The New England Wadeable Stream Survey (NEWS): Development of Common Assessments in the Framework of the Biological Condition Gradient

Prepared for:

USEPA Office of Science and Technology
Washington, DC
Susan K. Jackson
Work Assignment Manager

USEPA Office of Watersheds
Oceans and Wetlands
Washington, DC
Susan Holdsworth
Work Assignment Manager

Prepared by:

Hilary Snook
USEPA New England Regional Lab
Chelmsford, MA

Susan P. Davies
Maine DEP, Bureau of Water Quality Control
State House St. 17
Augusta, ME 04333

Jeroen Gerritsen
Tetra Tech, Inc.
400 Red Brook Blvd., Suite 200
Owings Mills, MD 21117

Benjamin K. Jessup
Tetra Tech, Inc.
15 State St., Suite 301
Montpelier, VT 05602

Richard Langdon
Vermont DEC
103 South Main St.
Waterbury, VT 05676

David Neils
New Hampshire DES
Concord, NH

Ernest Pizutto
Connecticut DEP
Bureau of Water Management
79 Elm Street
Hartford, CT 06106

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EXECUTIVE SUMMARY

In 2000, the United Sates Environmental Protection Agency implemented a stream monitoring project across the six New England states in order to uniformly assess the ecological condition of three hundred randomly selected wadeable stream segments across the region. The New England Wadeable Streams (NEWS) project was a collaborative effort between the USEPA Region 1, the USEPA Atlantic Ecology Division in Narragansett, Rhode Island, USEPA Office of Research and Development in Corvallis, Oregon, The New England Interstate Water Pollution Control Commission, five of six New England state environmental agencies, and key members of academia.

Randomized probability designs were used for selecting wadeable monitoring sites among second order and higher stream systems (Strahler 1964)[1] and for utilizing various geographic scales that would meet the needs of state and federal resource agencies. A large scale design encompassing the New England region was initially developed for reporting on regional conditions. State designs were confined to state geopolitical boundaries and “nested” within the regional probabilistic design with the intent of supporting 305b integrated assessment reporting requirements for individual states.

State and EPA field sampling crews collected stream macro-invertebrates and fish for biological assessments, evaluated in-stream and riparian habitat conditions, and collected ambient water samples for chemical analysis. Field methods for collecting samples and evaluating habitat conditions were consistent among partners with the exception of macro-invertebrate collections. NEWS monitoring locations were sampled for invertebrates using an adapted version of the Mid-Atlantic Integrated Assessment (MAIA) multi-habitat approach. Two states paired their macro-invertebrate methods alongside NEWS methods in order to make some determinations of comparability, while maintaining consistency in their 305b reporting efforts from previous years. The NEWS methods were applied at approximately three hundred locations across the region over the three year project period. CT and VT completed probability based assessments within their state boundaries, providing a statistical level of confidence acceptable for assessment purposes. Other participating states monitored at a lesser intensity, but at a level to compare their state monitoring methods with those of the NEWS project.

A Biological Condition Gradient (BCG) for New England was presented as a potentially favorable tool for categorizing levels of ecological condition and would serve as a potential vehicle for evaluating resource condition from samples collected with NEWS and a variety of state sampling methods. Sixty-six high gradient sites were initially selected for development of the BCG. BCG model defines “Tiers” of ecological condition within a resource population based upon a gradient of known stressors in a region. The model is firmly grounded in ecological science, and provides a means from which more refined and iterative resource management decisions can be made in working towards the objective of attainment of aquatic life uses.

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[1] Strahler, A.N., Quantitative geomorphology of drainage basins and channel networks, section 4-II, in *Handbook of Applied Hydrology,* ed. By V.T. Chow, pp. 4-39, 4-76, McGraw-Hill, New York, 1964.

The New England workgroup assigned sites to all but the uppermost BCG tier (Tier 1) that represents “pristine”, or least disturbed conditions. The explicit definition of a Tier 1 level initiated much discussion as to exactly what it constituted, and many biologists were reluctant to assign any site locations into this category. It was later agreed that Tier 1 and Tier 2 sites might be indiscernible based solely on biology, and that surrounding land use characteristics should probably be incorporated to discriminate between the two. The biological condition gradient was subsequently applied to the development of a quantitative fuzzy set model; a model that incorporates human decision making into quantitative “rules” that can be consistently applied in the context of a quantitative result. The model was developed in order to provide consistent assessments of resource condition across the region, based on the expertise of regional biologists. Workgroup biologists convened regularly to review and analyze the NEWS data and assign site taxa to specific attribute categories as defined in the BCG in order to develop the rules. The rules quantified the collective expertise from the regional biologists for input to the model.

Because of the close similarity between the macroinvertebrate NEWS regional sampling methods and CTDEP’s macroinvertebrate sampling methodology compared to other states, a dataset from the state of Connecticut that included the full range of BCG conditions was used for the fuzzy model calibration. The NEWS site data from within the state of Connecticut was used as a hold out dataset from which to test the model. After discrepancies were reconciled between the calibration and test datasets, the model was applied to sixty-six NEWS sites throughout the region, all with stream gradients at or above one percent. NEWS sites that were considered low gradient were not included, due to biologists concern over the biological comparability between highly dissimilar stream types. Assessment of low gradient systems is of high interest among regional biologists, but the decision was made to focus on higher gradient systems first.

Designated physical and chemical stressor categories, deemed to be anthropogenic in nature, displayed a population density threshold of approximately five people per square mile. While considered somewhat coarse due to discrepancies in census block information for differing population densities, it does demonstrate that physical and ecological impairment begins to occur at very low population densities; population density and subsequent land use activities may be the dominant stressor source. Some sites were identified as being chemically stressed from one or more of the various chemical indicators (high dissolved aluminum, copper, lead, low pH). Stressors that appear to be more region-wide and ubiquitous in nature are mercury, commonly found at 1-7 µg/l, well above the EPA chronic criteria threshold, and sediment. Sediment loading in streams was assessed using EPA’s semi-quantitative habitat assessment forms (RBP III) and appears to be a common stressor across the region. This observation is also consistent with findings from the National Wadeable Streams Assessment project (WSA). While the impacts of sedimentation and subsequent stream embedding are the same across the region, the sources are different. Field observations have revealed that historic legacy effects from more industrialized and urban areas are found to be more common in the southern parts of New England, while logging operations, ski areas, and agriculture commonly found farther north and west increase stream flows and road and stream bank erosion.

Results from the BCG indicated a distinct South to North stressor gradient for biological condition, with reference-like streams in the NEWS dataset occurring predominantly in the northern states of NH and ME. BCG attributes were similar among higher quality sites (Tier 2) in Connecticut and northern New England states, but the taxonomic composition was different and dominated by sensitive/intolerant species. Sites selected for BCG model development were predominantly located in the northern New England states, and were associated with steeper stream gradients as would be expected based upon the topography and surface geology of the region. The model had a tendency to assign better BCG condition tiers than what individual regional biologists assigned to the same sites. Despite the difference, the model provided consistency of assessment across all sites and holds promise as a reliable tool for regional assessments of resource condition. Further refinement could be accomplished with consistent sampling methods applied across the region.

A methods comparability exercise was initiated as part of the NEWS project for determining if common assessment endpoints could be attained among the various macroinvertebrate collections and subsampling methods used in the project along with common metrics. Attainment of common endpoints among data sets collected by different sampling methods would allow transferability of data among agencies and the ability to aggregate state data for regional assessment and reporting purposes. The exercise demonstrated consistencies, but transferability of datasets was limited and not recommended; a different approach is warranted in order to provide compatibility between regional and state assessments. BCG tier assignments consistently demonstrated a prominent high bias (better ecological quality) towards samples collected with state methods opposed to the NEWS methods. The result was attributed to the familiarity with traditional state methods and the specific riffle habitats that are targeted for assessment, whereas the NEWS multi-habitat approach often encompassed meso-habitat types that are populated by less sensitive species and relatively pollutant indifferent taxa.

Fish collection methods used by the states and EPA were identical, and an attempt was made to determine if a regional fish IBI could be developed for New England based on the project’s data, or whether an existing state’s IBI could be applied regionally. Vermont’s mixed water and cold water IBI’s were used to assist biologists in making BCG tier assignments and also tested to determine if either was applicable regionally. Vermont’s cold water IBI was applied to smaller cold water stream systems where expectations of finding from one to four fish species existed. Vermont’s nine-metric mixed water IBI included an additional metric for the proportion of non-native species captured, as these species are considered problematic in New England, outside of the state of Vermont. Non-native species were found at 43 percent of the sites and consisted predominantly of bluegill, brown trout, and largemouth bass, respectively. The three species most encountered at sites were blacknose dace, Eastern brook trout, and white sucker, respectively. Despite the presence of non-natives, which scored negatively for the IBI, 86 percent of 280 evaluated sites were still considered to be in “good” condition based on the BCG rankings. Comparisons between Vermont’s cold water IBI scores for the smaller high gradient stream systems showed IBI scores evenly distributed across the region, while for the same waterbodies the BCG tier assignments that rated excellent were found primarily in Vermont and New Hampshire. BCG assignments based on the fish data for Tier I and II sites were consistently lower than the IBI scores for the same sites, much like the invertebrate data. Fish IBI results more evenly distributed sites along the six condition tiers, while the BCG assigned sites more to “good” and “fair-poor” tiers. 71% of sites under BCG assignments fell in the “good and fair-poor range” while only 38% fell in these Tiers with scoring from the IBI. The mixed water IBI holds promise for use in the state of New Hampshire, but it is evident that to be applied elsewhere within New England, more and different metric development and testing will be warranted. Vermont's cold water IBI (CWIBI) however, could be applied in its present state in New Hampshire, Maine, and western Massachusetts.

The probabilistic design for the NEWS project led to the application of probability based assessments for the first time in 305(b) reporting for New England States. New Hampshire has utilized this design for determining aquatic life use support for 90 percent of its estimated wadeable stream miles based on macroinvertebrate IBI data. Of the streams that could be assessed 47% were considered fully supporting the state's aquatic life use designation, while 14% failed to meet the criteria. The remaining 39 percent of the stream miles that could be assessed were not included because they were low gradient systems for which the state's IBI is not applicable. Utilizing the BCG, the majority of sites were categorized as Tier 2 and Tier 3, with a minority in Tier 4 and 5. Tier 4 and 5 sites were located in the southern portion of the state, showing a similar south/north stressor gradient to the region-wide sites.

Connecticut DEP (CTDEP) utilized the NEWS project as a platform for meeting its 305(b) comprehensive reporting requirements and determining aquatic life use support. Utilizing a modified USEPA rapid bioassessment protocol (RBP III) as defined in the Consolidated Assessment Listing Methodology (CALM), attainment of aquatic life use support was determined and reported in the 2006 integrated assessment report. Using the state's assessment approach, 71% of the sites were fully supporting, 15% not supporting, and the remaining not included due to low gradient, non-riffle habitat. Utilizing the BCG, 19% of the sites were determined to be impaired (threshold halfway between Tier IV and V). Sixty-seven percent of the sites were determined to be fully supporting. 15%, 42%, and 10% of the sites were assigned to tiers II, III, and IV respectively. The same 14% were not assessed due to habitat limitations. The Tiered Aquatic Life use method increased assessment resolution within the fully supporting category for CTDEP.

This report represents the first time use of probability based survey data for incorporation into state integrated assessment reports in the region and demonstrates the benefits to state based water quality programs. The various products of the NEWS effort further demonstrate the utility and potential of large regional collaborative efforts between state and federal agencies, and the additional benefits that can be derived from close working relationships.

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This report is the product of five workshops hosted by the New England Interstate Water Pollution Control Commission and EPA New England Regional Laboratory. Core participants are listed in the table below. The participants in this effort invested significant time and commitment in the process. We are grateful for the hard work and enthusiasm that resulted in calibration of the Biological Condition Gradient to New England wadeable streams and the development of a predictive model.

LIST OF PARTICIPANTS
TIERED AQUATIC LIFE USE WORKSHOP

Name	Organization
David Armstrong (visitor)	USGS
Mike Beauchene	CT DEP
Doug Burnham	VT DEQ,
Susan Davies	ME DEP
Steve Fiske	VT DEP
Jeroen Gerritsen	Tetra Tech, Inc.
Jim Helsche (visitor)	EPA ORD, Narragansett Lab
Greg Hellyer (visitor)	EPA Region 1
Guy Hoffman	CT DEP
Ben Jessup	Tetra Tech, Inc.
Margaret Kearns (visitor)	MA Riverways
Rich Langdon	VT DEQ
David Neils	NH DES
Pete Nolan	EPA Region 1
Bob Nuzzo	MA DEP
Ernie Pizzuto	CT DEP
Todd Richardson	MA DEP
Hilary Snook	EPA Region 1
Leon Tsomides	ME DEP
Becky Weidman (visitor)	New England Interstate Water Pollution Control Commission

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1.0 Introduction

1.1 Problem Statement

In the late 1990's, criticism arose from various government and non-government organizations regarding the current efficiency and integrity of state and federal monitoring programs and their subsequent reporting accuracy on the condition of the Nation's waters. These criticisms consistently stated that state, regional, and national assessments of water quality could not be reliably determined or characterized due to monitoring program differences, and were not reliable sources for supporting water quality policy or for use in regulatory decision making (U.S. GAO 2000)[2]. Targeted monitoring efforts, different monitoring and analytical methods and index periods, and varying frequencies of sampling efforts among agencies were key factors as to why data could not be aggregated for consistent state, regional, or national reporting on resource condition. Current programs also demonstrated limitations for determining the geographic extent of problems and identifying those high quality resource waters potentially needing further protection. These differences among various agency monitoring programs limit EPA's ability to accurately make determinations on the efficiency of current federal water pollution control programs, appropriate allocations of Clean Water Act funds, the ability to identify national or regional trends in resource condition, or the ability to provide accurate reporting on water quality conditions at varying geographic and geopolitical scales.

State environmental agencies have been utilizing targeted monitoring approaches in their programs as the most efficient and value added use of program dollars. Targeted monitoring allows states flexibility in addressing suspect problem areas, and an ability to prioritize regulatory requirements such as discharge permitting and licensing, enforcement, known development and construction issues, and other activities. This traditional approach is logical from the standpoint of resource allocation among programs, but is limiting in its capacity for meeting some of the principle mandates of the Clean Water Act under section 305b, such as providing "a description of the water quality of all navigable waters," or "a description of the nature and extent of non-point sources of pollutants…" The 1997 USEPA guidelines on national 305(b) reporting highly recommended that states develop plans for providing valid comprehensive assessment coverage of their waters. The USEPA was designated to assist individual States, other jurisdictions, and participating tribes on the development of a design and reporting strategy for meeting this goal. The NEWS project was an initial step towards that end.

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[2] U.S. General Accounting Office. March 2000. *Water Quality – Key EPA and State Decisions Limited by Inconsistent and Incomplete Data.* Report to the Chairman, Subcommittee on Water Resources and Environment, Committee on Transportation and Infrastructure, House of Representatives.

1.2 Goals and Purpose of NEWS

The principal goal and purpose of the NEWS project was to provide unbiased regional and state level assessments of the ecological condition of wadeable streams across New England. A regional scale design would provide a large scale assessment of condition with utility for national water quality reporting purposes, and participating states would have the opportunity to incorporate state level probabilistic sampling designs into their current monitoring programs, testing its utility in supporting 305b requirements.

Another important goal of the NEWS effort was comparing individual state monitoring methods amongst one another for determining if differing methods resulted in different assessment outcomes. If assessment outcomes proved similar despite the various methods employed, then cross-state data could be pooled and resource condition assessed regionally with existing data. If State monitoring data proved not to be similar, then a unique region wide sampling methodology would have to be developed for future regional assessments to take place.

1.3 Reporting Audience

This report is directed to those individuals, organizations, and entities whose principal mission is the monitoring of lotic waterbodies, and those who are involved in the planning and management decision making processes that ultimately affect the use and sustainability of these resources. This report outlines a work in progress by regional scientists working intrinsically with the biology of running waters and the scientific method for decades. It is anticipated that through development of the products and working relationships evolving from this project, resource managers will have more refined tools from which to enhance existing monitoring programs and ultimately, formulate improved water quality policy.

1.4 Document Scope

This report is divided into several stand alone chapters and sections that document the initial processes of developing a comprehensive assessment methodology. The report compares the individual state and regional methods and their ability to move towards a common assessment endpoint. It also provides a dataset that summarizes biological, chemical, and habitat information on these waterbodies. Case studies from New Hampshire DES and Connecticut DEP demonstrate the utility of the design and its applicability to 305(b) reporting, as well as comparisons of assessments utilizing different methods. Collectively, they provide a compendium of the processes, approaches, and lessons learned in developing a region wide assessment capability.

This report is not considered an end all document, but rather a chronology of process from which further work can be directed. Data tables and summary statistics are included in the body of the document as examples to draw from, while the main data and project methods are provided via CD in the appendices. The database is provided for those who desire to utilize the data for other purposes such as individual site information, the continuance of biological condition gradient development, or any other use as deemed appropriate.

“The State water quality inventory reports serve an important function of requiring the States to assess at regular intervals the quality of their waters. In this way, information can be developed which will give the state, EPA, and Congress a measure of the effectiveness of the entire Federal water pollution control program. This report should be an important planning tool for the states,” (The Senate report 95-370 on the 1977 amendments to the Clean Water Act).

2.0 NEWS Sampling Methods

Various methods were used through the duration of the NEWS project in an effort to meet the needs of the individual states, providing utility to currently existing water quality monitoring programs. In order to meet the federal regional reporting needs, new methods unfamiliar to state monitoring programs were introduced. The following are brief summaries of the various methods used in the project.

2.1 Probabilistic Site Selection Process and Initial Site Screening

EPA's Office of Research and Development in Corvallis, Oregon and the EPA Atlantic Ecology Division in Narragansett, Rhode Island, jointly developed the probability based design for the NEWS project. A grid system of forty-two hexagonal shaped "cells" was laid over a 1:100,000 National Hydrographic Database (NHD) dataset. All streams greater than first order (Strahler 1964) were "clipped" at their intersection with cell grid lines and included into respective cells (Figure 2-1). First order stream systems were not included in the design for logistical considerations; many of these sites are ephemeral in nature, highly likely to be dry during the sampling index period. The intermittent nature of these stream types, combined with long drive times between sites, made it necessary to defer these systems to a later date when they could be given focused attention (Northern New England states experienced drought conditions through the first and second years of the project, leaving many second and some third order streams unable to be sampled due to no flow conditions).

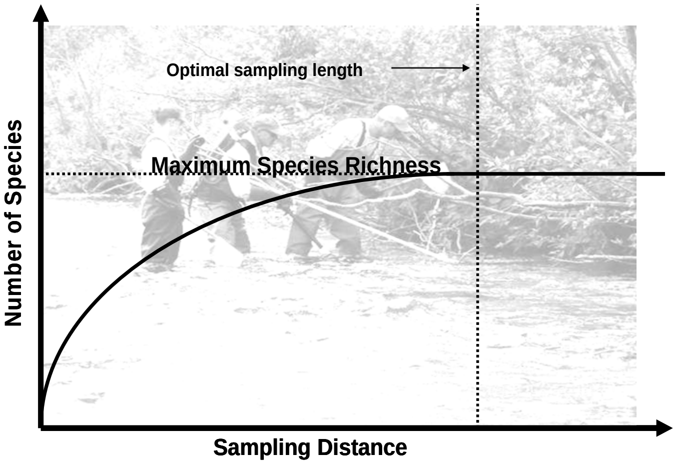


Figure 2-1. Relationship between number of species and sampling distance

After selecting an initial ten station locations within each hexagon, a two step screening evaluation process took place. Office based screening was step one, and step two was the selection of sites based on actual field observations. Step one evaluated site locations based on topographic and hydro-geologic GIS coverages and best professional judgment, from which each site was classified as a non-target or potential target site. Candidate target sites were visited in numeric order until a site was found that met the definition of the target population and considered accessible and able to be sampled. Candidate sites not meeting the criteria and the reasons why they were unacceptable were noted. If a stream appeared acceptable to survey with the exception of accessibility, a new location was selected from somewhere along the stream segment as close to the original site location as was possible. Surveying the topography and surrounding terrain and consulting topographic maps of the area ensured that there were no changes in the hydrogeologic characteristics of the stream segment or the riparian habitat.

State participation was initiated after the first year of the project period. Each fully participating state was provided the opportunity to complete a probability based design encompassing fifty sampling locations within their own state borders. Sampling was completed by the respective agencies at their own pace over the next three years; the remaining duration of the project period. The state of Connecticut's sampling effort was completed in a single year and consisted of fifty site locations. Vermont incorporated fifty site locations into their rotating basin sampling design, completing a few sites each year and finally completing their effort in the final sampling year of the regional design. New Hampshire and Maine chose to sample less than fifty sites due to staffing and logistics, completing their efforts in the second and third year of the project period. Massachusetts and Rhode Island chose not to participate in the project.

2.2 Biological Field Collection Methods

2.2.1 Fish

Although the number of fish species found in New England rivers and streams are considered to be relatively sparse compared to many other regions of the United States, they are still useful indicators of stream health and ecological condition and are important components of state and federal stream monitoring programs. Fish surveys can be especially informative when used in multi community aquatic assessments.

Collection efforts for fish were initiated at all site locations and were a principal component of this project. A single backpack electro-fishing unit was used in most cases. In larger wadeable streams, two backpack electro-fishers were used. Fish were collected to determine species presence and relative abundance. Young of year were noted as an indicator of fecundity within the system, and all fish were examined for external anomalies. The catch was enumerated and released, with the exception of individuals kept for further taxonomic identification or pathological purposes.

A 150 meter length of non block netted stream was sampled for the fish component of the project. This length of stream in the New England region has empirically been demonstrated to be an optimal length in which at least 95 percent of the species present in the waterbody will be captured (Figure 2-1). Distances beyond this length for wadeable streams rarely reveal new species, and only result in a higher level of effort expended for little informational gain.

Fish collection methods were the same among all participating agencies and were easily aggregated for assessment purposes. A full report on fish is included as a separate chapter in this document, and raw data tables are available in the CD accompanying this report.

Protocols utilized for survey efforts are a combination of those established by the OEME field monitoring team and EPA RBP methods **(**Barbour et al 1999**).**

2.2.2 Invertebrates

Assessing the ecological condition of the various wadeable stream types likely to be encountered through a probability based design in New England required that a unique sampling methodology be adopted, providing consistency across the region. The intent was to ensure that the samples collected would be representative of the type of streams from which they were sampled in their entirety, and that the maximum possible number of species could be represented.

The invertebrate collections utilized an adapted method from the Mid-Atlantic Highland REMAP project (adapted from Barbour et al 1999) in an effort to capture the heterogeneity within a sampling site segment. All habitat types within the 150 meter sampling reach were represented proportionately. This approach was utilized in order to capture all of the various stream types and meso-habitats that could possibly be encountered using a totally randomized wadeable stream selection process. This meant the method must be equally applicable to low gradient, slow winding fine substrate systems as it would be to high gradient hard bottomed streams.

The regional NEWS methodology used a one-fifth meter square quadrat randomly tossed within a particular mesohabitat of the stream reach. Twenty quadrats were collected at within a stream reach for a bottom surface area of four square meters. Quadrat collections were timed for one minute, during which all substrate was rubbed and the bottom fines disturbed to a depth of approximately 3 centimeters. Samples were collected at each site location in proportion to the existing habitat in the reach; if the reach consisted of half riffle and half pool habitat, then ten quadrats would be pulled from the pool and ten from the riffle areas.

Each individual state participating in the project conducted side by side comparisons of the NEWS multi-habitat sampling approach with their own existing state monitoring methods for data comparability purposes; if data from the various methods proved to be comparable, then data could be interchangeable and potentially shared among the various agencies. Method comparisons were made by Tetra Tech between state and NEWS methods, and among the various state methods. The results of the methods comparability work are covered in detail in chapter one of this report.

Assessment endpoints from analysis of NEWS methods and state methods was completed through several workgroup meetings utilizing best professional judgment, development of a biological condition gradient, and a “fuzzy set” model. The biological condition gradient is covered in chapter two of this document with information on fuzzy set model development in section 2.1.5.

The following provides a brief summary of the NEWS and State specific invertebrate sampling methodologies.

Connecticut

Connecticut Department of Environmental Protection's (CTDEP) invertebrate collection method follows USEPA Rapid Bioassessment Protocols (RBP) for Streams and Rivers (Plafkin 1989). CTDEP has slightly modified the approach and does not collect a CPOM sample, which results in 7 metrics instead of 8 metrics. RBP targets the richest habitat by collecting 12 kick samples (stops) throughout riffles at sampling sites using a rectangular net (18"x18"x10") with 800-900 micron mesh. The 12 stops equal a sampling area of approximately two square meters. The sampling goal is to obtain the best coverage (laterally and longitudinally) within the riffle habitat. Exact locations for these kicks are determined based on stream flow, depth and substrate characteristics. The resulting sample is meant to represent the community as a whole within the riffle. Benthic community samples are collected during the fall benthic community index period from October 1st through November 30th. Subsampling consists of a 200 organism minimum count randomly selected from a Caton grid.

Maine

Wire baskets of river run stone are placed in-stream for a period of 28 days +/- four days anywhere from late July through early September. Rock baskets are positioned in locations of similar habitat and usually deployed in triplicate. After the colonization period, an aquatic net or drift net (mesh size 600 microns) is positioned against the substrate immediately downstream of the basket which is then quickly lifted into the net. The contents of the basket and all net washings are emptied into a sieve bucket (600 microns); basket wires and then rocks are hand washed and the contents placed in sample jars. Samples are preserved to yield an approximate 70% alcohol solution. The entire sample is processed and identified. For greater detail see Maine's Quality Assurance Project Plan for the Biological Monitoring Program, available on the web at: http://www.maine.gov/dep/pubs/qapps/biomon.pdf

New Hampshire

Rock baskets are comprised of regionally indigenous bank run gravel ranging in size from 1.5 - 3.0 inches in diameter and are housed in a 6.5 inch diameter cylindrical plastic coated wire basket 11 inches in length. Baskets are placed in riffle habitats or at the base of riffles at depths that cover the artificial substrate by at least 5 inches. Each biomonitoring station uses three baskets that are anchored to the streambed by sinking ½ inch steel reinforcing rod and then attaching the baskets downstream in an array pattern with a loop of nylon coated steel cable. Substrates are left undisturbed at the site for a period of six to eight weeks in order for adequate colonization to take place. Rock baskets are placed in 600-µm 3-gallon sieve buckets and scrubbed thoroughly. Samples are preserved in 70% by volume alcohol. The index period is from late July through September. The New Hampshire sub-sampling procedure consists of a quarter sample that must contain a minimum of 100 organisms; if less than 100 organisms are found in the quarter sample, then the entire sample is processed.

Vermont

Macroinvertebrate samples are collected from September to mid-October. Samples are collected using an 18 inch wide x 12 inch high D-frame net with a 500 µm mesh size. The net is placed in riffle habitat and an area immediately upstream of the net is thoroughly disturbed by hand, ensuring that all pieces of substrate are moved and rubbed clean of attached organisms. This is repeated at 4 different locations moving upstream within the riffle habitat. Each location is sampled approximately thirty seconds until all the substrate in an 18” x 18” square area in front of net has been disturbed. The net contents are placed into a quart mason jar and preserved with 75% ethanol. Sub-sampling requires a quarter of the sample with a minimum of 300 organisms, otherwise the entire sample is identified.

2.3 Water Chemistry

Water column chemistry was completed at each site. A multi-probe was used for determining dissolved oxygen, temperature, and conductivity. Grab samples were collected for laboratory analysis. Nutrients, dissolved and total solids, low level dissolved metals (filtered), total organic carbon, alkalinity, and hardness. Water chemistry samples were collected at the very beginning of a survey to ensure that the water column was not disturbed prior to collection. Samples were preserved as necessary and placed on ice until transport back to the laboratory. Duplicate samples were collected at 10% - 15% of the site locations.

2.4 Habitat

In-stream and riparian habitat assessments were completed using standard RBP III protocols for habitat (Barbour et al 1999). This protocol uses ten different habitat variables, scored by BPJ through the sampling reach. Each variable is ranked from one to twenty, with twenty being the best condition. Each variable is representative of key factors that are either important components for ecological intactness, or are known stressors to aquatic biota in a running water body. The habitat assessments lend themselves to discriminating potential physical and/or chemical waterbody impairments that may reflect impacts on biological health.

A minimum of three field crew independently rank the habitat through the sampling reach. Habitat forms are the last of all tasks to be completed at a site, allowing for the entire sampling segment to be “surveyed” prior to assessment. Habitat scores are discussed among the team and then averaged. Each sampling location is photo documented upstream and downstream along the reach.

3.0 The Biological Condition Gradient – Background

3.1 Introduction

The Biological Condition Gradient (BCG) offers an interpretive framework for communicating technical findings about biological condition, in relation to human disturbance (Figure 3-1). It provides a means for standardizing communication about how much biological change has occurred in a sampled habitat. The BCG helps to communicate how closely the observed biological conditions in the receiving water match the state or federal goal conditions for the waterbody (Davies and Jackson 2006; USEPA 2005). The model consists of 6-tiers of progressively deteriorating conditions within which 10 ecological attributes are described as they change across an increasing stressor gradient (Table 3-1; Figure 3-1). It was developed by the USEPA and a national working group of state, federal and research aquatic biologists. The heuristic process (i.e., learning and discovery through experimental problem-solving) that was used to create the six operational tiers in the BCG has helped to clarify the extent of scientific consensus and the level of certainty associated with current interpretations of biological condition based on biomonitoring data. It offers states and EPA a template within which to report the "assessment endpoints" or outcomes of biological monitoring and provides a standardized means to communicate the results.

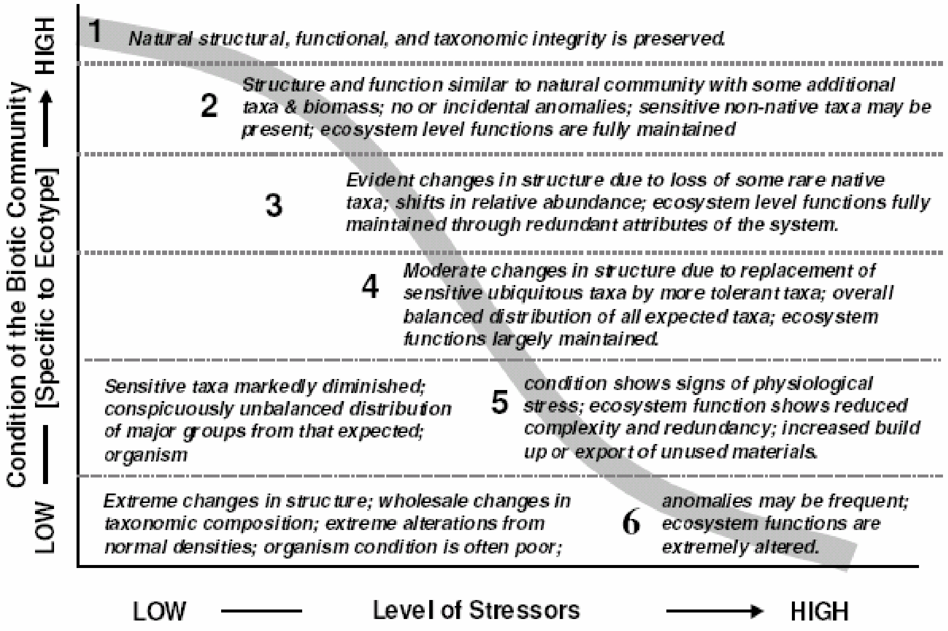


Figure 3-1. Conceptual model of the Biological Condition Gradient. (USEPA 2005)

Table 3-1. Narrative descriptions of the 10 attributes that distinguish the six tiers of the Biological Condition Gradient (Davies and Jackson 2006).

	Biological Condition Gradient Tiers					
	1 Natural or native condition	2 Minimal changes in structure of the biotic community and minimal changes in ecosystem function	3 Evident changes in structure of the biotic community and minimal changes in ecosystem function	4 Moderate changes in structure of the biotic community and minimal changes in ecosystem function	5 Major changes in structure of the biotic community and moderate changes in ecosystem function	6 Severe changes in structure of the biotic community and major loss of ecosystem function
	General Description of Biological Condition					
Attributes	Native structural, functional and taxonomic integrity is preserved; ecosystem function is preserved within the range of natural variability	Virtually all native taxa are maintained with some changes in biomass and/or abundance; ecosystem functions are fully maintained within the range of natural variability	Some changes in structure due to loss of some rare native taxa; shifts in relative abundance of taxa but sensitive-ubiquitous taxa are common and abundant; ecosystem functions are fully maintained through redundant attributes of the system	Moderate changes in structure due to replacement of some sensitive-ubiquitous taxa by more tolerant taxa, but reproducing populations of some sensitive taxa are maintained; overall balanced distribution of all expected major groups; ecosystem functions largely maintained through redundant attributes	Sensitive taxa are markedly diminished; conspicuously unbalanced distribution of major groups from that expected; organism condition shows signs of physiological stress; system function shows reduced complexity and redundancy; increased build-up or export of unused materials	Extreme changes in structure; wholesale changes in taxonomic composition; extreme alterations from normal densities and distributions; organism condition is often poor; ecosystem functions are severely altered

Table 3-1 (Continued).

	1 Natural or native condition	2 Minimal changes in structure of the biotic community and minimal changes in ecosystem function	3 Evident changes in structure of the biotic community and minimal changes in ecosystem function	4 Moderate changes in structure of the biotic community and minimal changes in ecosystem function	5 Major changes in structure of the biotic community and moderate changes in ecosystem function	6 Severe changes in structure of the biotic community and major loss of ecosystem function
I Historically documented, sensitive, long-lived or regionally endemic taxa	As predicted for natural occurrence except for global extinctions	As predicted for natural occurrence except for global extinctions	Some may be absent due to global extinction or local extirpation	Some may be absent due to global, regional or local extirpation	Usually absent	Absent
II Sensitive-rare taxa	As predicted for natural occurrence, with at most minor changes from natural densities	Virtually all are maintained with some changes in densities	Some loss, with replacement by functionally equivalent sensitive-ubiquitous taxa	May be markedly diminished	Absent	Absent
III Sensitive-ubiquitous taxa	As predicted for natural occurrence, with at most minor changes from natural densities	Present and may be increasingly abundant	Common and abundant; relative abundance greater than sensitive-rare taxa	Present with reproducing populations maintained; some replacement by functionally equivalent taxa of intermediate tolerance	Frequently absent or markedly diminished	Absent

Table 3-1 (Continued).

	1 Natural or native condition	2 Minimal changes in structure of the biotic community and minimal changes in ecosystem function	3 Evident changes in structure of the biotic community and minimal changes in ecosystem function	4 Moderate changes in structure of the biotic community and minimal changes in ecosystem function	5 Major changes in structure of the biotic community and moderate changes in ecosystem function	6 Severe changes in structure of the biotic community and major loss of ecosystem function
IV Taxa of intermediate tolerance	As predicted for natural occurrence, with at most minor changes from natural densities	As naturally present with slight increases in abundance	Often evident increases in abundance	Common and often abundant; relative abundance may be greater than sensitive-ubiquitous taxa	Often exhibit excessive dominance	May occur in extremely high or extremely low densities; richness of all taxa is low
V Tolerant taxa	As predicted for natural occurrence, at most minor changes from natural densities	As naturally present with slight increases in abundance	May be increases in abundance of functionally diverse tolerant taxa	May be common but do not exhibit significant dominance	Often occur in high densities and may be dominant	Usually comprise the majority of the assemblage; often extreme departures from normal densities (high or low)
VI Non-native or intentionally introduced taxa	Non-native taxa, if present, do not displace native taxa or alter native structural or functional integrity	Non-native taxa may be present, but occurrence has a non-detrimental effect on native taxa	Sensitive or intentionally introduced non-native taxa may dominate some assemblages (e.g., fish or macrophytes)	Some replacement of sensitive non-native taxa with functionally diverse assemblage of non-native taxa of intermediate tolerance	Some assemblages (e.g., fish or macrophytes) are dominated by tolerant non-native taxa	Often dominant; may be the only representative of some assemblages (e.g., plants, fish, bivalves)

Table 3-1 (Continued).

	1 Natural or native condition	2 Minimal changes in structure of the biotic community and minimal changes in ecosystem function	3 Evident changes in structure of the biotic community and minimal changes in ecosystem function	4 Moderate changes in structure of the biotic community and minimal changes in ecosystem function	5 Major changes in structure of the biotic community and moderate changes in ecosystem function	6 Severe changes in structure of the biotic community and major loss of ecosystem function
VII Organism condition (especially of long-lived organisms)	Any anomalies are consistent with naturally occurring incidence and characteristics	Any anomalies are consistent with naturally occurring incidence and characteristics	Anomalies are infrequent	Incidence of anomalies may be slightly higher than expected	Biomass may be reduced; anomalies increasingly common	Long-lived taxa may be absent; biomass reduced; anomalies common and serious; minimal reproduction except for extremely tolerant groups
VIII Ecosystem functions	All are maintained within the range of natural variability	All are maintained within the range of natural variability	Virtually all are maintained through functionally redundant system attributes; minimal increase in export except at high storm flows	Virtually all are maintained through functionally redundant system attributes though there is evidence of loss of efficiency (e.g., increased export or decreased import)	Apparent loss of some ecosystem functions manifested as increased export or decreased import of some resources, and changes in energy exchange rates (e.g., P/R, decomposition)	Most functions show extensive and persistent disruption
IX Spatial and temporal extent of detrimental effects	N/A A natural disturbance regime is maintained	Limited to small pockets and short duration	Limited to the reach scale and/or limited to within a season	Mild detrimental effects may be detectable beyond the reach scale and may include more than one season	Detrimental effects extend far beyond the reach scale leaving only a few islands of adequate conditions; effect extends across multiple seasons	Detrimental effects may eliminate all refugia and colonization sources within the catchment and affect multiple seasons

Table 3-1 (Continued).

	1 Natural or native condition	2 Minimal changes in structure of the biotic community and minimal changes in ecosystem function	3 Evident changes in structure of the biotic community and minimal changes in ecosystem function	4 Moderate changes in structure of the biotic community and minimal changes in ecosystem function	5 Major changes in structure of the biotic community and moderate changes in ecosystem function	6 Severe changes in structure of the biotic community and major loss of ecosystem function
X Ecosystem connectance	System is highly connected in space and time, at least annually	Ecosystem connectance is unimpaired	Slight loss of connectance but there are adequate local recolonization sources	Some loss of connectance but colonization sources and refugia exist within the catchment	Significant loss of ecosystem connectance is evident; recolonization sources do not exist for some taxa	Complete loss of ecosystem connectance in at least one dimension (i.e., longitudinal, lateral, vertical, or temporal) lowers reproductive success of most groups; frequent failures in reproduction and recruitment

3.1.1 Targeted Monitoring Designs versus Large-scale, Probability-based Monitoring Designs: Understanding Differences in Monitoring Perspective

The objectives of large-scale (i.e., region-wide or nation-wide_), probability-based monitoring programs are different from those of targeted monitoring approaches that seek to identify or define specific environmental problems at smaller spatial scales (i.e., watershed or state-wide). Typically, states direct most of their limited monitoring resources to assess site-specific conditions to carry out their mission to ensure compliance with state water quality standards and the designated uses that apply to the sampled reach. When the assessment indicates non-attainment of designated uses, these findings usually will invoke management interventions to bring the waterbody into compliance with state standards. The probability-based sampling of large-scale EPA regional monitoring projects such as NEWS or the Wadable Streams Assessment are designed to deliver a statistically valid summary of the percentage of stream miles of a sampled resource population that are in each reporting condition. These programs intentionally de-emphasizes local, site-specific findings in order to report region-wide or nation-wide overviews of resource condition. Such projects may also be applied at state-wide scales, by states themselves, in combination with targeted monitoring, because they enable states to report on 100% of waters at a fraction of the sampling effort that would be required through targeted monitoring designs alone.

In some cases the differing objectives of region-wide or national-wide sampling programs diminish the ability of states to fully utilize probability-based monitoring data in environmental decision-making because of difficulty relating large-scale assessment outcomes back to state-specific aquatic life use attainment criteria. On the other hand, states working in isolation and not employing probability-based designs may not be able to detect environmental perturbations that operate at large spatial scales. Communication of assessment results between and among states may be hampered by differing sampling and analytical methods and differing interpretations of data, thus making reporting at regional or national scales of assessment difficult or impossible. These considerations highlight the importance, for both states and EPA, of a collaborative approach to developing monitoring designs.

3.1.2 Purpose, Goals and Objectives of the NEWS-BCG Project

The NEWS project has attempted to bridge the gap in the objectives of region-wide, probability design sampling versus the targeted monitoring designs commonly used by states, by defining assessment endpoints for the NEWS project in terms of the six condition tiers described by the BCG. Multi-state monitoring results can be more easily used in state water resource management decisions when outcomes are reported in biologically descriptive condition tiers that enable states to place assessment outcomes into the context of their own aquatic life uses and management goals.

The overall purpose of the use of the Biological Condition Gradient in the NEWS project has been to create linkage and coordination between states' methods for determining attainment of aquatic life uses and the approach used by the Region 1 NEWS study. The goal of the partnership has been to facilitate an accurate and relevant assessment of biological condition in New England wadeable streams that is of equal use to the states and to EPA.

3.1.3 The Biological Condition Gradient

Biological condition tiers and associated attributes are narrative statements on presence, absence, abundance, and relative abundance of several groups of taxa that have been empirically observed to have differing responses to stressors caused by human disturbance, as well as statements on system connectivity and ecosystem attributes (e.g., production, material cycling) (Table 3-1). The USEPA Tiered Aquatic Life Uses national workgroup developed the statements out of consensus best professional judgments (Davies and Jackson, 2006; USEPA 2005). The attributes and transitions between the tiers that are described in the BCG model are based on years of biologists' field experience in a given region and reflect accumulated biological knowledge. The current generalized BCG model evolved from a prototype model that was adjusted following a series of exercises, conducted in several different regions of the United States, in which biologists attempted to place actual biomonitoring data into BCG tiers. Greater detail about the Biological Condition Gradient and the Tiered Aquatic Life Use Model may be found in Davies and Jackson 2006 and USEPA 2005.

3.1.3.1 Attributes of the Biological Condition Gradient

The BCG is presented as a 6 by 10 matrix of tiers and attributes that describe differences in the relative condition of the tiers (Table 3-1).
The attributes are:

I. Historically documented, sensitive, long-lived or regionally endemic taxa
II. Sensitive and rare taxa
III. Sensitive but ubiquitous taxa
IV. Taxa of intermediate tolerance
V. Tolerant taxa
VI. Non-native taxa
VII. Organism condition
VIII. Ecosystem functions
IX. Spatial and temporal extent of detrimental effects
X. Ecosystem connectance

The ten attributes presented in the BCG describe multiple aspects of ecological condition, including taxonomic and structural information at the site scale (Attributes I-VI), organism and system performance at the site scale (Attributes VII and VIII), and physical-biotic interactions at broader temporal and spatial scales (Attributes IX and X). Some of the attributes in the BCG represent core data elements that are commonly

measured in most state/tribal biological monitoring programs (e.g., Attributes II, III, IV, V, VI, VII) while others, though recognized as very important (e.g., Attributes I, VIII, IX and X), are not commonly measured due to resource limitations and technical complexity.

3.2 Approach

The organization of the following summary of methods used in the NEWS-BCG project roughly parallels the structure and logical progression of Chapter 3 in USEPA 2005.

3.2.1 Establish the Conceptual Foundation

As a first step to quantify and calibrate BCG assessment endpoints from the NEWS dataset, biologists needed to confirm that the national model and the Maine Example adequately represented local biologists' understanding of the response of New England biota to differing intensities of anthropogenic stress.

3.2.2 The Workgroup Process

The workgroup met five times between January 2004 and April 2005. A list of core workgroup members is on page vi and meeting agendas and summaries are provided in electronic Appendix K. Assessments of NEWS site biological condition using the Biological Condition Gradient was initially restricted to the use of information contained in Attributes II-V. Attributes II-V present a gradient of taxonomic sensitivity and summarize the majority of the assemblage-based biological condition information that is sampled by typical state biological monitoring programs. Raw assemblage data, in its most basic form, consists of lists of organism names with a quantitative or semi-quantitative measure of the abundance of each reported taxonomic unit. Information concerning Attributes I, and VI-X was not strictly required to develop a benthic macroinvertebrate fuzzy set model and data availability was limited for the remaining attributes in the BCG so further consideration was deferred.

To support BCG development, Connecticut DEP developed a relational database and Tetra Tech, Inc. analyzed and interpreted the data. Database development and data analysis steps are described in Appendices A-D. Appendix I is an analysis of data and assessment comparability among the different field methods used. The invertebrate taxa list, the MS-Access database, and site photos are in electronic Appendices L, M, and N.

To accomplish the goals stated in Section 3.1.2, New England state and EPA biologists began by assigning benthic macroinvertebrate taxa in the NEWS database to the taxonomic tolerance categories described in the BCG, based on best professional judgment. (See electronic Appendix L). Final assignments were made following graphical and statistical analysis of actual taxonomic distributions, in relation to gradients of physical-chemical stressors, in the NEWS and the Connecticut datasets. Tetra Tech, Inc. produced a standard biological sample report (See Appendix E) for each evaluated site that showed the distribution of taxa in each of four tolerance categories and also summarized basic site information about presence, absence, abundance, and relative abundance of all observed taxa. The panel of biologists assigned a subset of 111 (of 172) sites from the NEWS project into BCG tiers over the course of three workgroup meetings. The workgroup recorded consensus decision rules to guide further assignments. This exercise allowed the workgroup to develop decision rules for tier assignments from which Tetra Tech developed a non-linear fuzzy-set model for the assignment of the full dataset to BCG tiers (Section 4.0).

3.2.3 Describe Native Assemblages

The workgroup came to consensus that the description and example benthic macroinvertebrate taxa presented for Tiers 1 and 2 in the Maine Example represented a good fit for northern New

England states (VT, NH, ME) but that the Maine Example needed adjustment for Connecticut for macroinvertebrates. The fish sub-group also indicated a need for some adjustment of the BCG description for fish (Section 4.0). State biologists provided site data from known minimally disturbed reference locations within their states to stimulate further discussion about expected taxa.

3.2.4 Classification

For purposes of this discussion the term "classification" refers to differences in biota that are the result of natural environmental gradients (e.g., latitudinal and longitudinal, temperature, elevation, stream gradient, stream size, substrate characteristics, etc). The workgroup elected to assign samples to BCG tiers in separate assemblage-specific processes. Benthic macroinvertebrate data from high to mid-gradient streams were analyzed to develop the fuzzy set model (Section 4.0). The Fish sub-group process is described in Section 5.0. High-gradient streams are the stream type for which biologists had the most experience and for which the most data are available. Biologists in the region have significantly less experience interpreting benthic macroinvertebrate data from low gradient or soft-bottomed streams so the group decided to exclude them from the analysis.

3.2.5 Reference Condition

A regional BCG must be anchored in an understanding of the biological assemblages that can be expected to occur naturally, within a given stream-type in a locale, in the absence of significant human disturbance. Data from know minimally disturbed reference sites establish Tier 1 of the BCG and subsequent tiers represent departures from this expected reference condition.

3.2.6 Regional Conceptual Model

Development of a regional BCG model entails two tasks (USEPA 2005):

1. Establish a consensus conceptual understanding of patterns of benthic macroinvertebrate and fish assemblages that are characteristic of New England minimally disturbed streams; (i.e., "calibrate the biologists" by reaching workgroup agreements on expected native assemblage structure) and

2. Establish consensus workgroup expectations for the quantitative and taxonomic characteristics and indicators of minimally disturbed streams in the NEWS dataset (i.e., "calibrate method-specific, quantitative taxonomic expectations" for minimally disturbed samples).

To establish the consensus regional BCG conceptual model (#1 above), samples of existing benthic macroinvertebrate data from minimally disturbed streams in Vermont and Maine (collected using standard state-specific sampling methods) were provided to the workgroup. The selected streams were deemed, by the state biologist data providers, to be of minimally disturbed reference quality. The taxonomic name and count data fostered discussion of biologists' experience with relative sensitivities of the taxa observed in these streams and helped inform the assignment of taxa in the NEWS dataset to BCG taxonomic attribute groups.

