR not a "+"). Nest associates are species that are known to spawn on different-species minnow or sunfish nests. Tolerant species are shown as "T" and intolerant species as "I" for variable, TOL. See **Methods**, Chapter 1 for further explanation.

	TRO	NUM	BEN	GEN	AGE	SUB	CAR	LIT	ASS	TOL
Petromyzontidae										
<i>Ichthyomyzon greeleyi</i>	DAH	2	+		6	MIN	+			I
<i>Lampetra aepyptera</i>	DAH	2	+		6	MIN	+			
<i>Lampetra appendix</i>	DAH	2	+		5	MIN	+			
<i>Petromyzon marinus</i>	PIS	1			9	MIN	+			
Amiidae										
<i>Amia calva</i>	PIS	1			4	VEG	+			
Clupeidae										
<i>Alosa aestivalis</i>	INV	1			4	NON				
<i>Dorosoma cepedianum</i>	AHI	2			2	VAR				T
Salmonidae										
<i>Oncorhynchus mykiss</i>	IP	2			1	MIN	+			
<i>Salmo trutta</i>	IP	2			1	MIN	+			
<i>Salvelinus fontinalis</i>	IP	2			2	MIN	+			I
Anguillidae										
<i>Anguilla rostrata</i>	IP	2			5	NON				
Esocidae										
<i>Esox americanus</i>	PIS	1			2	VEG				
<i>Esox lucius</i>	PIS	1			2	VEG				
<i>Esox niger</i>	PIS	1			2	VEG				
Umbridae										
<i>Umbra pygmaea</i>	INV	1			1	VAR	+			

	TRO	NUM	BEN	GEN	AGE	SUB	CAR	LIT	ASS	TOL
Cyprinidae										
<i>Campostoma anomalum</i>	DAH	2	+		2	MIN	+			
<i>Clinostomus funduloides</i>	INV	1			2	MIN		+	+	
<i>Cyprinus carpio</i>	AHI	4		+	3	VAR				T
<i>Cyprinella analostana</i>	INV	2			1	VAR				
<i>Cyprinella galactura</i>	INV	2			2	VAR				
<i>Cyprinella spiloptera</i>	INV	3		+	1	VAR				
<i>Erimystax insignis</i>	AHI	3	+		1	MIN		+		
<i>Exoglossum laurae</i>	INV	1			2	MIN	+			I
<i>Exoglossum maxillingua</i>	INV	1			2	MIN	+			
<i>Hybognathus regius</i>	DAH	2			2	VAR				
<i>Luxilus albeolus</i>	INV	1			1	MIN		+	+	
<i>Luxilus cerasinus</i>	INV	2			2	MIN		+	+	
<i>Luxilus chrysocephalus</i>	INV	4		+	2	MIN		+	+	
<i>Luxilus coccogenis</i>	INV	1			2	MIN		+	+	
<i>Luxilus cornutus</i>	INV	4		+	2	MIN		+	+	
<i>Lythrurus ardens</i>	INV	3		+	1	MIN		+	+	
<i>Lythrurus lirus</i>	INV	1			1	MIN		+		
<i>Margariscus margarita</i>	INV	3		+	1	MIN	+			
<i>Nocomis leptocephalus</i>	AHI	3		+	3	MIN	+			
<i>Nocomis micropogon</i>	INV	3		+	3	MIN	+			
<i>Nocomis platyrhynchus</i>	INV	3		+	3	MIN	+			
<i>Nocomis raneyi</i>	INV	3		+	3	MIN	+			
<i>Notemigonus crysoleucas</i>	AHI	2			2	VAR			+	T
<i>Notropis alborus</i>	INV	2			1	MIN		+		
<i>Notropis altipinnis</i>	INV	2			1	VAR				
<i>Notropis amblops</i>	INV	1	+		1	MIN		+		I
<i>Notropis amoenus</i>	INV	1			1	MIN		+	+	
<i>Notropis buccatus</i>	AHI	3	+		1	MIN		+		T
<i>Notropis chalybaeus</i>	INV	2			1	VAR				
<i>Notropis chiliticus</i>	INV	2			1	MIN		+	+	
<i>Notropis hudsonius</i>	INV	2			2	VAR				
<i>Notropis leuciodus</i>	INV	1			1	MIN		+	+	
<i>Notropis photogenis</i>	INV	2			1	MIN		+		
<i>Notropis procne</i>	INV	2			2	MIN		+	+	