The BCG model is fundamentally a field-based stressor-response model that describes biological response as the dependent variable on the y-axis, and stressor or disturbance intensity as the independent variable on the x-axis (USEPA 2005). To accomplish #2 above, several workgroup meetings were dedicated to identifying NEWS reference sites from the stressor gradient data (i.e., x-axis physical, chemical site information and SPARROW land use information). Analysis of stressor and land cover data to define Tier 1 physical and biological conditions is presented in greater detail in Section 4.0 and Appendices B and C. Throughout the workgroup meetings, there was considerable discussion, but no consensus, on the existence or prevalence of Tier 1 sites, or minimally stressed reference sites (Stoddard et al. 2006) in New England. Consequently, Tier 1 could not be described in detail by expert judgment, and it was not included in the modeling exercise. Tier descriptions and the assessment model were initially limited to Tiers 2-6. Comprehensive land use/land cover data on delineated watersheds was not part of the original design but became available late in the project (Moore et al. 2004). The land use data revealed that several sites in northern New England states (Maine and New Hampshire) potentially met criteria for minimally stressed, and hence were candidate Tier 1 sites. Preliminary analysis of these data is also discussed in Section 4.2.7 but was not incorporated into the BCG exercise or model.

3.2.7 Identify Regional Stressors

The BCG and Maine Example follow a stressor gradient scenario of increasing temperature, sedimentation and loss of riparian cover secondary to changes in land use (agriculture, forest harvesting, urbanization). This stressor scenario is a good fit for the comparatively low population density, northern New England states (VT, ME and NH). The historical New England farming landscape has been replaced in many areas by second growth forest, masking many of the historic man-made structures of stone walls and freestone dams and impoundments that were common on many streams. In addition to being a source of water for power and livestock, these structures provided an efficient settling basin for large volumes of sediments that ran off historic farmlands and dirt roads. Most of these structures are now breeched and the large volumes of sediment once held back are finding their way into streams, as the waterbody incises once impounded sediments in its movement to attain historic base level conditions. Sedimentation stresses can also be found in the remote Maine woods and northernmost corners of Vermont and New Hampshire. Poor water diversion controls from logging roads, clearcutting practices adjacent to streams, and road drainage designs are all significant contributors. Clearcutting even in the most remote areas, accelerates snow melt and seasonal runoff, resulting in much higher streamflows that in turn induce excessive bank erosion and sediment influx to the waterbody. More information on sediment effects can be found on the EPA Watershed Assessment of River Stability and Sediment Supply Web site (**http://www.epa.gov/WARSSS/sedsource/sabs.htm**). Legacy and present day effects of toxics and large volume point sources of pollution are more common in southern New England. Impacts are currently evident as in-place contamination from industrial activities and extensively altered urban and sub-urban land use over long time periods. These differences in historical landscape alterations that affect the severity of the stressor scenario raised greater concern that the prototype BCG stressor scenario is not a good fit for Connecticut.

4.0 The Biological Condition Gradient – Benthic Macroinvertebrates

4.1 Methods

Following the development of the conceptual model, we quantified and calibrated an expert consensus model for assessing BCG Tiers 2 through 6, for benthic macroinvertebrate data. This section presents the approach used by the NEWS regional workgroup to identify reliable and accurate quantitative indicators of those expected biological responses to stressors. This section deals with in-hand, benthic macroinvertebrate data, specifically:

- NEWS Regional probability sites;
- NEWS state probability sites;
- State-sites/state-specific methods (CT, ME, NH, or VT)

4.1.1 NEWS Data

Data were checked for quality and errors, corrected as necessary, and migrated to a relational database by Connecticut DEP. Corrections included reconciling site locations and site identifiers among biological, chemical, and physical data; reconciling multiple labels for chemical parameters; and other tasks as described in Appendix A.

After initial data cleanup, it was also necessary to reconcile multiple biological sampling methods and replication protocols so that all data analysis represented single samples rather than fragments of samples or duplicate samples. Also, the master taxonomic list had to be reduced to an operational target level to avoid ambiguities of identification (Appendix A). The taxa list is in electronic Appendix I.

4.1.2 Reference Condition

A regional BCG must be anchored in an understanding of the biological assemblages that can be expected to occur naturally in a locale, in the absence of significant human disturbance. Data from known minimally disturbed reference sites establish Tier 1 of the BCG and subsequent tiers represent departures from this expected reference condition. We identified candidate reference sites from the NEWS data set, which were used to classify streams and to anchor the BCG. A summary of the reference site selection process and the criteria used are in Appendix B.

4.1.3 Classification

Ordination analysis of the NEWS data showed a distinct north-south gradient in the taxa of the region. All potential reference sites sampled by NEWS methods were in Maine and New Hampshire, all in the northern part of the region. Classification of the sites is discussed in detail in Appendix C; Table C-3 and shows differences between the north and south NEWS samples, and the Connecticut reference sites.

4.1.4 Quantify the Conceptual Model

Define and Assign Taxonomic Attribute Categories II-V

Ecological attributes are measurable characteristics of the system (USEPA 2005; Davies and Jackson 2006). Taxa identified in the New England samples were assigned to the taxonomic attributes of the BCG (Attributes I through VI; see above). This was an iterative process involving professional consensus of the workgroup on each taxon considered, coupled with analysis of two data sets to empirically examine the response of selected taxa to a generalized stressor gradient. Results of the data analysis were discussed by the group to refine the professional consensus decisions.

We used two data sets; in addition to the NEWS data, a data set was provided by Connecticut DEP consisting of 143 samples from 1976 to 2003 and spanning the worst to the best conditions observed in Connecticut, as well as the NEWS samples from the NEWS database. Several of the early Connecticut samples were examples of “worst” conditions – toxic sites with heavy industrial legacy pollution as well as untreated municipal wastewater. “Best” sites in the Connecticut database were identified by CT DEP personnel from CT reference sites. Recent sampling sites (both NEWS and CT DEP samples) no longer receive untreated wastewater. Several legacy industrial sites were hand-selected for NEWS sampling, to match existing CT sites. The potentially “worst” and “best” NEWS sites were identified from land use information. Best sites had at least 90 percent natural land cover, and were identified as potential reference sites.

We examined responses of taxa to the defined stressor gradients (best to worst sites) with both indicator species analysis and correlation of taxon abundance with the principal stressor gradient identified in ordination analysis. Indicator species analysis examines the probability that a species’ presence gives diagnostic information on the membership of a site to one of several *a priori* groups (Appendix D). The a priori groups were “best”, “intermediate”, and “worst” as identified above.

Graphical analysis of individual taxa on ordination plots was deemed to be the most useful for identifying attribute groups (Figures 4-1, 4-2). Sites were plotted in ordination space from a non-metric multidimensional scaling analysis (NMS), and identified with each of the a priori groups by symbol and color. For each taxon, symbol size was controlled by the relative abundance of the taxon at each site, so that taxa that were more abundant, or occurred more frequently in any one or two of the *a priori* groups could be readily identified. The graphical analysis was combined with correlation of each taxon abundance with site scores on each ordination axis. Initial analysis used the older Connecticut data (Fig. 4-1, 4-2), which had no chemical observations , so potential stressors associated with the ordination axes could not be identified.

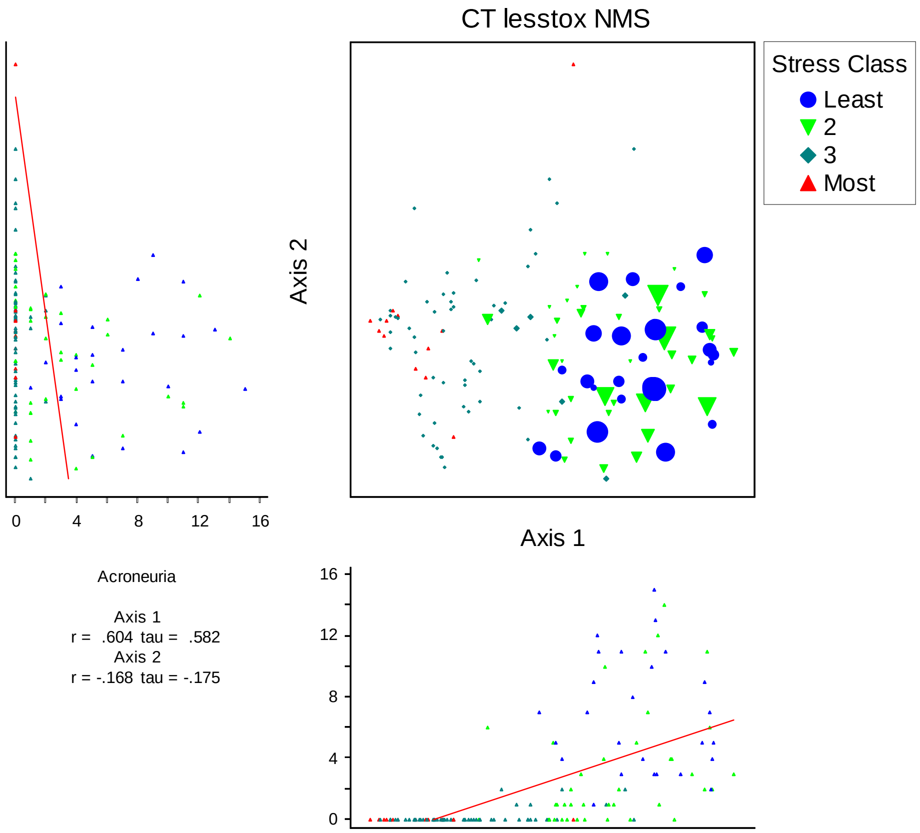


Figure 4-1. NMS ordination of CT DEP data. Upper right scatter plot is sites in ordination space, and bottom and left plots show relative abundance of the stonefly *Acroneuria* on the x- and y- axes, respectively. Size of symbols in upper right plot also shows relative abundance. Plot shows that *Acroneuria* occurs primarily in least-stressed and low-stress sites. It generally does not occur at all in the stressed sites.

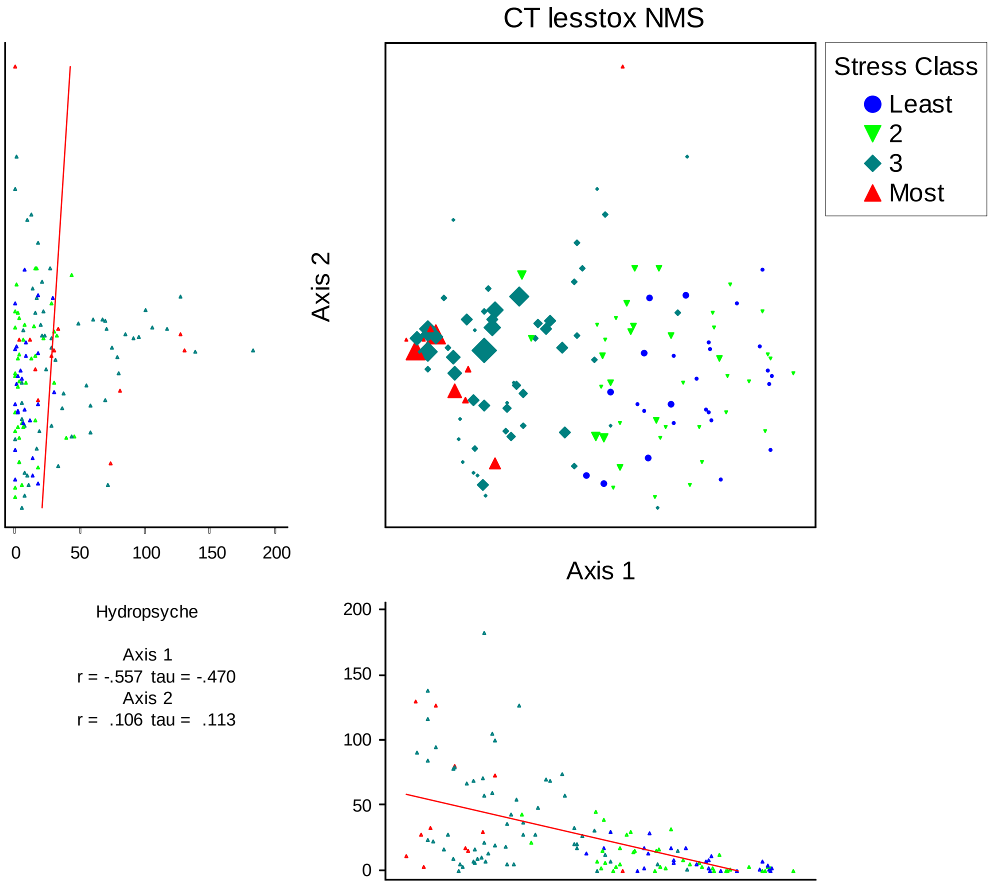


Figure 4-2. NMS ordination as in Figure 4-1, but showing the caddisfly *Hydropsyche*. Plot shows that *Hydropsyche* is most abundant in stressed sites.

Identify Rules to Assign Sites to Tiers

Tier descriptions in the conceptual model tend to be rather general (e.g., "reduced richness"). To allow for consistent assignments of sites to tiers, it was necessary to formalize the expert knowledge by codifying tier descriptions into a set of rules (e.g., Droesen 1996). If formalized properly, anyone can follow the rules to obtain the same tier assignments as the group of experts.

Rules are logic statements that experts use to make their decisions, for example: "If taxa richness is high, then biological condition is high." Rules on attributes can be combined, for example: "If the number of highly sensitive taxa (Attribute II) is high, and the number of tolerant individuals (Attribute V) is low, then the Tier is 2." In questioning individuals on how decisions are made in assigning sites to tiers, it became clear that rules are not "crisp." For example, there is no distinct number of highly sensitive taxa that would always distinguish Tier 2 from Tier 3. Rather, people use strength of evidence in allowing some deviation from their ideal for any individual attributes, as long as most attributes are in or near the desired range. Clearly, the definitions of "high," "moderate," "low," etc., are uncertain. These rules preserve the collective professional judgment of the expert group and set the stage for the development of models that reliably assign sites to tiers without having to reconvene the same group. In essence, the rules and the models capture the group's collective decision criteria.

Rule development required discussion and documentation of tier assignment decisions and the reasoning behind the decisions. During this discussion, we recorded:

- each participant's decision ("vote") for the site;
- the critical or most important information for the decision—for example, the number of taxa of a certain attribute, the abundance of an attribute, the presence of indicator taxa, etc.; and
- any confounding or conflicting information and how this was resolved for the eventual decision.

Rule development was iterative. Following the initial development phase, the draft rules were tested by the panel to ensure that new data and new sites are assessed in the same way. The test sites had not been used in the initial rule development and also spanned the range of anthropogenic stress. Any remaining ambiguities and inconsistencies from the first iterations were also resolved. Rules can be used directly for assessments, for calibrating one of the previous assessment methods (IBI, discriminant model), or as the basis of an expert system.

4.1.5 Fuzzy Set Model to Automate Tier Decisions

Consensus professional judgment used to describe the tiers can take into account nonlinear responses, uncommon stressors, masking of responses, and unequal weighting of attributes. This is in contrast to the commonly used biological indexes, which are typically unweighted sums of attributes (e.g., multimetric indexes; Karr and Chu 1999, Barbour et al. 1999), or a single attribute, observed to expected taxa (e.g., Wright 2000, Simpson and Norris 2000). Consensus assessments built from the professional judgment of many experts result in a high degree of confidence in the assessments, but the assessments are labor-intensive (several experts must rate each site). It is also not practical to reconvene the same group of experts for every site monitored in a long-term program for assessment and management. Since individuals may be replaced on a panel over time, assessments may in turn "drift" due to individual differences of new panelists. Management and regulation, however, require clear and consistent methods and rules for assessment, which do not drift unless deliberately reset.

Use of the BCG in operational monitoring, management and regulation thus requires a way to automate the consensus expert judgment so that the assessments are consistent until such time that they are explicitly altered due to new knowledge becoming available. Two options have been used in the past: the Maine DEP developed a set of multivariate linear discriminant models to imitate the expert consensus and predict a site assessment (Davies et al. 1995); and the UK Environmental Agency defined ranges of scores of two indexes (their RIVPACS index and a tolerance index) that corresponded to the expert consensus (Hemsley-Flint 2000). Both of these approaches require one or more multivariate statistical models to statistically predict the expert judgment in assessments.

Instead of a statistical prediction of expert judgment, we have chosen to use a methodology that directly, explicitly and transparently converts the expert consensus to automated site assessment. The method uses fuzzy set theory applied to rules developed by the group of experts. Fuzzy sets and fuzzy logic are directly applicable to environmental assessment; they have been used extensively in engineering applications worldwide (e.g., Demicco and Klir 2004) and environmental applications have been explored in Europe and Asia (e.g., Castella and Speight 1996; Ibelings et al. 2003) but environmental applications are still unusual in North America (e.g., Bosserman and Ragade 1982).

Fuzzy sets and fuzzy logic allow degrees of membership (in sets) and degrees of truth (in logic), compared to all-or-nothing in classical set theory and logic. This has immediate advantages in scientific classification, for example, “sand” and “gravel,” where a particle with diameter of 1.999 mm is classified as “sand” in classical set theory, and one with 2.001 mm diameter is classified as “gravel.” In fuzzy set theory, both particles may have nearly equal membership in both classes (Demicco 2004). Demicco and Klir (2004) proposed four reasons why fuzzy sets and fuzzy logic enhance scientific methodology:

- Fuzzy sets can capture “irreducible measurement uncertainty”, as in the sand/gravel example above;
- Fuzzy logic captures vagueness of linguistic terms, such as “many,” “large” or “few”;
- Fuzzy sets and logic can be used to manage complexity and computational costs of control and decision systems; and
- Fuzzy logic enhances the ability to model human reasoning and decision-making.

4.2 Results

4.2.1 Taxa Assignments to Attributes

Attribute groups of taxa, from the most sensitive to the most tolerant, were examined with a combination of Indicator Species Analysis (McCune and Grace 2002), graphical analysis, and biological knowledge of the taxa by state biological experts. Early in the process, we assigned New England taxa to the first six attribute groups. Participants discussed genera on the New England list (450 entries), and developed a consensus assignment. 130 taxa (29 percent) were left unassigned because participants felt there was insufficient information on the taxa, or they were too rare in the database; however, these unassigned taxa accounted for only 6 percent of all occurrences. Throughout, participants referred to the example developed for Maine (Davies and Jackson 2006).

Five attribute groups could be identified from the data:

Group II: Highly sensitive taxa.

These taxa may be common to uncommon, but typically only occur in good to very good sites, and occur only rarely in moderate to poor sites. Many occur in low abundances only, so occurrence (presence) is more informative than abundance. Examples: *Lepidostoma, Protoptila, Leuctra, Drunella, Blephariceridae.*

Group III. Sensitive taxa.

These taxa may occur in all sites, but they occur more frequently and are more abundant in high quality sites. Thus, they are somewhat tolerant of poor conditions, but prefer unpolluted and good quality habitat. Examples: *Psephenus, Eurylophella, Acroneuria, Glossosoma, Rhyacophila.*

Group IV: Broadly tolerant (indifferent) taxa:

These taxa may occur in any conditions and at any abundance. They appear to have no detectable preference for water quality or habitat, except for some decline in the most polluted sites (Tier 6). In general, they do not provide useful information. Examples: *Stenelmis, Baetis, Orthocladius, Hydroptila, Ceratopsyche, Perlesta.*

Group V. Tolerant taxa:

These taxa occur more frequently and especially at increased relative abundance in poor water quality and poor habitat. Many also occur in good quality conditions, but abundances are reduced. Examples: *Gammarus, Cricotopus, Hydropsyche (sensu stricto).*

Group Va. Highly tolerant specialists:

These taxa tend to occur primarily in severely degraded and polluted habitats, and they are typically not found in good quality waters. Many are specialized for thriving in hypoxic waters. Examples: Erpobdellidae, Glossiphoniidae, Tubificidae, *Cardiocladius*.

4.2.2 Tier Elicitation and Calibration

The entire workgroup discussed the conceptual model of the BCG for New England, and developed preliminary definitions of Tiers 2 - 6 of the gradient. Using these preliminary definitions, state workgroup members assigned sites in their respective states to the tiers, for each of the two sampling methods represented (each state, and NEWS). These BCG tier assignments were used to estimate attribute statistics among the tiers, and to help develop rules for assigning sites to BCG tiers.

Initial development of the BCG description and rules was based on data from Connecticut, because the Connecticut methods were deemed most compatible with the NEWS methods (riffle sample and fixed subsample of 200 organisms). Because Connecticut personnel were most familiar with data collected with Connecticut methods, and less familiar with the NEWS methods, we used ratings from Connecticut data as the calibration standard for the BCG rules, that is, NEWS samples were assigned to BCG tiers according to the ratings developed from the corresponding Connecticut samples. Metrics (number of taxa, percent of taxa, and percent of individuals) were calculated for each attribute, as well as some combined attributes, and characterized for each tier and method combination. See summary of Connecticut and NEWS attribute metrics, Tables 4-1 and 4-2.

Table 4-1. Ranges of attribute metrics in CT samples (CT Kick net), by assigned tiers.

Attributes	**CT kick net, high-gradient streams**					
	CT Tier Call (n=43)					
	1	**2 (n=8)**	**3 (n=23)**	**4 (n=7)**	**5 (n=5)**	**6 (n=0)**
0 General		rich 37-51	rich 24-52	rich 25-49	rich 10-21	
I *Endemics*						
II *Highly* sensitive taxa		4-8 taxa 5 - 22%	0-5 taxa 0-18%	0-1 taxa 0 - 3%	0-1 taxa 0-1%	0 taxa
III *Intermediate* ***Sensitive*** *taxa*		14 - 27 taxa 31 -62%	6 -21 taxa 13 - 70%	10 -18 taxa 23-63%	0-7 taxa 0-27%	
		II + III: 18-32 taxa II + III: 43-62% of taxa II + III: 40-78% of indiv	II+III: 9-25 taxa II+III: 29-58% of taxa II+III: 22-82% of indiv	II+III: 10-19 taxa II+III: 32-44% of taxa II+III: 23-63% of indiv	II+III: 0-7 taxa II+III: 0-39% of taxa II+III: 0-27% of indiv	
IV *Indifferent taxa*		13-19 taxa, 19-53%	11-26 taxa, 13 - 59%	12-22 taxa, 28 -74	3-13 taxa, 3 - 70%	
V.a *Tolerant taxa*		1-5 taxa, 2-7% Va+Vb: 1-5 taxa, Va+Vb 2-7% of indiv	2 -6 taxa, 2-35% Va+Vb: 2-6 taxa, Va+Vb: 2-35% of indiv.	2-6 taxa, 3 - 33% Va+Vb: 2-6 taxa, Va+Vb: 3-33% of indiv.	2-5 taxa 24 - 86% Va+Vb: 2-7 taxa, Va+Vb: 24-85% of indiv.	
V.b *Highly tolerant taxa*		0 taxa IV + V + Vb: 21-58%	0 taxa IV + V + Vb: 18-77%	0 taxa IV + V + Vb: 36-76%	0-2 taxa, 0-4% IV + V + Vb: 73-95%	
Indicator Taxa		E rich: 3-11 E %: 7-48 Hydro 5-12% Tubi: 0 Nonins: 0-11%	E rich: 1-8 E %: Hydro 2-38% Tubi: 0 Nonins: 1-24%	E rich: 1-5 E %: 1-51% Hydro 2-33% Tubi: 0 Nonins: 0.5-20%	E rich: 0-3 E %: 0-9% Hydro 22-76% Tubi: 0 Nonins: 0-4%	

Table 4-2. Ranges of attribute metrics in paired NEWS samples from Connecticut

Attributes	**NEWS Methods, High-Gradient Streams**					
	CT State Tier Call (n=24)					
	1	**2 (n=2)**	**3 (n=13)**	**4 (n=4)**	**5 (n=5)**	**6 (n=0)**
0 General		rich 36-60	rich 37-53	rich 37-55	rich 16-42	
I *Endemics*						
II *Highly* sensitive *taxa*		3-7 taxa 10-15%	0-6 taxa 0-9%	0-2 taxa 0 - 2%	0-1 taxa 0-1%	
III *Intermediate* ***Sensitive*** *taxa*		10-20 taxa 43-45%	12-20 taxa 24-64%	11-18 taxa 22-40%	1 - 8 taxa 1-31%	
		II + III: 20-27 taxa II + III: 45-56% of taxa II + III: 55-57% of indiv	II + III: 13-25 taxa II + III: 30-58% of taxa II + III: 29-67% of indiv	II + III: 12-20 taxa II + III: 32-37% of taxa II + III: 23-41% of indiv	II + III: 1-9 taxa II + III: 4-26% of taxa II + III: 1-31% of indiv	
IV *Indifferent taxa*		14-27 taxa 36-41%	12-24 taxa 28- 60%	13-24 taxa 28 -72%	5-23 taxa 30-80%	
V.a *Tolerant taxa*		1-4 taxa 1-3%	1-7 taxa 1 – 21%	3-9 taxa 5 - 40%	5-9 taxa 15-66%	
		Va+Vb: 1-5 taxa Va+Vb: 1-3%	Va+Vb: 2-7 taxa Va+Vb: 4-22%	Va+Vb: 3-10 taxa Va+Vb: 5-42%	Va+Vb: 7-9 taxa Va+Vb: 16-68%	
V.b *Highly tolerant taxa*		0-1 taxa, 0-1% IV + V + Vb: 36-44%	0 - 1 taxa, 0 - 2% IV + V + Vb: 31-72%	0 - 1 taxa, 0 - 8% IV + V + Vb: 57-77%	0-2 taxa, 0-7% IV + V + Vb: 69-98%	
Indicator Taxa		E rich 3-7 E % 10-21 Hydro 2-3% Tubi 0-0.5% Nonins 1-5%	E rich 2-7 E % 5-39 Hydro 0-25% Tubi 0-2% Nonins 1-42%	E rich 2-3 E % 2-13 Hydro 2-8% Tubi 0-8% Nonins 5-17%	E rich 1-3 E % 1-6 Hydro 8-40% Tubi 0-2% Nonins 3-41%	

Comparison of Tables 2-1 and 2-2 reveals that the NEWS and CT methods yielded remarkably similar patterns in number of taxa and relative abundance among the attribute groups. Individual taxa vary between the methods because the NEWS method samples more habitat types and captures slightly more taxa.

4.2.3 Tier Descriptions

Descriptions of tiers and rules are given in Table 4-3. We found that most biologists preferred to use taxa richness within the two sensitive attributes as the first and most important criterion for determining site tier assignments. Thus, the number of highly sensitive taxa was most often used to distinguish between Tier 2 and Tier 3 sites. Tier 2 should have several highly sensitive taxa (Attribute II), but their richness may be reduced in Tier 3. For example, a rule for Tier 2 was that highly sensitive taxa richness (Attribute II taxa richness) should be more than two to four taxa.

Based on the characterization of sites identified as belonging to different tiers (Tables 4-1, 4-2), we developed a set of linguistic rules for distinguishing tiers. Complete attribute descriptions, the linguistic rules, and the quantitative rules that follow from the linguistic rules are shown in Table 4-3, and are summarized below:

Tier 2
- Total Taxa Richness: moderately high to high
- Total abundance: near target for subsample (200)
- Highly sensitive taxa: At least some taxa are present
- All sensitive taxa (highly sensitive + intermediate sensitive): comprise nearly half or more of all taxa
- All sensitive individuals: comprise nearly half or more of all organisms
- Tolerant individuals (tolerant + highly tolerant): a small fraction or less of all organisms

Based on the group decisions, the Tier 2 rules are “all or nothing”, that is, all must be met for a sample to be considered Tier 2. Logically, they are combined with AND statements. The Tier 2 rules discriminate Tier 2 from Tier 3 (and lower).

Tiers 3 and 4

Tiers 3 and 4 overlapped almost entirely in the distributions of the attribute groups (Tables 1, 2) except that the upper ends of the ranges of the sensitive taxa attributes were slightly higher in Tier 3 sites, and the upper ends of the ranges of the tolerant attributes were slightly higher in Tier 4 sites. Accordingly, the only consistent basis for distinguishing Tier 3 from Tier 4 was an average function of the attributes. In contrast, a linguistic rule (above) distinguishes Tier 2 from Tier 3, and a linguistic rule was developed to distinguish Tier 4 from Tier 5 (below)

Tier 4 and better

- Total Taxa Richness: moderately high to high
- Total abundance: near target for subsample

- Highly sensitive taxa: may be absent (**no rule**)
- All sensitive taxa (highly sensitive + intermediate sensitive):
    - a moderate amount is present, AND
    - comprise more than a small fraction of all the taxa OR comprise more than a small fraction of all organisms
- Tolerant individuals (tolerant + highly tolerant): are less than half of all individuals

The Tier 4 and better rules are also combined with AND statements, with the exception of the 2 conditions for the sensitive taxa above. The Tier 4 and better rules discriminate Tier 4 from Tier 5 (and lower).

Tier 5 and better

Tier 5 was discriminated from Tier 4 by a significant reduction of sensitive taxa (Attributes II and III) to the point where they are merely incidental if present and are not a functional part of the community. To qualify as Tier 4, sensitive taxa had to be present in low diversity or higher, and comprise a low proportion, but more than negligible, of all organisms. The range of 5% to15% relative abundance was deemed the lower bound to qualify as “a functional part of the community” and “more than negligible”.

- Total Taxa Richness: intermediate to moderate
- Total abundance: near target for subsample
- Highly sensitive taxa: may be absent (**no rule**)
- All sensitive taxa (highly sensitive + intermediate sensitive): may be absent (**no rule**)
- All tolerant individuals (tolerant + highly tolerant): may be more than half of all individuals (**no rule**)
- Highly tolerant individuals: A small fraction or less

Tier 6 was discriminated from Tier 5 by increasing loss of all taxa or extreme dominance by tolerant taxa (Attribute 5). Tier 6 could also be indicated by extreme low numbers. The three rules for Tier 5 discriminate Tier 5 from Tier 6: failure of one of these rules means that a sample is assigned to Tier 6.

The rules are applied as a downward cascade: for a site to be rated as Tier 2 (the highest described tier), all attributes must meet the Tier 2 condition (Table 4-3). A Tier 3-4 rating requires one or more failures of Tier 2 rules, but the site must meet all minimum Tier 4 rules. Tiers 3 and 4 are discriminated based on averages of the Tier 3-4 rules. Tier 5 represents a failure of Tier 4 rules, and so on to Tier 6, such that Tier 6 is applied to sites that fail conditions for all higher tiers (Table 4-3).

Table 4-3. Biological Condition Gradient: description of gradient and rules for cold-water streams of New England. Modified after Davies and Jackson (2006). Rules apply to benthic macroinvertebrates sampled with CT DEP or NEWS methods (kick net, genus ID, 200 organism subsample). Grayed text not part of model development

Resource Condition Tiers	Biological Condition Characteristics (Effects)
1 **Natural or native condition** *Native structural, functional and taxonomic integrity is preserved; ecosystem function is preserved within the range of natural variability*	**I *Historically documented, sensitive, long-lived, or regionally endemic taxa*** ➔ Long-lived native species of fish-host specialist or long-term brooder mussels such as Brook floater- *Alasmodonta varicosa;* Triangle floater- *Alasmodonta undulata;* Yellow lampmussel- *Lampsilis cariosa* are present in naturally occurring densities ➔ **Fishes**: Brook stickleback, Swamp darter, accessible to migratory fish (Atlantic salmon, eel) **II *Highly Sensitive taxa*** ➔ The proportion of total richness represented by rare, specialist and vulnerable taxa is high, for example, without limitation, the following taxa are representative: **Plecoptera**: Peltoperlidae*, Amphinemura, Isogenoides, Neoperla, Pteronarcys, Leuctra*; **Ephemeroptera**: *Centroptilum, Heterocloeon, Brachycercus, Drunella, Rhithrogena, Epeorus, Leucrocuta*; **Trichoptera**: *Protoptila; Psilotreta, Lepidostoma, Ceraclea*; **Diptera**: Blephariceridae, *Stempellina, Limnophila* **III *Intermediate Sensitive taxa*** ➔ Densities of Intermediate Sensitive taxa are as naturally occur. The following taxa are representative of this group for Maine: **Plecoptera**: *Acroneuria*; **Ephemeroptera**: *Ephemerella, Baetisca, Procloeon;* **Coleoptera**: *Psephenus* **Diptera:** *Rheocricotopus, Stempelinella***; Fishes**: Brook trout, Burbot, Lake chub **IV *Taxa of Intermediate (indifferent) tolerance*** ➔ Densities of indifferent tolerance taxa are as naturally occur. The following taxa are representative of this category: **Trichoptera**: *Diplectrona, Hydroptila*, *Chimarra*, *Neureclipsis;* **Diptera**: *Tvetenia, Polypedilum ,Microtendipes, Simulium;* **Coleoptera**: *Stenelmis;* **Fishes**: Common shiner, Fallfish **V *Tolerant taxa*** ➔ Occurrence and densities of Tolerant taxa are as naturally occur. The following taxa are representative of this category: **Diptera**: *Cricotopus*, *Chironomus, Rheotanytarsus*; **Non-Insects**: *Caecidotea*, Isopoda*;* **Fishes***:* White sucker, Blacknose dace, Creek chub **Va *Highly Tolerant taxa*** ➔ Occurrence and densities of Highly Tolerant taxa are as naturally occur. Rare and sparse in high-gradient streams (usually absent from samples). The following taxa are representative of this category: **Diptera**: Psychodidae, *Dicrotendipes*; **Non-Insects**: Erpobdellidae, Tubificidae, Glossiphoniidae **VI *Non native or intentionally introduced taxa*** ➔ Non native taxa such as Brown trout, Rainbow trout, Yellow perch, are absent or, if they occur, their presence does not displace native biota or alter native structure and function **VII *Physiological condition of long-lived organisms*** ➔ Anomalies are absent or rare; any that occur are consistent with naturally occurring incidence and characteristics **VIII *Ecosystem Function*** ➔ Rates and characteristics of *life history (e.g., reproduction, immigration, mortality, etc.)*, and materials exchange processes *(e.g., production, respiration, nutrient exchange, decomposition, etc.)* are comparable to that of “natural” systems ➔ The system is predominantly heterotrophic, sustained by leaf litter inputs from intact riparian areas, with low algal biomass; P/R<1 (Photosynthesis: Respiration ratio) **IX *Spatial and temporal extent of detrimental effects*** ➔ Not applicable- disturbance is limited to natural events such as storms, droughts, fire, earth-flows. A natural flow regime is maintained. **X *Ecosystem connectance*** ➔ Reach is highly connected with groundwater, its floodplain, and riparian zone, and other reaches in the basin, at least annually. Allows for access to habitats and maintenance of seasonal cycles that are necessary for life history requirements, colonization sources, migration and *refugia* for extreme events.

Table 4-3 (Continued).

2

Minimal changes in structure of the biotic community and minimal changes in ecosystem function

Virtually all native taxa are maintained with some changes in biomass and/or abundance; ecosystem functions are fully maintained within the range of natural variability

Whole assemblage and sample

➔ Overall taxa richness and density is as naturally occurs

➔ **RULE 1:** Taxa richness is high and subsample density is near target

➔ **Quantitative Rule 1**: Total taxa > (30-35) genera and Total individuals > (45-55% of target)

I *Historically documented, sensitive, long-lived, regionally endemic taxa*

➔ Some endemic species (e.g., the Dwarf wedgemussel- *Alasmidonta heterodon*, and/or Brook stickleback are absent. Migratory species (eels, Atlantic salmon) may be absent due to dams; possible reduced recruitment of Unionid mussels.

II *Highly Sensitive taxa*

➔ Richness of rare and/or specialist invertebrate taxa is low to moderate though densities may be low : **Plecoptera**: Peltoperlidae*, Amphinemura, Isogenoides, Neoperla, Pteronarcys, Leuctra*; **Ephemeroptera**: *Centroptilum, Heterocloeon, Brachycercus, Drunella, Rhithrogena, Epeorus, Leucrocuta*; **Trichoptera**: *Protoptila; Psilotreta, Lepidostoma, Ceraclea*; **Diptera**: Blephariceridae, *Stempellina, Limnophila*

➔ Fish assemblage is predominantly native including Slimy sculpin, Longnose sucker, Longnose dace.

➔ **RULE 2:** At least some taxa are present

➔ **Quantitative Rule 2**: Taxa (II) > (2-4)

III *Intermediate Sensitive taxa*

➔ Richness and abundance of intermediate sensitive taxa is high. Some may have increased due to slightly elevated production (e.g., : **Plecoptera**: *Acroneuria*; **Ephemeroptera**: *Ephemerella, Baetisca, Procloeon;* **Coleoptera**: *Psephenus* **Diptera:** *Rheocricotopus, Stempelinella***;**

➔ Populations of such native fish taxa as Brook trout, Lake chub, Burbot are common.

➔ **RULE 3:** All sensitive taxa (highly sensitive + intermediate sensitive): comprise nearly half or more of all taxa

➔ **RULE 4** : All sensitive individuals: comprise nearly half or more of all organisms

➔ **Quantitative Rule 3**: Taxa (II + III) > (35 – 40%) of all taxa

➔ **Quantitative Rule 4**: Individuals (II + III) > (35-40%)

IV *Taxa of Intermediate (indifferent) tolerance*

➔ Possible Increased biomass of diatoms that respond to increased nutrients and temperatures, but sensitive diatom species are maintained. Diatom richness increased; filamentous forms are rare

➔ May be slight increases in densities of macroinvertebrate taxa such as : **Trichoptera**: *Diplectrona, Hydroptila*, *Chimarra*, *Neureclipsis;* **Diptera**: *Tvetenia, Polypedilum ,Microtendipes, Simulium* **Coleoptera**: *Stenelmis*. Common shiner and Fallfish are in good condition

➔ **RULE:** None

V *Tolerant taxa*

➔ Occurrence and densities of Tolerant taxa are as naturally occur. Typically present but a very small fraction of organisms. **Diptera**: *Chironomus, Cricotopus, Rheotanytarsus;* **Non-Insects**: Isopoda*, Physa* **Fishes**: White sucker; Creek chub, Blacknose dace

➔ **RULE 5:** Tolerant individuals (tolerant + highly tolerant) comprise a small fraction or less of all organisms

➔ **Quantitative Rule 5**: Individuals (V + Va) < (10-20%)

Va *Highly Tolerant taxa*

➔ Occurrence and densities of Highly Tolerant taxa are as naturally occur. . Rare and sparse in high-gradient streams (usually absent from samples). The following taxa are representative of this category: **Diptera**: Psychodidae, *Dicrotendipes*; **Non-Insects**: Erpobdellidae, Tubificidae, Glossiphoniidae

➔ **RULE:** see rule for Group V

VI-IX *Non-native taxa; Physiological condition; Ecosystem Function; Spatial and temporal extent*

➔ Not addressed for macroinvertebrates; See Davies and Jackson (2006).

X *Ecosystem connectance*

➔ Connectance on a local scale (floodplain, tributaries) remains good but dams and other flow obstructions downstream impede migration of fish and mussels (eels, salmonids, migration-dependent unionids)

COMBINATORIAL RULE

➔ To be considered Tier 2 for macroinvertebrates, all rules for Attributes II through V must apply; combined with AND.

Table 4-3 (Continued).

3

Evident changes in structure of the biotic community and minimal changes in ecosystem function

Some changes in structure due to loss of some rare native taxa; shifts in relative abundance of taxa but <u>sensitive-ubiquitous</u> taxa are common and abundant; ecosystem functions are fully maintained through redundant attributes of the system

Whole assemblage and sample

➔ Overall taxa richness and density is as naturally occurs

➔ **RULE 1:** Taxa richness is moderately high and subsample density is near target

➔ **Quantitative Rule 1**: Total taxa > (20-25) and Total individuals > (45-55% of target)

I *Historically documented, sensitive, long-lived, or regionally endemic* taxa

➔ Endemic mussels uncommon or absent due to extirpation

II *Highly Sensitive taxa*

➔ Some replacement of taxa having narrow or specialized environmental requirements, with functionally equivalent *intermediate-sensitive* taxa; coldwater obligate taxa are disadvantaged. Reduced richness; may be absent. Taxa such as **Plecoptera**: Capniidae, *Taeniopteryx, Isoperla, Perlesta, Pteronarcys, Leuctra*, *Agnetina*; **Ephemeroptera**: *Cinygmula, Rhithrogena, Epeorus, Serratella, Leucrocuta*; **Trichoptera**: *Glossosoma, Psilotreta, Brachycentrus*; **Diptera**: *Stempellina, Rheopelopia; Hexatoma, Probezzia*; **Coleoptera**: *Promoresia;* **Fishes**: Brook stickleback, Longnose sucker, Longnose dace are uncommonly or absent

➔ **RULE:** May be absent (no rule)

III *Intermediate Sensitive* taxa

➔ Intermediate sensitive or generalist taxa are common and abundant; taxa with broader temperature-tolerance range are favored (e.g., : **Plecoptera**: *Acroneuria*; **Ephemeroptera**: *Ephemerella, Baetisca, Procloeon;* **Coleoptera**: *Psephenus* **Diptera:** *Rheocricotopus, Stempelinella***)**

➔ Brook trout are reduced due to introduction of Brown trout and increased temperature

➔ **RULE 2:** All sensitive taxa (highly sensitive + intermediate sensitive) are moderately diverse

➔ **Quantitative Rule 2**: Taxa (II + III) > 10-12

➔ **RULE 3:** All sensitive taxa (highly sensitive + intermediate sensitive) comprise a substantial fraction of all taxa

➔ **Quantitative Rule 3**: Taxa (II + III) > (30 – 40%) of all taxa

➔ **RULE** 4: All sensitive individuals: comprise a substantial fraction of all organisms

➔ **Quantitative Rule 4**: Individuals (II + III) > (30-50%)

IV *Taxa of Intermediate (indifferent) tolerance*

➔ Filter-feeding blackflies (*Simulium*) and indifferent net-spinning caddisflies (e.g.,*Polycentropus, Neureclipsis*) may show increased densities in response to nutrient enrichment, but relative abundance of all expected major groups is well-distributed : **Trichoptera**: *Diplectrona, Hydroptila*, *Chimarra*, *Neureclipsis;* **Diptera**: *Tvetenia, Polypedilum ,Microtendipes, Simulium;* **Coleoptera**: *Stenelmis*

➔ Increased temperature and increased available nutrients result in increased algal productivity causing an increase in the thickness of the diatom mat. This results in a “slimy” covering on hard substrates.

➔ Fish assemblage exhibits increased occurrence of Common shiner and Fallfish

➔ **RULE:** None

V *Tolerant taxa*

➔ Richness of **Diptera**: Chironomidae is increased; relative abundance of Diptera and Non-insects is somewhat increased but overall relative abundance is well-distributed among taxa from Groups III, IV and V, with the majority of taxa represented from Groups III and IV. Blacknose dace, white sucker are more common.

➔ **RULE 5:** Tolerant individuals (tolerant + highly tolerant) comprise a moderately small fraction or less of all organisms

➔ **Quantitative Rule 5**: Individuals (V + Va) < (20-30%)

Va *Highly Tolerant taxa*

➔ Occurrence and densities of Highly Tolerant taxa are as naturally occur. Rare and sparse in high-gradient streams (usually absent from samples). The following taxa are representative of this category: **Diptera**: Psychodidae, *Dicrotendipes*; **Non-Insects**: Erpobdellidae, Tubificidae, Glossiphoniidae

➔ **RULE:** see rule for Group V, above

VI-X *Non-native taxa; Physiological condition; Ecosystem Function; Spatial and temporal extent; Connectance*

➔ Not addressed for macroinvertebrates. See Davies and Jackson (2006).

COMBINATORIAL RULE

Must fail Tier 2 and must meet minimum Rules for Tier 4 (Tier 4 Rules 1, 2, and 5, and either of Rules 3 or 4; See Tier 4 rules next page). To distinguish from Tier 4, an average of Tier 3 Rules 2, 3, 4, and 5 is used.

Table 4-3 (Continued).

4

Moderate changes in structure of the biotic community and minimal changes in ecosystem function

Moderate changes in structure due to replacement of some Sensitive-ubiquitous taxa by more tolerant taxa, but reproducing populations of some Sensitive taxa are maintained; overall balanced distribution of all expected major groups; ecosystem functions largely maintained through redundant attributes

Whole assemblage and sample

➔ Overall taxa richness is slightly reduced, and density may be high
➔ **RULE 1:** Taxa richness is moderately high and subsample density is near target
➔ **Quantitative Rule 1**: Total taxa > (20-25) and Total individuals > (45-55% of target)

I Historically documented, *sensitive, long-lived, regionally endemic* taxa

➔ Generalist mussel species are present (e.g., *Elliptio; Lampsilis radiata radiata or* Eastern floater-*Pyganodon cataracta*) but sensitive taxa (e.g., *Alasmodonta varicosa; Alasmodonta undulata; Lampsilis cariosa* are absent.

II *Highly Sensitive taxa*

➔ Richness of specialist and vulnerable taxa is notably reduced; if present, densities are low (e.g., **Plecoptera**: Capniidae, *Taeniopteryx, Isoperla, Perlesta, Pteronarcys, Leuctra*; *Agnetina*; **Ephemeroptera**: *Cinygmula, Rhithrogena, Epeorus, Serratella, Leucrocuta*; **Trichoptera**: *Glossosoma; Psilotreta; Brachycentrus*; **Diptera**: *Stempellina, Rheopelopia; Hexatoma, Probezzia*; **Coleoptera**: *Promoresia,* **Fishes**: Occurrence of Slimy sculpin, Longnose sucker and Longnose dace is reduced
➔ **RULE:** May be absent (no rule)

III *Intermediate Sensitive taxa*

➔ Densities of sensitive- ubiquitous scraper and gatherer insects (e.g., **Plecoptera**: *Acroneuria*; **Ephemeroptera**: *Ephemerella, Baetisca, Procloeon;* **Coleoptera**: *Psephenus* **Diptera:** *Rheocricotopus, Stempelinella*) are sufficient to indicate that reproducing populations are present but relative abundance is reduced due to increased densities of opportunist invertebrate taxa (Group IV)
➔ Overall mayfly taxonomic richness is reduced relative to the Tier 2 condition.
➔ Predatory stoneflies are reduced (e.g., *Acroneuria*)
➔ **RULE 2:** Sensitive taxa (highly sensitive + intermediate sensitive) are moderately diverse; may be less than Tier 3
➔ **Quantitative Rule 2**: Taxa (II + III) > (8-12)
➔ **RULE 3:** All sensitive taxa (highly sensitive + intermediate sensitive) comprise at least a moderate and functional fraction of all taxa
➔ **Quantitative Rule 3**: Taxa (II + III) > (20 -30%) of all taxa
➔ **RULE 4**: All sensitive individuals comprise at least a moderate and functional fraction of all organisms
➔ **Quantitative Rule 4**: Individuals (II + III) > (10-20%)

IV *Taxa of Intermediate (indifferent) tolerance*

➔ Possible increase of bryophytes and macro-algae due to increased nutrients.
➔ Increased loads of suspended particles favor collector-filterer invertebrates resulting in increased densities and relative abundance of filter-feeding caddisflies and chironomids (e.g., **Trichoptera**: Hydropsychidae, *Chimarra*, *Neureclipsis*, *Polycentropus;* **Diptera**: *Tvetenia, Polypedilum, Microtendipes, Rheocricotopus, Simulium;* **Fishes**: Common shiner and Fallfish are common and abundant
➔ **RULE:** None

V *Tolerant taxa*

➔ There is an increase in the relative abundance of tolerant generalists (for example, *Eukeifferiella, Cricotopus*) and tolerant net-spinning caddisflies (e.g., *Hydropsyche, Cheumatopsyche*) but they do not exhibit significant dominance
➔ Overall relative abundance is well distributed among taxa from Groups III, IV and V, with the majority of the total abundance represented from Group IV.
➔ Native fish such as White sucker, Blacknose dace, Creek chub are common.
➔ **RULE 5:** Tolerant individuals (tolerant + highly tolerant) comprise less than half of all organisms
➔ **Quantitative Rule 5**: Individuals (V + Va) < (40 - 50%)

Va *Highly Tolerant taxa*

➔ Occurrence and densities of Highly Tolerant taxa are as naturally occur. Often absent. The following taxa are representative of this category: **Diptera**: Psychodidae, *Dicrotendipes*; **Non-Insects**: Erpobdellidae, Tubificidae, Glossiphoniidae
➔ **RULE:** see rule for Group V, above

VI-X *Non-native taxa; Physiological condition; Ecosystem Function; Spatial and temporal extent; Connectance*

➔ Not addressed for macroinvertebrates. See Davies and Jackson (2006).

COMBINATORIAL RULE

Must fail Tier 2 and must meet Rules 1, 2, and 5, and either of Rules 3 or 4. To distinguish from Tier 3, an average of Rules 2, 3, 4, and 5 is used.