	TRO	NUM	BEN	GEN	AGE	SUB	CAR	LIT	ASS	TOL
<i>Notropis rubricroceus</i>	INV	2			1	MIN		+	+	
<i>Notropis rubellus</i>	INV	1			1	MIN		+	+	
<i>Notropis scabriceps</i>	INV	1			2	MIN		+		
<i>Notropis semperasper</i>	INV	1			2	MIN		+		
<i>Notropis spectrunculus</i>	INV	1			1	MIN		+		
<i>Notropis stramineus</i>	INV	3		+	1	MIN		+		
<i>Notropis telescopus</i>	INV	1			2	MIN		+		
<i>Notropis volucellus</i>	INV	3		+	1	VAR				
<i>Phenacobius teretulus</i>	INV	1	+		2	MIN		+		
<i>Phoxinus oreas</i>	DAH	3		+	1	MIN		+	+	
<i>Pimephales notatus</i>	AHI	3		+	1	VAR	+			T
<i>Pimephales promelas</i>	AHI	3		+	1	VAR	+			T
<i>Rhinichthys atratulus</i>	INV	3		+	2	MIN		+	+	T
<i>Rhinichthys cataractae</i>	INV	2			2	MIN		+		
<i>Semotilus atromaculatus</i>	IP	4		+	1	MIN	+			T
<i>Semotilus corporalis</i>	IP	4		+	2	MIN	+			
Catostomidae										
<i>Catostomus commersoni</i>	AHI	3	+		3	MIN		+		T
<i>Erimyzon oblongus</i>	INV	3	+		2	VAR				
<i>Hypentelium nigricans</i>	INV	2	+		3	MIN		+		
<i>Hypentelium roanokense</i>	INV	3	+		2	MIN		+		
<i>Moxostoma anisurum</i>	INV	3	+		5	MIN		+		
<i>Moxostoma ariommum</i>	INV	2	+		3	MIN		+		I
<i>Moxostoma cervinum</i>	INV	3	+		2	MIN		+		
<i>Moxostoma duquesnei</i>	INV	3	+		3	MIN		+		
<i>Moxostoma erythrurum</i>	INV	3	+		4	MIN		+		
<i>Moxostoma hamiltoni</i>	AHI	3	+		3	MIN		+		
<i>Moxostoma macrolepidotum</i>	INV	3	+		4	MIN		+		
<i>Moxostoma pappillosum</i>	INV	3	+		4	MIN		+		
<i>Moxostoma rhothoecum</i>	AHI	3	+		3	MIN		+		I
Ictaluridae										
<i>Ameiurus brunneus</i>	IP	3		+	3	VAR	+			
<i>Ameiurus catus</i>	IP	3		+	3	MIN	+			
<i>Ameiurus melas</i>	IP	3		+	2	MIN	+			T
<i>Ameiurus natalis</i>	IP	3		+	2	VAR	+			