Table 4-3 (Continued).

5

Major changes in *structure* of the biotic community and moderate changes in *ecosystem function*

Sensitive taxa are markedly diminished; conspicuously unbalanced distribution of major groups from that expected; organism condition shows signs of physiological stress; system function shows reduced complexity and redundancy; increased build-up or export of unused materials

Whole assemblage and sample

➔ Overall taxa richness is reduced, but density may be high
➔ **RULE 1:** Taxa richness is moderate and subsample density is near target
➔ **Quantitative Rule 1**: Total taxa > (8-12) and Total individuals > (45-55% of target)

I *Historically documented, sensitive, long-lived, or regionally endemic taxa*

➔ Mussel fauna, including commonly occurring, generalist taxa is markedly diminished due to poor water quality

II *Highly Sensitivetaxa*

➔ Only the rare occurrence of individual representatives of specialist and vulnerable taxa with no evidence of successful reproduction
➔ **RULE:** May be absent (no rule)

III *Intermediate Sensitive taxa*

➔ Either absent or present in very low numbers, indicating impaired recruitment and/or reproduction
➔ **RULE:** May be absent
➔ **Quantitative Rule:** Failure of Tier 4 rules (complement)

IV *Taxa of Intermediate (indifferent) tolerance*

➔ Filter-feeding invertebrates such as Hydropsychid caddisflies (e.g., *Cheumatopsyche*) and filter-feeding midges (e.g., *Rheotanytarsus, Microtendipes*) may occur in very high numbers
➔ **RULE:** None

V *Tolerant taxa*

➔ Frequent occurrence of tolerant collector-gatherers (e.g., Orthocladiini, *Micropsectra, Pseudochironomus,* Isopoda- *Caecidotea;* Amphipoda- *Hyalella, Gammarus*);
➔ Relative abundance of non-insects often equal to or higher than relative abundance of insects
➔ Deposit-feeders such as Oligochaeta are increased
➔ Numbers of tolerant predators are increased (Hirudinea, *Thienemannimyia, Cryptochironomus*)
➔ Native fish species are essentially absent with the exception of tolerant taxa like White sucker, Blacknose dace and Creek chub
➔ **RULE:** May be very abundant
➔ **Quantitative Rule:** Failure of Tier 4 rule (complement)

Va *Highly Tolerant taxa*

➔ Occurrence and densities of Highly Tolerant may be increased, but do not dominate taxa richness or abundance. The following taxa are representative of this category: **Diptera**: *Dicrotendipes*; **Non-Insects**: Erpobdellidae, Tubificidae, Glossiphoniidae
➔ **RULE 2:** Highly Tolerant individuals are less abundant than Tolerant Individuals
➔ **Quantitative Rule 2**: Individuals (Va) < Individuals (V)

VI-X *Non-native taxa; Physiological condition; Ecosystem Function; Spatial and temporal extent; Connectance*

➔ Not addressed for macroinvertebrates. See Davies and Jackson (2006).

COMBINATORIAL RULE

Failure of Tier 4 rules and must meet both Rules 1 and 2

Table 4-3 (Continued).

6 **Severe changes in structure of the biotic community and major loss of ecosystem function** *Extreme changes in structure; wholesale changes in taxonomic composition; extreme alterations from normal densities and distributions; organism condition is often poor; ecosystem functions are severely altered*	***Whole assemblage and sample*** ➔ Overall taxa richness is greatly reduced, but density may be high, or greatly reduced (indicating toxicity) ➔ **RULE:** Taxa richness may be extremely low or subsample density may be below target ➔ **Quantitative Rule**: Total taxa < (8-12) or Total individuals < (45-55% of target) (fails Tier 5) **I *Historically documented, sensitive, long-lived, regionally endemic taxa*** ➔ Poor water quality, compaction of substrate, elevated temperature regime and absence of fish hosts for reproductive functions preclude the survival of any mussel fauna **II *Highly Sensitive taxa*** ➔ These taxa are absent due to poor water quality, elevated temperature regime, alteration of habitat, loss of riparian zone, etc. **III *Intermediate Sensitive taxa*** ➔ Absent due to above listed factors, though an occasional transient individual, usually in poor condition, may be collected. **IV *Taxa of Intermediate (indifferent) tolerance*** ➔ Filter-feeding insects and other macroinvertebrate representatives of this group are severely reduced in density and richness, or are absent. **V *Tolerant taxa*** ➔ Low dissolved oxygen conditions preclude survival of most insect taxa except those with special adaptations to deficient oxygen conditions (e.g., *Chironomus*) ➔ The macroinvertebrate assemblage is dominated by tolerant non-insects (Planariidae, Oligochaeta, Hirudinea, Sphaeriidae, etc.) **Va *Highly Tolerant taxa*** ➔ Occurrence and densities of Highly Tolerant taxa are as naturally occur. The following taxa are representative of this category: **Diptera**: Psychodidae, *Dicrotendipes*; **Non-Insects**: Erpobdellidae, Tubificidae, Glossiphoniidae ➔ **RULE:** Highly Tolerant individuals may be dominant ➔ **Quantitative Rule**: Individuals (Va) > Individuals (V) (fails Tier 5) **VI-X *Non-native taxa; Physiological condition; Ecosystem Function; Spatial and temporal extent; Connectance*** ➔ Not addressed for macroinvertebrates. See Davies and Jackson (2006). **COMBINATORIAL RULE** **RULE:** Rule for Tier 6 is any failure of Tier 5 rule

4.2.4 Rule-based Fuzzy Inference

In order to develop the fuzzy inference model, each linguistic variable (e.g., “high taxa richness”) must be defined quantitatively as a fuzzy set (e.g., Klir 2004). A fuzzy set has a membership function, and the membership functions of different classes of taxa richness are shown in Figure 4-3. We used piecewise linear functions to assign membership of a sample to the fuzzy sets shown (Figure 4-3). Numbers below a lower threshold have membership of 0, and numbers above an upper threshold have membership of one, and membership is a straight line between the lower and upper thresholds. For example, in Figure 2-1, a sample with 15 taxa would have a membership of 0.75 in the set “Low-moderate Taxa” and a membership of 0.25 in the set “Moderate Taxa.”

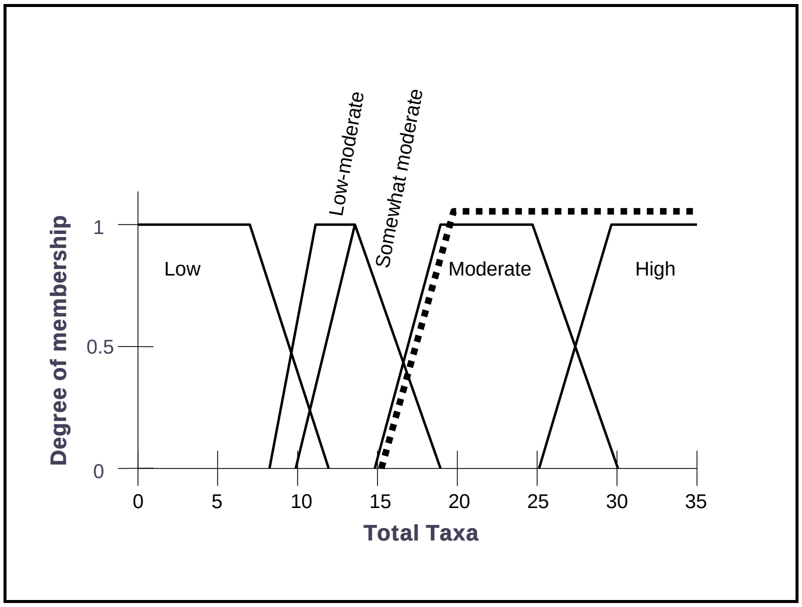


Figure 4-3. Fuzzy set membership functions assigning linguistic values of Total Taxa to defined quantitative ranges. Heavy dashed line shows membership of fuzzy set defined by "Total taxa are moderate to high."

Inference uses the logic statements developed by expert consensus. In "crisp" logic, an "and" statement is the same as "Intersection" in crisp set theory, and logical "or" is equivalent to set theory "union". These are the same in fuzzy logic, however, a fuzzy "and" uses the minimum membership of the two sets, and a fuzzy "or" uses the maximum (Klir 2004). For example, we may have a rule "If Highly Sensitive taxa are Moderate AND Sensitive Taxa are High, THEN Tier is 2." To illustrate this rule, suppose a sample has membership of 0.25 in the set: "Highly Sensitive taxa are Moderate" and membership of 0.75 in "Sensitive Taxa are High;" then its membership in Tier 2 is min(0.75, 0.25) = 0.25. Output of the inference model may include membership of a sample in a single tier only, ties between tiers, and varying memberships among two or more tiers. The tier with the highest membership value is taken as the nominal tier, called "defuzzyfication" of the output.

4.2.5 Model Performance

The models were initially developed using the rules discussed by the participants during the assessment sessions, and modified based on the assessments by CT DEP staff of the sites in Connecticut. The membership functions of the fuzzy sets were subsequently calibrated from the quantitative ranges of the attributes (Tables 4-1, 4-2) for each tier, and recalibrated according to performance of the models.

Because of the high similarity in attribute values between NEWS and CT methods, the same decision rules could be developed for both. Accordingly, we calibrated the decision rules from all CT sites rated by CT state biologists (N=43), and then adjusted the quantitative thresholds of the rules so that both the CT and the NEWS methods yielded the highest agreement. Resultant quantitative rules are shown in Table 4-3, alongside the descriptions and linguistic rules. The state assessment on CT-Kick samples was used as the calibration standard.

Overall agreement between the model and the CT state assessors was 71 percent. It could not entirely reproduce the decisions of the CT state biologists (Table 4-4). For the samples done with CT methods (column CT Kick; Table 4-4), the model assigned a higher quality tier to six sites and lower quality to five sites. For three samples, the model was tied with the tier selected by CT personnel and a tier either higher or lower. In all three cases, the tied tiers of the model were the nominal and minority choice of CT personnel, showing that the model agreed with the CT personnel on these three intermediate sites. In a further eight sites, the nominal tier selected by the model was one (N=7) or two (N=1) tiers different from the nominal choice of CT personnel. Reasons for disparities could include inconsistency by human assessors (the computerized model is not inconsistent – it may be incorrect, but it is always consistent), or incorrect assignment of taxa to an attribute in the master taxa list, or unresolved discrepancies in the rules.

There were seven paired site disagreements (Table 4-4) between the assessments done by CT personnel for the CT Kick method (the calibration standard), and the model results for the NEWS method (column “paired NEWS samples,” Table 4-4). At two of these sites the sample characteristics were clearly different between the NEWS and the CT method (possible random habitat effects in NEWS method). For four sites the model gave the same result for both the CT Kick and the NEWS samples but the state assessors did not; and in one site the model was marginal between agreement and one tier better.

The rightmost column of Table 4-4 shows the agreement between the model and CT personnel ratings of NEWS samples, where the CT assessors were not shown the CT kick net data. These results show a slight bias by CT personnel to downgrade NEWS samples compared to CT samples, most likely because NEWS samples consistently have larger relative proportions, and richness, of fine sediment, slow water taxa such as the Chironomidae.

4.2.6 BCG Assessment of NEWS Sites

Workgroup members of each respective state assigned consensus tiers to 111 NEWS sites within their state. A total of 66 high gradient sites (reach slope > 1 percent, as determined by USGS RF3 data; Moore et al. 2004) were rated by the group, and the fuzzy set model was applied to all 125 high gradient sites (Appendix F). A cross-tabulation of the nominal tiers assigned by each is shown in Table 4-5, and shown graphically in Figure 4-4. The fuzzy model tended to rate the NEWS sites to a higher BCG tier than the state assessors; for example, the model assigned a BCG Tier 2 to 16 sites (of 34) that state assessors had assigned to Tier 3 (Table 4-5).

Table 4-4. Agreement between fuzzy rule-based model and CT assessors for CT sites.

	Rated by CT personnel	Paired NEWS samples rated according to CT kick	NEWS samples, as independently rated by CT personnel
Model 1 tier higher (better)	5 (13%)	7 (29%)	12 (36%)
Model tied higher	1 (2.6%)	….	….
Agree with state personnel	27 (71%)	17 (71%)	16 (48%)
Model tied lower	2 (5.2%)	….	….
Model 1 tier lower (poorer)	2 (5.2%)	0	5 (15%)
Model 2 tiers lower	1 (2.6%)	0	0
N	38	24	33

Table 4-5. State and fuzzy set model ratings of 66 NEWS high gradient sites.

State Nominal BCG Tier	**Fuzzy set nominal BCG tier**					**Total**
	2	**3**	**4**	**5**	**6**	
2	12	1	0	0	0	13
3	16	14	2	2	0	34
4	1	10	2	4	0	17
5	0	0	0	2	0	2
Total	29	25	4	8	0	66

Table 4-5 and Figure 4-4 illustrate that state personnel, accustomed to riffle samples, tend to give biased assessments of the NEWS samples because the NEWS method was multihabitat, and yielded more organisms considered “tolerant” of sedimentation or embeddedness (See Appendix I; comparability). All states in Region 1 preferentially sample riffle habitat, whether by nets or with artificial substrates. Persons accustomed to riffle samples are highly likely to give multi-habitat samples poorer ratings than riffle samples because of the relatively greater proportion of fine-sediment organisms and relative lesser proportion of cobble organisms in the multi-habitat samples.

Based on land use, population, and chemical data, we divided the sites into a priori stress categories. The “Best” and “Near-best” sites were the same as those identified as candidate reference and candidate Tier 1 sites (Appendix B). “Medium” included sites that did not qualify as “Near best” but had less than 100 persons per square mile in the watershed. “High stress” included all sites with more than 100 persons per square mile. “Acid” sites were those with dissolved aluminum > 100 µg/L, and “Chemical” sites were those with more than eight chemical exceedances that were not otherwise in the “Medium” or High” group. The *a priori* best sites correspond to Least Disturbed reference sites as defined in Stoddard et al. (2006).

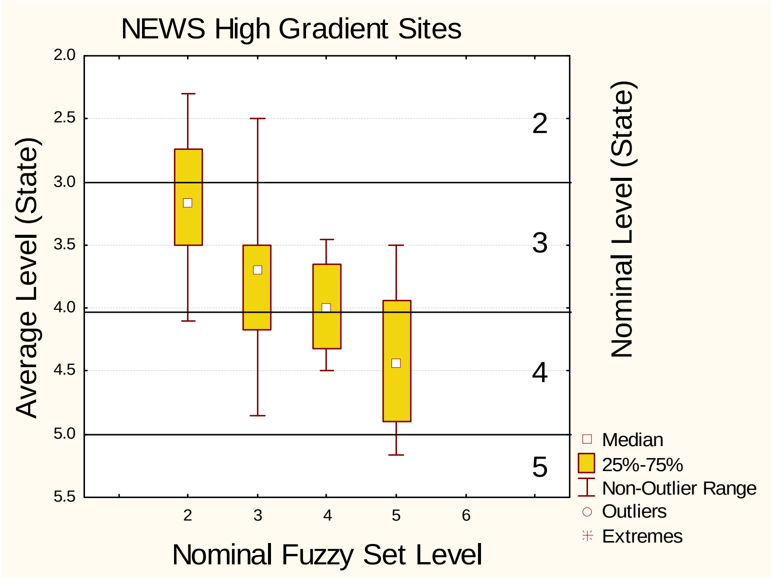


Figure 4-4. Agreement between state assessments of NEWS sites and fuzzy model assessments (see Table 4-5). Boxes and left axis show state assessment tier, average of 2-4 assessors for each site. Right-hand axis shows corresponding nominal tier (scoring convention was that scores of 2 to <3 corresponds to BCG Tier 2; etc.). X-axis shows nominal BCG Tier determined by the fuzzy-set model.

4.2.7 Minimally Stressed Sites

The SPARROW data (Moore et al. 2004) that became available late in the project included both land use and census data, delineated according to catchments of National Hydrography Dataset (NHD) stream reaches, defined from 1: 100,000 maps. A reach is defined as a stream segment bounded by upstream and downstream confluences, or by the watershed divide upstream and a downstream confluence. We used the entire reach-based catchment for each sampling site, so all catchments are attributed somewhat larger areas than would actually be the case for catchments delineated to the sampling site. Completion of the SPARROW data set allowed us to search for “Minimally Disturbed” reference sites (Stoddard et al. 2006), or sites that have a defined, minimal level of human disturbance and stressor, and hence would qualify as candidate Tier 1 sites.

We considered human population density, urban/suburban land use, and agricultural land use, as potential sources of stressors, and physical-chemical parameters measured in the NEWS project as actual measured stressors. Scatter plots of both stressors and biological responses on the potential sources of stress revealed an apparent threshold response in both stressors and biological responses in the range of 3-5 persons per square mile (Figures 4-5 to 4-13). The existence of several sites below this threshold suggests that these sites are “minimally disturbed” or “minimally stressed,” and may qualify as candidate Tier 1 sites. Before the SPARROW dataset was completely available, our initial a priori threshold for “best” sites was 50 persons per square mile. The initial *a priori* “best” (Appendix B) and the Least Disturbed Criteria are compared in Table 4-6.

Table 4-6. Minimally stressed background levels compared to “Best site” selection criteria. Parameters with too few data, or that were all or mostly below detection are not included. See Table B-1 (Appendix B).

Measure	“Best” *a priori* criteria (Appendix B)	Minimally Stressed Background
Chloride	< 20 mg/L	< 1 mg/L (high gradient) < 5 mg/L (low gradient)
Total Phosphorus	< 20 µg/L	< 20 ug/L
Sulfate	< 10 mg/L	< 5 mg/L
Aluminum, dissolved	< 100 µg/L	< 100 µg/L
pH	Not used	6.0 – 7.5
Copper	< 9 µg/L	< 9 µg/L
Iron, dissolved	< 400 µg/L	400 µg/L
Lead, dissolved	< 2.5 µg/L	0.4 µg/L
Mercury, dissolved	< 6 ng/L	< 7.5ng/L
Nickel, dissolved	< 2 µg/L	1.5 µg/L
Zinc, dissolved	< 120 µg/L	< 6 µg/L
Total habitat score	> 140	> 140
Urban/suburban land use	< = 5%	< = 0.1%
Cultivated land	< = 10%	< = 2%
Natural land cover	> 90%	> 90%
Population density	< 50 / mi^2	< 5 / mi^2
Permitted discharges	0	0
Alkalinity	Not used	< 30 mg/L as Ca
$NO_3 + NO_2$	“	< 0.25 mg/L
Potassium	“	< 0.75 mg/L
Sodium	“	< 2 mg/L
Specific conductivity	“	< 100 µS/cm
Total cations	“	< 1000 µeq/L
Total Dissolved Solids	“	< 90 mg/L

Both the chemical and biological data suggest an apparent threshold of chemical concentrations and biological responses at human population densities of 3-5 persons per square mile. Conditions below the threshold appear to represent the natural background, because there is no continuation of either of the associations between population density and stressor values or biological responses, which can be clearly seen above the threshold (Figures 4-8 to 4-13). Finally, the population threshold of 3-5 per square mile is the same for land use (Figures 4-5, 4-6), physical and chemical stressors (Figure 4-2), and biological responses (Figures 4-8 to 4-13).

Because of errors in catchment delineation, and data resolution at low population density, we do not believe that the “thresholds” visible in Figures 4-5 to 4-13 are an actual response threshold; instead, we believe the threshold represents an effective quantitation limit for population density. All catchments used in the SPARROW data set are defined on a downstream confluence, and thus are larger than the actual catchment for a sampling point. Furthermore, census blocks are large in sparsely populated areas, and have reduced spatial resolution compared to densely populated areas, and their boundaries do not coincide with catchment boundaries. Thus, population density estimates less than 5 per square mile are at or below the quantitation limit for catchments in the SPARROW data.

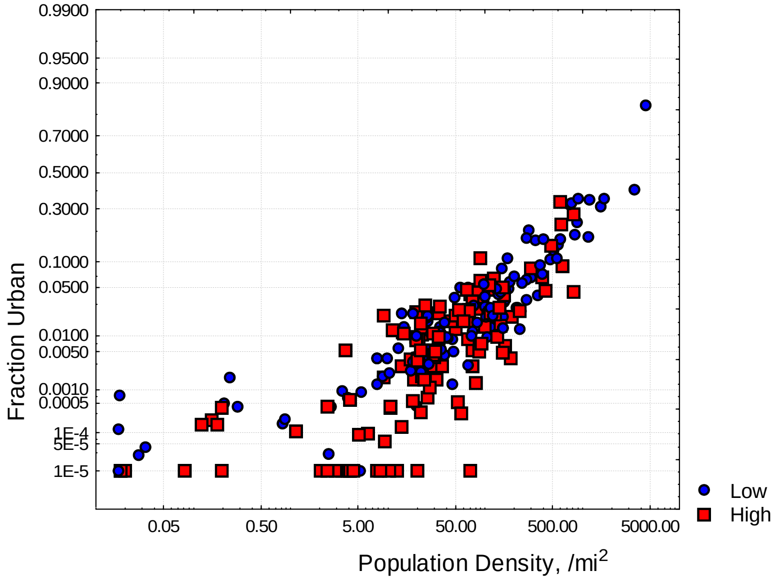


Figure 4-5. Fraction urban/suburban land use and population density, all NEWS sites.

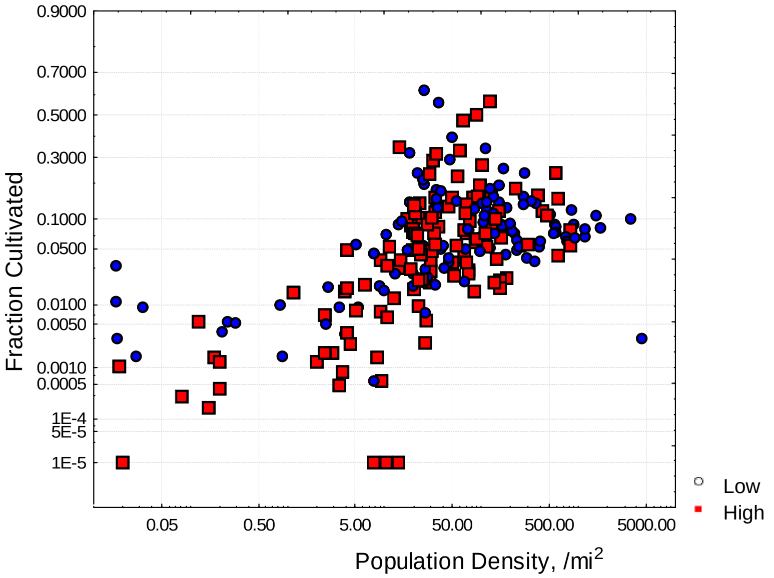


Figure 4-6. Fraction cultivated land and population density, all NEWS sites.

We considered the minimally stressed sites described in Table 4-6 to meet the criteria for Tier 1 sites in New England. The physical and chemical data show that there are no or few anthropogenic stressors acting on these sites (Table 4-6). It should be noted that some sites with population density below 4 persons per square mile nevertheless indicated stress, as several sites had high dissolved aluminum concentrations (> 100 µg/L) and pH less than 6.5, possibly due to acid deposition or quarrying activities. One was noted to have logging activity nearby and also had anomalously high conductivity as well as a very high proportion of tolerant taxa (Carrying Place Stream, ME, sampled in 2002). One stream had moderately high dissolved lead, and two others had copper concentrations at or near the EPA chronic criteria. Nearly all streams had mercury concentrations of 1 - 7 µg/L (Figure 4-7e), well above the EPA chronic criteria of 0.77 µg/L (USEPA 2002). Consequently, we did not screen Tier 1 sites for mercury. We found 11 sites that met the selection criteria of Table 4-6 for minimally stressed sites.

The Tier 1 sites from Maine and New Hampshire did not differ in terms of the BCG attributes from the Tier 2 sites of Connecticut (Table 4-7). This is in spite of known taxonomic differences on the north-south gradient; for example, the Baetidae and Leuctridae are found more frequently in Maine and New Hampshire than in Connecticut, but the Polycentropodidae and Taeniopterygidae are found more frequently in Connecticut than in Maine and New Hampshire (Appendix C; Table C-3). All four of these families are moderately to highly sensitive.

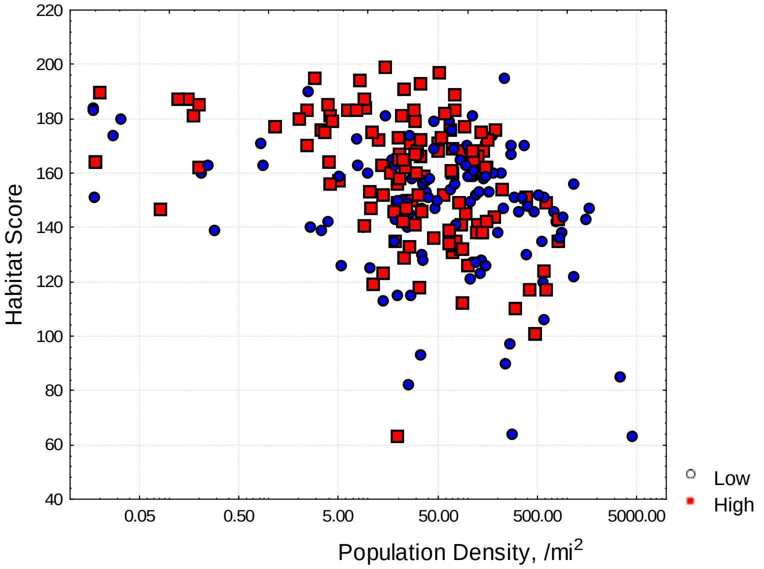


Figure 4-7a. Stressor indicators (habitat) and population density; high and low gradient streams

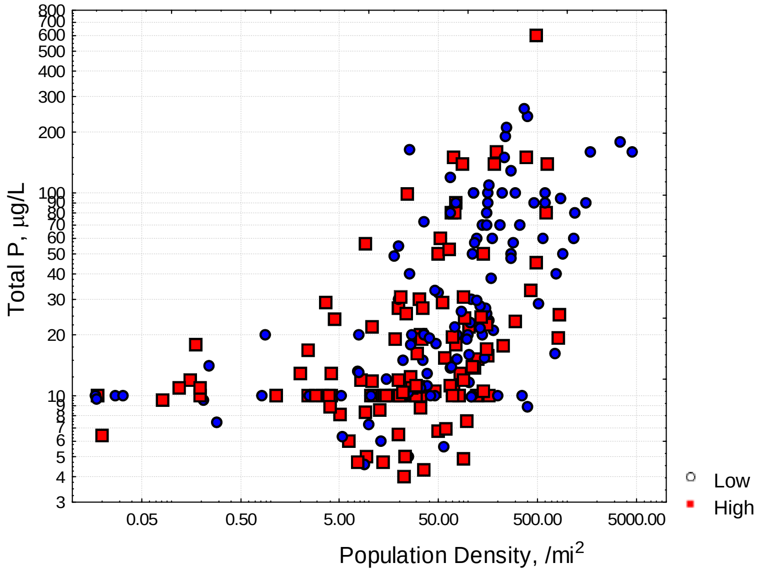


Figure 4-7b. Stressor indicators (Total phosphorus) and population density; high and low gradient streams

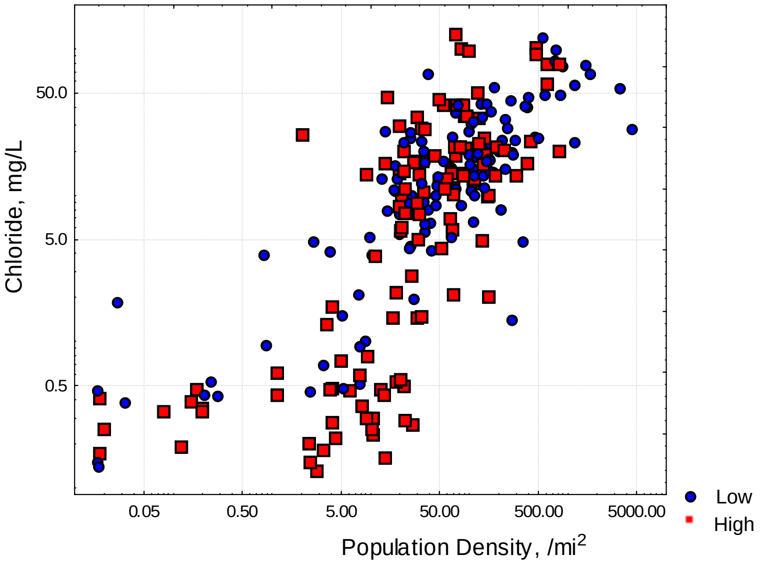


Figure 4-7c. Stressor indicators (chloride) and population density; high and low gradient streams

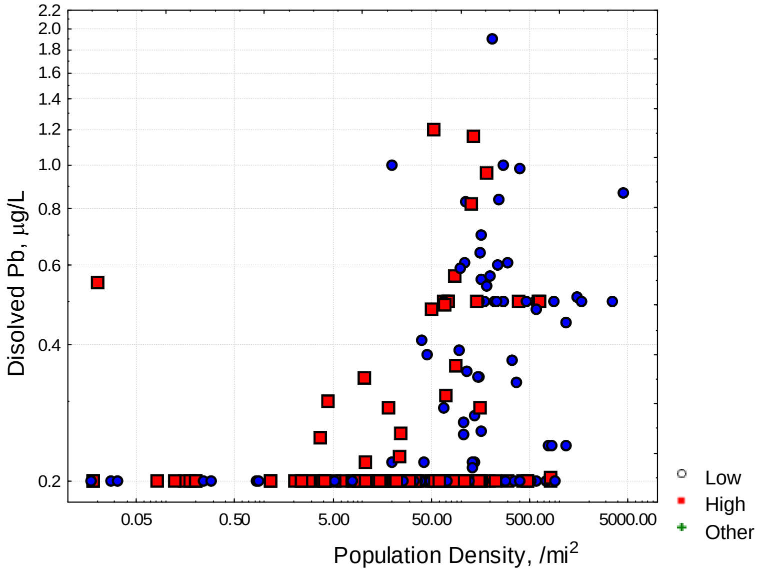


Figure 4-7d. Stressor indicators (dissolved lead) and population density; high and low gradient streams

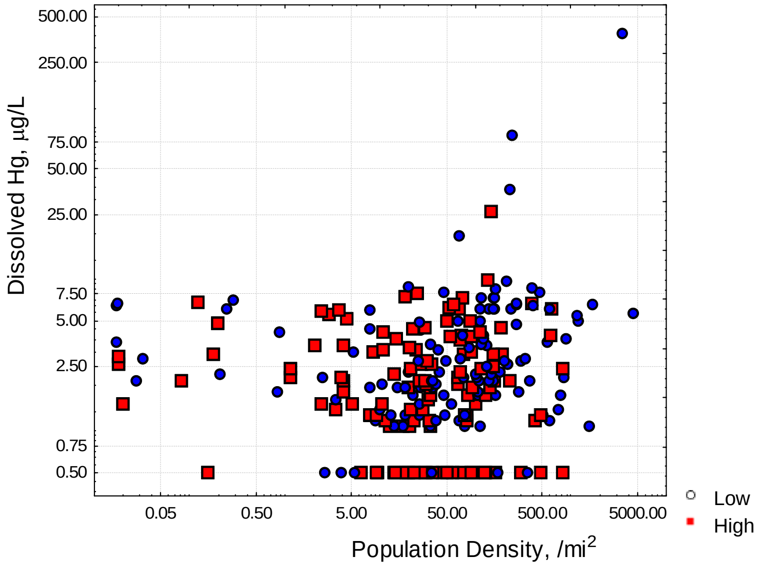


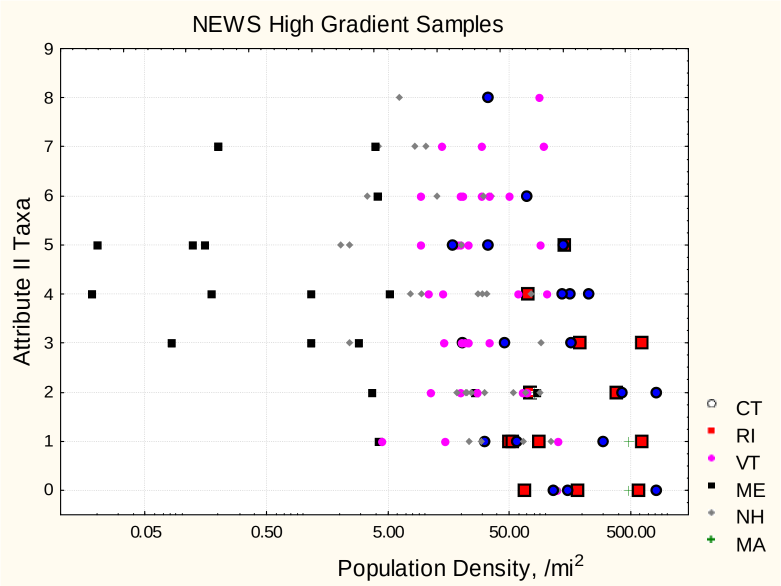
Figure 4-7e. Stressor indicators (dissolved mercury) and population density; high and low gradient streams

Examination of the physical-chemical data, and the response of the BCG attribute metrics to population density, suggests that Tier 1 sites in New England are dilute, highly oligotrophic streams with low nutrient concentrations, low dissolved solids, and especially low chloride concentrations (< 1 mg/L). Moderate disturbance and enrichment may result in increases in richness and relative abundances of Attributes IV and V (indifferent and tolerant taxa), without reducing richness of the two sensitive attributes (cf. Figures 4-8 to 4-13). At many sites, the richness and relative abundance of the most sensitive taxa , Attribute II, also increased under moderate levels of stress (Figure 4-8). These results may indicate a subsidy stress, or an overall increase in absolute abundance of all taxa under mild to moderate stress.

These results suggest that while minimally stressed sites can be identified in New England from land use and physical-chemical data, we cannot separate Tier 1 sites from Tier 2 sites using the benthic macroinvertebrate samples of the NEWS program. The Tier 1 invertebrate data from Maine fit neatly into the Tier 2 description developed from sites in Connecticut. Additional attributes already identified for the BCG, such as productivity (Attribute VII; ecosystem function attributes), extent of rare and endemic taxa (Attribute I), or connectivity (Attribute X), may be necessary to discriminate Tier 1 from Tier 2.

Table 4-7. Tier 1 Attributes compared to Tiers 2 and 3. Tiers 2 and 3 from Table 4-2.

Attributes	**NEWS Methods, High-Gradient Streams**		
	ME, NH Tier 1 (n=11)	**CT Tier 2 (n=2)**	**CT Tier 3 (n=13)**
0 General	Richness: 32-52	Richness: 36-60	Richness: 37-53
I *Endemics*			
II *Highly sensitive taxa*	3-7 taxa 3-21%	3-7 taxa 10-15%	0-6 taxa 0-9%
III *Intermediate Sensitive taxa*	10-19 taxa 27-67%	10-20 taxa 43-45%	12-20 taxa 24-64%
	II + III: 14-26 taxa II + III: 27-56% of taxa II + III: 35-71% of indiv	II + III: 20-27 taxa II + III: 45-56% of taxa II + III: 55-57% of indiv	II + III: 13-25 taxa II + III: 30-58% of taxa II + III: 29-67% of indiv
IV *Indifferent taxa*	12-28 taxa 19-58%	14-27 taxa 36-41%	12-24 taxa 28- 60%
V.a *Tolerant taxa*	1-8 taxa 1.4 – 9.7%	1-4 taxa 1-3%	1-7 taxa 1 – 21%
		Va+Vb: 1-5 taxa Va+Vb: 1-3%	Va+Vb: 2-7 taxa Va+Vb: 4-22%
V.b *Highly tolerant taxa*		0-1 taxa, 0-1% IV + V + Vb: 36-44%	0 - 1 taxa, 0 - 2% IV + V + Vb: 31-72%
Indicator Taxa	E rich 4-9 E% 8-33 Hydro: 0.5-4% Nonins: 2-21%	E rich: 3-7 E %: 10-21 Hydro: 2-3% Tubi: 0-0.5% Nonins: 1-5%	E rich 2-7 E % 5-39 Hydro 0-25% Tubi 0-2% Nonins 1-42%



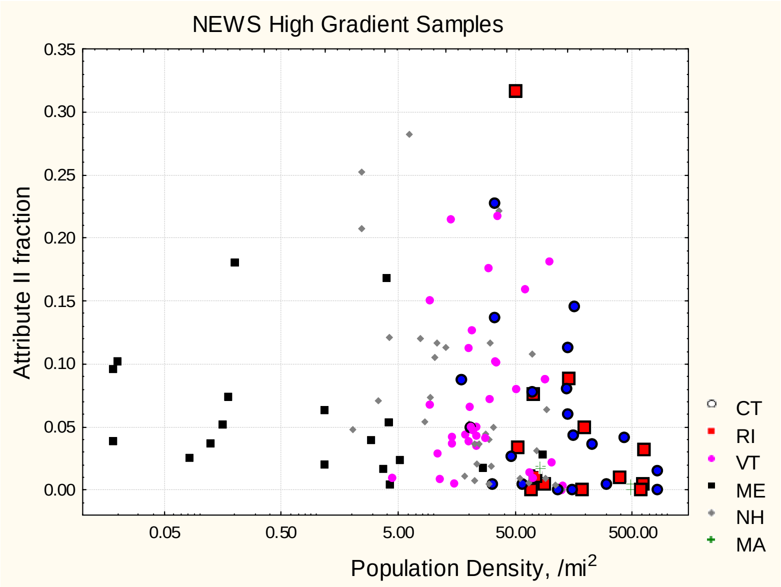


Figure 4-8. Attribute 2 taxa and population density

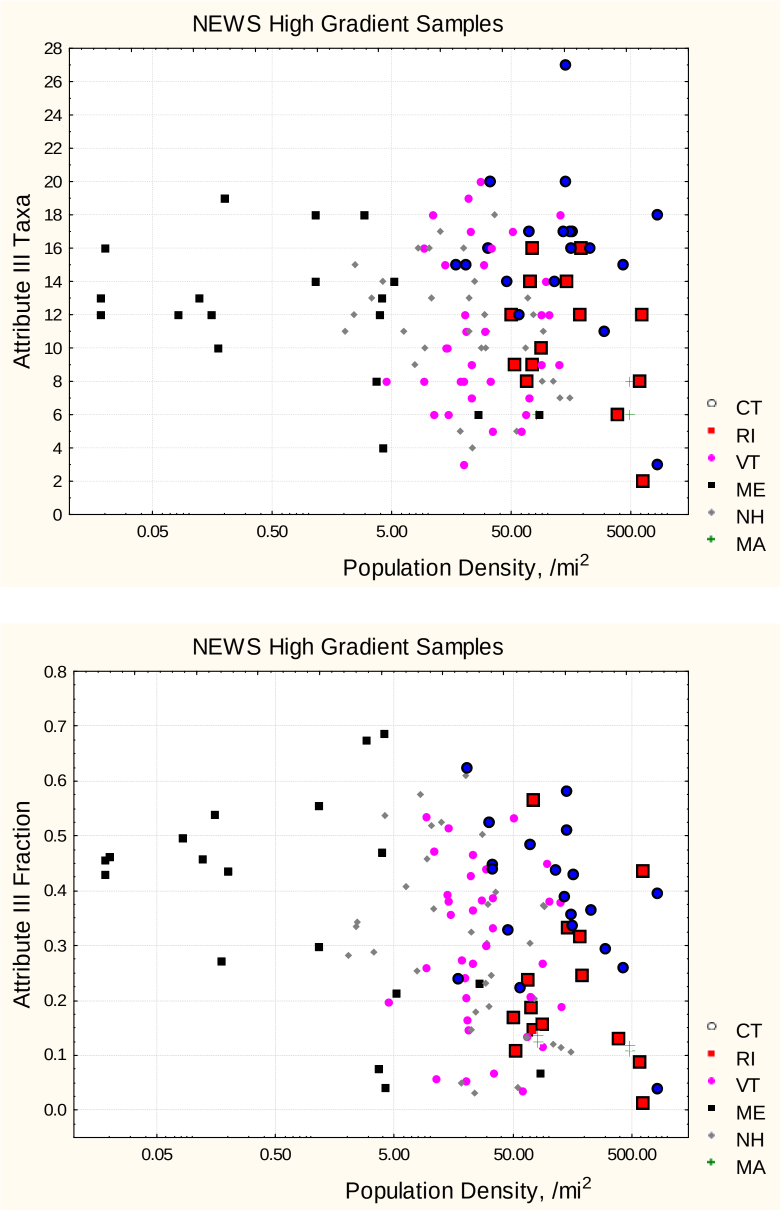
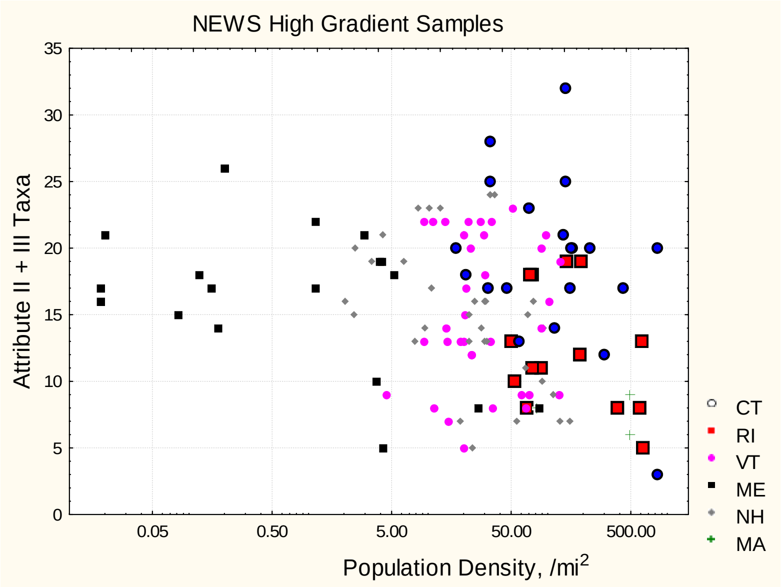


Figure 4-9. Attribute 3 taxa and population



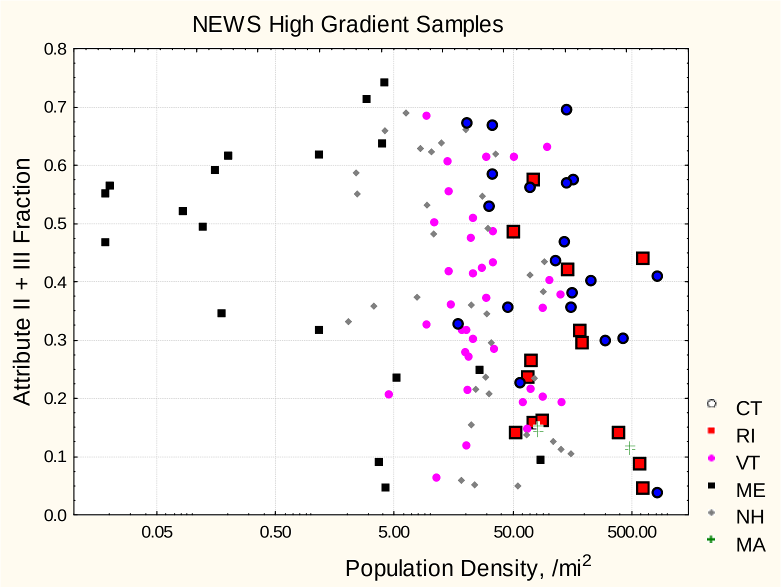


Figure 4-10. Atrributes II + III (all sensitive taxa) summed, and population

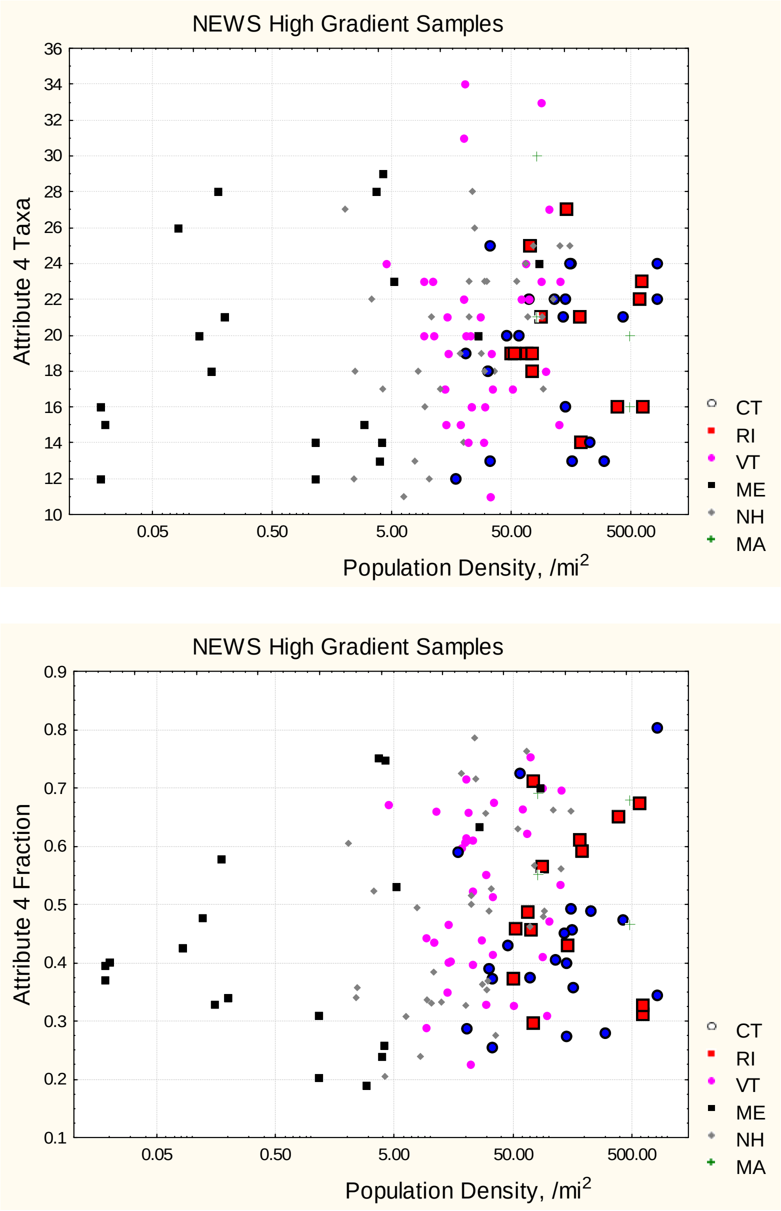


Figure 4-11. Intermediate (indifferent) taxa and population

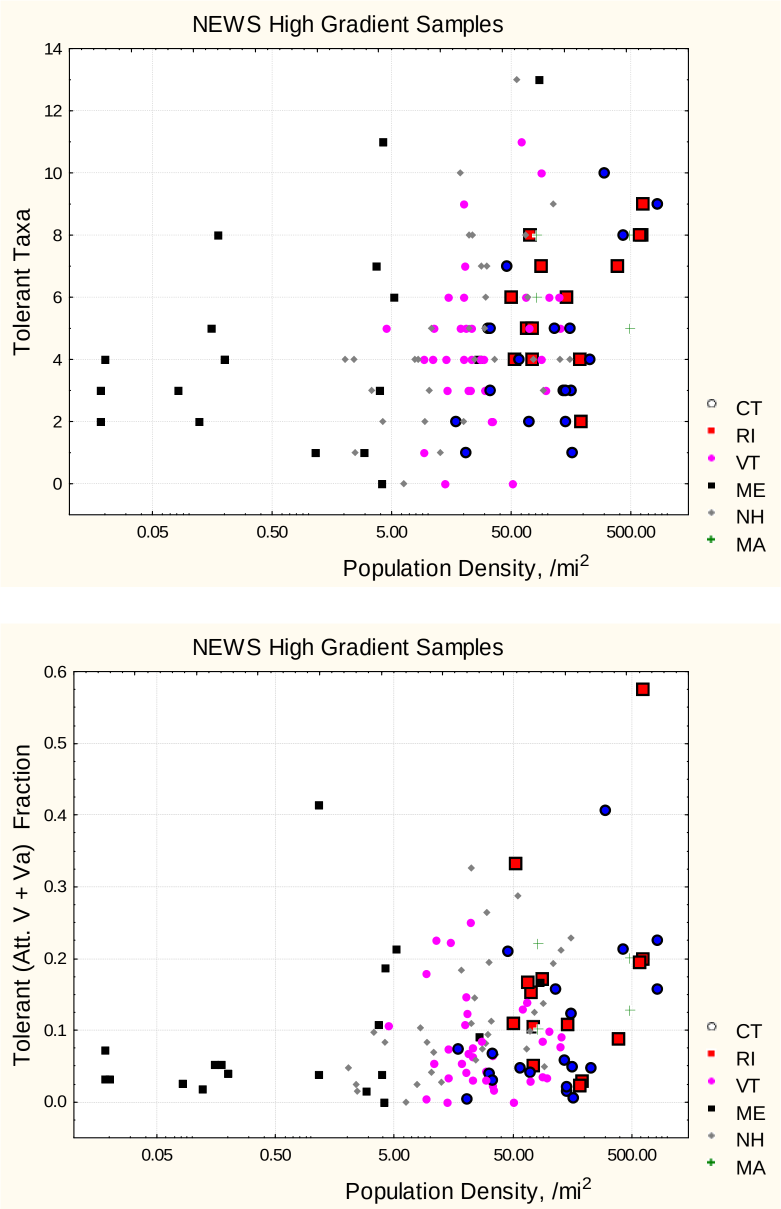


Figure 4-12. Tolerant taxa (Attributes 5 + 5a) and population

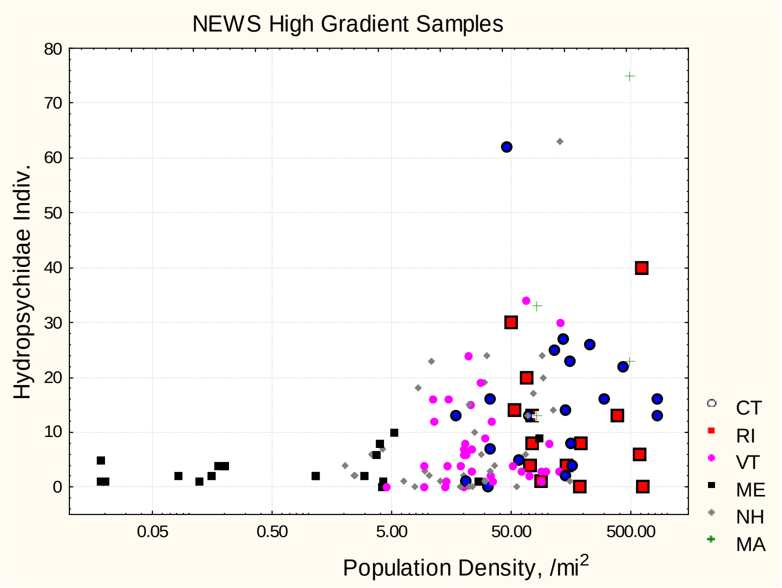


Figure 4-13. Number of Hydropsychidae (from 200-organism subsample) and population.