	TRO	NUM	BEN	GEN	AGE	SUB	CAR	LIT	ASS	TOL
<i>Ameiurus nebulosus</i>	IP	3		+	3	VAR	+			
<i>Ameiurus platycephalus</i>	IP	3		+	3	VAR	+			
<i>Ictalurus punctatus</i>	IP	3		+	3	VAR	+			
<i>Noturus flavus</i>	INV	2			3	MIN	+			
<i>Noturus gyrinus</i>	INV	1	+		2	VAR	+			
<i>Noturus insignis</i>	INV	2			3	MIN	+			
Aphredoderidae										
<i>Aphredoderus sayanus</i>	INV	2			1	VAR	+			
Cyprinodontidae										
<i>Fundulus catenatus</i>	INV	1			1	MIN		+		
<i>Fundulus diaphanus</i>	INV	1			1	VAR				
<i>Fundulus heteroclitus</i>	INV	2			1	VAR				
<i>Fundulus rathbuni</i>	INV	1			1	VAR				
Poeciliidae										
<i>Gambusia affinis</i>	INV	1			0	NON				T
Cottidae										
<i>Cottus bairdi</i>	INV	1	+		2	VAR	+			
<i>Cottus baileyi</i>	INV	1	+		2	VAR	+			
<i>Cottus carolinae</i>	INV	1	+		2	VAR	+			
<i>Cottus cognatus</i>	INV	1	+		2	VAR	+			
<i>Cottus girardi</i>	INV	1	+		2	VAR	+			
<i>Cottus sp.</i>	INV	1	+		2	VAR	+			
<i>Cottus sp.</i>	INV	1	+		2	VAR	+			
Moronidae										
<i>Morone americana</i>	IP	2			2	MIN		+		
Centrarchidae										
<i>Acantharchus pomotis</i>	INV	2			2	VAR	+			
<i>Ambloplites cavifrons</i>	IP	2			2	MIN	+			I
<i>Ambloplites rupestris</i>	IP	2			2	MIN	+			
<i>Centrarchus macropterus</i>	INV	2			2	VAR	+			
<i>Enneacanthus gloriosus</i>	INV	1			2	VAR	+			
<i>Enneacanthus obesus</i>	INV	1			2	VAR	+			
<i>Lepomis auritus</i>	IP	2			2	MIN	+			
<i>Lepomis cyanellus</i>	IP	2			1	VAR	+			T
<i>Lepomis gibbosus</i>	INV	1			1	VAR	+			

	TRO	NUM	BEN	GEN	AGE	SUB	CAR	LIT	ASS	TOL
<i>Lepomis gulosus</i>	IP	2			1	VAR	+			
<i>Lepomis macrochirus</i>	INV	1			1	VAR	+			T
<i>Lepomis megalotis</i>	INV	1			2	MIN	+			
<i>Lepomis microlophus</i>	INV	1			2	VAR	+			
<i>Micropterus dolomieu</i>	IP	2			2	MIN	+			
<i>Micropterus punctulatus</i>	IP	2			2	VAR	+			
<i>Micropterus salmoides</i>	PIS	1			2	VAR	+			
<i>Pomoxis annularis</i>	IP	2			2	VAR	+			
<i>Pomoxis nigromaculatus</i>	IP	2			2	VAR	+			
<i>Percidae</i>										
<i>Etheostoma blennioides</i>	INV	1	+		2	VAR				
<i>Etheostoma caeruleum</i>	INV	1	+		1	MIN		+		
<i>Etheostoma collis</i>	INV	1	+		1	VAR				
<i>Etheostoma flabellare</i>	INV	1	+		2	MIN	+			T
<i>Etheostoma fusiforme</i>	INV	1	+		1	VAR				T
<i>Etheostoma kanawhae</i>	INV	1	+		2	MIN		+		
<i>Etheostoma longimanum</i>	INV	1	+		1	MIN	+			
<i>Etheostoma nigrum</i>	INV	1	+		1	VAR	+			T
<i>Etheostoma olmstedii</i>	INV	1	+		1	VAR	+			T
<i>Etheostoma podostemone</i>	INV	1	+		1	MIN	+			
<i>Etheostoma rufilineatum</i>	INV	1	+		1	MIN		+		
<i>Etheostoma serrifer</i>	INV	1	+		1	VAR				
<i>Etheostoma simoterum</i>	INV	1	+		1	VAR				
<i>Etheostoma vitreum</i>	INV	1	+		1	VAR				
<i>Etheostoma zonale</i>	INV	1	+		1	VAR				
<i>Percina caprodes</i>	INV	1	+		2	MIN		+		
<i>Percina gymnocephala</i>	INV	1	+		2	MIN		+		
<i>Percina notogramma</i>	INV	1	+		2	MIN		+		
<i>Percina oxyrhynchus</i>	INV	1	+		1	MIN		+		
<i>Percina peltata</i>	INV	1	+		2	MIN		+		
<i>Percina roanoka</i>	INV	1	+		2	MIN		+		
<i>Perca flavescens</i>	IP	2			3	VAR				

Vita

Roy A. Smogor was born in 1959. He went to school for a very long time and ended up with this thesis in 1996.