5.0 The Biological Condition Gradient – Fish

5.1 Fish Methods

5.1.1 The Biological Condition Gradient

Biocondition gradient values were assigned to a total of 280 of 282 sites by a panel of biologists. Two hundred and twenty-six state sites were assessed by two or three biologists, and 56 regional EPA sites were assessed by all seven. The group assigned tiers to the fish data using the following approach: a borderline evaluation was designated as 0.2 for borderline lower tier, and 0.8 for a borderline upper tier with 0.5 being a mid-tier assignment. For example a mid-Tier 2 site was numerically represented as a 2.5, a Tier 2 value that bordered on Tier 1 was given a 2.2, and correspondingly a borderline evaluation with Tier 3 was rated as 2.8.

The group discussed at length, the potential for identifying Tier 1 sites. It was believed by some that no Tier 1 sites exist, since historic widespread human activities have imparted varying degrees of impact on nearly all riverine communities. Others maintained that simple fish assemblages of small unimpacted coldwater streams support the same species, e.g. brook trout and slimy sculpin that probably existed pre-European colonization. It was recognized by all that defining a Tier 1 stream assemblage for larger streams and rivers with more species becomes problematic because so few examples of reference conditions exist in larger watersheds, especially those in low elevation regions. An expeditious view of this issue evolved that, for scoring considerations, sites may only score as high quality as Tier 2. Implicit with this approach is that the differences between a Tier 1 and Tier 2 assemblages are so fine as to be undetectable with the resolution afforded by the current sampling technique. The essential distinction between Tier 1 and 2 is that some changes in density and/or biomass occur in Tier 2. Since the parameters of density and biomass vary temporally, especially in small New England streams, speculating on what the slight but significant change in density/biomass would be between the Tier 1 and Tier 2 is difficult, if not impossible. Consequently the group concluded that the best BCG score attainable in this exercise would be Tier 2. It should be understood, however, that this scoring approach does not preclude the possibility that within the list of Tier 2 sites are some assemblages that are in actuality Tier 1. While assemblage differences between Tier 1 and Tier 2 may be unapparent, measures of current and past watershed disturbance may be a useful Tier indicator.

The first three ecological attributes describing sensitive species, 1) *historically documented, sensitive long-lived or regionally endemic taxa,* 2) *sensitive rare taxa,* and 3) *sensitive ubiquitous taxa* were combined into a single "sensitive" category for the purposes of classifying species in this analysis. The current distributional range of many Attribute I and II species in New England is restricted to certain watersheds or specific coastal regions. It is difficult to know whether their rareness is due to natural zoogeographic and habitat requirements, or a result of a history of human disturbance. It therefore seems unreasonable to assume that all of these species *should* potentially occur at most sites and that their absence is necessarily an indication of disturbance. Consequently, combining the three attributes removes the question of original range and simply credits a site for having *any* sensitive species. It may be feasible to differentiate between attribute II and III species in certain restricted locations, such as low gradient coastal streams. Where an attribute II species did occur, extra consideration was given in determining the final BCG Tier designation for that site.

Table 5-1 lists ecological attribute classification of fishes potentially inhabiting New England running waters. This table is based largely on (Halliwell et al. 1999). Modifications were made to fit the BCG model attributes by the workgroup.

Table 5-1. Biocondition Axis of ecological attribute classification for running water New England fish species.

Ecological Attributes	Species	
I. *Historically Documented **Sensitive**, Long-lived or Regionally-endemic Taxa*	American brook lamprey Atlantic sturgeon Shortnose sturgeon Lake sturgeon (VT Champlain Basin)	
II. ***Sensitive** Rare Taxa*	Atlantic salmon East. silvery minnow Cutlips minnow Northern redbelly dace (MA) Blacknose shiner (VT Champlain Drainage) Bridle shiner Lake chub (MA)	Creek chubsucker Burbot (MA, NH, CT) Slimy sculpin (CT) Banded sunfish Swamp darter Longnose sucker (MA, NH, RI, and CT)
III. ***Sensitive**-Ubiquitous Taxa*	Brook trout Slimy sculpin	Longnose sucker (VT, NH and ME) Burbot (VT, ME)
IV. *Taxa of **Intermediate** Tolerance*	Northern pike (VT Champlain Drainage) Redfin pickerel Chain pickerel Longnose dace Fallfish Common shiner Lake chub Pearl dace Spottail shiner Northern redbelly dace (ME, NH, and VT)	Brook stickleback (VT Champlain Drainage) Three-spine stickleback Four spine stickleback Nine spine stickleback Smallmouth bass (VT Champlain Drainage) Pumpkinseed Bluegill (VT Champlain Drainage) Redbreast Sunfish White Perch (where native) Tessellated Darter
V. ***Tolerant** taxa*	American Eel Central Mudminnow (VT Champlain Drainage) Blacknose Dace Creek Chub Golden Shiner Bluntnose Minnow (VT Champlain Drainage)	Fathead Minnow (VT Champlain Drainage) Brown Bullhead Yellow bullhead (VT Champlain Drainage) White Sucker Banded Killifish
VI. *Nonnative or intentionally Introduced Taxa*	Brown trout Rainbow trout Carp Goldfish Black crappie White crappie Green sunfish Largemouth bass White perch (where nonnative)	*The Following species are native only to VT. Champlain drainage and are considered as nonnative to the remainder of New England:* Northern pike Central mudminnow Fathead minnow Bluntnose minnow Yellow bullhead Bluegill Smallmouth bass

5.1.2 The Index of Biotic Integrity

The Index of Biotic Integrity (IBI) was originally designed to be applied to Midwestern U.S. streams. In order for the IBI to be used successfully elsewhere, the index requires modification to account for regional changes in species composition. Modifying the index for streams in New England has been especially challenging because waters in this region are characterized by low species richness. Modifications for Northeast wadeable running waters have been offered by Miller et. al (1988), Langdon (1988, 2001), Halliwell et al. (1999) and Daniels et al. (2001).

The working group elected to use two IBI's employed by the State of Vermont as tools in assisting biologists in making BCG determinations. The nine-metric Mixed Water IBI (MWIBI) was applied to warm and cold water streams naturally supporting five or more species, and the six-metric Cold Water IBI (CWIBI) was used to evaluate small coldwater streams naturally supporting two to four species. Because nonnative species were viewed by the group as a more prevalent problem in running waters in New England outside Vermont, the MWIBI was modified to include the addition of a metric that accounted for proportion of catch comprised of nonnative species. This metric would score negatively with an increasing proportion as nonnative species. For current purposes, the modified IBI was known as the New England IBI (NEIBI).

The primary approach in evaluating stream sites was to apply the BCG tier descriptions to sampled fish assemblages. It was the consensus of the working group that the IBI's should be used only as one tool in assessing fish community health. Several metrics contained in the Vermont IBI's are reflected as BCG tier-specific attributes, so examination of the component metrics as well as the final IBI score proved helpful in developing BCG Tier assignments.

5.2 Results

5.2.1 Species Information

Fifty-six species and two hybrids were identified from 282 sites. Table 5-2 shows frequency of occurrence for each species. The three most often collected species were blacknose dace (181 sites -64 percent), brook trout (179 sites - 63 percent) and white sucker (159 sites-56 percent). Eleven nonnative species were recorded. Nonnative species were collected at 43.5 percent (126) of the sites and made up 5.2 percent of all fish collected. The most common nonnative species observed were bluegill (66 sites-23 percent), brown trout (57 sites-20.1 percent) and largemouth bass (49 sites-17..3 percent).

Eleven of the 56 species collected were considered intolerant to human disturbance; nine are native to New England. The two most common sensitive species encountered were brook trout and slimy sculpin, occurring at 63 percent and 25 percent of the sites sampled respectively. Brook trout is considered sensitive ubiquitous (Attribute III). Slimy sculpin is classified as *sensitive rare* in Maine, Connecticut and Rhode Island, and *sensitive ubiquitous* in New Hampshire and Vermont. Stocked Atlantic salmon were present at 14 percent of the sites and is also considered a *sensitive ubiquitous* species. There were no species collected that were classified as Attribute I, *Historically documented, sensitive, long-lived or regionally endemic.*

Table 5-2. Common and scientific names of fishes collected at 282 NEWS sites 2000-2003 and number of sites where recorded. Common names of nonnative species are in italics.

Species		No. Of Sites	% of Total
Blacknose Dace	*Rhinichthys atratulus*	181	64.0
Eastern Brook Trout	*Salvelinus fontinalis*	179	63.3
White Sucker	*Catostomus commersoni*	159	56.2
Creek Chub	*Semotilus atromaculatus*	101	35.7
Common Shiner	*Notropis cornutus*	94	33.2
Longnose Dace	*Rhinichthys cataractae*	84	29.7
American Eel	*Anguilla rostrata*	83	29.3
Pumpkinseed	*Lepomis gibbosus*	79	27.9
Slimy Sculpin	*Cottus cognatus*	70	24.7
Bluegill	*Lepomis macrochirus*	66	23.3
Tessellated Darter	*Etheostoma olmstedi*	59	20.8
Fallfish	*Semotilus corporalis*	58	20.5
Brown Trout	*Salmo trutta*	57	20.1
Largemouth Bass	*Micropterus salmoides*	49	17.3
Atlantic Salmon	*Salmo salar*	40	14.1
Chain Pickerel	*Esox niger*	39	13.8
Redbreast Sunfish	*Lepomis auritus*	33	11.7
Redfin Pickerel	*Esox americanus americanus*	32	11.3
Brown Bullhead	*Ameiurus nebulosus*	31	11.0
Golden Shiner	*Notemigonus crysoleucas*	28	9.9
Rainbow Trout	*Oncorhynchus mykiss*	26	9.2
Yellow Perch	*Perca flavescens*	21	7.4
Smallmouth Bass	*Micropterus dolomieu*	19	6.7
Burbot	*Lota lota*	18	6.4
Unknown sp.		14	4.9
Longnose Sucker	*Catostomus catostomus*	13	4.6
Rock Bass	*Ambloplites rupestris*	13	4.6
Yellow Bullhead	*Ameiurus natalis*	12	4.2
Northern Redbelly dace	*Phoxinus eos*	10	3.5
Fathead Minnow	*Pimephales promelas*	8	2.8
Spottail Shiner	*Notropis hudsonius*	7	2.5
Creek Chubsucker	*Erimyzon oblongus*	6	2.1
Bluntnose Minnow	*Pimephales notatus*	6	2.1
Lake Chub	*Couesius plumbeus*	6	2.1
Banded Killifish	*Fundulus diaphanus*	5	1.8
Cutlips Minnow	*Exoglossum maxillingua*	5	1.8
Finescale Dace	*Phoxinus neogaeus*	4	1.4
Fourspine Stickleback	*Apeltes quadracus*	4	1.4
Nine-spined Stickleback	*Pungitius pungitius*	4	1.4
Stickleback sp.		4	1.4
Black Crappie	*Pomoxis nigromaculatus*	3	1.1
Rosyface Shiner	*Notropis rubellus*	3	1.1
Threespine Stickleback	*Gasterosteus aculeatus*	3	1.1
Brook Stickleback	*Culaea inconstans*	2	0.7

Green Sunfish	*Lepomis cyanellus*	2	0.7

Table 5-2 (Continued).

Species		No. Of Sites	% of Total
Hybrid Sunfish	*Lepomis*	2	0.7
Alewife	*Alosa pseudoharengus*	1	0.4
Blueback Herring	*Alosa aestivalis*	1	0.4
Carp	*Cyprinus carpio*	1	0.4
Log Perch	*Percina caprodes*	1	0.4
Margined Madtom	*Noturus insignis*	1	0.4
Mimic Shiner	*Notropis volucellus*	1	0.4
Mudminnow	*Umbra limi*	1	0.4
Mummichog	*Fundulus heteroclitus*	1	0.4
Northern Pike	*Esox lucius*	1	0.4
Sea Lamprey	*Petromyzon marinus*	1	0.4
Spotfin Shiner	*Cyprinella spiloptera*	1	0.4
Swamp Darter	*Etheostoma fusiforme*	1	0.4
Tiger Trout	*Salvelinus fontinalis X Salmo trutta*	1	0.4
Trout Perch	*Percopsis omiscomaycus*	1	0.4

5.2.2 Biological Condition Gradient Evaluations

Of the 282 sites sampled, 280 were evaluated using the BCG. Two sites in Vermont were not evaluated because the sampling was judged as non representative; one site was not wadeable and the other was a deep section of a low gradient reach. Biocondition Gradient values from all sites are given in Appendix G.

Nearly 86 percent of all sites were scored Tier 4 (*fair-good*)or better. Seventy-two % of sites were determined to be Tier 3 or 4. Thirty-nine sites (13.9 percent) were assigned a Tier 2 rating. The group making the evaluations believed that some of the Tier 2 sites could have actually been designated as Tier 1 (see discussion in Methods section). Nearly 14 percent of sites were cited as Tier 5. Less than 1 percent were designated as Tier 6; both sites were in Vermont. The dominance of Tier 3 and 4 sites was observed in every state dataset (Table 5-3, Figure 5-1)**.**

Table 5-3. Distribution of Biocondition Gradient scores by state (includes state and regional data).

BCG Tier	Connecticut	Maine	New Hampshire	Vermont	Rhode Island	Massachusetts	Total	%
2	2 (3.0)	11 (20.0)	9 (14.8)	11 (19.0)	5 (13.9)	1	39	13.9
3	27 (40.9)	16 (29.0)	17 (27.9)	18 (31.0)	11 (30.6)	0	89	31.7
4	29 (43.9)	23 (41.8)	22 (36.1)	22 (37.9)	15 (41.7)	1	112	40.0
5	8 (12.1)	5 (9.1)	13 (21.3)	5 (8.6)	5 (13.9)	2	38	13.6
6	0	0	0	2 (3.4)	0	0	2	0.7

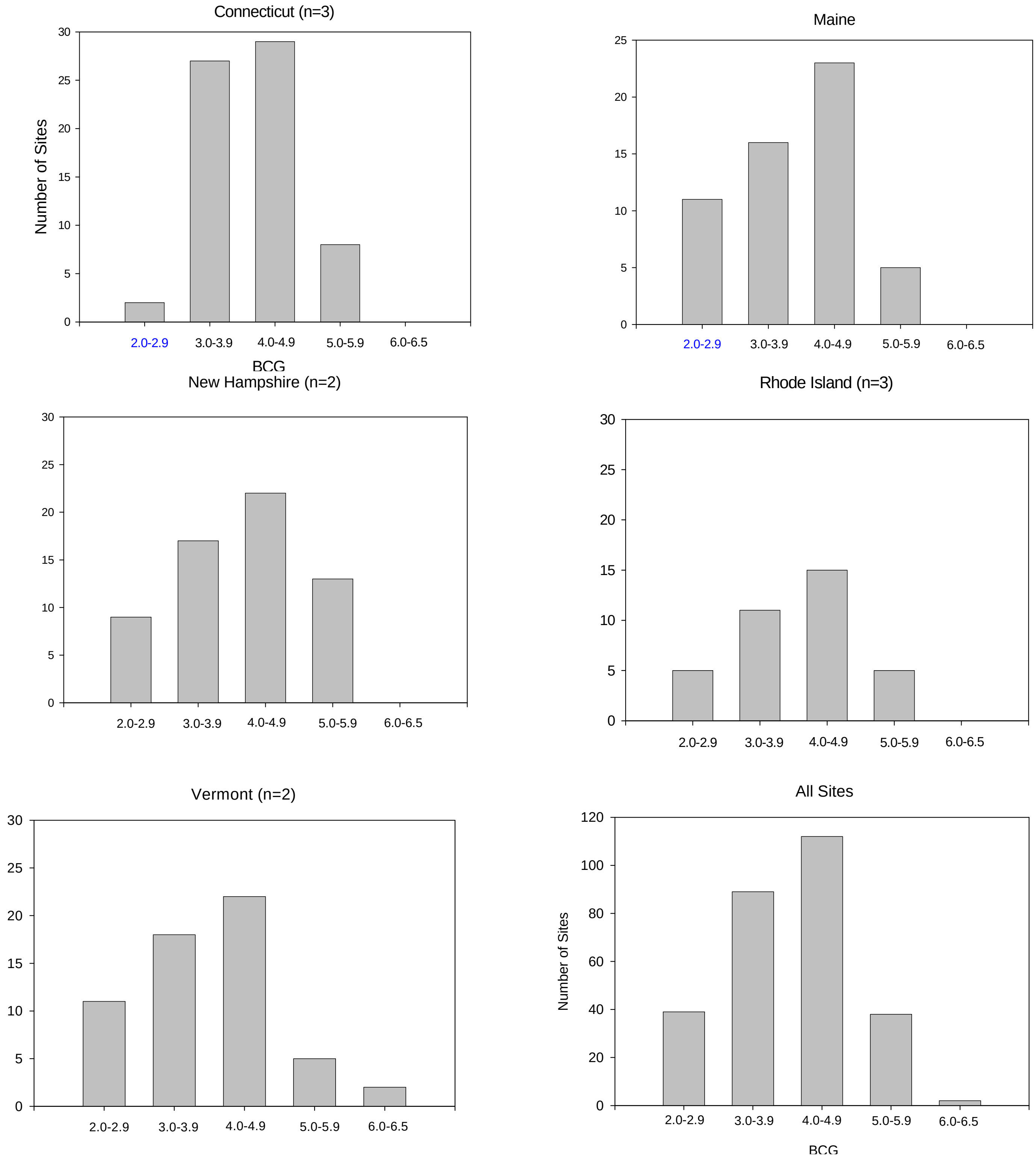


Figure 5-1. Distribution of Biocondition Gradient scores by state and total (includes state and regional data), and n-number of evaluators.

BCG values for all sites spanned the entire 14 step range of 2.2 to 6.5 (see Methods section for discussion on the assignment of scores). Between-biologist variation in scores increased with the number of individuals rating a site (Table 5-4). All possible two-biologist combinations from two, three, and seven biologist assessments were contrasted. The mean difference per site in steps (one tier =3 steps) is 2.1. Rhode Island and Connecticut sites were evaluated by three biologists generating a mean difference of 2.5 steps. A 5.2 mean step difference resulted from seven biologists evaluating EPA regional sites. Given that there are a total of 14 steps from 2.2 to 6.5, the 5.2 step variation per site evaluation is a sizable disparity. A nine step difference (3 tiers) in BCG determinations was seen at three sites.

Table 5-4. Variation in BCG tier step assignments between biologists

	Number of Evaluators		
	2	3	7
Step difference Means and (number of evaluations)	1.7 (54) - N.H. 2.0 (49) - VT. 2.6 (28) - Maine Grand Mean -2.1	2.2 (56) - (Conn.) 3.0 (36) - (R.I.) Grand Mean -2.6	5.4 (55)- EPA Regional
All 27 combinations of two biologists from all data	2.1 (1,557)	-	-

A preliminary attempt to explain reasons for the larger differences in tier assignments between biologists yielded no clear explanations. A typical NEWS sample is represented by high to moderate gradient, hard bottomed sites with moderate to high fish density and good representation of benthic insectivores and top carnivores. The panel of biologists are more familiar evaluating these stream types. Additionally, IBIs can be scored for this stream type as a tool in aiding the evaluator. Conversely, low gradient, soft-bottomed streams in New England will often naturally support the same species as similarly sized hard-bottomed streams, but dominance of species within the assemblage will be shifted away from benthic insectivores and towards pool species which are usually tolerant to disturbance. Also lower densities may be recorded from low gradient streams. Applying current modifications of IBI's to soft bottomed streams then, often results in artificially lower index scores (reflecting increased dominance of tolerant, generalist species). As a result, no IBI has been devised for New England streams that can be consistently applied to low gradient stream reaches. Biologists may differ then in their evaluation to site population data from low gradient sites because without a clear idea of the stream type, a BCG evaluation may be in error, much in the same way as a conventional IBI evaluation would. A problem for both assessment approaches is that no clear and widely known reference condition for this stream reach type exists.

To examine the role of site density an diversity in high site variation in biologist BCG scores, all sites evaluated by two biologists with more than a two step difference, all three-evaluator sites with more than a 3-step difference, and all EPA Regional sites (seven biologists) with more than a 5-step variation were examined. The ratio of sites with high BCG score variation to those with low variation in BCG assessments was contrasted between two groups of sites: one low density/diversity data and the other "normal" data. The proportion of low density/diversity sites in the high variation site group was similar to low density diversity sites in the lower variation group indicating that the characteristics of low density and diversity probably did not explain a significant extent of the observed variation in BCG scores. A more detailed investigation of the data would be helpful in explaining reasons for variation between biologists.

Since reference expectations are essentially different between soft and hard bottomed streams it is imperative that the evaluator have foreknowledge of the type of stream to be evaluated. Variation in biologist's evaluations should be minimized in the future by providing each biologist with the full description of the physical habitat for all sampling sections, particularly of substrate composition. Providing photos of every sampled section to all evaluators would also prove useful in this regard. Other site attributes relating to ecoregion or other regional faunal classification may also serve to inform the evaluating biologist.

5.2.3 Biotic Integrity Indexes

The two Vermont IBIs and the modification were designed to be applied to wadeable hard bottomed streams with at least two naturally occurring native species present. A total of 209 of the 282 sites sampled (74 percent) met the requirements for application of an IBI. The NEIBI and Vermont MWIBI were scored for 160 sites, and the CWIBI was applied to 49 sites (Appendix H). The NEIBI is identical to the MWIBI with the exception that it contains an extra metric that scores negatively for an increasing proportion of nonnative species. Both have the same criteria for application: a population that naturally supports at least five native species. Small coldwater sites naturally supporting from two to four species were assessed using the CWIBI. Table 5-5 shows the approximate relationship between BCG Tiers and IBI-based evaluations. It should be noted that changes in condition between categories are best described as gradual rather than discrete demarcations.

Table 5-6 shows the distribution of scores from 209 NEWS sites for each of the three IBIs. The MWIBI and NEIBI showed a nearly identical distribution of scores between the rating categories. Approximately 25 percent of sites scored *excellent* and 66-67 percent scored *good* or above for both indices. As expected, the MWIBI and NEIBI were highly correlated with each other: r=0.98, p<0.001 (Spearman Correlation). Biocondition gradient values showed a moderate correlation with NEIBI scores (Spearman r= -0.71, p<0.001**)** (Figure 5-2).

Of the 49 small coldwater streams evaluated by the CWIBI, 53 percent fell into the *excellent* category compared to 46 percent with BCG scores 2.0-2.9. There was a strong correlation between BCG scores and CWIBI scores (Spearman r= -0.89, p<0.001) (Figure 5-3).

Table 5-5. Biocondition gradient tier and suggested corresponding ratings to three versions of the IBI. IBI ranges associated with BCG tiers are for guidance only. Some BCG determinations of NEWS sites are outside ranges in corresponding IBIs presented here.

BCG Tier	Corresponding IBI Evaluation	IBI Scores		
		NEIBI	VT CWIBI	VTMWIBI
1. Natural or native condition *and* **2**. Minimal changes in *structure* of the biotic community and minimal changes in *ecosystem function*	Excellent	44-50	42-45	41-45
3. Evident changes in *structure* of the biotic community and minimal changes in *ecosystem function*	Very Good	40-42	36	37-39
4. Moderate changes in *structure* of the biotic community with minimal changes in *ecosystem function*	Good	34-38	33	31-35
5. Major changes in *structure* of the biotic community and moderate changes in *ecosystem function*	Fair-Poor	28-32	27-30	27-29
6. Severe changes in *structure* of the biotic community and major loss of *ecosystem function*	Very Poor	10-26	<27	<27

Table 5-6. Biological integrity evaluations as determined by three IBIs. Number of sites is followed by % of total in parenthesis.

Rating	New England IBI	Vermont MWIBI	Vermont CWIBI	**Combined NE and CW IBI**
Excellent	40 (24.7)	44 (24.9)	26 (53.1)	**66 (31.7)**
Very Good	28 (17.3)	26 (14.7)	7 (14.3)	**35 (16.6)**
Good	40 (24.7)	46 (26.0)	8 (16.3)	**47 (22.3)**
Fair-Poor	42 (25.9)	39 (22.0)	4 (8.2)	**46 (21.8)**
Very Poor	12 (7.4)	22 (12.4)	4 (8.2)	**16 (7.6)**

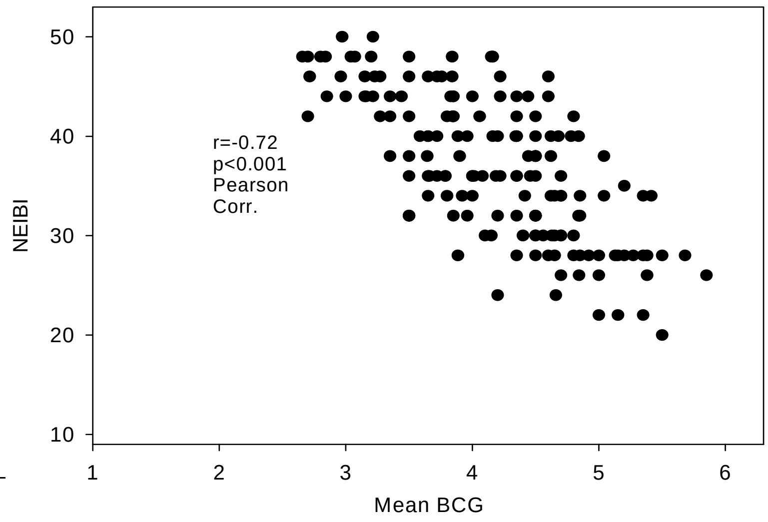


Figure 5-2. Plots of and Biocondition Gradient scores for New England IBI.

The consensus from the group was that the CWIBI would be more applicable across New England's small coldwater streams than would the MWIBI for streams where that index could be applied. The CWIBI, as currently used in Vermont is thought to be appropriate for all small New England trout streams. This would be especially true for Maine and New Hampshire. Since Connecticut has fewer native brook trout streams, the index may see less use there. It is also assumed that while few Massachusetts sites were included in the study that the CWIBI would also work effectively in small cold water streams in Western portion of the state.

While showing more promise in New Hampshire, the MWIBI appears to need additional modification beyond the nonnative metric included in the NEIBI for use in other New England states. Certain MWIBI metrics could be modified, or new metrics can be added for sites falling into the *Northeastern Coastal Zone* ecoregion that contain slightly different species assemblages than found in Vermont. Threats to fish assemblage health may also differ in areas of New England outside of Vermont and New Hampshire. For example, many rivers in Massachusetts and Connecticut have been impounded, resulting in replacement of riverine species with standing water species. A metric accounting for proportion of lotic species may be appropriate for assessing these streams.

The relationship between the BCG concept and the IBI approach in bioassessments of fish communities in New England is unclear. One could just as easily be an adjunct to the other in an assessment, depending on who is doing the evaluating and the stream type being evaluated. Regardless, both tools appear to show promise in bioassessment of running water fish assemblages, and in the best case should probably be applied in tandem to maximize evaluative power.

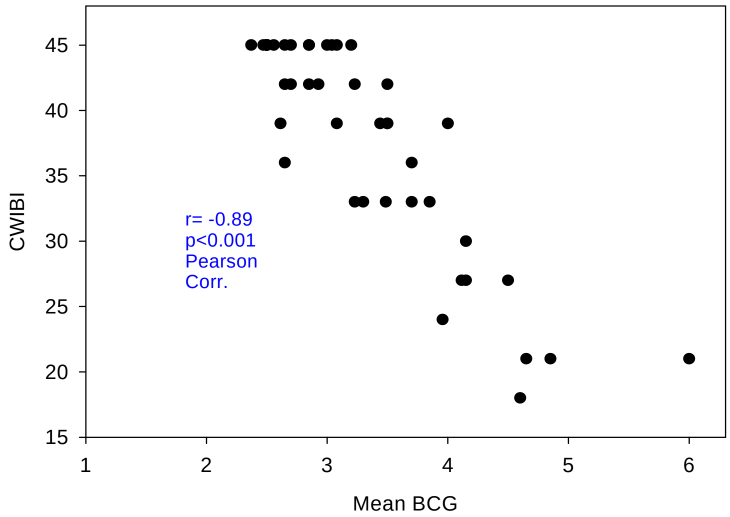


Figure 5-3. Plots of Biocondition Gradient scores for Vermont Coldwater IBI.

6.0 State Applications

6.1 The New Hampshire 305b Report

6.1.1 Introduction

Probability based monitoring uses randomly assigned stations to take an unbiased sample of a natural resource. Statistics from the sample can be used to make inferences about conditions throughout the resource. The major advantage of this approach is that 100 percent of the resource can be assessed at minimal cost. The biggest disadvantage is that the specific locations of water quality violations cannot be inferred from the statistical sample. Therefore, the results of the probabilistic assessment must be used in concert with the deterministic assessments of individual assessment units in the New Hampshire Department of Environmental Services (DES) Assessment Database. Probabilistically selected stations that were sampled in New Hampshire are shown in Figure 6-1.

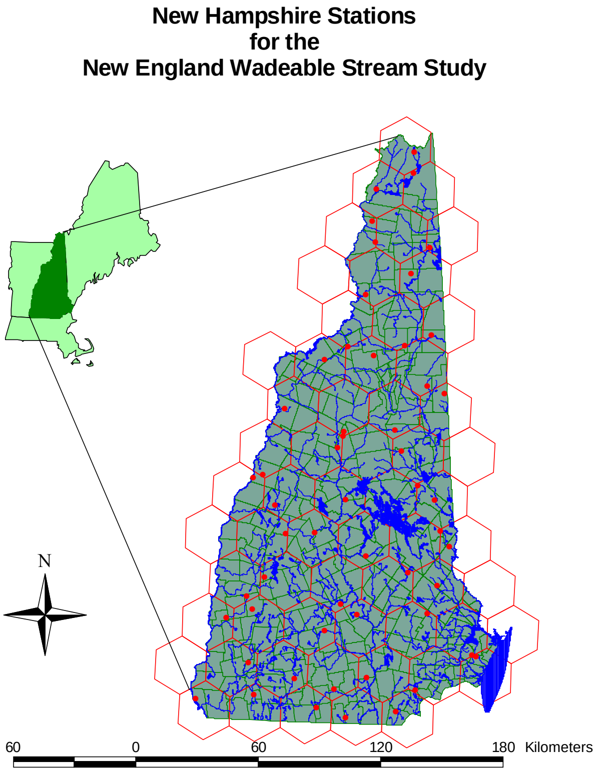


Figure 6-1. New Hampshire stations for the New England Wadeable Stream Study

6.1.2 Environmental Indicators

Wadeable streams were the target resource for a probabilistic-based assessment in the New Hampshire 2006 305(b) water quality report. Defined as 1st through 4th order streams, the DES Assessment Database indexed 9,050 stream miles based on the 1:100,000 national hydrography dataset. In order to evaluate the condition of the resource, benthic macroinvertebrates served as the core indicator. At each station the aquatic life designated use was classified as fully supporting, insufficient information, or not supporting based on the core indicator.

For aquatic life use support, the DES Biomonitoring Program assessed benthic macroinvertebrate data using a modified index of biological integrity (IBI). Placement of sites into aquatic life use support categories using macroinvertebrates was completed utilizing an assessment tool that differed from standard techniques outlined in the DES Consolidated Assessment and Listing Methodology (CALM) (DES 2006). Deviation from DES' wadeable stream aquatic life use assessment tool, as detailed in the CALM, was necessary because macroinvertebrate samples collected using the NEWS field protocols differed dramatically from standard DES field techniques. The modified IBI consisted of 6 metrics (EPT taxa richness, % EPT taxa, Scraper taxa richness, % Clinger taxa, % intolerant taxa, % Individuals in top 5 taxa). The selection of IBI metrics was consistent with an IBI constructed for the northeastern United States as part of the ongoing National Wadeable Stream Assessment (WSA) Project being completed by the USEPA and cooperating states. The metrics contained in WSA IBI were applied to the NEWS macroinvertebrate data because both projects utilized similar field collection protocols. The WSA IBI was subsequently recalibrated using regional reference sites from Maine, New Hampshire, and Vermont (northern New England, NNE). A total of 40 reference sites were selected as a subset of the WSA NNE reference sites. The threshold for Fully Supporting or Not Supporting aquatic life use categories was set at 68 out of a possible score of 100. Sites scoring less than 68 were considered Not Supporting while sites scoring 68 or greater were considered Fully Supporting. The aquatic life use reference threshold for the IBI was defined as the 25th percentile of all reference site IBI scores. Following calibration, IBI scores were computed for individual NEWS sites that were judged to be within either medium or high gradient streams. Low gradient streams for which biomonitoring data were collected were classified as Insufficient Information. The exclusion of low gradient streams from the probabilistic assessment differs from targeted wadeable stream aquatic life use assessments covered under the current DES CALM, but is consistent with the use and recalibration of WSA IBI. DES felt it was more important to be consistent with concurrent probabilistic data collection protocols and assessment indices than the assessment techniques developed specific to DES data collection protocols.

For demonstration purposes, the Biocondition Gradient (BCG) model developed by EPA was implemented for macroinvertebrate data collected from wadeable streams. The model consists of 6 tiers that incorporate several community ecological attributes across increasing levels of human disturbance (USEPA 2005). The BCG model allows for the placement of sites into a tier based on community composition and abundance of specific taxa or ecological groups. The development of the BCG model was instigated by a recognized need for a common tool in communicating the condition of ecological communities. Unlike traditional IBIs, which generally support a single endpoint (i.e. above or below an established threshold), the BCG model provides multiple possible endpoints that are not strictly based on “use-support” thresholds and can be used to set management goals.

As part of the NEWS project, USEPA Region 1 and state cooperators worked in conjunction with a contractor (Tetra Tech, Inc.) to develop an objective tool to place individual sites into a BCG tier. The state’s role was to provide input on the biological and ecological attributes of individual or groups of macroinvertebrate taxa and the decisions used to place sites into BCG tiers. The private contractor then took this information and constructed an objective non-linear, logic based (Fuzzy Set) model that predicted the BCG tier (Chapter 4). The model was calibrated from regional reference and test sites (N=43), then applied to the remaining regional NEWS sites to predict each site’s BCG tier assignment. The results given herein are presented solely for demonstration purposes and not intended for regulatory interpretation. Results of the BCG and existing IBI assessments are shown in Table 6-1.

For primary and secondary contact recreation, DES measured *E. coli* concentrations from three visits within a 60 day period at each of the stations. The assessment methods from the CALM could be used to assess attainment of the *E. coli* water quality standard without modification. Three of the NEWS stations fell within Class A waters. The *E. coli* standards for Class A waters were used to evaluate the data from the stations in Class A waters. Class B standards were used for the remainder of the stations.

6.1.3 Results

Table 6-1 provides detailed information regarding each site’s IBI scores, aquatic life use status, and BCG tier assignment. Figures 6-2 and 6-3 provide the probability-based results for the IBI assessment and BCG tier assignment, respectively. For Figures 6-2 and 6-3 the summary table shows the percent of wadeable stream in each designated use category, including those streams that were not assessed. The tabular results are mimicked in the middle pie chart. The lower pie chart shows the percent of the resource in each category, excluding those sites that were not assessed. Sites listed as “not assessed” were anomalies resulting from the hexagonal grid overlay and represented only a small portion of the resource.

6.1.4 Discussion

Data were available for the indicators for 95.4% of the wadeable stream miles. There was no information on the remaining 4.6% of the resource because the several hexagons in the original design were not sampled. The total miles of wadeable streams in New Hampshire was estimated to be 9,050. Therefore, these assessments cover 8,634 stream miles, which is 90% of the 9,628 stream miles for all the NHRIV assessment units.

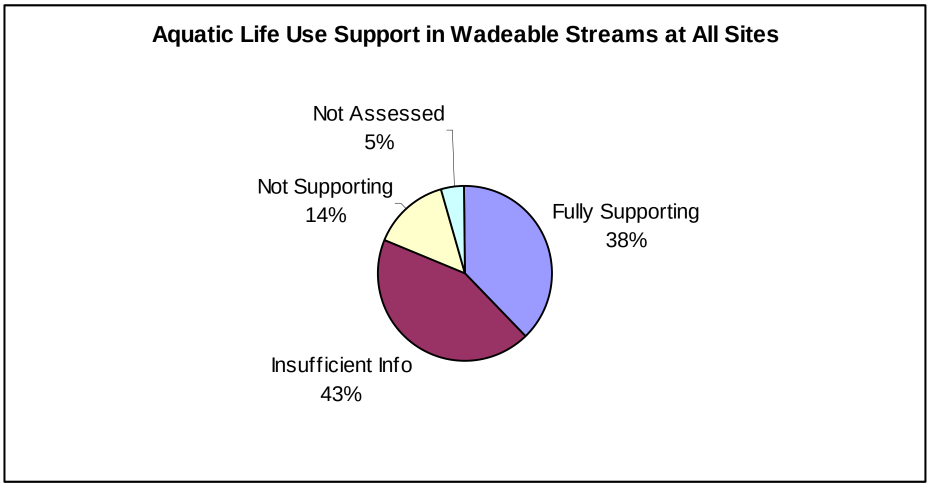
Table 6-1 Comparison of assessment endpoints for NEWS benthic macroinvertebrate sites: NH-aquatic life use attainment; NH IBI and NEWS Fuzzy Set BCG Tier

StationID	Waterbody Name	Grad type[1]	MWSA IBI score[2]	MWSA IBI Status[3]	BCG tier[4]	NH IBI score[5]	NH ALU status[6]	NH ALU narrative category[7]
NH HEX 1.03	Connecticut River	h	92.80	FS	2	-------	-------	-------
NH HEX 10.02	Bog Brook	m	84.39	FS	3	68.27	FS	FS-Marginal
NH HEX 11.01	Bumpus Brook	h	77.98	FS	2	73.67	FS	FS-Marginal
NH HEX 12.02	Peabody Brook	h	59.62	NS	3	-------	-------	-------
NH HEX 14.02	Ammonoosuc River	m	81.90	FS	3	-------	-------	-------
NH HEX 15.01	Appleby Brook	h	86.27	FS	2	-------	-------	-------
NH HEX 16.02	East Branch Saco River	h	90.68	FS	2	71.03	FS	FS-Good
NH HEX 17.03	Clark Brook	h	71.63	FS	2	70.71	FS	FS-Good
NH HEX 18.01	Eastman Brook	h	85.84	FS	3	75.84	FS	FS-Good
NH HEX 2.05	Indian Stream	m	77.73	FS	3	-------	-------	-------
NH HEX 20.01	Langdon Brook	h	80.02	FS	2	-------	-------	-------
NH HEX 21.05	Grant Brook	h	76.51	FS	3	80.65	FS	FS-Good
NH HEX 23.01	Johnson Brook	h	76.70	FS	2	85.25	FS	FS-Good
NH HEX 24.02	Paugus Brook	h	63.80	NS	2	-------	-------	-------
NH HEX 26.05	Hewes Brook	h	95.18	FS	2	60.64	FS	FS-Marginal
NH HEX 28.03	Ames Brook	m	68.39	FS	3	66.55	FS	FS-Good
NH HEX 29.03	Dan Hole River	h	78.56	FS	3	-------	-------	-------
NH HEX 3.05 (A)	Connecticut River	h	82.61	FS	3	-------	-------	-------
NH HEX 36.01	Branch River	m	86.95	FS	3	-------	-------	-------
NH HEX 37.02	Cold River	h	76.07	FS	4	-------	-------	-------
NH HEX 4.04	North Branch River	h	64.61	NS	3	-------	-------	-------
NH HEX 40.02	Webster Stream	h	62.19	NS	5	-------	-------	-------
NH HEX 43.03	Cold River	h	91.85	FS	2	-------	-------	-------
NH HEX 45.04	Piscataquog River	h	80.12	FS	4	59.01	FS	FS-Marginal
NH HEX 47.02	North River	h	52.66	NS	4	-------	-------	-------
NH HEX 5.04	Simms Stream	h	85.94	FS	3	-------	-------	-------
NH HEX 51.04	Otter Brook	m	66.31	NS	3	62.08	FS	FS-Marginal
NH HEX 52.01	Contoocook River	m	80.27	FS	3	67.38	FS	FS-Good
NH HEX 53.01	Purgatory Brook	h	72.78	FS	3	68.36	FS	FS-Good
NH HEX 58.03	Mill Brook	m	65.00	NS	5	-------	-------	-------
NH HEX 59.03	Souhegan River	m	66.29	NS	5	37.14	NS	NS-Poor
NH HEX 6.01 (RD)	Unnamed Brook	h	72.69	FS	3	-------	-------	-------
NH HEX 8.02	Roaring Brook	h	74.85	FS	3	-------	-------	-------
NH HEX 9.05	Newell Brook	m	73.46	FS	2	86.51	FS	FS-Good

1 - Stream gradient; h = high, m=medium (low gradient sites excluded)
2 - Modified National Wadable Stream study NAP region IBI score
3 - FS = full support; NS non-support (threshold = 68)
4 - Number corresponds to BCG tier as determined by Fuzzy Set model
5 - New Hampshire DES benthic IBI score (2006 CALM bioregional thresholds applied)
6 - New Hampshire DES aquatic life use (ALU) status
7 - New Hampshire DES ALU narrative category

Aquatic Life Use Support in Wadeable Streams

	Percent of Resource		Stream Miles	
	Value	Error	Value	Error
Fully Supporting	37.9%	12.9%	3,429	1,171
Insufficient Info	43.2%	13.2%	3,910	1,196
Not Supporting	14.3%	9.3%	1,298	846
Not Assessed	4.6%		413	
Total	100.0%		9,050	



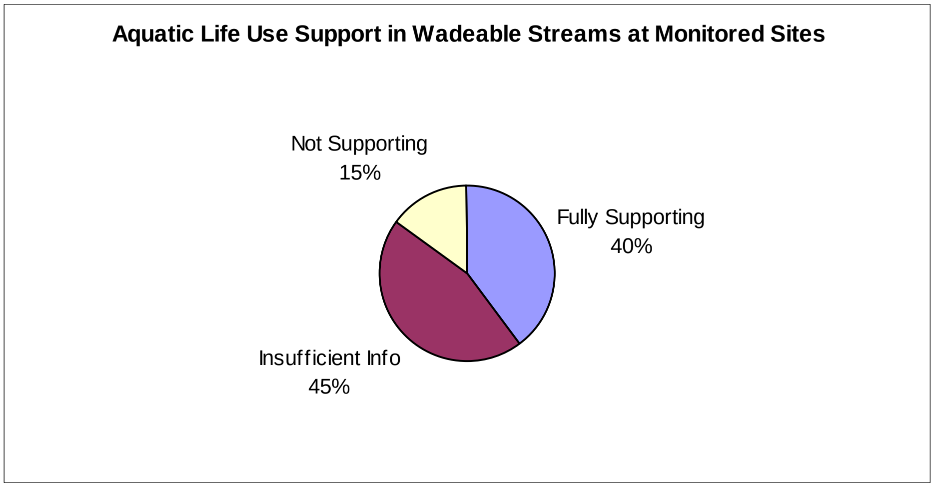
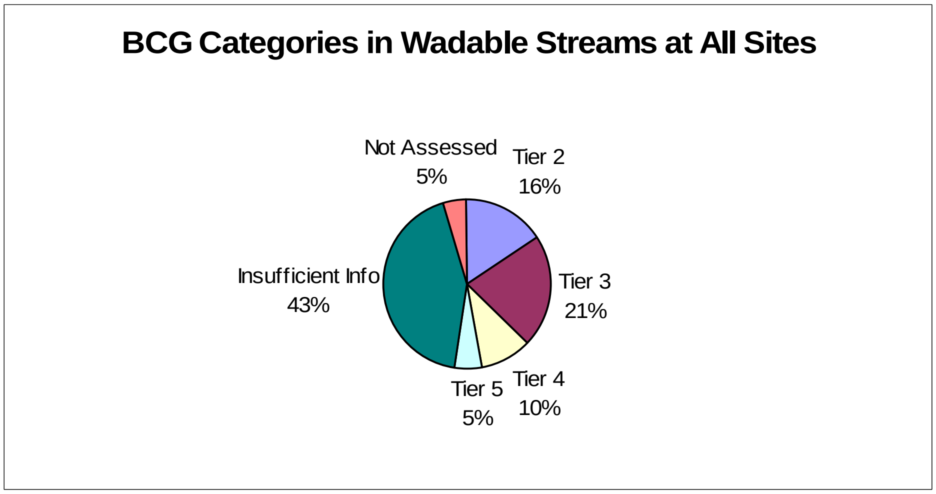


Figure 6-2. Aquatic Life Use Support

	Percent of Resource		Stream Miles	
Tier	**Value**	**Error**	**Value**	**Error**
Tier 2	16.1%	9.8%	1,457	887
Tier 3	21.2%	10.9%	1,915	986
Tier 4	9.7%	7.9%	877	714
Tier 5	5.3%	6.0%	478	540
Insufficient Info	43.2%	13.2%	3,910	1,196
Not Assessed	4.6%		413	
Total	100.0%		9,050	



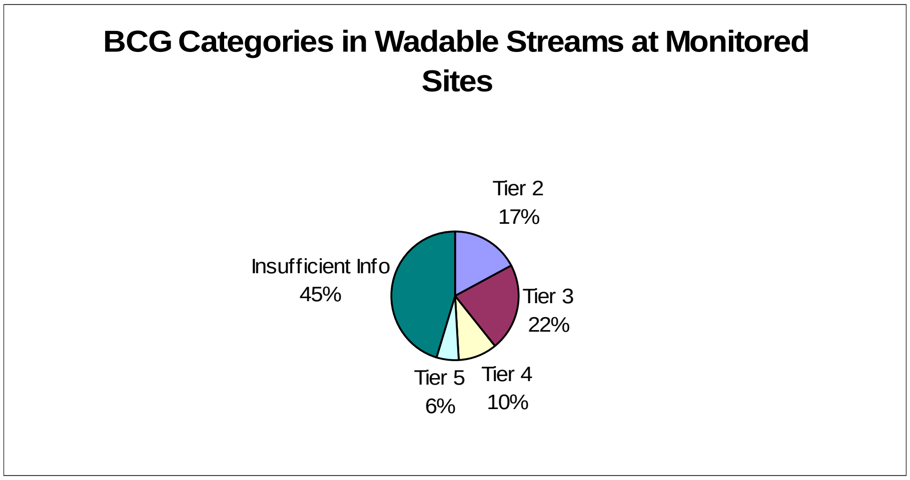
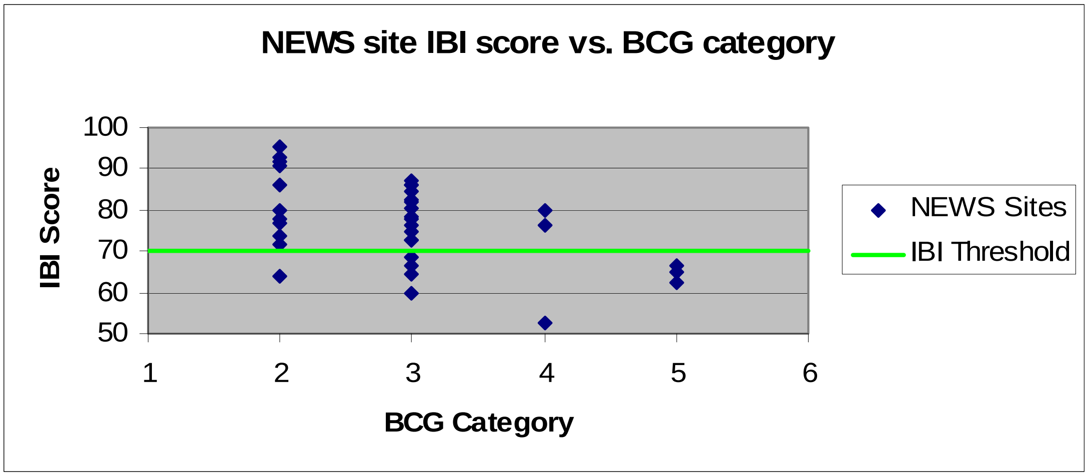


Figure 6-3. Biological Condition Gradient Categories

For aquatic life use support, the indicator showed that 37.9% of the wadeable streams were fully supporting, while 14.3% of the streams were not supporting. A large percentage of the streams had insufficient information to make the assessment because the sites were low gradient and the benthic IBI could not be applied to the data. The stations that were categorized as not supporting were not concentrated in any particular part of the state.

When applied to the BCG model, the majority of wadeable streams were in Tiers 2 (17%) and 3 (22%), categories characteristic of streams in good to excellent condition (Table 6-1). A minority of wadeable streams were in Tiers 4 (10%) and 5 (6%), categories indicative of streams in intermediate to poor condition, respectively. All of the Tier 4 and 5 stations were located in the southern part of the state. In contrast, all of the stations in the northern part of the state were either Tier 2 or 3. Similar to the aquatic life use results obtained for the IBI, a large percentage of the streams had insufficient information for placement into a BCG category because several sites were excluded from the analysis due to the low gradient nature of the sites that were sampled.

The level of correspondence between IBI scores and BCG Tiers indicated that percentage of sites attaining full support aquatic life use status based on IBI scores dropped consistently from 90% of sites in Tier 2 (10 out of 11 sites) to 0% of sites in Tier 5 (0 out of 3 sites). IBI score distributions also declined from Tier 2 through Tier 5 sites (Figure 6-4). As indicated in the box and whisker plots, the IBI threshold probably lies within Tier 4 of the BCG model, however, the dataset tested here lacks sufficient numbers of sites in these lower Tiers to make a definitive conclusion. The results demonstrate there is a moderate level of correspondence between IBI scores and BCG Tiers. To our knowledge, this is the first application of an IBI-based aquatic life use determination concurrent with the BCG model for a probabilistic monitoring network. Repetitive applications of the BCG model to a probabilistic monitoring network over time have the added advantage of showing incremental changes in resource condition, while IBI aquatic life use determinations are most helpful in determining the percentages of the resource characterized as fully supporting and non-supporting.



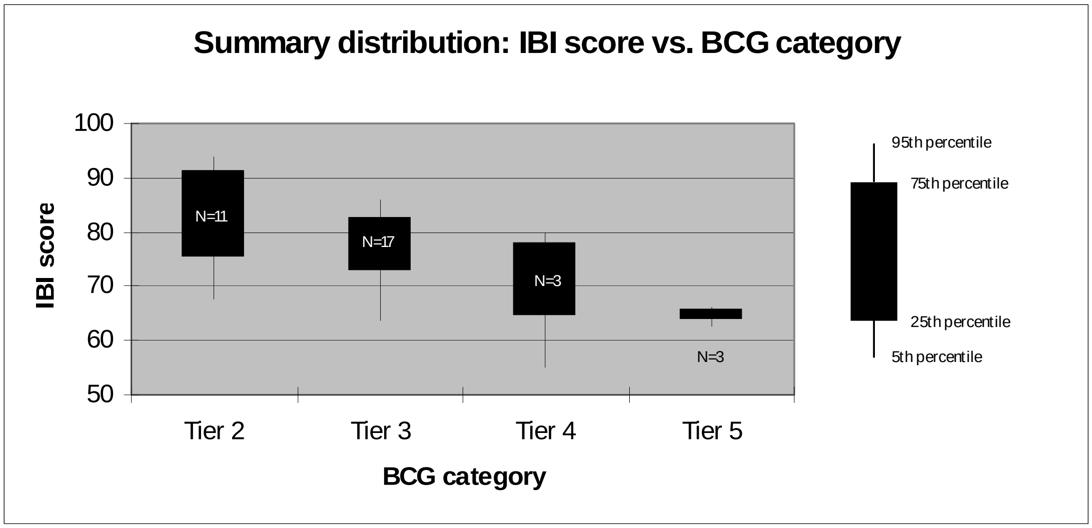


Figure 6-4a and b. Tiered Aquatic Life Use Categories vs. IBI Scores

6.2 Connecticut's Use of Probabilistic Monitoring in 305b Reporting

6.2.1 Purpose

The purpose of this project was to conduct a probabilistic survey of the environmental quality of wadeable streams in Connecticut. Ecological and water quality assessments were based on the community structure of resident biota, and water chemistry parameters. Data were collected at locations selected by a statistically valid, random sampling design, so that the results would reflect statewide conditions with 90% confidence. The biological and water quality data were used to provide a comprehensive assessment of wadeable streams in partial fulfillment of reporting requirements under sec 305(b) of the CWA

6.2.2 Scope

The Connecticut probabilistic wadeable stream-monitoring project was conducted over a three-year period beginning in January 2002. The primary funding for this project was a USEPA Regional-EMAP (Environmental Monitoring and Assessment Program) grant. Periphyton monitoring was funded under a USEPA grant for nutrient criteria development. The sampling design was based on a probabilistic draw developed by two USEPA laboratories—the Office of Ecosystem Measurement and Evaluation (OEME-Chelmsford), and the Office of Research and Development, Atlantic Ecology Division (ORD-AED-Narragansett). The CT project was a component of a larger multi-year probabilistic survey conducted by the USEPA to assess the environmental quality of wadeable streams throughout New England (NEWS Project). Data from the Regional Project was evaluated by a workgroup made up of biologists from the NE states and EPA using a Tiered Aquatic Life Use (TALU) model along a human disturbance gradient. Tetra Tech, Inc provided technical support for this workgroup.

To work toward the goal of a comprehensive assessment, the Department accepted the opportunity to participate with the EPA Region 1 OEME Laboratory in a two-year monitoring project following completion of the CT five-year rotating basin strategy in 2001. This project was conducted from 2002 to 2004 and assessed wadeable streams based on a statewide probabilistic design. Sample coverage included biological monitoring of fish, invertebrate and periphyton communities, and quarterly water chemistry at the fifty-nine sites shown in the map below in Figure 6-5.

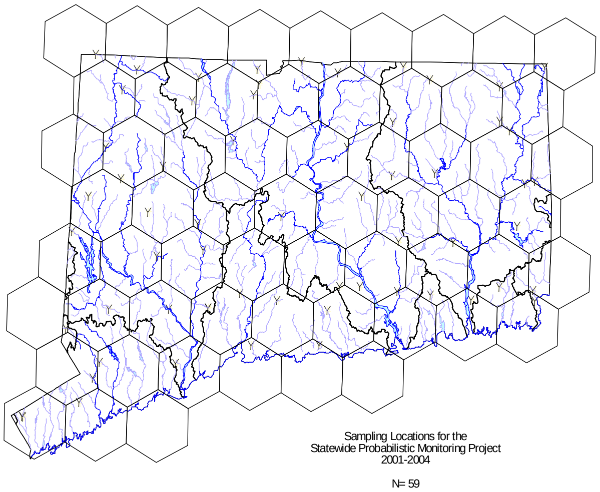


Figure 6-5. Sampling Locations for the Statewide Probabilistic Monitoring Project 2001-2004

6.2.3 Methods

- The probabilistic draw was determined by the use of a hexagonal overlay pattern as shown in Figure 6-5. One hexagon (#11) was randomly selected and used as the starting point. Sites located in odd numbered hexagons were sampled in 2002 and sites from even numbered hexagons were sampled in 2003. This provided broad geographical coverage each year to avoid bias from extreme weather conditions, etc.
- Using a combination of REMAP funds, EPA laboratory support, and existing State resources, the DEP sampled approximately sixty randomly selected sites for water chemistry, as well as benthic invertebrate and fish community structure in 2002 and 2003. Habitat evaluations were conducted at each site using RBP III habitat forms.
- Benthic invertebrate community sampling was conducted during a fall index period. Multiple samples were collected at each site using a combination of sampling methods as described below, including the REMAP protocol.

- All sampling was completed at the end of the second year. However, due to an unfortunate confluence of errors, benthic samples from year two of the project (2003) were compromised. All year two sites were re-sampled during year three (2004) for benthic invertebrates only. Sampling was conducted within the fall index period and the CT RBP III method was used.
- Fish community sampling was conducted during a summer index period using REMAP methods (modified RBP V).
- Surface water samples were collected during spring, summer and fall. Summer sampling mirrored the REMAP sampling methods and parameter list. Samples were delivered to the USEPA-OEME Laboratory in Chelmsford, MA for analysis. Water samples were also collected during site reconnaissance in the spring and benthic sampling in the fall. Spring and fall samples were not funded under the REMAP project. The CTDEP contracted with the CTDPH laboratory to analyze these samples. Analyses were conducted for general water chemistry, nutrients, and heavy metals.
- Field measurements for temperature, dissolved oxygen, pH, and specific conductance were conducted during all sampling trips.
- Indicator bacteria samples were collected along with all water samples. *E. coli* analysis was conducted by the CTDPH Laboratory.
- Periphyton sampling was conducted during the summer sampling using USEPA RBP Periphyton methods. Periphyton work was funded with a separate nutrient criteria grant under sec. 104(b)(3) of the CWA.

6.2.4 Benthic Assessment

Aquatic Life Use Support Assessment (ALUS) was conducted using a modified USEPA RBP III as described in the CT CALM document. The impairment threshold is between fifty and fifty-four percent of reference. Results for the fifty-nine sites sampled are shown in Table 6-2 . Forty-two sites (71%) were determined to be fully supporting including four sites that scored below the impairment threshold. These sites were included in the full support category due to fish sampling results indicating full support. Nine sites (15%) were classified as not supporting and eight sites (14%) were classified as insufficient data because a suitable riffle habitat was not available. See Figure 6-6 below.

Table 6-2. CTDEP Probabilistic Benthic Invertebrate ALUS Assessments for 2006 305(b) Report Data collected in 2002 and 2004 Comparison to BCG tiers

CT STATION ID		Stream_Name	CT 305(b) ALUS	CT RBP3 %REF	CT RBP3 BCG	Fuzzy set BCG
189		Natchaug River	reference	reference	2.7	2.0
316	21	Sandy Brook	reference	reference	2.8	2.0
317	17a	Salmon River	reference	reference	2.9	2.0
606	245	Green Fall River	reference	reference	3.2	3.0
63	18	Eight Mile River	reference	reference	3.4	3.0
612	250	Whitford Brook	reference	reference	3.4	3.0
627	234	Quaker Brook	reference	reference	3.7	3.0
325	25	Shepaug River	reference	reference	3.9	3.0
628	235	Titicus River	reference	reference	3.9	3.0
319	50	Saugatuck River	reference	reference	4.5	5.0
743	CT HEX 3.01	Sandy Brook	FS	100	2.3	2.0
778	CT HEX 17.08	Mashamoquet Brook	FS	100	3.3	2.0
742	CT HEX 11.02	Indian Meadow Brook	FS	95	2.5	2.0
746	CT HEX 35.05	Sawmill Brook	FS	95	3.2	2.0
925	CT HEX 42.03	Seth Williams Brook	FS	95	3.6	3.0
607	CT HEX 52.07	Shunock River	FS	95	3.6	3.0
766	CT HEX 7.06	Stickney Hill Brook	FS	94	3.2	2.0
907	CT HEX 4.01	East Branch Salmon Brook	FS	91	2.4	2.0
744	CT HEX 19.02	Lake Waramaug Brook	FS	90	3.8	3.0
923	CT HEX 38.01	Mill River	FS	89	4.6	4.0
933	CT HEX 28.01	Wood Creek	FS	86	2.5	2.0
915	CT HEX 20.02	Bantam River	FS	86	2.8	3.0
917	CT HEX 24.02	Sawmill Brook	FS	86	2.9	2.0
927	CT HEX 46.03	Five Mile Brook	FS	86	3.2	3.0
573	CT HEX 2.05	Blackberry River	FS	86	3.6	3.0
914	CT HEX 18.01	Housatonic River	FS	86	3.9	4.0
930	CT HEX 50.02	Eight Mile River	FS	86	3.2	3.0
756	CT HEX 49.05	Pond Meadow Brook	FS	85	3.9	3.0
750	CT HEX 47.02	Bladdens River	FS	85	4.1	
913	CT HEX 16.01	Wappoquia Brook	FS	81	3.1	2.0
758	CT HEX 31.02	Flat Brook	FS	76	3.5	2.0
741	CT HEX 21.02	Farmington River	FS	76	3.7	3.0
759	CT HEX 43.01	Shunock River	FS	76	3.7	3.0
754	CT HEX 57.04	Neck River	FS	75	3.7	2.0
745	CT HEX 27.02	Bull Mountain Brook	FS	75	4.1	4.0
751	CT HEX 59.01	East Branch Byram River	FS	75	4.2	4.0
740	CT HEX 13.02	Mountain Brook	FS	71	2.7	2.0
922	CT HEX 36.02	Pomperaug River	FS	67	3.4	3.0
918	CT HEX 26.04	Moosup River	FS	67	3.5	3.0
789	CT HEX 25.03	Ekonk River	FS	67	3.6	3.0
761	CT HEX 41.05	Latimer Brook	FS	67	3.9	3.0
928	CT HEX 48.01	Farm River	FS	63	5.0	5.0
909	CT HEX 8.02	North Running Brook	FS	62	2.9	3.0
924	CT HEX 40.01	Clark Creek	FS	62	3.3	3.0

Table 6-2. (Continued).

CT STATION ID		Stream_Name	CT 305(b) ALUS	CT RBP3 %REF	CT RBP3 BCG	Fuzzy set BCG
911	CT HEX 12.01	Beach Brook	FS	57	2.8	3.0
757	CT HEX 39.01	Beaver Meadow Brook	FS	57	3.4	3.0
762	CT HEX 33.04	Bentley Brook	FS	56	4.2	3.0
760	CT HEX 51.02	Flat Brook	FS	56	4.8	4.0
931	CT HEX 54.02	West Branch Saugatuck River	FS	55	4.7	5.0
780	CT HEX 1.08	Sages Ravine Brook	FS	50	3.3	4.0
763	CT HEX 9.02	Rocky Brook	FS	50	3.8	5.0
749	CT HEX 45.04	Limekiln Brook	FS	50	5.4	5.0
908	CT HEX 6.06	Still Brook	NS	48	4.3	5.0
753	CT HEX 53.04	Norwalk River	NS	47	5.3	5.0
752	CT HEX 55.01	Pumpkin Ground Brook	NS	41	5.5	5.0
920	CT HEX 32.01	Cabin Brook	NS	41	3.9	4.0
77	CT HEX 60.01	Five Mile River	NS	40	5.4	5.0
921	CT HEX 34.02	Crooked Brook	NS	38	4.2	3.0
748	CT HEX 37.01	Naugatuck River	NS	35	5.9	6.0
191	CT HEX 29.03	Naugatuck River	NS	33	5.8	5.0
916	CT HEX 22.03	Hockanum River	NS	29	5.1	5.0
932	CT HEX 56.08	Farm River	ID	ID	ID	ID
906	CT HEX 14.04	Freshwater Brook	ID	ID	ID	ID
910	CT HEX 10.02	Hollenbeck River	ID	ID	ID	ID
779	CT HEX 23.01	Hop River	ID	ID	ID	ID
424	CT HEX 30.03	Mattabesset River	ID	ID	ID	ID
739	CT HEX 5.02	Muddy Brook	ID	ID	ID	ID
765	CT HEX 15.02	Skungamaug River	ID	ID	ID	ID
926	CT HEX 44.01	Titicus River	ID	ID	ID	ID

Fuzzy set BCG values from Chapter 4
ID insufficient data (riffle habitat not present)

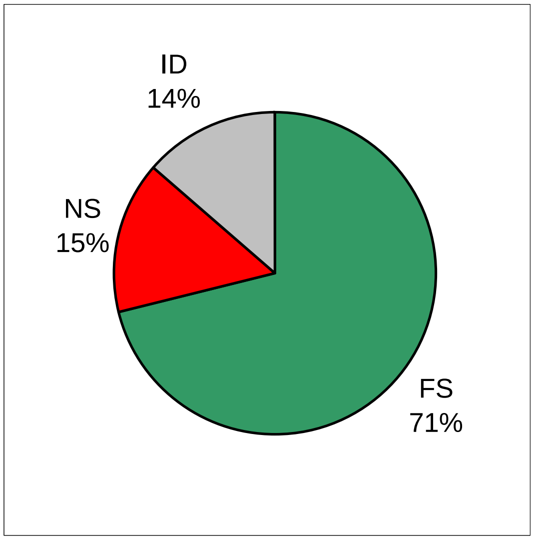


Figure 6-6. Aquatic Life Use Support Assessment (ALUS) results

Biocondition gradient tiers were also scored by CT biologists for the fifty-nine benthic samples using the TALU model. The impairment threshold was set half way between tier four and five. Using this model eleven sites (19%) were determined to be impaired. Nine (15%), twenty-five (42%), and six (10%) sites were assigned to tiers two, three, and four respectively. This information is shown in the Figure 6-7 below.

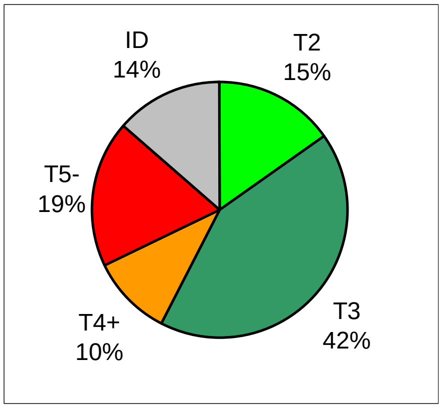


Figure 6-7. Biocondition gradient scores using TALU model.

The NEWS probabilistic draw was done using the National Hydrological Database (NHD) coverage at 1:100,000 scale. NEWS protocol required screening out first order streams, which was done for the CT sites using 1:24,000 scale coverage. Since stream order is not an attribute associated with the NHD coverage used, a degree of uncertainty was introduced into the estimate of total miles that the probabilistic sample was drawn from. CT biologists are currently working with personnel from the EPA ORD Laboratory at Narragansett to resolve this problem so that sample-weighting factors can be applied and confidence levels calculated.

7.0 Discussion, Lessons Learned, and Recommendations

In this report we present separate, assemblage-specific assignment of NEWS and state-collected samples to BCG tiers and provide examples of some resulting policy applications. There is still the need to develop an overall site assessment that integrates the results of fish data analysis (Section 3.0) with macroinvertebrate data results and physical-chemical data analysis (Section 2.0).

7.1 Challenges

7.1.1 Monitoring Design

While the NEWS project design offered an opportunity to collect biological survey data not commonly collected by state biomonitoring programs, the design itself introduced obstacles to the stated goal of assessing biological condition to address state and EPA reporting requirements (e.g., 305b and 303d status). State biomonitoring programs are designed to answer specific questions about biological condition of monitored sites relative to state aquatic life use threshold conditions, for purposes of 305b reporting, 303d listing, etc. Most state bioassessment programs apply model-based sampling designs (i.e., an a priori interpretive model exists that directs targeted monitoring and that also directs how sampled sites are to be assigned to different assessment categories, e.g., “attaining” vs. non-attaining”). Significant to the task of decision-making about attainment status, the NEWS multi-state survey was faced with the aquatic life uses and numeric biocriteria thresholds of four different states. Further, the newly introduced NEWS methods themselves lacked aquatic life use or biocriteria thresholds. Below is a summary of problems encountered, solutions, and results of our effort to reconcile fundamental differences encountered between NEWS probability-based design and state and BCG model-based designs.

Problem1: Assessment Endpoints
No framework to report assessment results existed at the start of the NEWS project. The range of possible assessment outcomes in probability-based design is relative to the range of measured conditions in the dataset. Assessment endpoints are computed relative to the characteristics of the dataset, for example, as percentiles of departure from the best measured condition. Because some regions exhibit a more complete gradient than others the gradient may be truncated, either towards very good conditions (i.e., towards BCG Tiers 1-4 but lacking very poor conditions) or towards poorer conditions (i.e., towards BCG Tiers 3-6 but lacking very good conditions), or towards the middle (i.e., uniformly mediocre). By definition, a gradient of environmental quality it is a value-based judgment. There are no commonly employed, objective analytical techniques to account for differences in the range of measured gradients such that states and regions can be compared in a standardized or “absolute value” context.

The Biological Condition Gradient presents a standardized assessment through use of a conceptually complete range of six possible outcomes from “natural” to “severely disturbed”. In the BCG, a minimally stressed reference condition (Stoddard et al. in press) is narratively defined a priori in the model as Tier 1 or Tier 2. Assessment endpoints of conditions that have declined from “natural” correspond to BCG tier descriptions for Tiers 3-6. The underlying stressor-response model corrects for circularity by considering biological and physical-chemical-landscape factors.

If the dataset used to calibrate the BCG lacks either end of the stressor gradient, then it is not possible to fully calibrate the BCG, or any other assessment model that requires the full stressor range. This is analogous to developing a regression model from a partial range of the explanatory (X-axis) variable. In New England, the NEWS probability-based sampling design did not find enough severely stressed sites to fully calibrate the BCG model because the design was not stratified on overall stress or disturbance; i.e., the stress-response model was not incorporated into the design. Model-based designs are, in effect, a form of experimental design (e.g., regression or ANOVA; Snedecor and Cochran 1973), with the objective of experimental design: to test a hypothesis or a model. The objective of probabilistic sampling design is unbiased estimation of population parameters (e.g., Thompson 1992), but not the development or testing of models.

Results and benefits
To enhance the transferability of NEWS results to meet states' need to report on ALU attainment status biologists used a combination of expert judgment and statistical and graphical analysis to develop a non-linear predictive model to assign NEWS biological data to tiers of the BCG[3]. The NEWS workgroup aligned the sampled sites against the conceptually complete gradient of the BCG and determined that the NEWS gradient was truncated in an upward direction (i.e., an insufficient number of Tiers 5-6 sites occurred in the NEWS dataset). We addressed the issue of a truncated gradient by introducing historical data from poor quality sites (Tiers 5-6 sites) from CT to the NEWS dataset to calibrate the taxonomic characteristics of the low end of the BCG for the northeast. We corrected for circularity in the biologically-based tier assignments by checking results against physical-chemical and land use findings that indicated levels of stressors to which the site was subjected.

For every NEWS site, macroinvertebrate data were assigned to a condition tier of the BCG by the resulting fuzzy set model. A fuzzy set model has not been developed for the fish data but all fish data were assigned to a BCG tier by expert judgment (Section 2). These assignments can be directly related to each state's biocriteria thresholds for reporting purposes.

Problem 2: Methods
To compare results within and across regions assessment endpoints must be transferable. Because all numeric biocriteria thresholds are specific to the sampling methods used to develop them, ecologically transparent correspondence is not possible among the numeric biocriteria thresholds of different programs. Assessment endpoints are the summary judgment about the significance of a given biocriteria value, in terms of attainment of state-specific aquatic life uses, or in general terms of biological condition, but because of methodological obstacles it is rarely possible to make meaningful comparisons among inter-state assessment results (Davies and Jackson 2006).

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[3] The NEWS model development process (utilizing a combination of expert judgment and multivariate analysis) was similar to that used by the Maine Department of Environmental Protection to develop a linear discriminant model to predict attainment of tiered biological criteria (Courtemanch 1993; Shelton and Blocksom 2004).

The NEWS probability-based sampling design utilized a statistically valid site selection process, free of assumptions about how to interpret the ecological characteristics encountered in the region. In customary practice, in the absence of a BCG model, method-specific biocriteria would be developed *a posteriori* as percentiles of the observed distribution of site conditions. Assessment results derived from this process are commonly reported in qualitative terms (e.g., "good-fair-poor") rather than ecological terms (USEPA 2006). Had NEWS followed this usual practice it would have introduced a fifth assessment context into the regional mix, one not directly transferable to any of the four established state assessment endpoints or numeric biocriteria.

Results and Benefits

The combined approach, using statistically valid site selection with the BCG fuzzy set model allowed New Hampshire (Section 4.1) and Connecticut (Section 4.2) to include in their Integrated Reports (Section 4.1) the aquatic life use attainment status, specific to their state biocriteria thresholds, for 100% of state river miles. Pearson correlation was 51% for the state of New Hampshire benthic macroinvertebrate Index of Biotic Integrity attainment results as compared to fuzzy set model tiers. Pearson correlation results were better for Vermont fish when comparing BCG tiers assigned by a panel of fish experts, to computed assessments generated by the state analytical approach:

- Vermont Coldwater IBI (fish)=89%
- New England IBI (fish)=72%

Biologists in the Region found the exchange of technical expertise and assessment perspectives to be of significant benefit to their programs. While the methods comparison effort (See Chapter 1) showed that differing state and NEWS sample collection methods confound direct comparisons of computed metric data (across methods), the NEWS project demonstrated the utility of the BCG to provide universal assessment endpoints and thus to serve as a translator among programs.

7.1.2 Classification and Methodological Issues

Analysis and interpretation of NEWS biological data presented significant classification challenges due to the large geographic area assessed and the overall complexity of the dataset. The latitudinal gradient represented in the data ranged from 41 degrees N latitude in southern Connecticut to 47 degrees N latitude at the northern-most monitored site in Maine. Elevation ranged from less than 20 to over 2,100 feet above sea level. The dataset was further complicated because latitudinal gradients in the northeastern United States are associated with co-varying stressor gradients from south to north due to differences in cultural patterns of settlement, population density and economic factors. These challenges were addressed by using the more simplified Connecticut dataset to calibrate the NEWS regional BCG (Section 2.0).

In addition, the methodological contexts (i.e., "interpretive cultures") of four different states and 10-12 biologists introduced complexity well beyond that encountered in typical state biocriteria development efforts. Many issues were raised by the state biologists' lack of experience with the newly introduced NEWS benthic macroinvertebrate sampling protocol. Riffle samples of benthic macroinvertebrates, as opposed to pool samples, have greater information content to detect biological responses to differences in water quality (Hynes 1970; Rabeni 1977; and Cummins 1996). For this reason biologists' experience in the region is with interpretation of stressor response signatures of riffle samples. Early in the project biologists expressed concern that NEWS methods that called for compositing of pool and riffle samples would flood the riffle sample signal with low-information content pool organisms that were not sensitive to the expected stressor scenarios (e.g., increased temperature and levels of anions and cations, lowered dissolved oxygen levels, sedimentation, etc.). The states' requested to perform side-by-side state sampling methods, along with the NEWS methods, in order to strengthen confidence in the assessment outcomes and to calibrate them, relative to well established state methods (Davies Appendix F).

7.1.3 Tier 1 Issues

Despite a large investment of time and effort the regional workgroup remained unwilling to assign any NEWS sites to Tier 1 by expert judgment. Ultimately detection of sites that appear to be consistent with Tier 1 concepts described by the BCG required the combination of expert judgment and additional objective statistical and graphical analysis of combined biological and stressor gradient data (Section 2.2.6). In the first tier assignment exercise biologists assigned 92 percent of the first 24 NEWS sites to Tiers 3 or 4, based on biological data alone. Accurate assignment of biological site data to BCG tiers is strengthened by interpretive experience with the sampling methods used to collect the data and it also requires checks against the circularity that is generated by an exclusive focus on one type of information (e.g., "the biology looks good therefore this must be what good biological condition looks like"). In the absence of methodological experience we found that biologists are conservative in tier assignments, tending to rate sites lower than their actual condition (Type 1 error) when rated by non-biological evidence (i.e., physical, chemical, land use condition). Tendency to Type 1 error in the NEWS project was exacerbated by methods that called for compositing pool and riffle samples because pool habitats tend to be populated by macroinvertebrates from groups that are tolerant to common stressors like fine sediment and low dissolved oxygen.

Tier 1 Conclusions

We found that sites consistent with Tier 1 are detectable in the NEWS dataset based on plots of taxonomic indicators against population density and other stressor gradient variables that indicate minimally disturbed conditions. The analysis indicates that departure of biological variables from Tier 1 characteristics appears to be non-linear and appears to support subsidy-stress gradient hypotheses (Odum et. al 1979; Odum 1985).

Identification of Tier 1 sites requires objective analysis of non-biological variables that reflect stressor or x-axis attributes. Detection of extant Tier 1 conditions is probably not possible at the reach-level of assessment. Consideration of data from multiple spatial and temporal scales provides knowledge of the status of Attributes IX and X in the BCG. For example, consideration of Attribute X Ecosystem Connectance, can provide information about whether a stream is fishless due to human disturbance (dams or improperly placed downstream culverts) or due to natural barriers to migration/colonization.

Sampling methods significantly influence biologists' assignment of sites to Tier 1. NEWS methods. Compositing pool and riffle samples within a site contributed to biologists' reluctance to assign sites to Tier 1. NEWS macroinvertebrate laboratory and analytical methods yielded insufficient information on taxa densities to adequately apply the BCG Tier 1 descriptions of density changes in Attributes I-V. This is an issue common to many state sample work-up methods that rely on fixed counts (e.g., 200 organism sub-samples) and relative abundance estimates of taxa but do not account for differences in straight densities of individual taxa. The BCG characterizes differences among the lowest tiers (i.e., 1-2 -3) in terms of shifts in presence, absence and density of taxonomic groups of differing sensitivity to stressors. Retention of density information requires application of fixed level of effort sampling and whole sample or proportional sample counts of observed taxa (Courtemanch 1996).

7.2 Lessons Learned

This project was an effort between state environmental agencies and the USEPA to collaboratively assess the condition of wadeable streams across New England and report out a region-wide assessment of condition. It was anticipated through the course of the project that a process would evolve closing the disparity between monitoring programs, providing a mechanism for large scale and non-targeted uniform assessments across geopolitical boundaries. Another anticipated goal was that the approach would reconcile some of the sharp criticisms by the GAO and others of the incomparability of agency monitoring data (GAO March 2000, PEER May 1999) and realign data reporting into a more manageable framework. The framework could then be used to assist in future national funding allocations and regional/national project and program assignments at EPA headquarters and congressional levels.

The NEWS project demonstrated that regional assessments of condition can be accomplished using existing state and federal data, but developing a regional model with these resources is complex and arduous, and a more proficient approach is warranted. A next step would involve investigating more potentially amenable strategies for regional assessments through collaborative workgroup efforts. Development of new methods directed towards currently under assessed resources (i.e. low gradient soft bottomed streams) could be extremely beneficial to current monitoring programs. If new methods are developed, they should be constructed so that they can be applied without undermining current state monitoring priorities.

The fish survey component and sampling methodology of the NEWS effort was consistent among all of the state partners and EPA, providing useful information on regional distributions of native and non-native fish species. However, clear relationships did not evolve between fish IBI's and the BCG model for fish. These data provide important information when looking at distributions of non-salmonid and non-gamefish populations, as these species are often overlooked due to their non-importance from a socio-economic viewpoint. This community can provide additional information on things such as endocrine disrupting chemical impacts, mercury levels in fish, and effects from pharmaceuticals and personal care products. A probabilistic design has potential to reveal the ubiquity of some of these constituents and add much information with modest additional effort.

7.2.1 NEWS Dataset can Provide Insight into Low Gradient Streams

Low gradient streams could provide much new information to state and federal water quality programs. A small but significant portion of the streams selected in the NEWS probabilistic design were low gradient, systems that are not normally addressed in state water quality assessments or monitoring efforts (unless riffle habitats are present). The NEWS dataset presents an opportunity to begin looking into this under assessed New England stream type for biological monitoring and interpretation purposes. There is presently a high interest among state monitoring programs to look into approaches for assessing low gradient systems, but with currently limited resources this will probably remain a low priority. The NEWS dataset can provide a cost affective means to begin looking into the ecological aspects of this stream type.

7.2.2 NEWS Project Sampling Dataset can Supplement Existing Data Sources

The focus of most state water quality monitoring efforts has been on problem areas and rarely have there been opportunities to develop comprehensive statewide information regarding regional distributions of invertebrates or other taxa. The NEWS project presented the opportunity to acquire this data. The sampling design lent itself for determining geographic ranges of taxa and their ability to span various habitats. The NEWS dataset can also contribute to existing data sources of other agencies such as the nature conservancy, state endangered species programs, and other groups having an interest in aquatic communities and ecology. Many of the site locations are acceptable to be utilized as reference conditions for future stream assessment activities, for development of stream classification systems, or for other random probability based monitoring efforts.

7.3 Recommendations

The workgroup recognized an unfinished aspect of the project in that information from multiple assemblages, as well as generalized stressor gradient information, should be considered to identify of overall Tier 1 sites. Our identification of Tier 1 was assemblage specific (i.e., Tier 1 fish sites and Tier 1 macroinvertebrate sites). Improvements could be made in the accurate assessment of the attributes described by the BCG with the addition of biological and physical chemical information at watershed and regional scales. Most of this information is not currently collected by typical state monitoring programs though it may be available from other institutions (for example historical distributions of endemic species, historical land use alterations, presence and intensity of stressors, species-specific response to stressors).

It is recommended that the participants in this project continue to formulate an ongoing work group. More information can be acquired from this project's data in regards to assessments of low gradient waters, species assemblages, methods development for larger surveys, and other topics. The data acquired and ongoing workgroup discussions can provide improved management for future large scale surveys at state and federal levels.

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APPENDIX A

NEWS DATABASE

APPENDIX A

NEWS DATABASE

A.1. Data Quality Assurance and Data Management

Mike Beauchene – QA site locations, correct incorrect locations, site Ids, etc.

Clean up chem. data.

- QA of lab chem data – Mike B
- Identifiers – several chemical parameters had multiple identifiers for the same parameter. These were reconciled to a common identifier for a single parameter and measurement method. Detection limits were often mixed; samples at the detection limit are reported as detection limit, with a flag identifying that the measurement was below detection.

Develop relational database

A.2. Geographic Coverage

A.3. Target Taxa list

Taxonomic identification often results in discrepancies of taxonomic level among samples, because some samples may have been less-well preserved, some samples are dominated by very small juveniles or early instars, which are less certain to identify to genus, and taxonomists have differing levels of expertise on damaged specimens or small juveniles. Therefore, the raw taxonomic information must be standardized so that taxa are identified to a target level.

Most insect identifications were standardized to genus level, but aquatic mites and leeches were identified to family only. Among Oligochaetes, the Naidae were identified to genus, but all other Oligochates were identified to family only. Similarly, beetle families that are not primarily benthic were identified to family only (e.g., Curculionidae, Carabidae, Dryopidae, Dytiscidae, Gyrinidae, Haliplidae). Among the Diptera, the Ceratopogonidae, Simuliidae, and Tabanidae were identified to family because of the difficulty in reliably identifying genera. Among the Hemiptera, the Nepidae and Veliidae were identified to genus, but Corixidae and Gerridaea were identified to family only.

Distinct taxa

When counting taxa in a sample, it is necessary to count only distinct taxa (Stoddard et al. 200_?), meaning that ambiguous identifications to family level are not counted as distinct taxa when one or more genera of that family have been identified in the same sample. Conversely, a family is counted as a distinct taxon if no genera within the family are identified in the same sample. For example, suppose the taxa in a sample were identified as *Baetis*, *Procloeon*, and unidentified Baetidae. This sample has 2 distinct taxa. A second sample which has only unidentified Baetidae would have one distinct taxon.

Operational Taxonomic Units for ordination

Further aggregation of taxa is necessary for ordination analysis. Ordination techniques do not recognize that genera are subsets of their respective families, so the inclusion of a family and genera within that family in the same ordination artificially creates more taxonomic entities than actually exist. Ordination and similarity analysis require definition of operational taxonomic units (OTUs; Hawkins 2000_) such that no ambiguous families occur with their component genera. OTUs are determined on a family-by-family basis. Either the family observations are eliminated from the analysis, or the component genera are all summed into the family. The rules-of-thumb for summing genera into a family include:

- Only one genus observed (no information loss)
- All genera rare (low frequency) in the database, or only one genus is common and the rest are rare (occur at 1-3 sites each). Rare taxa are unimportant in ordination, and their information content may be better preserved by lumping into genera.
- Family-level identification is 50% or more of all observations, but there are 2 or more relatively common genera. This indicates a family which, for a variety of reasons, may be difficult for taxonomists to identify to genus.

APPENDIX B

IDENTIFYING CANDIDATE REFERENCE AND TIER 1 SITES

APPENDIX B

IDENTIFYING CANDIDATE REFERENCE SITES (NEWS DATA)

Methods

Identification of a priori "Best" sites

Sites in which measured environmental conditions indicate minimal human influence in the watershed were identified through comparison of site characteristics to a table of environmental criteria (Table B-1). The criteria were established through a number of methods. Criteria for habitat and landscape characteristics were established through collective professional judgment (opinions of the workgroup and analysts). Sites were considered "best" if they met all criteria. Some sites were excluded from the "best" category based on judgments of the regional biologists familiar with the sites and surroundings.

Water chemistry was evaluated based on published criteria, stressor-response thresholds inferred from the NEWS data, and collective professional judgment. An inferred stressor-response threshold was used if NEWS data supported a threshold (see below). Otherwise, the threshold was the Criteria Continuous Concentration (CCC; U.S. EPA 2002) or ½ the Criteria Maximum Concentration (CMC; EPA acute criteria) if there was no CCC, or the drinking water criteria if there was no CMC or CCC. If a permitted discharge was within the watershed, a site would not be considered "best". The column in Table B-1 labeled "Minimally Stressed Background" reports values of these parameters observed in sites that were minimally stressed (candidate Tier 1 sites; see below); these values were not used to select the *a priori* best sites.

Table B-1. "Best site" selection criteria.

Measure	**"Best" *a priori* criteria**	**Published criteria**	**notes**
Chloride	20 mg/L	CCC: 230 mg/L	S-R*
Total Phosphorus	20 µg/L		S-R
Sulfate	10 mg/L		S-R
Turbidity	5 NTU		S-R (few obs)
Aluminum, dissolved	<100 µg/L	CCC: 87 µg/L	S-R (weak)
pH	(>6.5 if TOC <2.0 mg/L)		Not used (dissolved Al considered diagnostic)
Antimony, dissolved	5.6 µg/L	5.6 µg/L (human health)	No hits**
Arsenic, dissolved	150 µg/L	CCC: 150 µg/L	No hits
Barium, dissolved	1000 µg/L	1000 µg/L (human health)	No hits

Table B-1. Continued.

Cadmium, dissolved	0.25 µg/L	CCC: 0.25 µg/L	Insufficient hits
Copper	9 µg/L	CCC: 9 µg/L	No discernible S-R
Iron, dissolved	400 µg/L	CCC: 1000 µg/L	S-R (weak)
Lead, dissolved	2.5 µg/L	CCC: 2.5 µg/L	No discernible S-R
Mercury, dissolved	6 µg/L	CCC: 0.77 µg/L	S-R (weak)
Nickel, dissolved	2 µg/L	CCC: 52 µg/L	S-R
Selenium, dissolved	5 µg/L	CCC: 5 µg/L	1 hit
Silver, dissolved	1.6 µg/L	CMC: 3.2 µg/L	½ of CMC; no hits
Thallium, dissolved	1.7 µg/L	1.7 µg/L (human health)	No hits
Zinc, dissolved	120 µg/L	CCC: 120 µg/L	S-R too weak; use CCC
Total habitat score	> 140		S-R
Urban/suburban land use	< = 5 %		S-R
Cultivated land	< = 10%		S-R
Natural land cover	> 90 %		S-R
Population density	< 50 / mi^2		S-R
Discharges	0		

*Stressor-Response threshold used

** No hits = no observations above detection limit

Stressor-Response Thresholds

Stressor-response thresholds were inferred from scatterplots of biological response metrics (e.g., total taxa, EPT taxa, Ephemeroptera taxa, etc.) and measured stressor variables. We estimated thresholds of response and non-response from graphic analysis of scatter plots of biological indicator values and measured stressors values (Figure B-1).

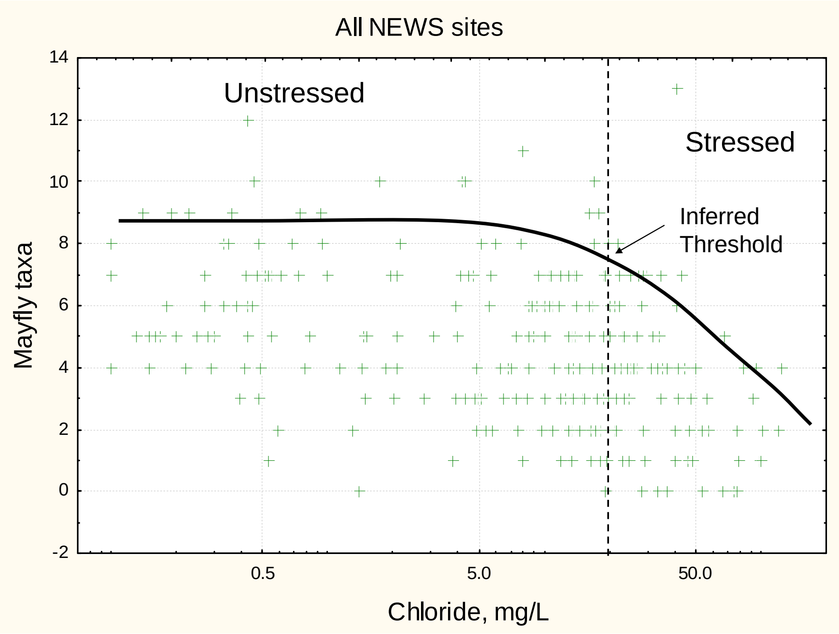


Figure B-1. Scatterplot of Mayfly taxa and chloride concentration. The threshold for potential effects was inferred at about 20 mg/L chloride (estimated by eye).

These scatterplots (Fig. B-1) often show a "wedge-shaped" plot such that when stress is low (as measured by the stressor variable), there are both high and low values of the biological indicator, but when stress is high, there are typically only low values of the biological indicator. For each stressor, we determined an upper limit of the biological indicator, or the best indicator value that can be obtained at that value of the stressor. This is given by the solid curve in Figure B-1 (fitted by eye; excluding outliers). This process assumes that near the solid curve, other stressors (not shown) are minimal, and the plotted stressor defines the limits of biological condition.

Because many of the stressors are collinear with land use and with each other, we can not infer that any given stressor causes impairment at values above the threshold, only that the stressor does not cause impairment at values below the threshold. Thus, we can only use these thresholds for screening potential reference sites.

“Best” and “Near-Best” Samples

Application of the criteria in Table B-1 resulted in 38 “best” samples. We also defined “near-best” samples where a single chemical observation was allowed to exceed its criterion up to twice the value of the criterion, and habitat score was allowed down to 120. This resulted in an additional 24 “near-best” samples. Some samples in these 2 groups were resamples at the same site in two years. The 62 best and near best samples were used for the classification analysis of the reference sites.

APPENDIX C

CLASSIFICATION OF REFERENCE SITES

APPENDIX C

CLASSIFICATION OF REFERENCE SITES

C.1. Methods

Differences in the natural biological assemblages among sites with relatively few environmental impacts were investigated so that these differences could be recognized and accounted for while assessing sites that may have greater degrees of environmental impact. The investigation entailed identification of the "best" sites within the NEWS data set, definition of those sites in terms of the structures of their biological samples, and determination of the environmental variables that are closely associated with the biological sample characteristics. Through this analysis, we hope to establish natural biological expectations that can be used in assessing all the sites in the NEWS data set.

At the outset of the analysis, we expected to find some differences in biological communities based on stream gradient or wetland influences in the watershed. These preconceptions came from previous analyses based on habitat gradient classes (high, mid, and low) and from discussions with New England biologists regarding unexpected assessment results. We used a combination of ordination, cluster analysis, and linear discriminant function analysis for the classification. Ordination arranges sites in ordination space according to their similarity of species compositions, such that sites near each other have similar species composition. Correlation of environmental variables with the axes of the ordination identifies environmental variables that may influence species distributions. Cluster analysis identifies discrete clusters of sites, and discriminant function analysis develops a model that predicts which cluster a site should belong to based on its environmental information.

NMS Ordination

Identifying classes among least-stressed New England stream sites requires identification of biological gradients or assemblage types and association of the biological gradient with natural variables. The biological gradients were explored using non-metric multidimensional scaling (NMS), a comparison of taxa within each sample and an arrangement of the samples so that similar samples plot closer together than dissimilar samples in multiple dimensions. Natural environmental variables can be associated with the biological gradient through correlations with the biologically defined axes of the NMS diagram. NMS is a robust method for detecting similarity and differences among ecological community samples (McCune and Mefford 1999). NMS analyses were performed using PC-Ord software (McCune and Mefford 1999).

A site-by-taxa matrix was compiled using sites that met the "best" criteria and taxa at levels that were consistently identified over all samples. Similarity among reference biological samples was made using the Bray-Curtis similarity measure based on relative abundances of organisms in each taxon. The Bray-Curtis (BC) formula is sometimes written in shorthand as

$$BC = 1-2W/(A+B)$$

where W is the sum of shared abundances, and A and B are the sums of abundances in individual sample units. The ordination software (PC-Ord, McCune and Mefford 1999) calculates a site by site matrix of BC similarity from which the arrangement of samples in the ordination diagram is derived. Multiple dimensions are compressed into two or three dimensions that can be plotted.

A site-by-environmental variable matrix included location information, physical habitat information, and land cover statistics. The environmental variables were related to the biologically defined NMS axes either categorically or continuously. Those variables that showed a smooth gradient or category distinction along one or more axes were considered important in defining the biological structure.

Clustering

A cluster analysis was conducted to determine distinct site classes based on biological data. The same site-by-taxa matrix that was used in the NMS analysis was used for clustering. Clustering used Bray-Curtis distances and a Flexible Beta group linkage method, with Beta set at -0.25. By dividing the cluster diagram into groups with similar linkage distances, multiple groups can be defined. Groups determined through cluster analysis were superimposed on the NMS diagram to determine which group distinctions (how many groups) are biologically meaningful.

Discriminant Function Analysis (DFA)

Discriminant Function Analysis (DFA) was used to identify environmental variables that dependably identified group membership. The environmental variables entered into the DFA were those that showed correlation with the NMS axes, were mostly or entirely independent of human influence, and would not categorize sites solely based on the regional North-South gradient.

This last consideration intends to allow extrapolation of the patterns observed in the “best” sites to sites of all environmental qualities. As will be discussed in more detail below, the “best” sites were all found in the northern New England states, excluding Connecticut, Massachusetts, and Rhode Island from any analysis of “best” sites. Thus, the latitudinal gradient is confounded with a stressor gradient, in that southern sites are more likely to be stressed than northern sites. Patterns observed in the northern states that, when extrapolated, would necessarily categorize all the southern sites into a single group were avoided. The latitudinal gradient was examined in a separate analysis (See 3.2.2 below). The variables that co-varied with north-south location included latitude, temperature, and elevation.

The DFA was performed using a forward stepwise method with consideration of the additional discrimination ability of each added variable. Percent correct prediction from a classification matrix of groups and group membership predicted by the DFA was a good measure of the additional discrimination ability of each variable. Predicted groups were also imposed on the NMS diagram to confirm sufficient agreement between NMS and DFA results. After selection of the best classification model, it was applied to all NEWS sites.

Indicator taxa

The groups determined through cluster analysis were further examined to identify the taxa that were consistently different among groups. Frequency of taxa occurrence within sites of each group and relative abundance of taxa within each group were used to determine the indicator taxa. The most important indicator taxa were identified through ratings of four analytical variables, as follows:

> Frequency of occurrence > 50% in either class
> Difference in Relative Abundance >= 70% between classes
> Difference in Frequency >= 30% between classes
> Analytical p value <= 0.02

Taxa that met three or more of the criteria were highlighted. Indicator analysis was conducted in PC-Ord using methods of (Dufresne and Legendre 1997).

C.2. Results

Of the 280 sites in the NEWS data set, 63 were used in the analyses of "best" and "near best" sites. These are sites that met the criteria in Table 1 (or met all but one of the water chemistry criteria), were not judged inadequate by the regional biologists, had data related to all the criteria, and had biological samples collected using NEWS methods. All of the "best" sites were in Maine, New Hampshire, and Vermont.

C.2.1 "Best" sites analysis – Stream Slope

The NMS analysis resulted in a configuration of sites in three dimensions of the multidimensional taxa space. The first axis explained most of the variance and the environmental variables related to that axis included elevation, stream slope, and % water and wetland in the local watershed (local hydrological unit). Variables related on the first and second axis (diagonal) included land slope, % local deciduous forest of all forest types, and % water and wetland in the total watershed. No environmental variables of consequence were related to the third axis.

The **cluster analysis** identified two groups that were associated with the first axis of the NMS, related to elevation, wetland coverage, and gradient (Figure 1). When three groups were considered, the lower gradient group was split, with little in the NMS diagram to account for the difference. Therefore, two groups were considered in subsequent analyses.

The **DFA** revealed that the best discrimination of the two groups of the cluster analysis was possible using a single variable. Little or no discrimination ability was added when adding a second variable to the models. For the purposes of NEWS classification, stream gradient alone can be used to classify sites. Elevation, latitude, % wetlands, and temperature were also reasonable classification variables. However, elevation and temperature are also associated with latitude, which is an incomplete gradient when considering only the "best" sites, which all occurred in northern New England. Of the remaining variables, stream slope alone was a better determinant of group membership than % wetlands or the combination of both. In the classification matrix, stream gradient correctly identified 73% of sites. The predicted groups displayed in the NMS diagram showed reasonable separation and minimal overlap (Figure C-1). The threshold value between high and low gradient streams was 1% stream slope.

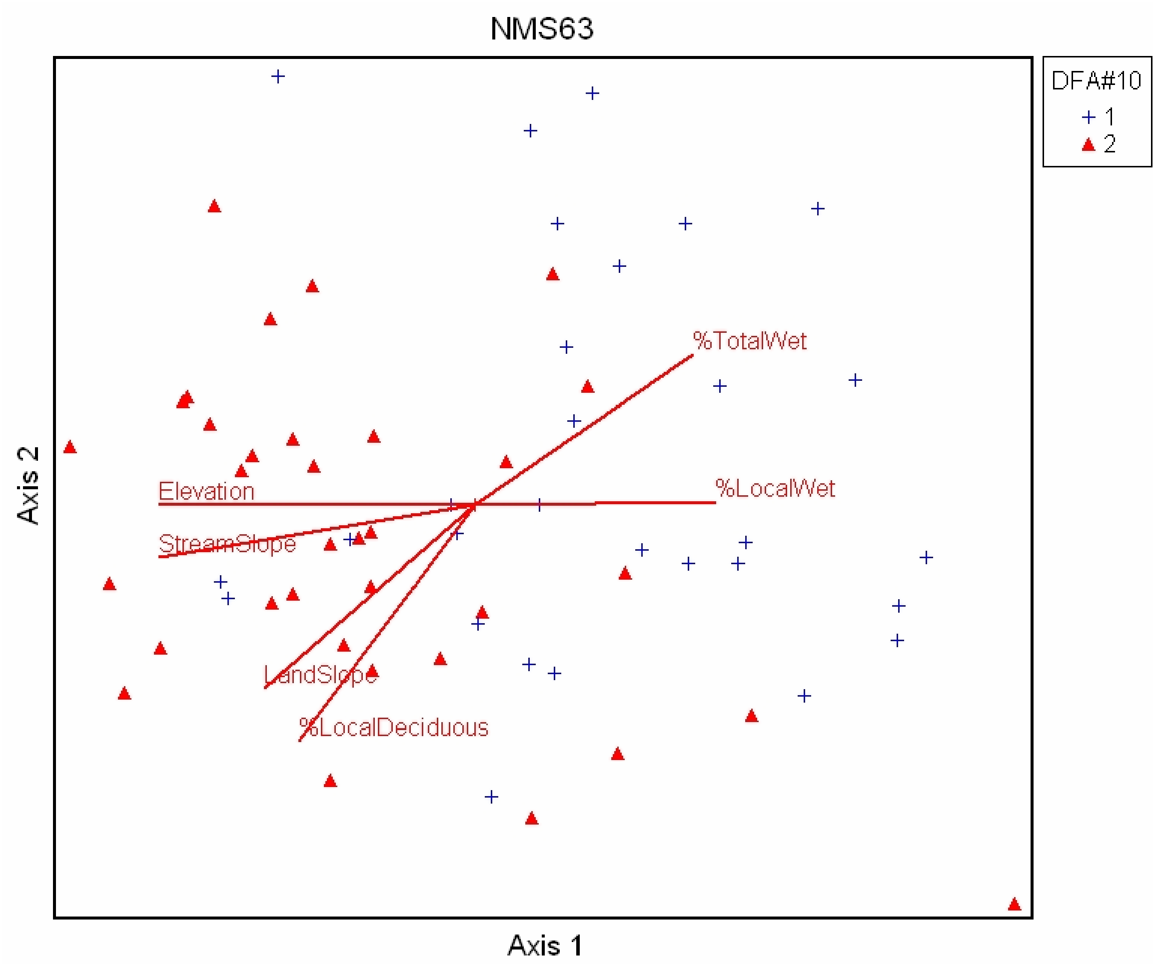


Figure C-1. Ordination diagram of "best" sites in taxa space. Vectors display the environmental variables associated with the axes. Symbols display group membership based on the cluster analysis.

Several ***indicator taxa*** were identified through indicator analysis. Thirty taxa met at least two of the criteria listed above. Those that met three or more criteria are listed in Table C-2, along with the high or low gradient group indicated by greater abundance and frequency of each taxon. All 18 of the most indicative taxa are insects. These are indicators within “best” sites, so they may not be reliable indicators in sites with greater degrees of environmental stress. Taxa that did not occur in at least 20% of one or the other site groups were not considered as reliable indicators.

Table C-2. Taxa that showed the greatest differences between high gradient and low gradient reference groups.

Order	Family	Taxon	Indication
Coleoptera	Elmidae	*Oulimnius*	High
Coleoptera	Elmidae	*Stenelmis*	Low
Diptera	Chironomidae	*Eukiefferiella*	High
Diptera	Chironomidae	*Micropsectra*	High
Diptera	Chironomidae	*Microtendipes*	Low
Diptera	Chironomidae	*Parakieferiella*	Low
Diptera	Chironomidae	*Rheocricotopus*	High
Diptera	Chironomidae	*Tanytarsus*	Low
Ephemeroptera	Baetidae	*Baetis*	High
Ephemeroptera	Ephemerellidae	*Drunella*	High
Ephemeroptera	Leptohyphidae	*Tricorythodes*	Low
Plecoptera	Leuctridae	*Chloroperlidae*	High
Plecoptera	Leuctridae	*Leuctridae*	High
Trichoptera	Glossosomatidae	*Glossosoma*	High
Trichoptera	Lepidostomatidae	*Lepidostomatidae*	High
Trichoptera	Leptoceridae	*Leptoceridae*	Low
Trichoptera	Philopotamidae	*Chimarra*	Low
Trichoptera	Philopotamidae	*Dolophilodes*	High

C.2.2 Latitude Gradient

Latitude of the NEWS sampling sites is strongly confounded with stressor gradients. Northern Maine and New Hampshire are primarily forested, sparsely populated, with extensive forest tracts that have not been logged for more than 50 years. Rhode Island and Connecticut are largely urban and suburban, with high road densities and high commuter traffic throughout. Furthermore, they both have a great deal of “legacy” effects from the early industrial period before 1900, as well as legacy pollution from the heavy industrial era of 1900-1970.

The least stressed sites (Appendix B) were all in Maine, New Hampshire, and Vermont, and the most stressed sites, in terms of urban land use, population density, chemical concentrations, etc. were all in Connecticut , Rhode Island, and Massachusetts. Consequently, we could not examine a latitudinal gradient using least stressed sites only.

To examine a latitude gradient, we identified a set of moderately stressed sites, that met neither “least stressed”, “near-best” nor “most stressed” conditions, assuming that these would be more equitably distributed throughout the region. This would allow us to examine latitudinal differences without confounding by a collinear stress gradient. We identified 78 “moderately stressed” sites, defined as having more than 20 and less than 200 persons per square mile in the watershed, and no more than 11% urban/suburban land use, and 60% agricultural land use. There were only 2 sites each in this category in Maine and Massachusetts, but sites in the other states were more equitably distributed (NH 22; VT 27; RI 10; CT 15). It should be noted that many of these sites belong to the "least stressed" group in Connecticut and Rhode Island.

Ordination of the "moderately stressed" sites revealed separation in ordination space by state (Figure C-2). The 5 most strongly correlated environmental variables were latitude, pH, longitude, calcium, and total phosphorus. Latitude is comprised of both temperature and elevation gradients in New England, with higher elevations occurring in the north. Vermont streams tended to have higher pH, calcium, alkalainity and conductivity than the streams of other states, and Rhode Island streams tended to have higher total phosphorus. The latitude (north-south) gradient in the ordination is readily explainable, but the longitude interstate differences may result from several factors:

- pH-alkalinity – pH of Vermont and Connecticut sites were higher than in their eastern neighbors (New Hampshire, Rhode Island). There are marble formations in extreme western Connecticut, and Vermont has calcareous rock (limestone, dolomite, marble) scattered throughout much of the state.
- Total Phosphorus – all Rhode Island sites had higher total phosphorus than the other states (50 to 160 µg/L), raising the possibility of systematic data entry or transcription errors.
- Sampling differences among states – we can not rule out methodological differences in field practices and lab protocols among the states. Sampling was carried out by USEPA personnel in Maine, New Hampshire, and Rode Island; but Vermont and Connecticut were sampled by their respective state agency personnel. Vermont and Connecticut samples were also sent to different contract laboratories than the other states for sorting and identification.

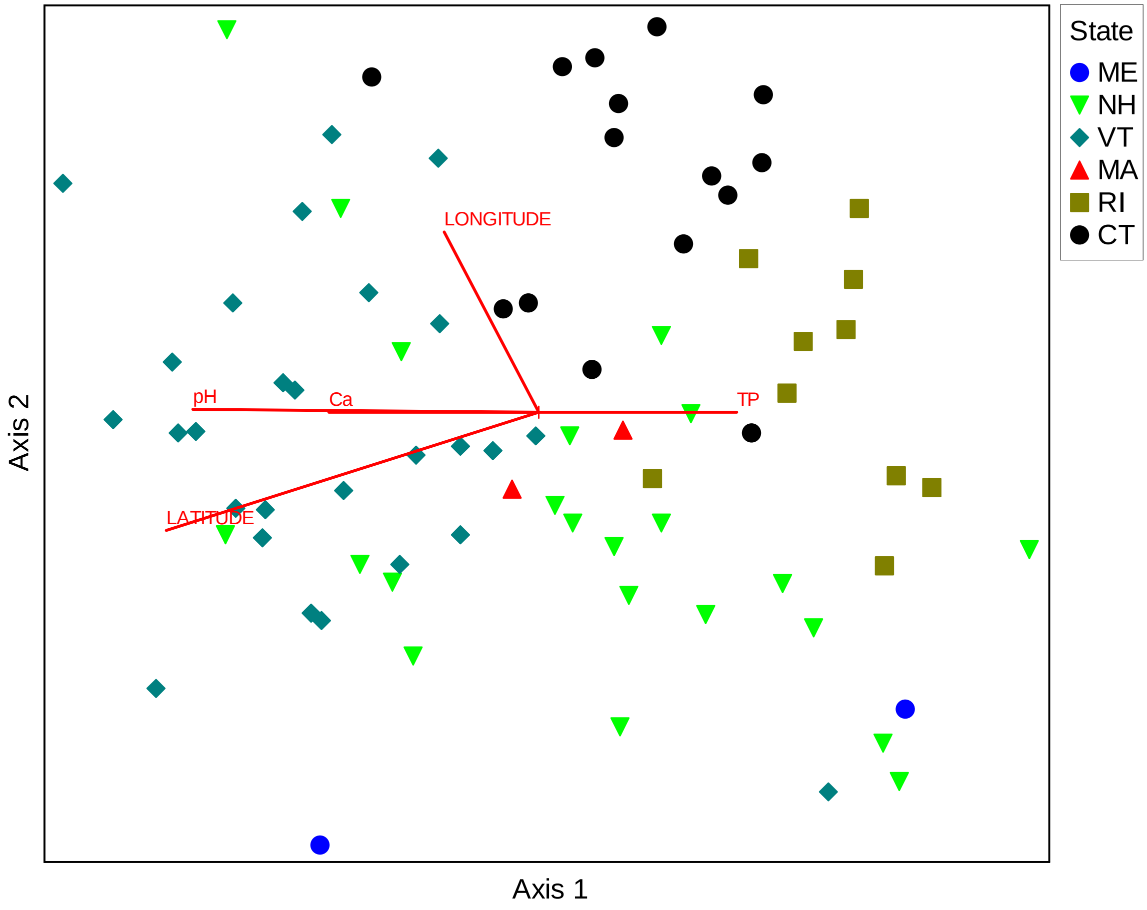


Figure C-2. "Moderately stressed" sites in NMS ordination space, and vectors showing correlation of 5 environmental variables (biplot).

Several taxa were found primarily in either the northern or southern states, in the moderately stressed sites. Table C-3 lists taxa found preferentially in one region or the other, by a factor of 2 or more. The families Baetidae (Ephemeroptera), Simuliidae (Diptera), and Leuctridae (Plecoptera), while relatively common throughout all of New England, all occurred at higher frequency (<40% of sites) in New Hampshire and Vermont than in Connecticut and Rhode Island. Common genera (<40%) in the north that were less common in the south included *Cricotopus* (Diptera: Chironomidae), *Glossosoma* (Trichoptera: Glossomatidae), and *Micropsectra* (Diptera: Chironomidae). Common families in the south that were less common in the north included the Psephenidae (Coleoptera), Polycentropodidae (Trichoptera), Taeniopterygidae (Plecoptera), and Tubificidae (Oligochaeta). Common southern genera that were less common in the north included *Parachaetocladius* (Diptera: Chironomidae), *Diplectrona* (Trichoptera: Hydropsychidae), and *Stilocladius* (Diptera: Chironomidae).

Table C-3. Taxa with large differences in percent occurrence between southern and northern sites, moderately stressed. Listed in order of increasing ratio N:S.

Order	Family	Target taxon	Frequency (%) South (N=27)	 North (N=51)	Total sites
Diptera	Chironomidae	*Pagastia*	0.0%	31.4%	16
Ephemeroptera	Leptohyphidae	Leptohyphidae	0.0%	23.5%	12
Trombidiformes (acari)	Lebertiidae	Lebertiidae	3.7%	31.4%	17
Diptera	Athericidae	*Atherix*	3.7%	19.6%	11
Diptera	Chironomidae	*Diplocladius*	3.7%	17.6%	10
Diptera	Chironomidae	*Cricotopus*	18.5%	62.7%	37
Trichoptera	Glossosomatidae	*Glossosoma*	18.5%	47.1%	29
Diptera	Chironomidae	*Phaenopsectra*	14.8%	35.3%	22
Diptera	Chironomidae	*Ablabesmyia*	14.8%	33.3%	21
Diptera	Chironomidae	*Micropsectra*	37.0%	80.4%	51
Ephemeroptera	Baetidae	Baetidae	44.4%	92.2%	59
Diptera	Simuliidae	Simuliidae	33.3%	66.7%	43
Plecoptera	Leuctridae	Leuctridae	29.6%	58.8%	38
Coleoptera	Psephenidae	*Ectopria*	29.6%	11.8%	14
Coleoptera	Psephenidae	*Psephenus*	55.6%	21.6%	26
Haplotaxida	Tubificidae	Tubificidae	40.7%	15.7%	19
Diptera	Chironomidae	*Parachaetocladius*	44.4%	15.7%	20
Trichoptera	Polycentropodidae	Polycentropodidae	63.0%	15.7%	25
Odonata	Calopterygidae	Calopterygidae	37.0%	7.8%	14
Trichoptera	Psychomyiidae	Psychomyiidae	33.3%	3.9%	11
Trichoptera	Hydropsychidae	*Diplectrona*	51.9%	5.9%	17
Plecoptera	Capniidae	Capniidae	37.0%	3.9%	12
Diptera	Chironomidae	*Stilocladius*	40.7%	2.0%	12
Plecoptera	Taeniopterygidae	Taeniopterygidae	59.3%	0.0%	16

C.4. Discussion

Similarities in biological composition of NEWS samples can be explained by stream gradient and latitude. This was expected and was even implemented in earlier analyses, in which the gradient category was derived from habitat analysis field sheets. The current analysis was based on remotely calculated gradient of the stream within the entire stream reach (upper stream elevation - lower stream elevation / stream length). The threshold between gradient groups is 1%.

The classification of "best" samples into two biologically dissimilar groups results from analysis of the relative abundances of taxa within each sample (cluster analysis). The best explanation for the differences in the groups is that gradient, elevation, and % wetlands and open water within the watershed affect the stream habitat in terms of temperature, velocity, and probably substrate (which was not analyzed). The organisms suited to high-gradient, fast and cold water are not the same as those suited to the lower-gradient, slower and warmer water (all in relative terms).

Literature Cited

Dufrene, M. and P. Legendre. 1997. Species assemblages and indicator species: the need for a flexible asymmetrical approach. Ecological Monographs 67:345-366.

McCune, B. and M. J. Mefford. 1999. PC-ORD. Multivariate Analysis of Ecological Data. Version 4.36. MjM Software, Gleneden Beach, Oregon, U.S.A.

USEPA 2002. National Recommended Water Quality Criteria: 2002. US EPA, Office of Water. EPA-822-R-02-047

APPENDIX D

EXAMINING ATTRIBUTES

APPENDIX D

EXAMINING ATTRIBUTES

D.1. Methods

Biologists have long observed that taxa differ in their sensitivity to pollution and disturbance. While biologists largely agree on the relative sensitivity of taxa, there may be subtle differences among stream types (high vs. low gradient) or among geographic regions (northern vs. southern New England).

We empirically examined the sensitivities of the benthic macroinvertebrates to a generalized stressor gradient. The gradient was defined by ordination analysis, where the least stressed and the most stressed sites (Appendix B) were identified in the ordination, and where the least and most stressed sites were well-separated in the ordination. These sites thus represent the endpoints of the stressor gradient in ordination space, and the stressor gradient is typically parallel to one of the axes of NMS ordination, or (less frequently) a slope defined by 2 ordination axes. The assumption here is that once natural classification and variability are accounted for (Section A.II), that remaining differences in taxonomic composition are caused by stressors. The principal natural classifications for the NEWS streams were slope and latitude, so we examined only high gradient streams (>1% slope), and analyzed separately by latitude groups.

Groups of sites identified by the stressor gradient (least stressed; intermediate, most stressed) were further examined to identify the taxa that were consistently different among groups. Frequency of taxa occurrence within sites of each group and relative abundance of taxa within each group were used to determine the indicator taxa. The most important indicator taxa were identified through ratings of four analytical variables, as follows:

Frequency of occurrence > 50% in either class
Difference in Relative Abundance >= 70% between classes
Difference in Frequency >= 30% between classes
Analytical p value <= 0.02

Taxa that met three or more of the criteria were highlighted. Indicator analysis was conducted in PC-Ord using methods of Dufresne and Legendre (1997).

Indicator species analysis examines the probability that a species's presence gives diagnostic information on the membership of a site to one of several a priori groups (Appendix B). Two difficulties arose with Indicator Species Analysis: 1) rare taxa that occurred exclusively in a single group (of 3) tended to be highly significant in spite of their low probability of occurrence; and 2) taxa that were absent from a single group, but not from other groups were not statistically significant because the analysis only considers diagnostic information for single groups. Thus, the indicator species analysis would not identify moderately sensitive taxa that are absent from the most stressed sites, nor would it identify tolerant taxa that are absent from the minimally stressed sites.

Graphical analysis of individual taxa on ordination plots was deemed to be the most useful for identifying attribute groups, coupled with correlation coefficient of each taxon with the principal gradient defining least and most stressed sites. Sites were plotted in ordination space from a non-metric multidimensional scaling analysis (NMS) (Fig. D-1), and identified with each of the a priori groups by symbol and color. For each taxon, symbol size was controlled by the relative abundance of the taxon at each site, so that taxa that were more abundant, or occurred more frequently in any 1 or 2 of the a priori groups could be readily identified (Figs. D2 - D15). The graphical analysis was combined with correlation of each taxon abundance with site scores on each ordination axis.

D.2 Connecticut Data

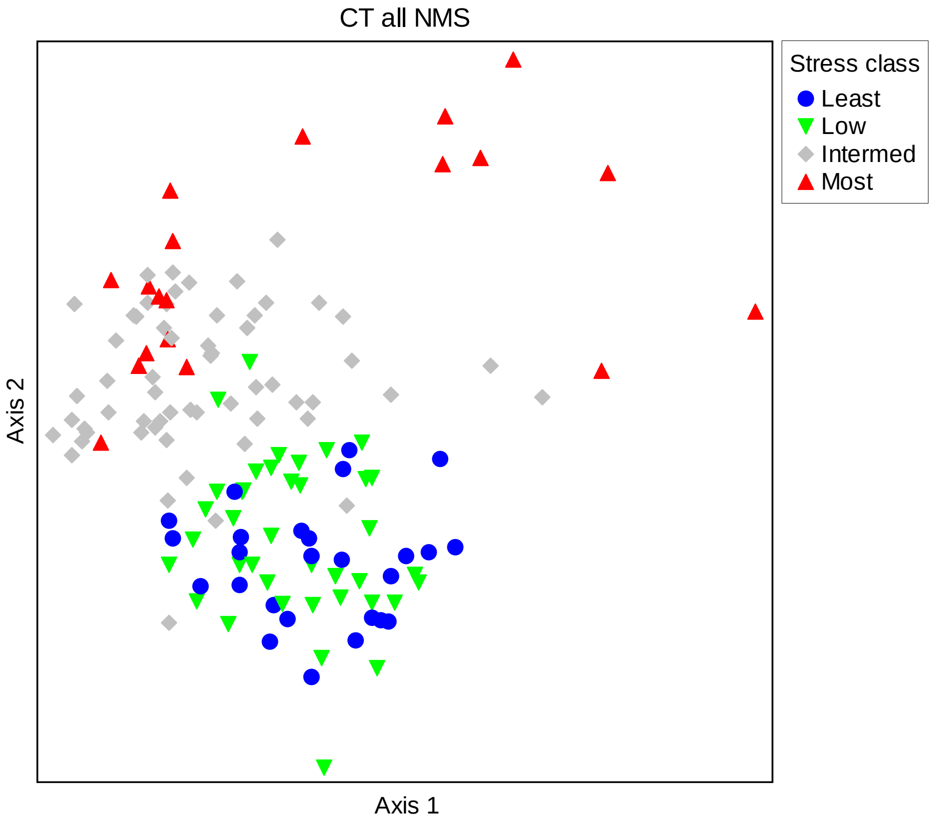
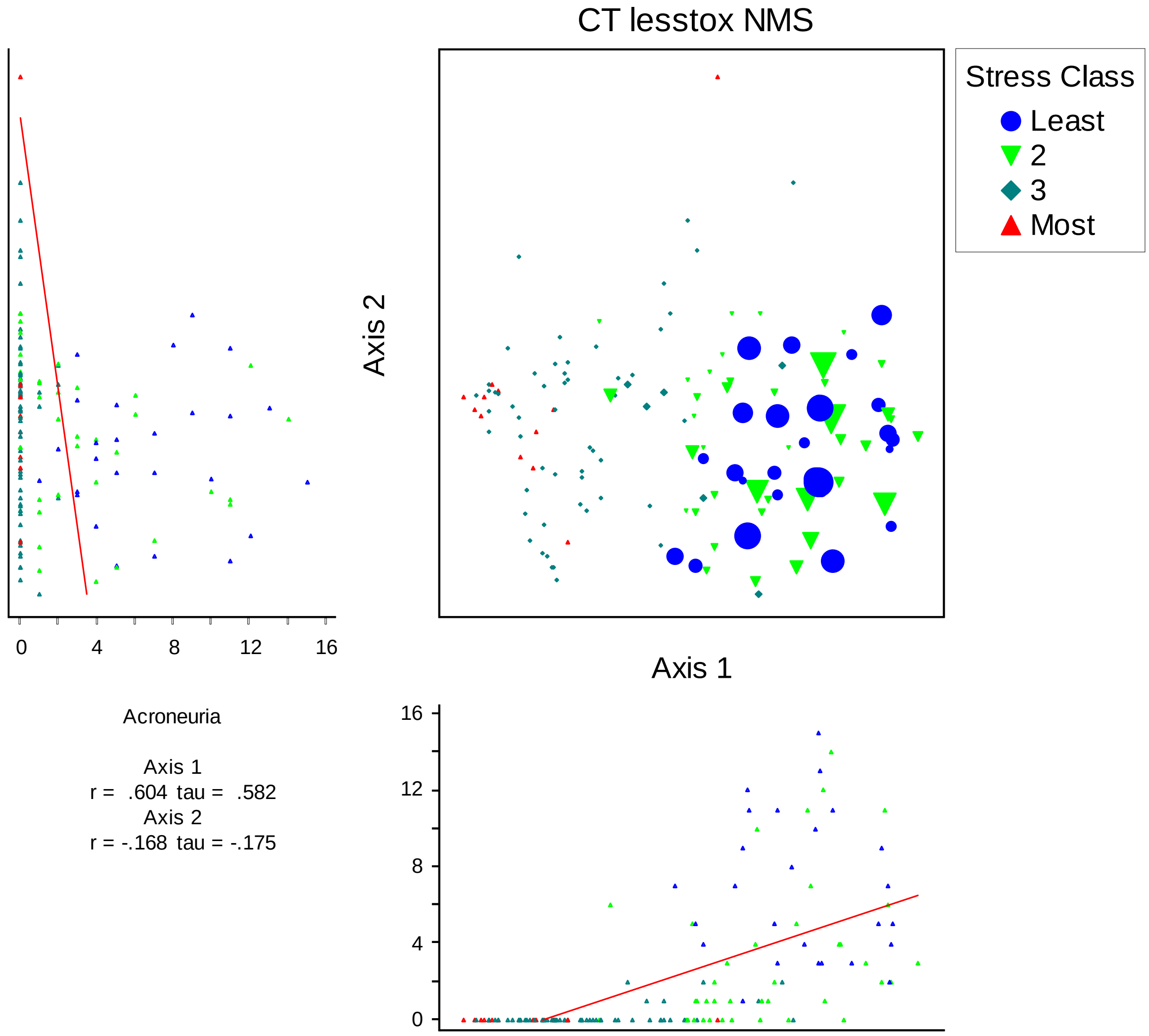


Figure D-1. NMS ordination of CT DEP data. Several of the “most stressed” sites (red triangles) showed evidence of severe toxic stress – very small samples (< 50 organisms, <10 taxa) and were in areas of heavy industrial legacy pollution, sampled in the 1970s and 80s (e.g., Naugatuck River). These sites are often dominated by either Tubificidae or highly tolerant genera of Chironomidae. The extremely degraded sites are outliers in the ordination and tend to confound display of the rest of the biological condition gradient.



Figures D-2 to D-15. NMS ordination of CT DEP data, but with the toxic legacy sites removed. With the effect of the anomalous toxic sites removed, the rest of the BCG gradient is displayed better in the ordination, allowing examination of individual taxa.

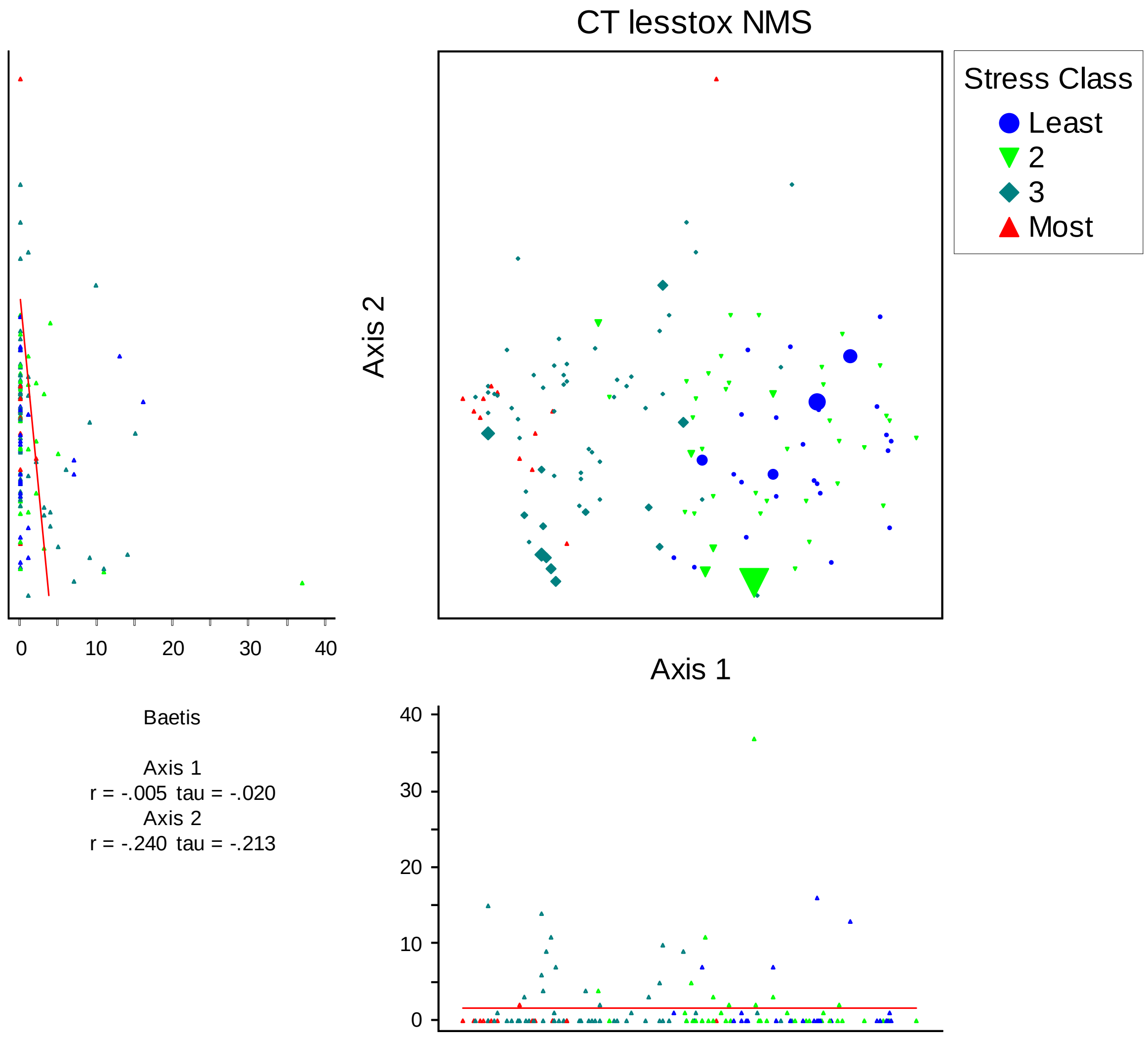
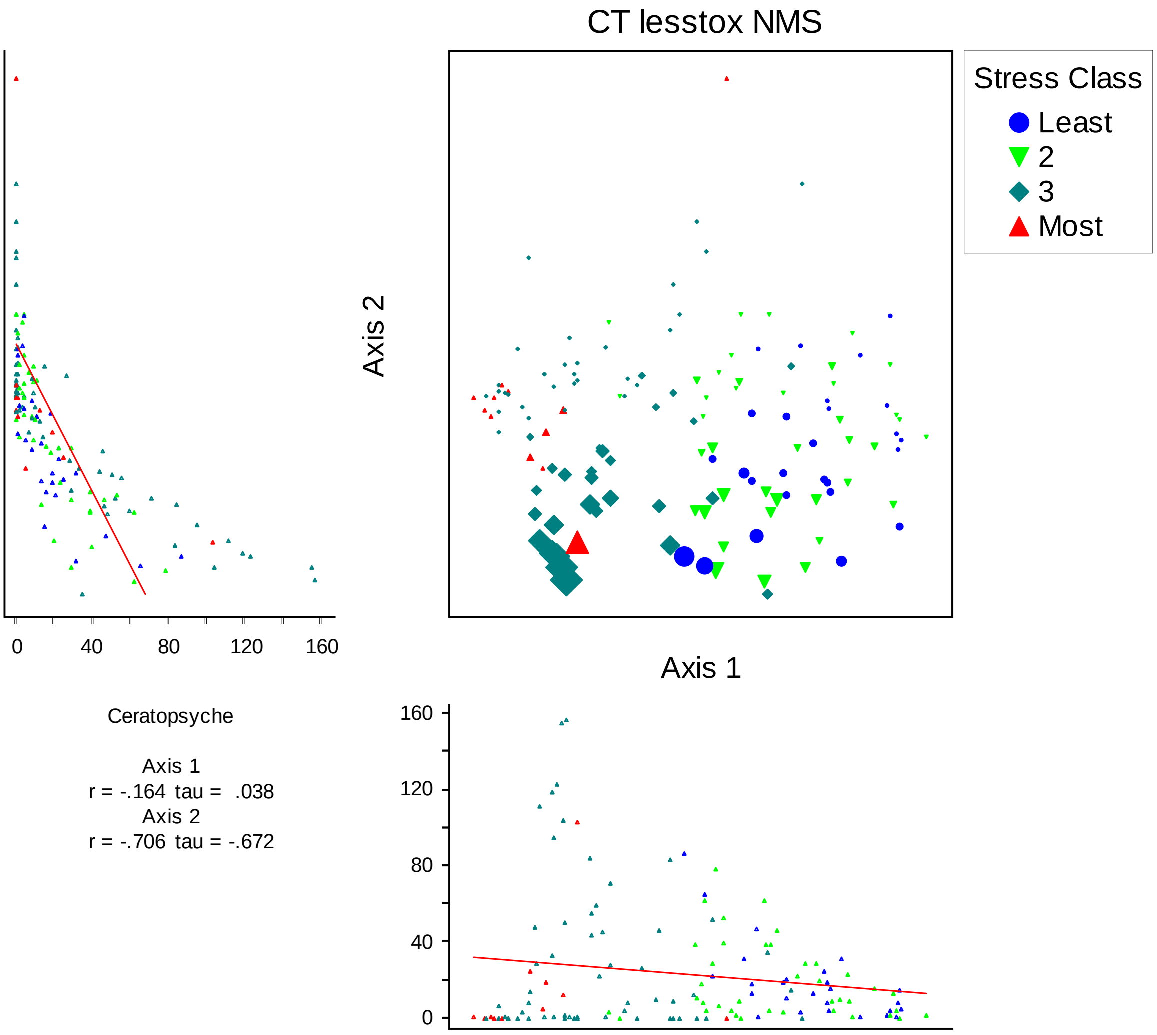

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
10
20
30
40
Baetis
Axis 1
r = -.005 tau = -.020
Axis 2
r = -.240 tau = -.213

Figure D-3



Ceratopsyche

Axis 1
r = -.164 tau = .038
Axis 2
r = -.706 tau = -.672

Figure D-4

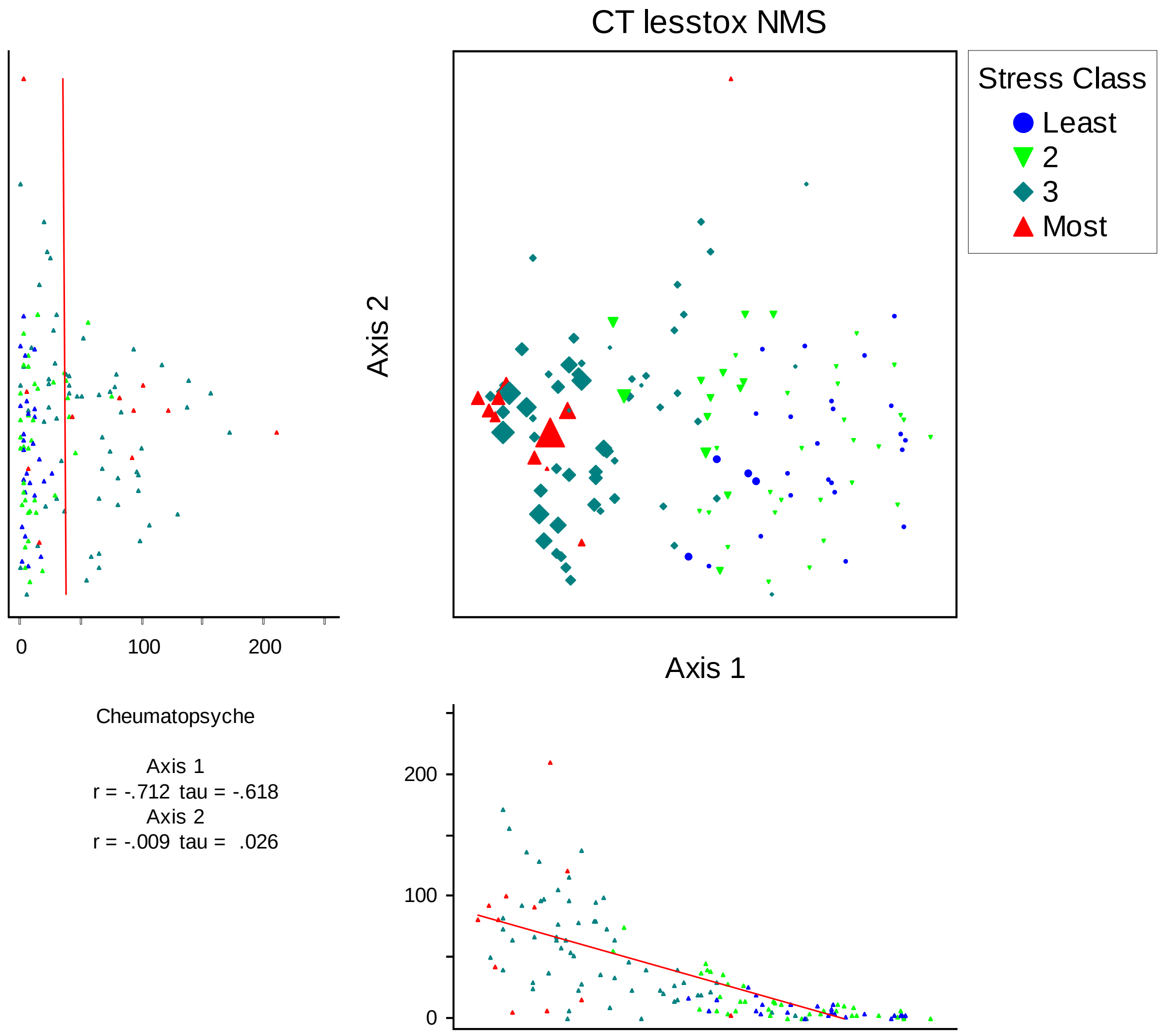

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
100
200
Cheumatopsyche
Axis 1
r = -.712 tau = -.618
Axis 2
r = -.009 tau = .026
200
100
0

Figure D-5

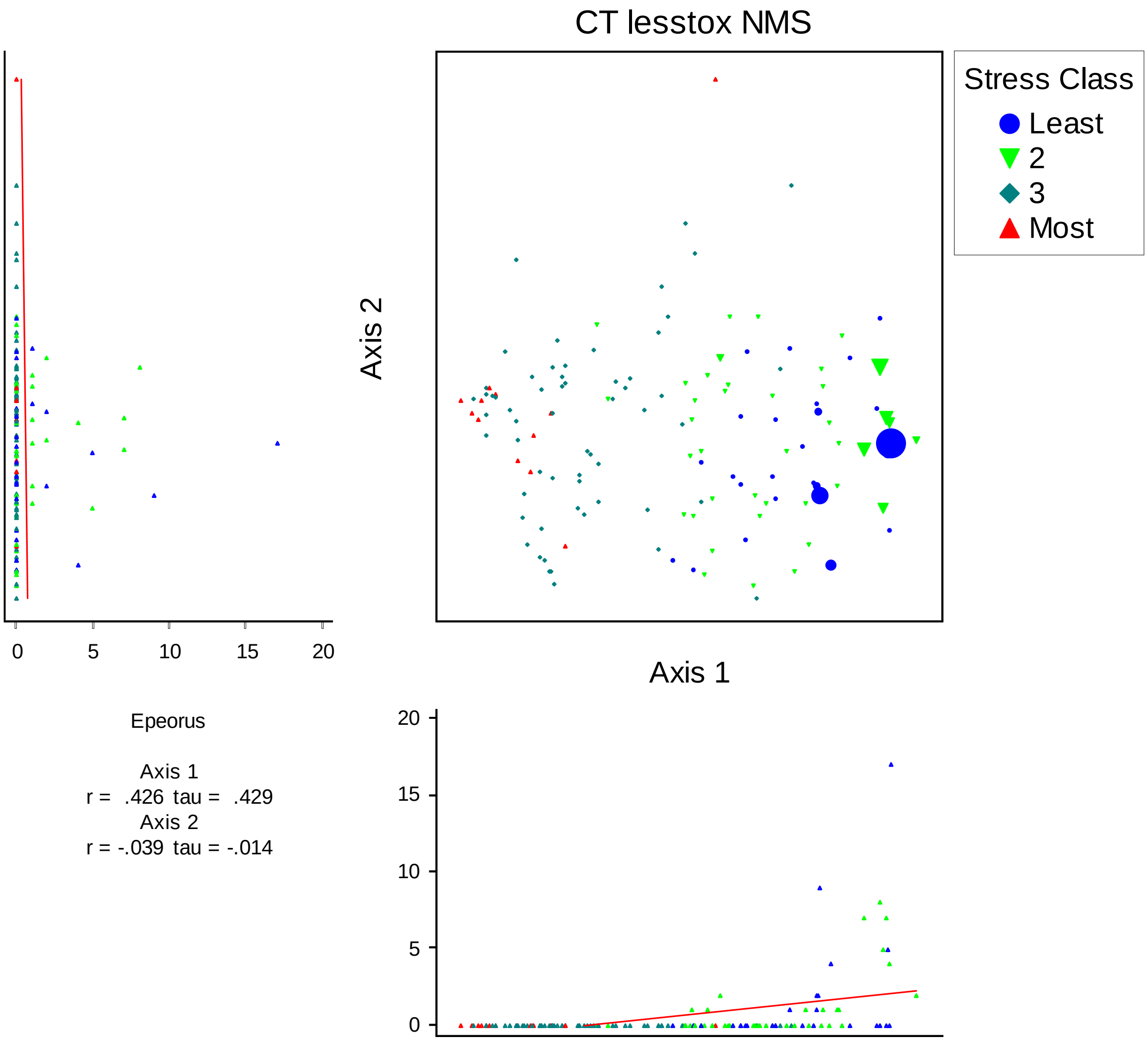

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
5
10
15
20
20
15
10
5
0
Epeorus
Axis 1
r = .426 tau = .429
Axis 2
r = -.039 tau = -.014

Figure D-6

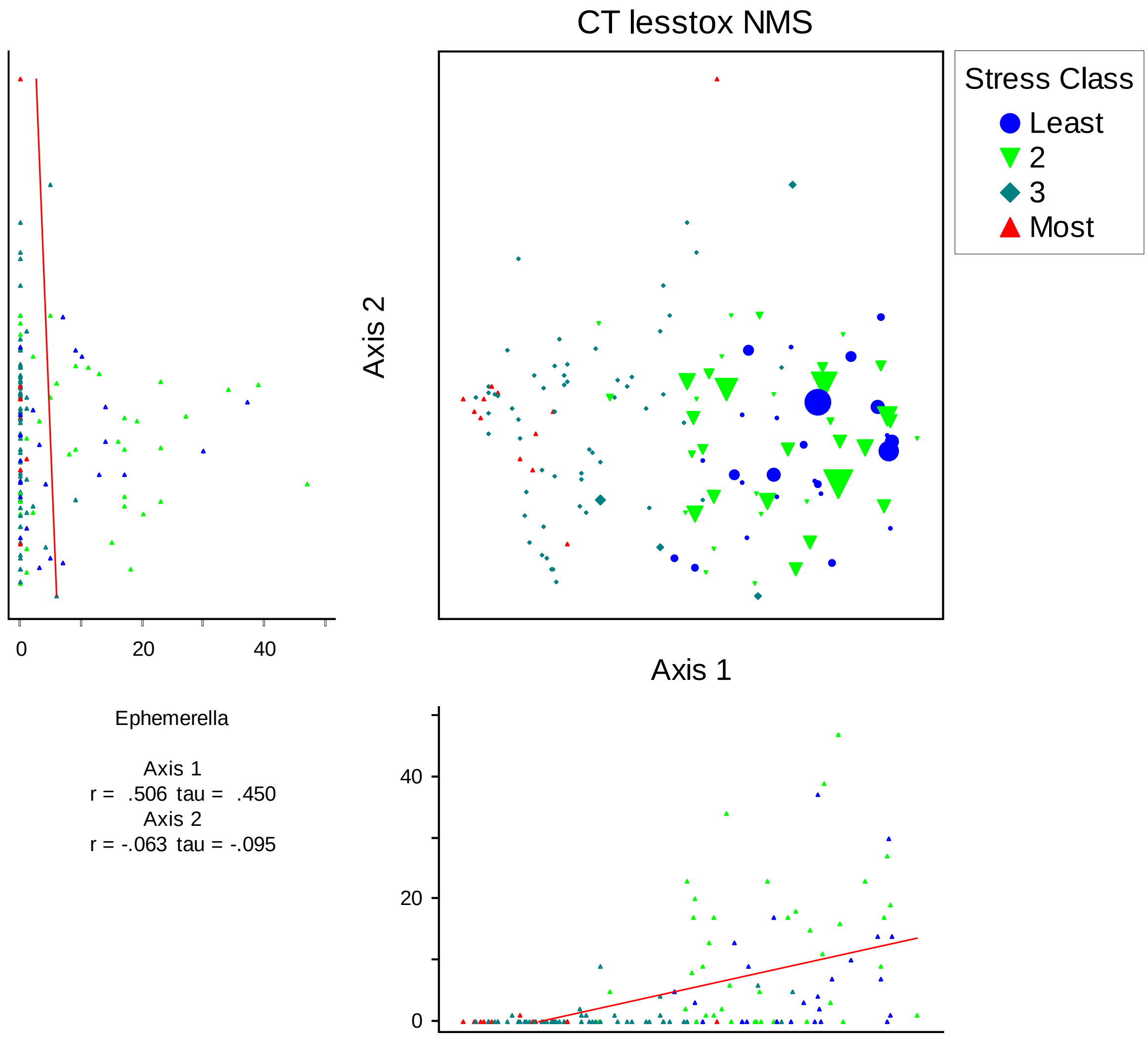

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
20
40
Ephemerella
Axis 1
r = .506 tau = .450
Axis 2
r = -.063 tau = -.095
40
20
0

Figure D-7

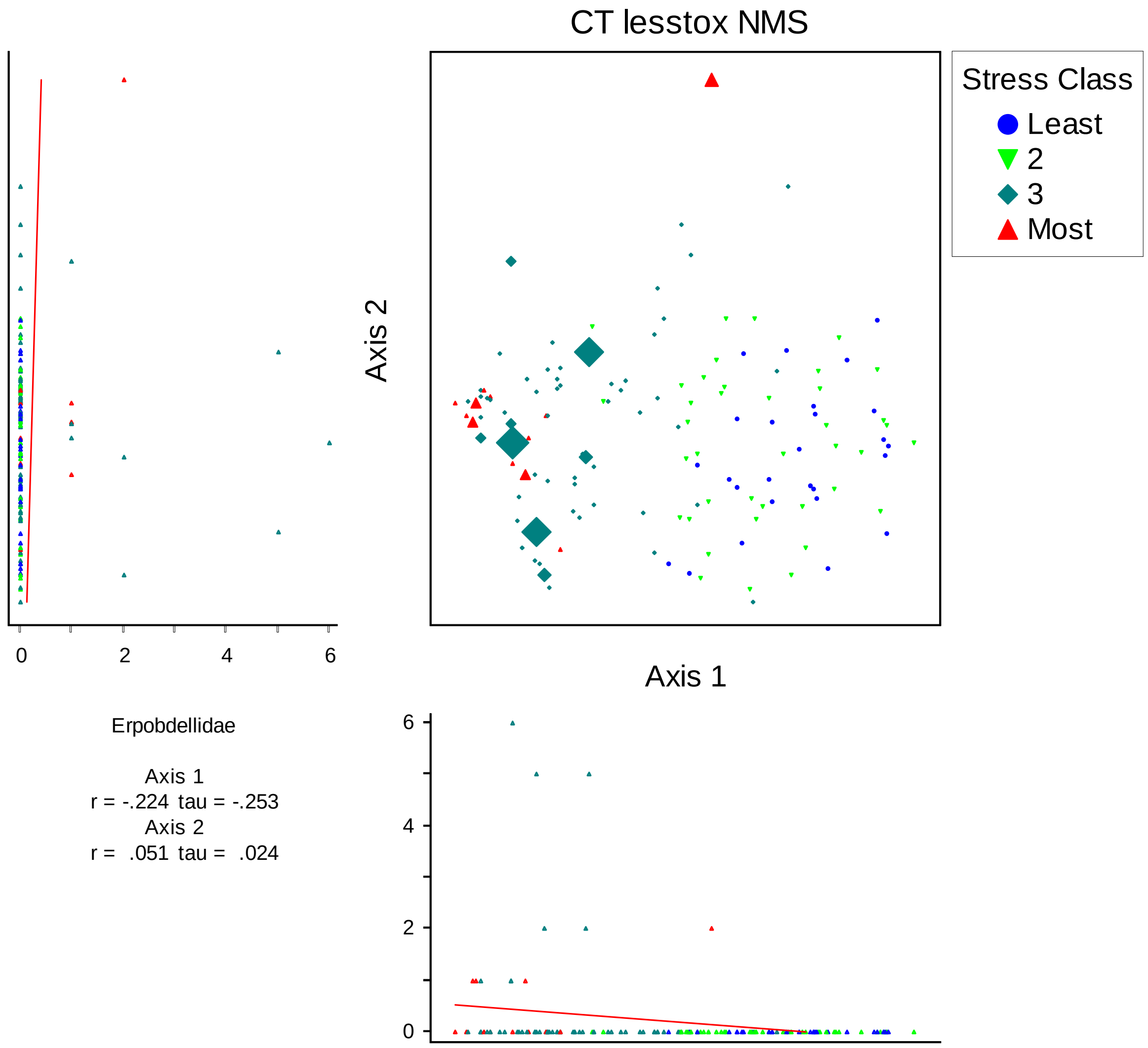

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
2
4
6
Erpobdellidae
Axis 1
r = -.224 tau = -.253
Axis 2
r = .051 tau = .024
6
4
2
0

Figure D-8

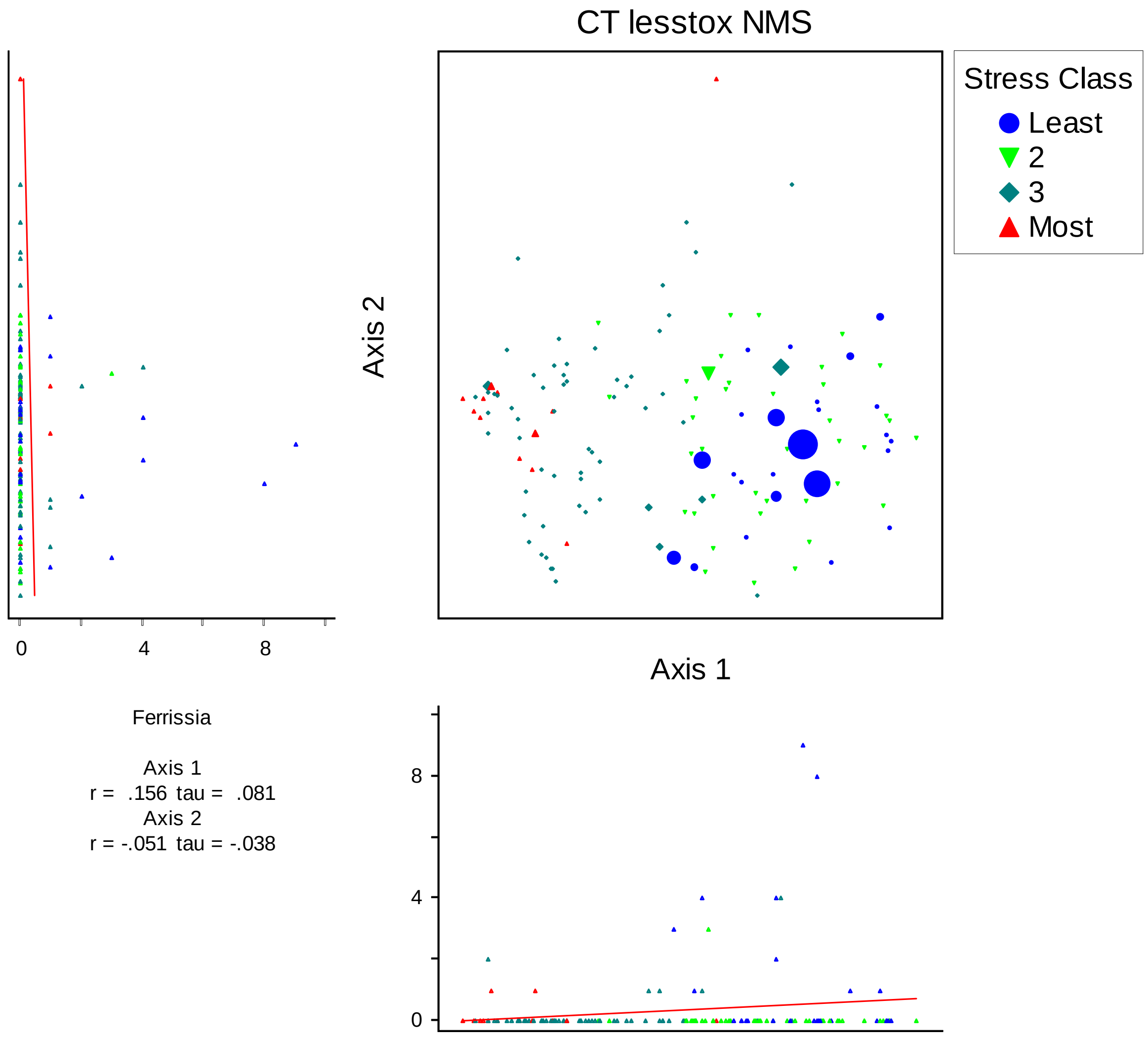

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
4
8
Ferrissia
Axis 1
r = .156 tau = .081
Axis 2
r = -.051 tau = -.038
8
4
0

Figure D-9

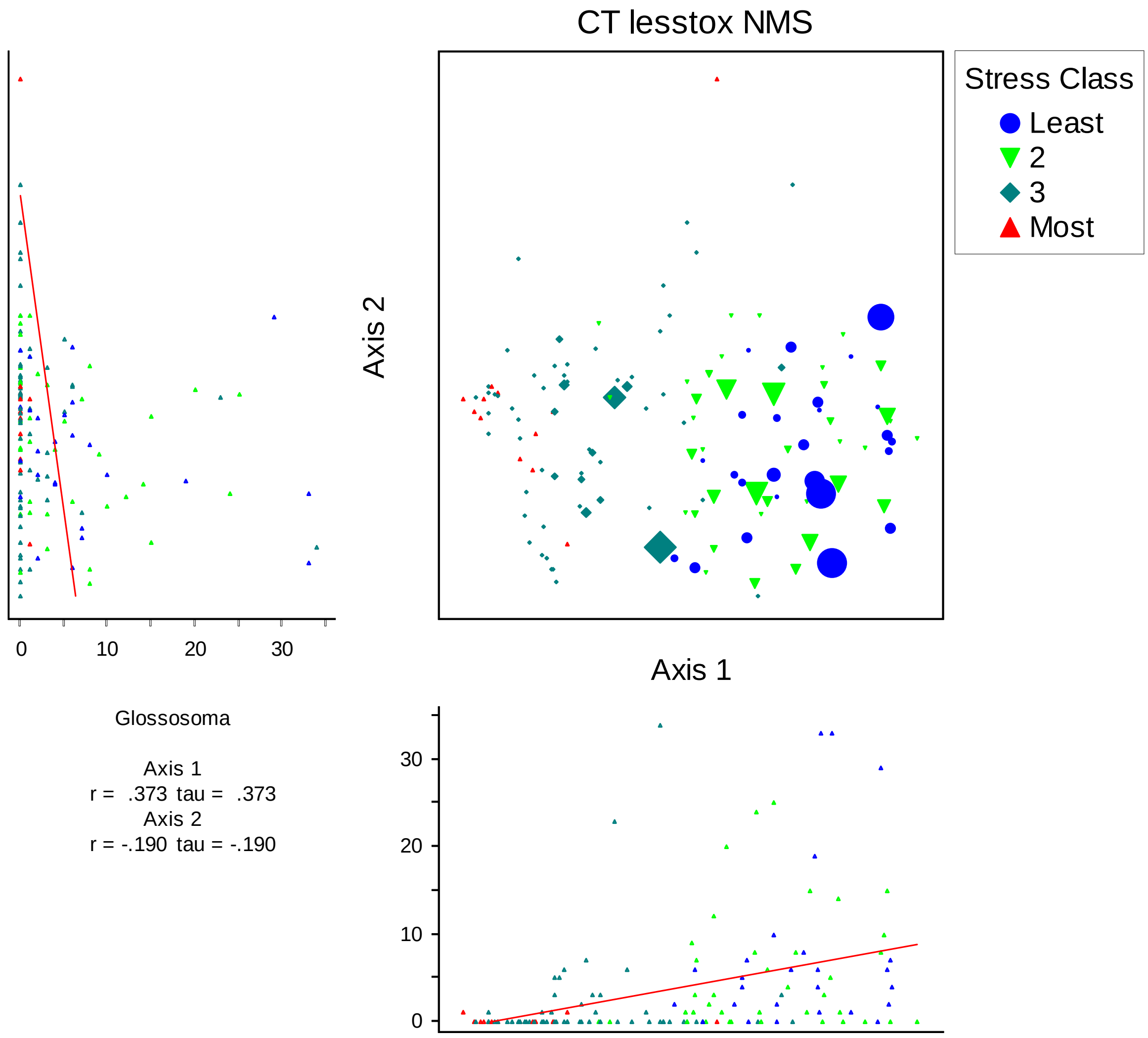

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
10
20
30
Glossosoma
Axis 1
r = .373 tau = .373
Axis 2
r = -.190 tau = -.190
30
20
10
0

Figure D-10

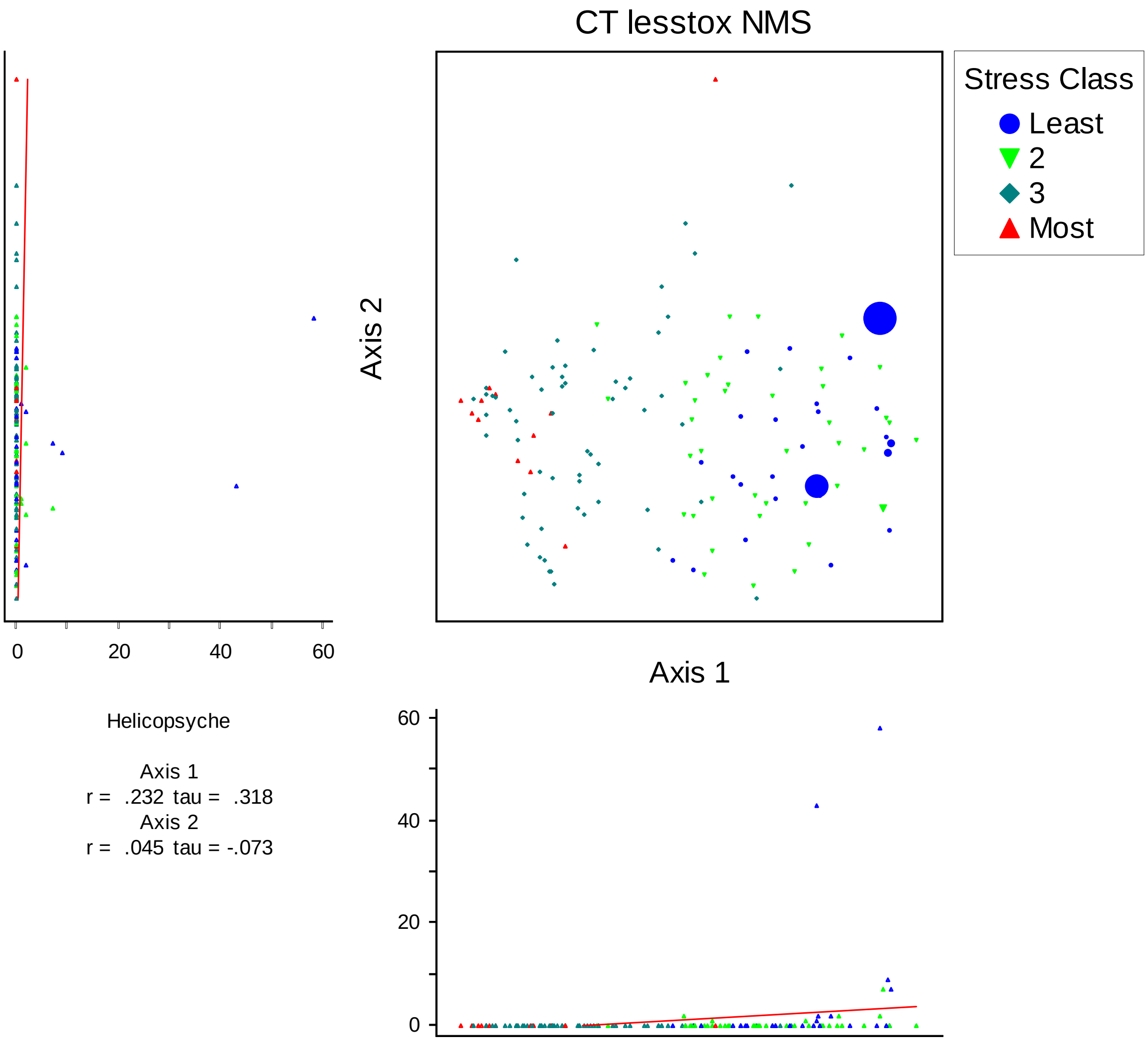

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
20
40
60
Helicopsyche
Axis 1
r = .232 tau = .318
Axis 2
r = .045 tau = -.073
60
40
20
0

Figure D-11

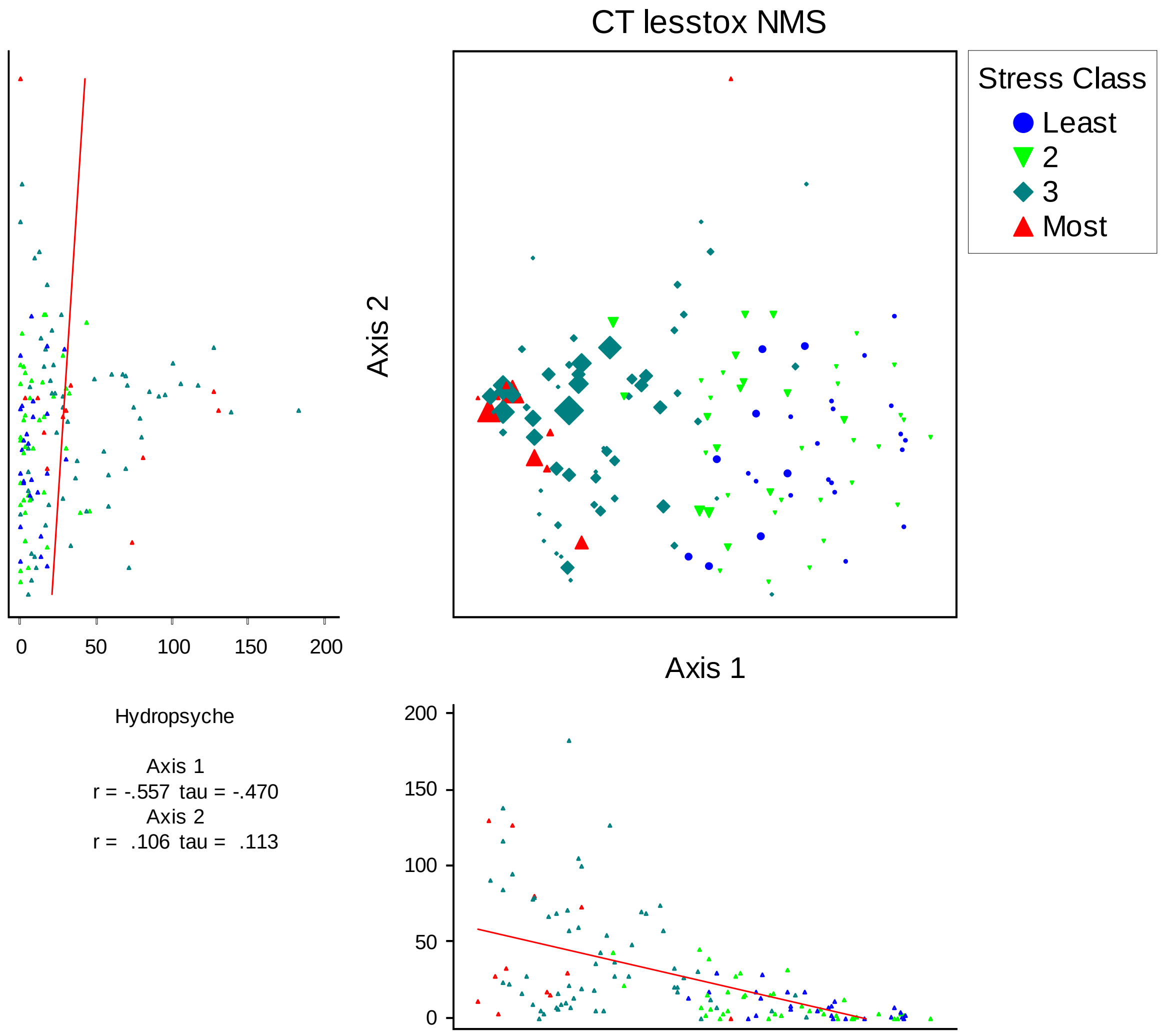

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
50
100
150
200
Hydropsyche
Axis 1
r = -.557 tau = -.470
Axis 2
r = .106 tau = .113
200
150
100
50
0

Figure D-12

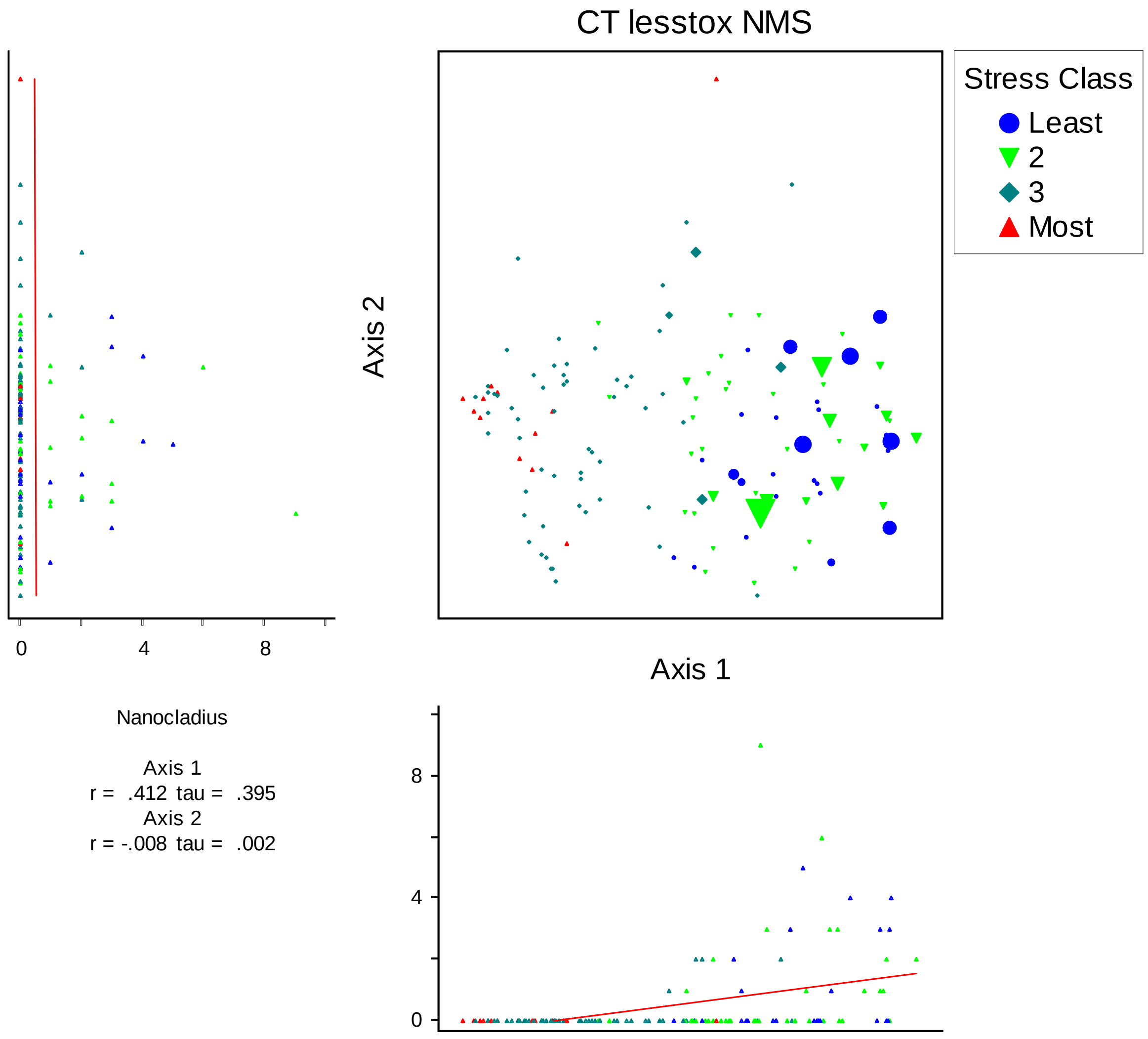

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
4
8
0
4
8
Nanocladius
Axis 1
r = .412 tau = .395
Axis 2
r = -.008 tau = .002

Figure D-13

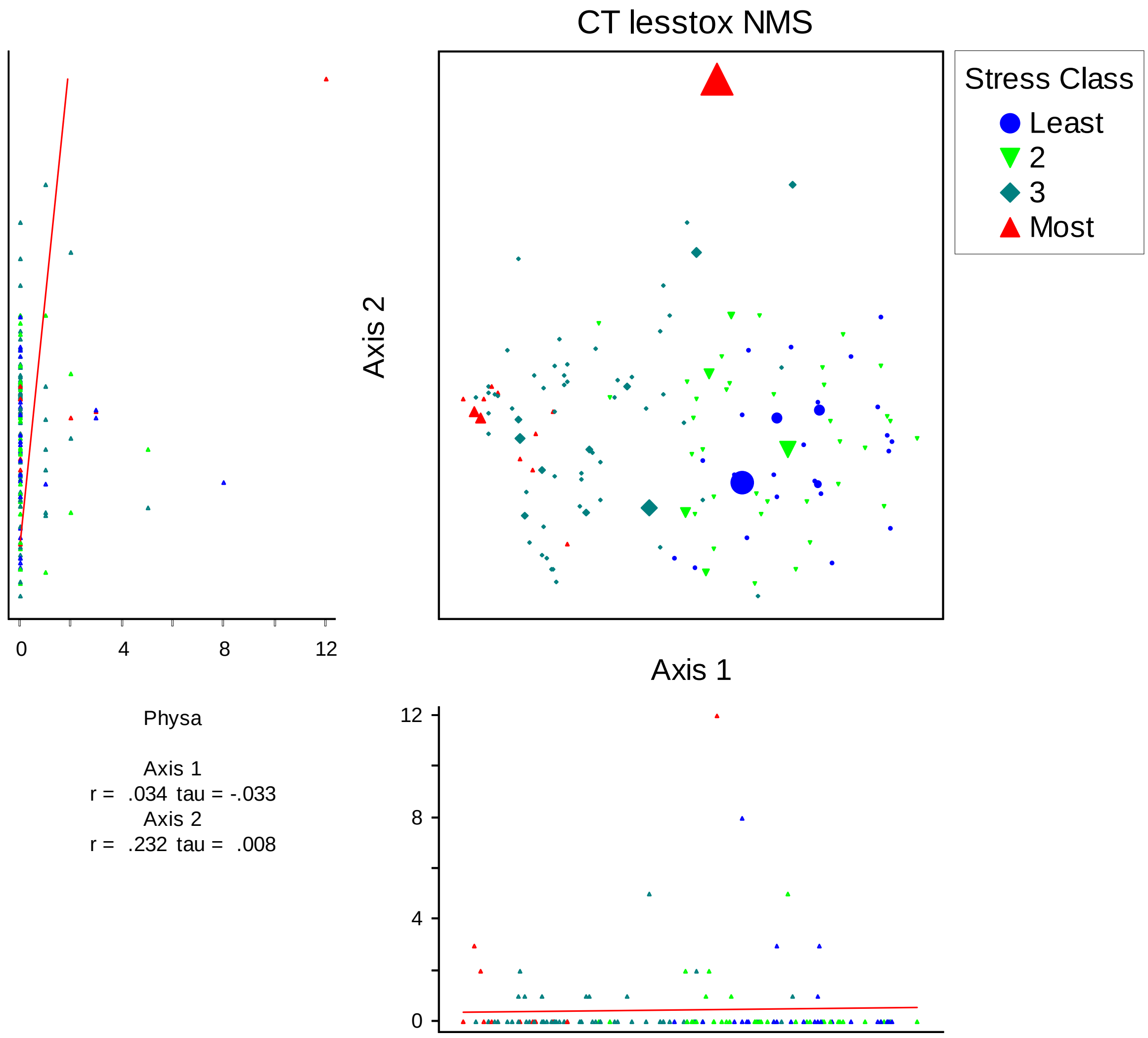

CT lesstox NMS
Stress Class
● Least
▼ 2
◆ 3
▲ Most
Axis 2
Axis 1
0
4
8
12
Physa
Axis 1
r = .034 tau = -.033
Axis 2
r = .232 tau = .008
12
8
4
0

Figure D-14

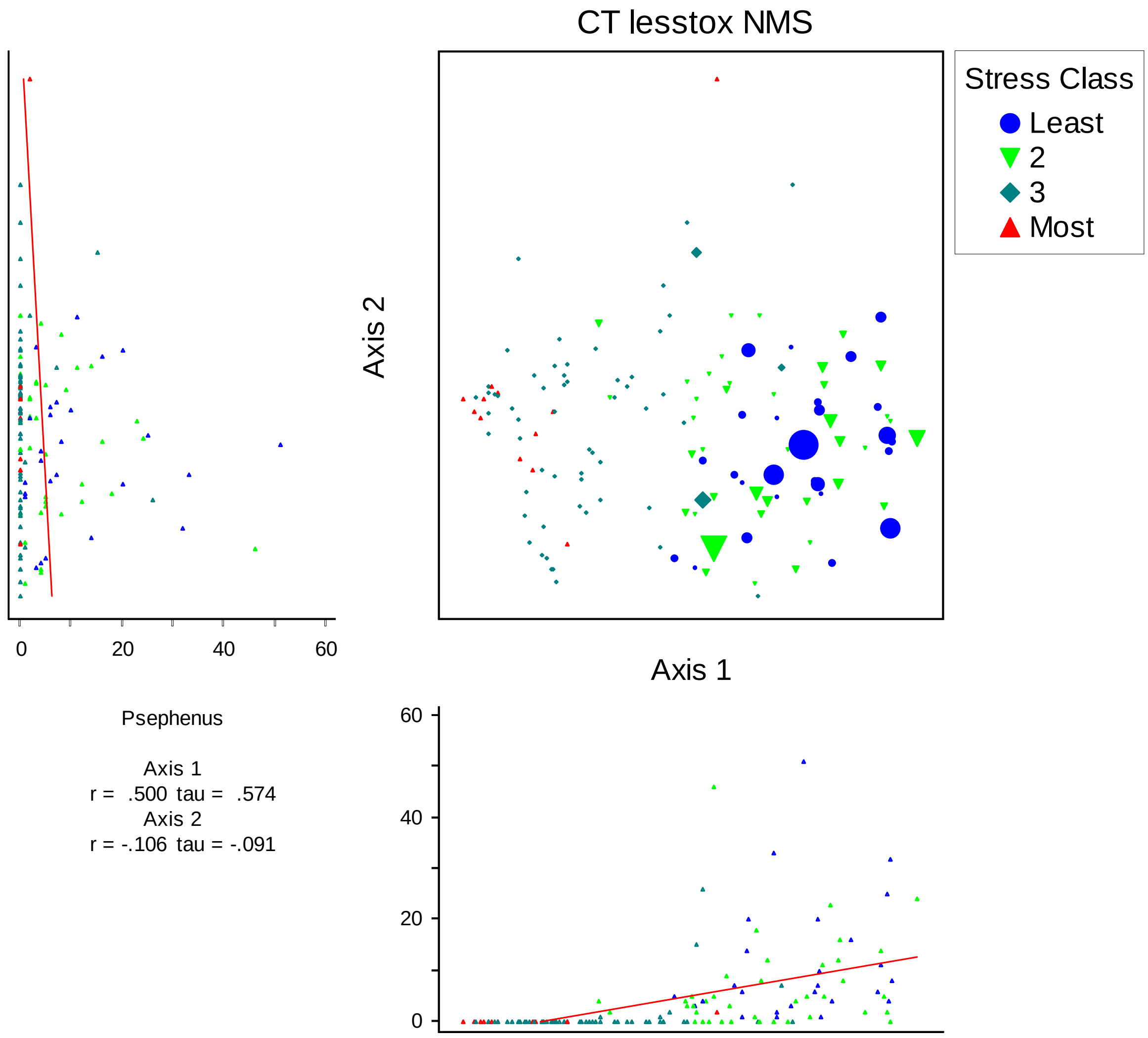

CT lesstox NMS
Stress Class
Least
2
3
Most
Axis 2
Axis 1
0
20
40
60
Psephenus
Axis 1
r = .500 tau = .574
Axis 2
r = -.106 tau = -.091
60
40
20
0

Figure D-15

SampID	97	**Tier**	**Reasoning**	
Site_ID	CT 01-08		enter_text_here	
Attribute	**Taxa**	**Individuals**	**Taxa**	**Individuals**
1	0	0	0	0
2	5	18	21	67
3	16	49		
4	12	121	12	121
5	2	15	2	15
5a	0	0		
x	1	2	1	2
Total	*36*	*205*		

State	CT
Station_ID	CT 01-08
Site_Name	Sages Ravine Brook
Method	1NEWS_HG
Average_Tier	
Model_Tier	
Runnerup	

Attribute	**FinalID**	**Individuals**	**Order**	**Family**	**Notes**
4	Physa	2	Basommatophor	Physidae	
3	Oulimnius	13	Coleoptera	Elmidae	
3	Promoresia	8	Coleoptera	Elmidae	
3	Atherix	1	Diptera	Athericidae	
4	Eukiefferiella	3	Diptera	Chironomidae	
4	Orthocladiinae	1	Diptera	Chironomidae	
3	Parachaetoclad	1	Diptera	Chironomidae	
4	Parametriocner	1	Diptera	Chironomidae	
4	Polypedilum	87	Diptera	Chironomidae	
3	Rheocricotopus	1	Diptera	Chironomidae	
5	Rheotanytarsus	11	Diptera	Chironomidae	
3	Stempellinella	1	Diptera	Chironomidae	
4	Tanytarsus	7	Diptera	Chironomidae	
4	Thienemannimy	1	Diptera	Chironomidae	
3	Hexatoma	2	Diptera	Tipulidae	
4	Baetis	3	Ephemeroptera	Baetidae	
2	Epeorus	2	Ephemeroptera	Heptageniidae	
4	Stenonema	6	Ephemeroptera	Heptageniidae	
3	Leptophlebiidae	1	Ephemeroptera	Leptophlebiidae	
4	Lumbricina	1	Lumbricina		
3	Nigronia	2	Megaloptera	Corydalidae	
3	Gomphidae	1	Odonata	Gomphidae	
3	Chloroperlidae	1	Plecoptera	Chloroperlidae	
2	Leuctra	1	Plecoptera	Leuctridae	
2	Peltoperlidae	1	Plecoptera	Peltoperlidae	
3	Taeniopterygid	1	Plecoptera	Taeniopterygidae	
3	Adicrophleps	2	Trichoptera	Brachycentridae	
4	Ceratopsyche	1	Trichoptera	Hydropsychidae	
4	Diplectrona	8	Trichoptera	Hydropsychidae	
5	Hydropsyche	4	Trichoptera	Hydropsychidae	
2	Lepidostomatid	13	Trichoptera	Lepidostomatidae	
x	Limnephilidae	1	Trichoptera	Limnephilidae	
2	Psilotreta	1	Trichoptera	Odontoceridae	
3	Dolophilodes	3	Trichoptera	Philopotamidae	
x	Philopotamidae	1	Trichoptera	Philopotamidae	

3	Polycentropus	1	Trichoptera	Polycentropodidae
3	Rhyacophila	10	Trichoptera	Rhyacophilidae

					Group Consensus		Fuzzy Model Results			
State	Stream Name	EPA site number	Date	Method	Average Tier	Nominal Tier	Nominal tier	Member-ship	Runner-up tier	Notes
CT	Eight Mile River	18	10/17/2003	CT riffle kick	3.40	3	3	0.60	4	
CT	Sandy Brook	21	11/12/2003	CT riffle kick	2.83	2	2	1.00		
CT	Shepaug River	25	10/20/2003	CT riffle kick	3.93	3	3	0.60	4	
CT	Quaker Brook	234	10/20/2003	CT riffle kick	3.70	3	3	1.00		
CT	Titicus River	235	10/20/2003	CT riffle kick	3.93	3	3	0.86	4	
CT	Green Fall River	245	10/7/2003	CT riffle kick	3.17	3	3	0.53	2	close
CT	Whitford Brook	250	10/9/2003	CT riffle kick	3.40	3	3	0.75	4	
CT	Mashamoquet Brook	224_CT	10/22/2002	CT riffle kick	3.27	3	2	0.50	3	tie
CT	Neck River	259_CT	10/9/2002	CT riffle kick	3.70	3	2	0.60	3	
CT	Saugatuck River	50_CT	10/14/2003	CT riffle kick	4.50	4	5	0.50	4	
CT	Saugatuck River	50_CT	11/5/2002	CT riffle kick	4.27	4	5	0.50	4	close
CT	Sages Ravine Brook	CT HEX 1.08	10/21/2002	CT riffle kick	3.27	3	4	0.55	3	close
CT	Sages Ravine Brook	CT HEX 1.08	10/21/2002	NEWS	3.70	3	3	0.78	4	
CT	Indian Meadow Brook	CT HEX 11.02	10/8/2002	CT riffle kick	2.50	2	2	1.00		
CT	Indian Meadow Brook	CT HEX 11.02	10/22/2003	NEWS	2.63	2	2	1.00		
CT	Indian Meadow Brook	CT HEX 11.02	10/8/2002	NEWS	3.07	3	2	1.00		
CT	Beach Brook	CT HEX 12.01	10/22/2003	Composite NEWS + C	3.37	3	3	0.82	4	
CT	Beach Brook	CT HEX 12.01	11/1/2004	CT riffle kick			3	0.82	4	
CT	Mountain Brook	CT HEX 13.02	10/28/2002	CT riffle kick	2.70	2	2	1.00		
CT	Mountain Brook	CT HEX 13.02	10/28/2002	NEWS	3.17	3	2	0.50	3	tie
CT	Wappoquia Brook	CT HEX 16.01	10/1/2003	Composite NEWS + C	3.53	3	3	0.93	4	
CT	Wappoquia Brook	CT HEX 16.01	10/29/2004	CT riffle kick			2	1.00		
CT	Salmon River	CT HEX 17A	10/18/2002	Composite NEWS + C	2.93	2	2	1.00		
CT	Salmon River	CT HEX 17A	10/8/2003	CT riffle kick	2.93	2	2	1.00		
CT	Housatonic River	CT HEX 18.01	11/17/2003	Composite NEWS + C	3.50	3	3	0.60	4	
CT	Housatonic River	CT HEX 18.01	10/27/2004	CT riffle kick			4	0.61	3	
CT	Lake Waramaug Brook	CT HEX 19.02	10/30/2002	CT riffle kick	3.83	3	3	0.40	4	close
CT	Lake Waramaug Brook	CT HEX 19.02	10/30/2002	NEWS	3.50	3	3	0.74	4	
CT	Blackberry River	CT HEX 2.05	11/3/2003	Composite NEWS + C	3.83	3	3	0.70	4	
CT	Blackberry River	CT HEX 2.05	10/27/2004	CT riffle kick			3	0.74	4	
CT	Natchaug River	CT HEX 20	10/18/2002	CT riffle kick	2.73	2	2	1.00		
CT	Natchaug River	CT HEX 20	10/18/2002	Low gradient	3.97	3	5	1.00		
CT	Bantam River	CT HEX 20.02	11/10/2003	Composite NEWS + C	3.60	3	3	0.95	4	
CT	Bantam River	CT HEX 20.02	10/27/2004	CT riffle kick			3	1.00		
CT	Farmington River	CT HEX 21.02	10/21/2002	CT riffle kick	3.70	3	3	0.52	4	close
CT	Farmington River	CT HEX 21.02	10/21/2002	NEWS	3.50	3	3	0.74	4	
CT	Hockanum River	CT HEX 22.03	11/12/2003	Composite NEWS + C	4.73	4	5	1.00		
CT	Hockanum River	CT HEX 22.03	10/29/2004	CT riffle kick			5	1.00		
CT	Sawmill Brook	CT HEX 24.02	10/8/2003	Composite NEWS + C	2.60	2	2	1.00		
CT	Sawmill Brook	CT HEX 24.02	10/29/2004	CT riffle kick			2	1.00		
CT	Ekonk River	CT HEX 25.03	10/17/2002	CT riffle kick	3.60	3	3	0.75	4	
CT	Ekonk River	CT HEX 25.03	10/6/2003	NEWS	3.83	3	3	0.82	4	
CT	Ekonk River	CT HEX 25.03	10/17/2002	NEWS	3.93	3	3	0.73	2	
CT	Moosup River	CT HEX 26.04	8/21/2003	Composite NEWS + C	3.50	3	3	0.78	2	
CT	Moosup River	CT HEX 26.04	10/27/2004	CT riffle kick			3	0.77	2	
CT	Bull Mountain Brook	CT HEX 27.02	10/29/2002	CT riffle kick	4.07	4	4	0.59	3	close
CT	Bull Mountain Brook	CT HEX 27.02	10/29/2002	NEWS	4.60	4	3	0.53	4	close
CT	Wood Creek	CT HEX 28.01	11/10/2003	Composite NEWS + C	3.37	3	2	0.50	3	tie
CT	Wood Creek	CT HEX 28.01	10/27/2004	CT riffle kick			2	1.00		
CT	Naugatuck River	CT HEX 29.03	10/1/2002	CT riffle kick	5.83	5	5	1.00		
CT	Naugatuck River	CT HEX 29.03	10/1/2002	NEWS	6.03	6	5	1.00		
CT	Sandy Brook	CT HEX 3.01	10/8/2002	CT riffle kick	2.30	2	2	1.00		
CT	Sandy Brook	CT HEX 3.01	11/12/2003	NEWS	2.97	2	2	1.00		
CT	Sandy Brook	CT HEX 3.01	10/8/2002	NEWS	2.83	2	2	1.00		
CT	Flat Brook	CT HEX 31.02	10/7/2002	CT riffle kick	3.50	3	2	0.94	3	
CT	Flat Brook	CT HEX 31.02	10/7/2002	NEWS	3.50	3	2	0.51	3	close
CT	Cabin Brook	CT HEX 32.01	10/8/2003	Composite NEWS + C	4.73	4	5	0.75	4	
CT	Cabin Brook	CT HEX 32.01	10/27/2004	CT riffle kick			4	0.65	3	
CT	Bentley Brook	CT HEX 33.04	8/14/2002	CT riffle kick	4.17	4	3	0.92	4	
CT	Bentley Brook	CT HEX 33.04	8/14/2002	NEWS	4.50	4	3	0.74	4	
CT	Crooked Brook	CT HEX 34.02	10/6/2003	Composite NEWS + C	4.73	4	4	0.65	3	
CT	Crooked Brook	CT HEX 34.02	10/27/2004	CT riffle kick			3	0.61	4	
CT	Sawmill Brook	CT HEX 35.05	10/29/2002	CT riffle kick	3.17	3	2	0.50	3	tie
CT	Sawmill Brook	CT HEX 35.05	10/29/2002	NEWS	2.73	2	2	1.00		
CT	Pomperaug River	CT HEX 36.02	11/10/2003	Composite NEWS + C	3.40	3	3	0.77	4	
CT	Pomperaug River	CT HEX 36.02	10/27/2004	CT riffle kick			3	0.50	4	tie
CT	Naugatuck River	CT HEX 37.01	10/2/2002	CT riffle kick	5.93	5	6	0.75	5	

					Group Consensus		Fuzzy Model Results			
State	Stream Name	EPA site number	Date	Method	Average Tier	Nominal Tier	Nominal tier	Member-ship	Runner-up tier	Notes
CT	Naugatuck River	CT HEX 37.01	10/2/2002	NEWS	5.83	5	5	1.00		
CT	Mill River	CT HEX 38.01	11/5/2003	Composite NEWS + C	4.17	4	4	0.47	3	close
CT	Mill River	CT HEX 38.01	10/28/2004	CT riffle kick			4	0.50	5	tie
CT	Beaver Meadow Brook	CT HEX 39.01	10/9/2002	CT riffle kick	3.40	3	3	0.92	4	
CT	Beaver Meadow Brook	CT HEX 39.01	10/9/2002	NEWS	4.17	4	3	0.70	2	
CT	East Branch Salmon Broo	CT HEX 4.01	10/22/2003	Composite NEWS + C	2.70	2	2	1.00		
CT	East Branch Salmon Broo	CT HEX 4.01	11/1/2004	CT riffle kick			2	1.00		
CT	Clark Creek	CT HEX 40.01	10/9/2003	Composite NEWS + C	3.93	3	3	0.90	4	
CT	Clark Creek	CT HEX 40.01	10/28/2004	CT riffle kick			3	0.67	4	
CT	Latimer Brook	CT HEX 41.05	10/5/2002	CT riffle kick	3.93	3	3	0.68	4	
CT	Latimer Brook	CT HEX 41.05	10/5/2002	NEWS	4.27	4	3	0.60	4	
CT	Seth Williams Brook	CT HEX 42.03	10/7/2003	Composite NEWS + C	4.93	4	4	0.61	5	
CT	Seth Williams Brook	CT HEX 42.03	10/28/2004	CT riffle kick			3	0.92	4	
CT	Shunock River	CT HEX 43.01	10/24/2002	CT riffle kick	3.70	3	3	0.90	4	
CT	Shunock River	CT HEX 43.01	10/24/2002	Low gradient	3.60	3	4	0.75	5	
CT	Shunock River	CT HEX 43.01	10/24/2002	NEWS	4.50	4	3	0.65	4	
CT	Limekiln Brook	CT HEX 45.04	10/5/2002	CT riffle kick	5.40	5	5	1.00		
CT	Limekiln Brook	CT HEX 45.04	10/5/2002	NEWS	4.97	4	5	1.00		
CT	Five Mile Brook	CT HEX 46.03	10/17/2002	Composite NEWS + C	3.97	3	2	1.00		
CT	Five Mile Brook	CT HEX 46.03	10/27/2004	CT riffle kick			3	1.00		
CT	Farm River	CT HEX 48.01	11/4/2003	Composite NEWS + C	4.30	4	4	0.60	3	
CT	Farm River	CT HEX 48.01	10/28/2004	CT riffle kick			5	1.00		
CT	Pond Meadow Brook	CT HEX 49.05	10/9/2002	CT riffle kick	3.87	3	3	1.00		
CT	Pond Meadow Brook	CT HEX 49.05	10/9/2002	NEWS	4.40	4	3	0.73	4	
CT	Eight Mile River	CT HEX 50.02	10/9/2003	Composite NEWS + C	3.50	3	3	0.70	4	
CT	Eight Mile River	CT HEX 50.02	10/28/2004	CT riffle kick			3	0.87	4	
CT	Flat Brook	CT HEX 51.02	10/15/2002	CT riffle kick	4.83	4	4	0.58	3	close
CT	Flat Brook	CT HEX 51.02	10/15/2002	NEWS	3.60	3	3	0.75	4	
CT	Shunock River	CT HEX 52.07	10/7/2003	CT riffle kick	3.60	3	3	0.82	4	
CT	Shunock River	CT HEX 52.07	10/7/2003	NEWS	3.37	3	3	0.62	4	
CT	Norwalk River	CT HEX 53.04	10/3/2002	CT riffle kick	5.27	5	5	1.00		
CT	Norwalk River	CT HEX 53.04	10/3/2002	NEWS	4.63	4	5	0.80	4	
CT	West Branch Saugatuck R	CT HEX 54.02	10/14/2003	Composite NEWS + C	4.40	4	4	0.66	3	
CT	West Branch Saugatuck R	CT HEX 54.02	10/26/2004	CT riffle kick			5	0.75	4	
CT	Pumpkin Ground Brook	CT HEX 55.01	10/31/2002	CT riffle kick	5.50	5	5	1.00		
CT	Pumpkin Ground Brook	CT HEX 55.01	10/31/2002	NEWS	5.17	5	5	1.00		
CT	Neck River	CT HEX 57.04	10/9/2002	Low gradient	4.40	4	5	1.00		
CT	Neck River	CT HEX 57.04	10/9/2002	NEWS	4.93	4	4	0.80	3	
CT	East Branch Byram River	CT HEX 59.01	10/3/2002	CT riffle kick	4.17	4	4	0.75	5	
CT	East Branch Byram River	CT HEX 59.01	10/3/2002	NEWS	4.50	4	4	0.84	3	
CT	Still Brook	CT HEX 6.06	10/21/2003	Composite NEWS + C	4.93	4	5	0.80	4	
CT	Still Brook	CT HEX 6.06	10/29/2004	CT riffle kick			5	0.75	4	
CT	Five Mile River	CT HEX 60.01	10/14/2003	Composite NEWS + C	5.60	5	5	1.00		
CT	Five Mile River	CT HEX 60.01	10/26/2004	CT riffle kick			5	1.00		
CT	Stickney Hill Brook	CT HEX 7.06	10/10/2002	CT riffle kick	3.17	3	2	1.00		
CT	Stickney Hill Brook	CT HEX 7.06	10/10/2002	NEWS	3.70	3	2	0.50	3	tie
CT	North Running Brook	CT HEX 8.02	10/1/2003	Composite NEWS + C	4.27	4	3	0.60	4	
CT	North Running Brook	CT HEX 8.02	10/29/2004	CT riffle kick			3	0.91	4	
CT	Rocky Brook	CT HEX 9.02	10/17/2002	CT riffle kick	3.83	3	5	0.75	4	
CT	Rocky Brook	CT HEX 9.02	10/17/2002	NEWS	3.50	3	2	1.00		
CT	Mill Brook	EPA HEX 35.04	10/23/2001	NEWS	3.62	3	3	1.00		
CT	West Branch Shepaug	EPA HEX 35.05	8/8/2002	NEWS	3.72	3	2	0.50	3	tie
CT	Willimantic River	EPA HEX 36.03	10/9/2001	NEWS	4.62	4	5	1.00		
CT	Saugatuck River	EPA HEX 39.01	10/24/2001	NEWS	3.85	3	5	0.75	4	
CT	Comstock Brook	EPA HEX 39.10	8/14/2002	NEWS	4.17	4	3	0.62	4	
CT	Steele Brook	EPA HEX 40.03	10/10/2001	NEWS	3.33	3	3	0.74	4	
CT	Pease Brook	EPA HEX 41.04	10/22/2001	NEWS			3	0.60	4	
CT	Eight Mile River	EPA HEX 41.05	8/13/2002	NEWS	3.85	3	3	0.51	4	close
MA	Sawmill River	EPA HEX 32.06	8/30/2001	NEWS	3.88	3	5	1.00		
MA	Sawmill River	EPA HEX 32.06	7/18/2002	NEWS			5	1.00		
ME	Aegan Brook	EPA HEX 1.01	7/18/2001	NEWS	3.28	3	2	0.50	3	tie
ME	Petite Brook	EPA HEX 1.08	7/24/2002	NEWS	3.18	3	3	0.72	2	
ME	Horseshoe Stream	EPA HEX 11.05	7/25/2001	NEWS	4.35	4	4	0.63	3	
ME	Carrying Place Stream	EPA HEX 12.02	7/26/2001	NEWS	3.58	3	2	1.00		
ME	Carrying Place Stream	EPA HEX 12.02	7/27/2002	NEWS			3	0.52	4	close

					Group Consensus		Fuzzy Model Results			
State	Stream Name	EPA site number	Date	Method	Average Tier	Nominal Tier	Nominal tier	Member-ship	Runner-up tier	Notes
ME	Spruce Brook	EPA HEX 14.10	7/22/2001	NEWS	3.78	3	3	0.96	4	
ME	Hardy Stream	EPA HEX 19.01	9/21/2001	NEWS	2.67	2	2	1.00		
ME	Halfway Brook	EPA HEX 2.03	7/17/2001	NEWS	3.05	3	3	0.60	4	
ME	Halfway Brook	EPA HEX 2.03	7/23/2002	NEWS			2	0.63	3	
ME	Higgins Brook	EPA HEX 20.02	7/23/2001	NEWS	4.13	4	4	0.63	3	
ME	Jellison Meadow Brook	EPA HEX 21.01	9/17/2002	NEWS	3.45	3	3	0.76	4	
ME	Togus Stream	EPA HEX 26.11	9/24/2001	NEWS	3.45	3	3	0.62	4	
ME	Medomak River	EPA HEX 27.01	8/4/2001	NEWS	4.07	4	5	0.52	4	close
ME	Medomak River	EPA HEX 27.01	9/18/2002	NEWS			4	0.70	5	
ME	Chemquasabamticook Br	EPA HEX 3.04	7/24/2002	NEWS	3.28	3	2	1.00		
ME	Snare Brook	EPA HEX 4.01	7/19/2001	NEWS	3.82	3	3	0.80	2	
ME	Youngs Brook	EPA HEX 5.02	7/23/2002	NEWS	3.90	3	3	0.81	4	
ME	North Branch Meduxneke	EPA HEX 5.07	7/28/2003	NEWS	3.05	3	2	1.00		
ME	Logan Brook	EPA HEX 7.03	7/26/2001	NEWS	3.50	3	2	0.60	3	close
ME	Logan Brook	EPA HEX 7.03	7/26/2002	NEWS			2	0.82	3	
ME	Musquash Brook	EPA HEX 7.07	7/30/2003	NEWS	3.62	3	2	1.00		
ME	Spruce Brook	ME HEX 14.03	7/29/2003	NEWS			2	1.00		
ME	Kimball Brook	ME HEX 16.04	7/28/2003	NEWS			3	0.51	4	close
ME	Dole Brook	ME HEX 19.01	7/27/2002	NEWS			2	1.00		
ME	Katahdin Stream	ME HEX 22.05	7/29/2003	NEWS			2	0.55	3	close
ME	West Branch Gulf Stream	ME HEX 24.01	7/31/2003	NEWS			3	0.77	4	
ME	Henderson Brook	ME HEX 26.03	7/30/2003	NEWS			2	1.00		
ME	West Branch Pleasant Riv	ME HEX 27.08	7/31/2003	NEWS			3	0.50	4	tie
ME	Cupsuptic River	ME HEX 30.01	8/19/2003	Artificial Subs	2.27	2	2	0.60	3	close
ME	Cupsuptic River	ME HEX 30.01	7/16/2003	NEWS	3.50	3	2	0.89	3	
ME	Sag Brook	ME HEX 31.01	7/16/2003	NEWS			2	1.00		
ME	Wilson Stream	ME HEX 33.06	7/17/2003	NEWS			4	0.56	3	close
ME	Main Stream	ME HEX 35.03	7/24/2003	NEWS			5	1.00		
ME	Swift River	ME HEX 38.03	7/16/2003	NEWS			2	0.60	3	close
ME	Crooked Stream	ME HEX 41.05	7/15/2003	NEWS			4	0.60	3	
ME	Ellis River	ME HEX 46.02	7/16/2003	NEWS			5	1.00		
ME	Martin Stream	ME HEX 47.09	7/23/2003	NEWS			5	1.00		
ME	Halfmoon Stream	ME HEX 48.05	8/12/2003	Artificial Subs	3.40	3	3	0.58	4	close
ME	Halfmoon Stream	ME HEX 48.05	7/15/2003	NEWS	3.77	3	4	0.65	3	
ME	Pleasant Stream	ME HEX 51.08	7/22/2003	NEWS			3	0.67	4	
ME	Mill Brook	ME HEX 55.02	8/15/2003	Artificial Subs	3.60	3	4	0.69	5	
ME	Mill Brook	ME HEX 55.02	7/17/2003	NEWS	3.00	3	3	0.50	4	tie
ME	Little Ossipee Stream	ME HEX 56.04	7/18/2003	NEWS			3	0.50	4	tie
NH	Cedar Stream	EPA HEX 11.06	7/28/2002	NEWS	3.05	3	3	0.82	4	
NH	Indian River	EPA HEX 24.01	8/12/2001	NEWS	4.67	4	3	0.53	4	close
NH	Beech River	EPA HEX 25.02	8/9/2001	NEWS	3.50	3	3	0.50	4	close
NH	Warren Brook	EPA HEX 29.07	8/8/2001	NEWS	3.38	3	3	0.75	4	
NH	Connecticut River	NH HEX 1.03	7/29/2002	NEWS	2.57	2	2	1.00		
NH	Bog Brook	NH HEX 10.02	9/17/2003	Artificial Subs	2.50	2	2	0.62	3	
NH	Bog Brook	NH HEX 10.02	7/24/2003	NEWS	3.00	3	3	0.72	4	
NH	Bumpus Brook	NH HEX 11.01	9/23/2002	Artificial Subs	4.75	4	5	0.50	6	tie
NH	Bumpus Brook	NH HEX 11.01	7/22/2002	NEWS	2.70	2	2	1.00		
NH	Peabody Brook	NH HEX 12.02	7/28/2003	NEWS			3	0.60	4	
NH	Ammonoosuc River	NH HEX 14.02	8/6/2003	NEWS			3	0.56	4	close
NH	Appleby Brook	NH HEX 15.01	8/2/2002	NEWS			2	1.00		
NH	East Branch Saco River	NH HEX 16.02	9/30/2003	Artificial Subs	2.80	2	2	0.59	3	close
NH	East Branch Saco River	NH HEX 16.02	7/29/2003	NEWS	3.50	3	2	0.96	3	
NH	Clark Brook	NH HEX 17.03	9/25/2002	Artificial Subs	3.15	3	3	0.75	4	
NH	Clark Brook	NH HEX 17.03	7/26/2002	NEWS	4.10	4	2	0.50	3	tie
NH	Eastman Brook	NH HEX 18.01	8/22/2003	Artificial Subs	3.50	3	2	0.95	3	
NH	Eastman Brook	NH HEX 18.01	6/24/2003	NEWS	2.50	2	3	0.80	2	
NH	Indian Stream	NH HEX 2.05	9/17/2003	Artificial Subs	4.20	4	3	0.50	4	tie
NH	Indian Stream	NH HEX 2.05	7/23/2003	NEWS	4.80	4	3	0.50	4	tie
NH	Langdon Brook	NH HEX 20.01	7/29/2003	NEWS			2	1.00		
NH	Grant Brook	NH HEX 21.05	9/25/2002	Artificial Subs	2.75	2	2	1.00		
NH	Grant Brook	NH HEX 21.05	7/25/2002	NEWS	4.00	4	3	0.78	4	
NH	Johnson Brook	NH HEX 23.01	9/18/2002	Artificial Subs	2.35	2	3	1.00		
NH	Johnson Brook	NH HEX 23.01	7/30/2002	NEWS	3.65	3	2	1.00		
NH	Paugus Brook	NH HEX 24.02	7/10/2003	NEWS			2	0.50	3	tie
NH	Hewes Brook	NH HEX 26.05	9/2/2003	Artificial Subs	3.80	3	3	0.75	4	
NH	Hewes Brook	NH HEX 26.05	6/29/2003	NEWS	2.70	2	2	1.00		

					Group Consensus		Fuzzy Model Results			
State	Stream Name	EPA site number	Date	Method	Average Tier	Nominal Tier	Nominal tier	Member-ship	Runner-up tier	Notes
NH	Ames Brook	NH HEX 28.03	9/5/2003	Artificial Subs	3.50	3	2	0.50	3	tie
NH	Ames Brook	NH HEX 28.03	7/7/2003	NEWS	3.80	3	3	0.57	4	close
NH	Dan Hole River	NH HEX 29.03	7/12/2002	NEWS			3	0.58	4	close
NH	Connecticut River	NH HEX 3.05 (	7/29/2002	NEWS			3	0.56	4	close
NH	Branch River	NH HEX 36.01	7/17/2003	NEWS			3	0.50	4	tie
NH	Cold River	NH HEX 37.02	7/17/2002	NEWS			4	0.60	3	
NH	North Branch River	NH HEX 4.04	7/30/2002	NEWS	3.12	3	3	0.78	2	
NH	Webster Stream	NH HEX 40.02	8/5/2003	NEWS			5	0.75	4	
NH	Cold River	NH HEX 43.03	7/17/2002	NEWS			2	1.00		
NH	Piscataquog River	NH HEX 45.04	9/16/2002	Artificial Subs	3.90	3	5	0.75	4	
NH	Piscataquog River	NH HEX 45.04	7/10/2002	NEWS	3.85	3	4	0.56	5	close
NH	North River	NH HEX 47.02	7/11/2002	NEWS			4	0.50	5	tie
NH	Simms Stream	NH HEX 5.04	7/30/2002	NEWS	3.35	3	3	0.64	2	
NH	Otter Brook	NH HEX 51.04	9/30/2002	Artificial Subs	3.00	3	4	0.54	3	close
NH	Otter Brook	NH HEX 51.04	8/6/2002	NEWS	4.00	4	3	0.68	4	
NH	Contoocook River	NH HEX 52.01	11/25/2003	Artificial Subs	3.65	3	4	0.43	3	close
NH	Contoocook River	NH HEX 52.01	9/10/2003	NEWS	3.75	3	3	0.57	4	close
NH	Purgatory Brook	NH HEX 53.01	9/16/2002	Artificial Subs	3.65	3	5	1.00		
NH	Purgatory Brook	NH HEX 53.01	7/19/2002	NEWS	3.50	3	3	0.76	4	
NH	Mill Brook	NH HEX 58.03	9/4/2003	Artificial Subs	3.20	3	4	0.57	3	close
NH	Mill Brook	NH HEX 58.03	6/25/2003	NEWS	4.80	4	5	0.73	4	
NH	Souhegan River	NH HEX 59.03	9/30/2002	Artificial Subs	4.50	4	5	1.00		
NH	Souhegan River	NH HEX 59.03	8/8/2002	NEWS	4.00	4	5	1.00		
NH	Unnamed Brook	NH HEX 6.01 (	7/24/2003	NEWS			3	0.64	4	
NH	Roaring Brook	NH HEX 8.02	7/30/2002	NEWS	3.93	3	3	0.60	4	
NH	Newell Brook	NH HEX 9.05	9/23/2002	Artificial Subs	2.50	2	3	0.60	2	close
NH	Newell Brook	NH HEX 9.05	7/24/2002	NEWS	2.75	2	2	0.50	3	tie
RI	Pawcatuck River	RSD1	10/5/2000	NEWS			5	0.75	4	
RI	Pawcatuck River	RSD2	10/5/2000	NEWS			5	1.00		
RI	Borden Brook	RSD28	9/20/2000	NEWS			5	0.75	4	
RI	Pawcatuck River	RSD3	10/5/2000	NEWS			5	1.00		
RI	Latham Brook	RSD52	9/8/2000	NEWS			4	0.61	3	
RI	Hawkins Brook	RSD53	9/19/2000	NEWS			5	1.00		
VT	Willoughby River	EPA HEX 10.01	9/22/2001	NEWS	2.95	2	2	1.00		
VT	Miller Run	EPA HEX 18.01	9/23/2001	NEWS	3.50	3	3	0.62	4	
VT	3rd Branch White River	EPA HEX 23.01	8/15/2001	NEWS	4.57	4	4	0.73	3	
VT	Huntington River	EPA HEX 23.02	8/1/2002	NEWS	2.30	2	2	1.00		
VT	Mettawee River	EPA HEX 28.05	8/16/2001	NEWS	3.05	3	3	0.60	4	
VT	Black River	EPA HEX 29.03	8/1/2002	NEWS	3.45	3	4	0.54	3	close
VT	Trout Brook	EPA HEX 9.01	7/31/2002	NEWS	4.22	4	5	1.00		
VT	Wells Brook	VT HEX 1.01	7/2/2002	NEWS			3	0.77	2	
VT	Hancock Brook	VT HEX 1.01 (	7/29/2003	NEWS	4.15	4	4	0.51	3	close
VT	Hancock Brook	VT HEX 1.01 (	7/29/2003	VT riffle kick	3.50	3	3	0.75	4	
VT	North Branch Hoosic Rive	VT HEX 1.02	7/10/2003	NEWS			4	0.54	3	close
VT	Mill Brook (Rupert VT)	VT HEX 10.01 (	7/9/2003	NEWS	4.85	4	3	0.60	4	
VT	Mill Brook (Rupert VT)	VT HEX 10.01 (	7/9/2003	VT riffle kick	3.65	3	3	0.75	4	
VT	Browns River	VT HEX 10.02	7/16/2002	NEWS			5	0.75	4	
VT	Ryder Brook Trib #5	VT HEX 11.01	6/25/2002	NEWS			5	1.00		
VT	Mount Tabor Brook	VT HEX 11.01	7/1/2003	NEWS	3.65	3	2	1.00		
VT	Mount Tabor Brook	VT HEX 11.01	7/11/2003	VT riffle kick	2.85	2	2	1.00		
VT	Lamoille River	VT HEX 12.01	7/17/2002	NEWS			3	0.75	4	
VT	Andover Branch	VT HEX 12.01	9/2/2003	NEWS	2.85	2	3	1.00		
VT	Lamoille River	VT HEX 12.01	12/13/2002	VT riffle kick			3	0.64	2	
VT	Andover Branch	VT HEX 12.01	9/2/2003	VT riffle kick	2.65	2	2	1.00		
VT	Blood Brook	VT HEX 13.03	7/10/2003	NEWS	3.35	3	2	1.00		
VT	Blood Brook	VT HEX 13.03	7/3/2003	VT riffle kick	3.00	3	2	1.00		
VT	Mill Brook (Fairfax VT)	VT HEX 13.03 (	7/3/2002	NEWS	4.15	4	4	0.70	5	
VT	Mill Brook (Fairfax VT)	VT HEX 13.03 (RD) (2004)		VT riffle kick	3.65	3	3	0.64	4	
VT	Hubbard Brook	VT HEX 14.02	7/8/2003	NEWS	3.00	3	2	1.00		
VT	Hubbard Brook	VT HEX 14.02	7/8/2003	VT riffle kick	3.35	3	2	1.00		
VT	Gihon River	VT HEX 14.02 (	7/3/2002	NEWS	3.35	3	2	1.00		
VT	Gihon River	VT HEX 14.02 (RD)		VT riffle kick	3.35	3	3	0.79	4	
VT	Johns River	VT HEX 14.03 (	8/19/2003	NEWS			5	1.00		
VT	Mosher Meadow Brook	VT HEX 15.01 (	8/21/2003	NEWS	3.65	3	2	0.99	3	
VT	Mosher Meadow Brook	VT HEX 15.01 (	8/21/2003	VT riffle kick	2.85	2	2	1.00		

					Group Consensus		Fuzzy Model Results			
State	Stream Name	EPA site number	Date	Method	Average Tier	Nominal Tier	Nominal tier	Member-ship	Runner-up tier	Notes
VT	Morril Brook	VT HEX 15.01 (	8/21/2003	NEWS	3.65	3	3	0.91	4	
VT	Morril Brook	VT HEX 15.01 (	8/21/2003	VT riffle kick	2.65	2	2	1.00		
VT	Kilburn Brook	VT HEX 15.02	8/7/2003	NEWS			3	0.66	4	
VT	Morrison Brook	VT HEX 15.03 (	7/23/2002	NEWS			2	0.62	3	
VT	East Branch North River	VT HEX 2.01	7/14/2003	NEWS	3.65	3	2	1.00		
VT	East Branch North River	VT HEX 2.01	7/14/2003	VT riffle kick	3.00	3	2	1.00		
VT	Seaver Brook	VT HEX 2.03	7/2/2002	NEWS	4.20	4	3	0.74	4	
VT	Seaver Brook	VT HEX 2.03		VT riffle kick	3.85	3	3	0.75	4	
VT	Green River	VT HEX 3.01	7/14/2003	NEWS			2	0.79	3	
VT	Poultney River	VT HEX 3.02	6/28/2002	NEWS			4	0.64	5	
VT	Sunny Brook	VT HEX 3.03 (2	6/25/2003	NEWS			3	0.53	4	close
VT	Mill River (Georgia VT)	VT HEX 4.01 (2	8/6/2002	NEWS	5.00	5	5	0.75	4	
VT	Mill River (Georgia VT)	VT HEX 4.01 (2004)		VT riffle kick	3.50	3	3	1.00		
VT	Kent Pond Outlet	VT HEX 4.01 (R	7/30/2002	NEWS	4.80	4	5	1.00		
VT	Kent Pond Outlet	VT HEX 4.01 (RD)		VT riffle kick	5.00	5	5	1.00		
VT	Whitman Brook Trib # 1	VT HEX 5.01 (R	7/25/2002	NEWS	3.65	3	3	0.84	4	
VT	South Stream	VT HEX 5.01 (R	7/15/2003	NEWS			4	0.55	3	close
VT	Whitman Brook Trib # 1	VT HEX 5.01 (RD)		VT riffle kick	3.65	3	2	0.54	3	close
VT	Burgess Branch	VT HEX 6.01	8/7/2002	NEWS			5	1.00		
VT	Trib To Green River	VT HEX 6.02	7/14/2003	NEWS	3.80	3	2	1.00		
VT	Trib To Green River	VT HEX 6.02	7/14/2003	VT riffle kick			2	1.00		
VT	Batten Kill River	VT HEX 7.01	9/3/2003	NEWS	4.35	4	3	0.69	4	
VT	Batten Kill River	VT HEX 7.01	9/3/2003	VT riffle kick	3.50	3	3	0.75	4	
VT	Old City Brook	VT HEX 7.03	8/1/2002	NEWS			3	0.60	4	
VT	Wardsboro Brook Trib #5	VT HEX 8.01	6/30/2003	NEWS	2.50	2	2	1.00		
VT	Wardsboro Brook Trib #5	VT HEX 8.01	8/1/2003	VT riffle kick	2.50	2	2	1.00		
VT	Pherrins River	VT HEX 8.01 (2	8/20/2003	NEWS	3.65	3	3	0.71	4	
VT	Pherrins River	VT HEX 8.01 (2	8/20/2003	VT riffle kick	2.85	2	2	1.00		
VT	Ompompanoosuc River	VT HEX 8.02	7/11/2002	NEWS			5	1.00		
VT	North Branch Ball Mountai	VT HEX 8.02	9/2/2003	NEWS	3.65	3	2	0.54	3	close
VT	North Branch Ball Mountai	VT HEX 8.02	9/2/2003	VT riffle kick	2.85	2	2	1.00		
VT	Miles Stream	VT HEX 8.02 (2	8/6/2003	NEWS	3.65	3	3	0.52	2	close
VT	Miles Stream	VT HEX 8.02 (2	8/6/2003	VT riffle kick	2.85	2	2	1.00		
VT	Goshen Brook Trib #2	VT HEX 8.02 (2	7/9/2003	NEWS	2.80	2	2	1.00		
VT	Goshen Brook Trib #2	VT HEX 8.02 (2	7/9/2003	VT riffle kick	2.35	2	2	1.00		
VT	Joe's Brook	VT HEX 8.03 (2	8/19/2003	NEWS			2	0.50	3	tie
VT	Meadow Brook	VT HEX 9.01	7/26/2002	NEWS			3	0.40	4	close
VT	Williams River Trib #11	VT HEX 9.01	7/1/2003	NEWS			2	1.00		
VT	Rock River	VT HEX 9.01 (2	8/5/2002	NEWS			5	1.00		
VT	Second Branch White Rive	VT HEX 9.02 (2	7/2/2002	NEWS			4	0.66	5	

Appendix G. Biocondition Gradient values, based on the fish assemblage, for the 56 Regional NEWS sites. BCG values are provided by seven biologists; three from Connecticut, and one each from the USEPA, Maine, New Hampshire and Vermont..

Appendix Table G-1

EPA Regional Sites

Hex	Location	Connecticut			US EPA	Maine	New Hampshire	Vermont	**Mean BCG**	min	max	Step Range
EPA HEX 1.01 ME	Aegan Brook	2.2	2.5	2.5	2.5	2.2	2.5	2.2	**2.5**	2.2	2.5	*1*
EPA HEX 1.08 ME	Petite Brook	2.5	3.5	2.5	2.2	2.2	2.2	2.2	**3.5**	2.2	3.5	*4*
EPA HEX 5.02 ME	River DeChute	2.5	3.5	2.2	2.5	2.5	2.5	2.2	**3.5**	2.2	3.5	*4*
EPA HEX 23.02 VT	Huntington River	2.2	2.8	2.2	2.2	3.2	3.2	2.2	**3.2**	2.2	3.2	*3*
EPA HEX 19.03 ME	Bear River	2.5	2.2	2.2	2.8	3.2	3.2	2.2	**3.2**	2.2	3.2	*3*
EPA HEX 7.03 ME	Logan Brook	2.5	2.8	2.5	2.8	2.2	3.8	2.2	**3.8**	2.2	3.8	*5*
EPA HEX 2.03 ME	Halfway Brook	2.5	2.8	2.5	3.2	3.5	2.2	2.2	**3.5**	2.2	3.5	*4*
EPA HEX 23.01 VT	Third Branch White River	2.5	2.5	2.2	3.2	2.2	3.2	2.2	**3.2**	2.2	3.2	*3*
EPA HEX 32.06 MA	Sawmill River	2.8	3.5	2.2	3.2	2.5	3.8	2.2	**3.8**	2.2	3.8	*5*
EPA HEX 12.02 ME	Carrying Place Stream	2.5	2.5	4.8	2.5	2.8	3.2	2.5	**4.8**	2.5	4.8	*7*
EPA HEX 5.02 ME	Young Brook	3.2	3.8	2.2	3.2	3.8	3.8	2.2	**3.8**	2.2	3.8	*5*
EPA HEX 8.06 ME	Greenleaf Brook	3.2	3.5	4.5	2.5	3.2	3.2	2.5	**4.5**	2.5	4.5	*6*
EPA HEX 24.01 NH	Indian River	3.2	3.8	2.2	3.2	3.5	3.5	2.2	**3.8**	2.2	3.8	*5*
EPA HEX 7.07 ME	Musquash Brook	2.5	3.5	2.2	2.8	3.2	4.2	2.2	**4.2**	2.2	4.2	*6*
EPA HEX 11.06 NH	Cedar Stream	3.2	3.5	2.2	3.5	3.5	3.8	2.2	**3.8**	2.2	3.8	*5*
EPA HEX 28.05 VT	Mettawee River	2.8	3.5	2.5	3.2	2.2	4.8	2.2	**4.8**	2.2	4.8	*7*
EPA HEX 29.07 NH	Warren Brook	3.2	3.5	2.2	3.2	2.5	4.5	2.2	**4.5**	2.2	4.5	*7*
EPA HEX 4.01 ME	Snare Brook	3.2	3.5	4.5	2.8	2.8	3.8	2.8	**4.5**	2.8	4.5	*5*
EPA HEX 41.04 CT	Pease Brook	2.8	3.8	3.2	3.5	3.5	4.5	2.8	**4.5**	2.8	4.5	*5*
EPA HEX 3.04 ME	Chemquasabamticook Bk.	3.2	3.8	2.2	3.2	3.8	4.8	2.2	**4.8**	2.2	4.8	*8*
EPA HEX 5.07 ME	No.Br. Meduxnekeag River	2.8	3.8	2.2	2.8	4.2	5.2	2.2	**5.2**	2.2	5.2	*9*
EPA HEX 13.02 ME	Schoodic Brook	2.8	3.8	4.2	2.2	3.2	4.8	2.2	**5.2**	2.2	5.2	*9*
EPA HEX 14.10 ME	Spruce Brook	3.2	3.8	4.5	2.5	3.2	4.5	2.5	**4.8**	2.5	4.8	*7*
EPA HEX 25.03 ME	Teddy's Brook	3.2	3.8	4.8	3.5	3.8	4.2	3.2	**4.8**	3.2	4.8	*5*
EPA HEX 35.05 CT	West Branch Shepaug	3.8	3.8	3.2	3.5	3.8	5.5	3.2	**5.5**	3.2	5.5	*7*
EPA HEX 18.01 VT	Miller Run	4.5	4.5	2.2	4.5	3.2	3.8	2.2	**4.5**	2.2	4.5	*7*
EPA HEX 21.10 ME	Naraguagus River	3.2	3.8	4.5	3.8	3.5	4.2	3.2	**4.5**	3.2	4.5	*4*
EPA HEX 39.10 CT	Comstock Brook	3.8	4.2	4.2	3.2	3.2	5.2	3.2	**5.2**	3.2	5.2	*6*
EPA HEX 41.05 CT	Eight Mile River	4.2	4.2	3.2	4.2	4.5	3.5	3.2	**4.5**	3.2	4.5	*4*
EPA HEX 10.01 VT	Willoughby River	3.2	3.8	3.2	3.8	2.5	4.2	2.5	**5.2**	2.5	5.2	*6*

EPA HEX 19.01 ME	Hardy Stream	3.5	3.8	4.5	3.5	3.8	4.8	3.5	**4.8**	3.5	4.8	*4*
EPA HEX 3.05 ME	Depot Stream	3.2	4.5	4.2	3.2	3.2	4.8	3.2	**5.2**	3.2	5.2	*6*
EPA HEX 22.09 ME	New Brook	3.8	3.8	4.5	3.5	3.8	4.5	3.5	**4.5**	3.5	4.5	*3*
EPA HEX 29.03 VT	Black River	4.5	4.5	2.2	5.5	2.8	4.2	2.2	**5.5**	2.2	5.5	*7*
EPA HEX 6.02 ME	Churchill Stream	3.5	3.8	4.5	2.8	4.2	5.5	2.8	**5.5**	2.8	5.5	*8*
EPA HEX 9.0 VT	Trout Brook	3.5	3.8	4.2	3.5	3.5	4.8	3.5	**4.8**	3.5	4.8	*4*
EPA HEX 11.05 ME	Horseshoe Stream	3.8	3.8	4.2	3.2	3.5	4.8	3.2	**5.5**	3.2	5.5	*5*
EPA HEX 31.02 ME	Kennebunk River	4.5	4.5	4.8	4.2	4.8	3.2	3.2	**4.8**	3.2	4.8	*5*
EPA HEX 39.01 CT	Saugatuck River	5.5	4.5	4.2	4.8	4.8	2.8	2.8	**5.5**	2.8	5.5	*8*
EPA HEX 36.03 CT	Willamantic River	5.2	4.5	5.2	4.8	4.2	2.8	2.8	**5.2**	2.8	5.2	*7*
EPA HEX 42.01 MA	Great Swamp Brook	4.8	4.5	4.8	3.8	4.8	4.2	3.8	**4.8**	3.8	4.8	*3*
EPA HEX 34.06 NH	Beaver Brook	5.5	4.5	5.2	4.8	4.2	3.5	3.5	**5.5**	3.5	5.5	*6*
EPA HEX 26.11 ME	Togus Stream	4.5	4.5	5.2	4.5	4.2	3.8	3.8	**5.2**	3.8	5.2	*4*
EPA HEX 25.02 NH	Beech River	4.8	4.5	4.2	3.2	4.2	5.5	3.2	**5.8**	3.2	5.8	*7*
EPA HEX 33.03 NH	Dinsmore Stream	4.8	4.5	4.2	4.8	4.5	4.8	4.2	**4.8**	4.2	4.8	*2*
EPA HEX 27.01 ME	Medomak River	4.5	4.5	5.5	5.5	3.5	4.2	3.5	**5.5**	3.5	5.5	*6*
EPA HEX 30.04 NH	Cocheco River	4.5	4.8	5.2	4.8	5.2	4.2	4.2	**5.2**	4.2	5.2	*3*
EPA HEX 17.02 VT	Winooski River (east)	5.5	5.2	3.2	5.5	3.5	4.5	3.2	**5.5**	3.2	5.5	*7*
EPA HEX 9.05 VT	Black Creek	5.5	5.2	3.5	5.2	4.5	5.2	3.5	**5.5**	3.5	5.5	*6*
EPA HEX 38.02 MA	Longwater Brook	3.2	4.8	6.2	5.2	5.5	4.8	3.2	**6.2**	3.2	6.2	*9*
EPA HEX 15.02 ME	Boyden Stream	4.5	4.5	5.5	5.5	4.5	5.5	4.5	**5.5**	4.5	5.5	*3*
EPA HEX 35.04 CT	Mill Brook	4.5	5.5	5.2	5.2	5.2	4.8	4.5	**5.5**	4.5	5.5	*3*
EPA HEX 20.02 ME	Higgins Brook	4.5	5.5	5.2	5.2	5.2	6.5	4.5	**6.5**	4.5	6.5	*6*
EPA HEX 40.03 CT	Steele Brook	5.5	5.2	5.2	5.8	5.2	5.8	5.2	**5.8**	5.2	5.8	*2*
EPA HEX 37.01 MA	Stop River	4.5	5.5	5.8	6.2	5.8	4.8	4.8	**6.5**	4.5	6.5	*6*
EPA HEX 16.07 VT	Winooski River*											
Region Mean , range and mean range in BCG steps		**3.7**	**4.0**	**3.7**	**3.7**	**3.8**	**4.2**	**4.0**	**3.9 (2.2 - 6.5)**			**5.3** **(0-9)**

Appendix Table G-2.

Connecticut

Hex Number	Location	Biologist A	Biologist B	Biologist C	Mean BCG	Min.	Max.	Step Range
CT HEX 12.01	Beach Brook	2.5	2.8	2.8	2.7	*2.5*	*2.8*	2
CT HEX 4.01	East Branch Salmon Bk.	3.2	2.5	2.8	2.8	*2.5*	*3.2*	4
CT HEX 31.02	Flat Brook	3.2	2.8	2.8	2.9	*2.8*	*3.2*	3
CT HEX 9.02	Rocky Brook	3.5	2.5	2.8	2.9	*2.5*	*3.5*	3
CT HEX 1.08	Sages Ravine Brook	3.5	2.5	2.8	2.9	*2.5*	*3.5*	3
CT HEX 28.01	Wood Creek	3.8	2.5	2.8	3.0	*2.5*	*3.8*	1
CT HEX 46.03	Five Mile Brook	3.5	2.8	2.8	3.0	*2.8*	*3.5*	3
CT HEX 35.05	Sawmill Brook	3.5	2.5	3.2	3.1	*2.5*	*3.5*	3
CT HEX 39.01	Beaver Meadow Brook	2.5	3.5	3.2	3.1	*2.5*	*3.5*	3
CT HEX 11.02	Indian Meadow Brook	3.5	3.2	2.5	3.1	*2.5*	*3.5*	4
CT HEX 51.02	Flat Brook	3.8	2.8	2.8	3.1	*2.8*	*3.8*	4
CT HEX 24.02	Sawmill Brook	3.8	2.5	3.2	3.2	*2.5*	*3.8*	4
CT HEX 13.02	Mountain Brook	4.2	2.8	3.2	3.4	*2.8*	*4.2*	2
CT HEX 45.04	Limekiln Brook	4.2	3.2	2.8	3.4	*2.8*	*4.2*	4
CT HEX 8.02	North Running Brook	4.2	2.8	3.5	3.5	*2.8*	*4.2*	1
CT HEX 47.02	Bladdens River	2.8	4.2	3.5	3.5	*2.8*	*4.2*	4
CT HEX 49.05	Pond Meadow Brook	3.8	3.2	3.5	3.5	*3.2*	*3.8*	2
CT HEX 10.02	Hollenbeck River	3.5	4.2	3.2	3.6	*3.2*	*4.2*	3
CT HEX 53.04	Norwalk River	4.2	3.8	3.2	3.7	*3.2*	*4.2*	3
CT HEX 36.02	Pomperaug River	4.2	3.2	3.8	3.7	*3.2*	*4.2*	3
CT HEX 7.06	Stickney Hill Brook	4.2	3.2	3.8	3.7	*3.2*	*4.2*	3
CT HEX 38.01	Mill River	4.2	3.8	3.5	3.8	*3.5*	*4.2*	2
CT HEX 3.01	Sandy Brook	4.2	3.5	3.8	3.8	*3.5*	*4.2*	2
CT HEX 48.01	Farm River	4.5	3.8	3.5	3.9	*3.5*	*4.5*	3
CT HEX 41.05	Latimer Brook	4.5	3.8	3.5	3.9	*3.5*	*4.5*	2
CT HEX 34.02	Crooked Brook	4.2	4.2	3.5	4.0	*3.5*	*4.2*	2
CT HEX 40.01	Clark Creek	3.8	4.5	3.8	4.0	*3.8*	*4.5*	2
CT HEX 50.02	Eight Mile River	4.2	4.5	3.8	4.2	*3.8*	*4.5*	2
CT HEX 17.08	Mashamoquet Brook	4.5	3.5	4.5	4.2	*3.5*	*4.5*	3
CT HEX 42.03	Seth Williams Brook	4.5	3.8	4.2	4.2	*3.8*	*4.5*	2
CT HEX 16.01	Wappoquia Brook	4.5	3.8	4.2	4.2	*3.8*	*4.5*	2
CT HEX 19.02	Lake Waramaug Brook	4.5	3.8	4.5	4.3	*3.8*	*4.5*	2
CT HEX 43.01	Shunock River	4.5	3.8	4.5	4.3	*3.8*	*4.5*	2
CT HEX 44.01	Titicus River	4.5	3.5	4.8	4.3	*3.5*	*4.8*	4
CT HEX 27.02	Bull Mountain Brook	4.2	4.8	4.2	4.4	*4.2*	*4.8*	2
CT HEX 23.01	Hop River	4.5	4.5	4.5	4.5	*4.5*	*4.5*	0
CT HEX 54.02	West Br. Saugatuck R.	4.5	4.5	4.5	4.5	*4.5*	*4.5*	0
CT HEX 32.01	Cabin Brook	4.2	5.2	4.2	4.5	*4.2*	*5.2*	3
CT HEX 21.02	Farmington River	4.2	5.2	4.2	4.5	*4.2*	*5.2*	3
CT HEX 20.02	Bantam River	4.5	4.8	4.5	4.6	*4.5*	*4.8*	1
CT HEX 22.03	Hockanum River	4.8	4.8	4.2	4.6	*4.2*	*4.8*	2
CT HEX 26.04	Moosup River	4.5	4.5	4.8	4.6	*4.5*	*4.8*	1
CT HEX 5.02	Muddy Brook	4.5	4.8	4.5	4.6	*4.5*	*4.8*	1
CT HEX 56.08	Farm River	4.5	5.2	4.2	4.6	*4.2*	*5.2*	3

CT HEX 57.04	Neck River	4.5	4.8	4.8	4.7	*4.5*	*4.8*	1
CT HEX 37.01	Naugatuck River	4.5	5.2	4.5	4.7	*4.5*	*5.2*	2
CT HEX 25.03	Ekonk River	4.8	4.8	4.8	4.8	*4.8*	*4.8*	0
CT HEX 14.04	Freshwater Brook	4.8	5.2	4.5	4.8	*4.5*	*5.2*	2
CT HEX 18.01	Housatonic River	4.5	5.2	4.8	4.8	*4.5*	*5.2*	2
CT HEX 30.03	Mattabesset River	4.5	5.2	5.2	5.0	*4.5*	*5.2*	2
CT HEX 2.05	Blackberry River	5.2	5.2	4.8	5.1	*4.8*	*5.2*	1
CT HEX 55.01	Pumpkin Ground Bk.	5.2	4.8	5.2	5.1	*4.8*	*5.2*	1
CT HEX 59.01	East Branch Byram R.	5.5	5.5	5.2	5.4	*5.2*	*5.5*	1
CT HEX 15.02	Skungamaug River	5.5	5.5	5.2	5.4	*5.2*	*5.5*	1
CT HEX 33.04	Bentley Brook	5.5	5.5	5.8	5.6	*5.5*	*5.8*	1
CT HEX 6.06	Still Brook	5.8	5.8	5.5	5.7	*5.5*	*5.8*	1
		4.2	**4.0**	**4.0**	**4.1**	**2.5-5.8**		**2.2**

Appendix Table G-3.

Maine

Hex Number	Location	Biologist A	Biologist B	Mean BCG	Step Range
ME HEX 50.02	Tunk Stream	2.2	3.2	2.7	3
ME HEX 51.08	Pleasant Stream	2.2	3.2	2.7	3
ME HEX 14.03	Spruce Brook	2.2	3.5	2.9	4
ME HEX 31.01	Sag Brook	4.2	2.8	3.5	4
ME HEX 19.01	Dole Brook	2.2	3.8	3.0	5
ME HEX 33.06	Wilson Stream	2.2	4.8	3.5	8
ME HEX 22.05	Katahdin Stream	2.5	2.5	2.5	0
ME HEX 27.08	West Branch Pleasant R.	2.5	4.2	3.4	5
ME HEX 24.01	West Branch Gulf Str.	2.8	2.5	2.7	1
ME HEX 26.03	Henderson Brook	2.8	2.5	2.7	1
ME HEX 16.04	Kimball Brook	4.2	4.8	4.5	2
ME HEX 3.03	Little River	4.2	4.8	4.5	2
ME HEX 48.05	Halfmoon Stream	4.2	5.5	4.9	4
ME HEX 49.03	Flood Stream	4.2	5.5	4.9	4
ME HEX 55.02	Mill Brook	4.5	4.2	4.4	1
ME HEX 57.01	Mill Stream	4.5	4.2	4.4	1
ME HEX 60.02	Neddick River	4.8	4.2	4.5	2
ME HEX 56.04	Little Ossipee Stream	4.8	4.5	4.7	1
ME HEX 30.01	Cupsuptic River	4.8	4.5	4.7	1
ME HEX 52.01	Little River	5.2	4.5	4.9	2
ME HEX 29.10	Tolman Brook	5.2	4.2	4.7	3
ME HEX 35.03	Main Stream	5.2	4.2	4.7	3
ME HEX 41.05	Crooked Stream	5.2	4.2	4.7	3
ME HEX 42.03	Birch Stream	5.2	4.2	4.7	3
ME HEX 58.04	Little River	5.2	4.2	4.7	3
ME HEX 47.09	Martin Stream	5.2	5.5	5.4	1
ME HEX 11.04	Pratt Lake Stream	5.2	5.8	5.5	2
ME HEX 12.05	St. Croix Stream	5.2	5.5	5.4	1
Means		**4.0**	**4.2**	**4.1 (2.2-5.8)**	**2.6**

Appendix Table G-4.

New Hampshire

Hex Number	Location	Biologist A	Biologist B	Mean	Step Range
NH HEX 4.04	North Branch River	2.8	2.2	2.5	2
NH HEX 15.01	Appleby Brook	2.8	2.2	2.5	2
NH HEX 9.05	Newell Brook	2.8	2.2	2.5	2
NH HEX 24.02	Paugus Brook	3.2	2.2	2.7	3
NH HEX 12.02	Peabody Brook	3.2	2.2	2.7	3
NH HEX 11.01	Bumpus Brook	3.5	2.2	2.9	4
NH HEX 18.01	Eastman Brook	3.5	2.2	2.9	4
NH HEX 23.01	Johnson Brook	3.5	2.2	2.9	4
NH HEX 20.01	Langdon Brook	3.5	2.2	2.9	4
NH HEX 61.04	Beaver Brook	3.5	3.5	3.5	3
NH HEX 8.02	Roaring Brook	4.2	3.5	3.9	2
NH HEX 5.04	Simms Stream	3.2	3.5	3.4	1
NH HEX 6.01	Unnamed Brook	3.5	3.5	3.5	0
NH HEX 2.05	Indian Stream	3.8	3.5	3.7	1
NH HEX 45.04	Piscataquog River	3.5	3.8	3.7	1
NH HEX 59.03	Souhegan River	3.8	3.5	3.7	1
NH HEX 21.05	Grant Brook	4.2	3.2	3.7	3
NH HEX 57.03	Ash Swamp Brook	3.8	3.8	3.8	0
NH HEX 3.05	Connecticut River	4.2	3.8	4.0	1
NH HEX 28.03	Ames Brook	4.5	3.2	3.9	4
NH HEX 14.02	Ammonoosuc River	4.2	3.5	3.9	2
NH HEX 52.01	Contoocook River	4.2	3.5	3.9	2
NH HEX 16.02	East Branch Saco River	4.2	3.5	3.9	2
NH HEX 19.01	Swift River	4.2	3.5	3.9	2
NH HEX 35.01	Churchill Brook	4.2	3.8	4.0	1
NH HEX 43.03	Cold River	3.8	4.2	4.0	1
NH HEX 53.01	Purgatory Brook	3.8	4.5	4.2	2
NH HEX 22.05	Hubbard Bk	4.5	3.8	4.2	2
NH HEX 1.03	Connecticut River	3.2	4.2	4.2	3
NH HEX 39.01	Dolf Brook	4.2	4.2	4.2	0
NH HEX 46.02	Turkey River	4.2	4.2	4.2	0
NH HEX 51.04	Otter Brook	4.2	4.5	4.4	1
NH HEX 10.02	Bog Brook	4.5	4.5	4.5	1
NH HEX 30.02	Poland Brook	4.2	4.5	4.4	1
NH HEX 56.01	Cornelius Brook	5.8	3.5	4.7	7
NH HEX 26.05	Hewes Brook	4.8	4.5	4.7	1
NH HEX 34.03	Tioga River	4.8	4.5	4.7	1
NH HEX 37.02	Cold River	4.8	4.5	4.7	1
NH HEX 44.02	Dodge Brook	4.8	4.8	4.8	0
NH HEX 31.01	Taylor Brook	4.8	4.8	4.8	0
NH HEX 58.03	Mill Brook	4.2	5.5	4.9	4
NH HEX 41.04	Berry Brook	5.2	4.8	5.0	1
NH HEX 27.04	Mascoma River	4.8	5.2	5.0	1
NH HEX 38.05	Trask Brook	4.8	5.2	5.0	1
NH HEX 36.01	Branch River	4.8	5.5	5.2	2
NH HEX 17.03	Clark Brook	4.5	5.8	5.2	4
NH HEX 29.03	Dan Hole River	5.2	5.2	5.2	0
NH HEX 32.05	Smith Brook	5.2	5.2	5.2	0
NH HEX 48.04	Winnicut River	5.2	5.2	5.2	0
NH HEX 47.02	North River	5.2	5.5	5.4	1

NH HEX 54.01	Riddle Brook	5.2	5.8	5.5	2
NH HEX 40.02	Webster Stream	5.5	5.5	5.5	0
NH HEX 60.03	Rocky Pond Brook	5.5	5.8	5.7	1
NH HEX 55.02	Island Pond Stream	5.5	5.8	5.7	1
		1.7		**4.1 (2.2-5.8)**	**1.7**

Appendix Table G-5.

RHODE ISLAND and MASSACHUSETTS

Hex Number	Location	Biologist A	Biologist B	Biologist C	Mean	Min	Max	Step Range
RSD36	Scituate Reservoir Tribs	2.5	2.2		2.4	2.2	2.5	1
RSD18	Cocumcussoc Brook	2.5	2.2	2.5	2.4	2.2	2.5	1
RSD21	Cole Brook	3.5	2.5	2.5	2.5	2.5	2.5	0
RSD12	Saugatucket River	2.5	2.8	2.8	2.7	2.5	2.8	1
Q001	Queens River	2.5	2.5	3.2	2.8	2.5	3.2	2
RSD39	Furnace Hill Brook	2.8	3.2	3.2	3.0	2.8	3.2	1
RSD17	Queens River	2.8	2.8	3.5	3.1	2.8	3.5	2
RSD7	Beaver River	3.2	3.2	3.2	3.2	3.2	3.2	0
RSD11	Glen Rock Brook	2.8	2.8	3.8	3.2	2.8	3.8	3
RSD37	Hemlock Brook	2.8	3.5	3.5	3.2	2.8	3.5	2
RSD23	Quidneck Brook	3.2	3.2	3.5	3.3	3.2	3.5	1
RSD38	Cork Brook	3.5	3.8	3.2	3.5	3.2	3.8	2
RSD33	Lockwood Brook	2.5	4.5	3.5	3.5	2.5	4.5	6
RSD59	Abbott Run Brook	3.5	3.2	4.5	3.8	3.2	4.5	4
RSD51	Clear River	4.2	3.5	3.8	3.8	3.5	4.2	2
RSD61	Burnt Swamp Brook	3.5	4.2	4.2	3.9	3.5	4.2	2
RSD57	Branch River	4.5	3.5	4.5	4.1	3.5	4.5	3
RSD9	Brushy Brook	6.2	5.5	3.2	4.1	3.2	5	8
RSD10	Wood River	4.8	3.2	4.5	4.1	3.2	4.8	5
RSD53	Hawkins Brook	3.8	4.3	4.5	4.2	3.8	4.5	2
RSD47	Pocasset River	4.5	3.8	4.5	4.2	3.8	4.5	2
RSD58	Crookfall Brook	4.5	3.8	4.8	4.3	3.8	4.8	3
RSD16	Nooseneck River	4.5	3.8	4.8	4.3	3.8	4.8	3
RSD29	Moosup River	4.8	3.8	4.8	4.4	3.8	4.8	3
RSD56	Nipmuc River	4.8	3.8	4.8	4.4	3.8	4.8	3
RSD54	Moshassuck River	4.2	4.2	4.8	4.4	4.2	4.8	2
RSD40	Pocasset River	5.2	3.8	4.8	4.6	3.8	5.2	4
RSD46	Pocasset River	4.5	4.5	4.8	4.6	4.5	4.8	1
RSD48	Ten Mile River	5.2	4.2	4.5	4.7	4.2	5.2	3
RSD8	Saugatucket River	5.2	4.2	4.8	4.7	4.2	5.2	3
RSD31	Pawtuxet River So. Branch	6.3	3.5	4.8	4.9	3.5	6.3	8
RSD52	Latham Brook	5.5	4.5	4.8	5.0	4.5	5.5	3
RSD4	Cedar Swamp Brook	6.5	4.5	5.5	5.0	4.5	5.5	3
RSD32	Pawtuxet River Main Stem	5.5	4.5	5.2	5.0	4.5	5.5	3
RSD45	Peeptoad Brook	6.3	3.8	5.5	5.1	3.8	6.3	7
RSD50	Clear River	5.8	4.8	4.8	5.2	4.8	5.8	3
Means		**4.1**	**3.7**	**4.2**	**3.9**	**(2.2-6.3)**		**2.8**

Appendix Table G-6.

Vermont

Hex Number	Location	Biologist A	Biologist B	Mean	Step Range
VT HEX 13.03	Blood Brook	2.2	2.8	2.5	2
VT HEX 8.02	Goshen Brook Trib #2	2.2	2.8	2.5	2
VT HEX 14.02	Hubbard Brook	2.2	2.8	2.5	2
VT HEX 15.02	Kilburn Brook	2.2	2.8	2.5	2
VT HEX 7.03	Old City Brook	2.2	2.8	2.5	2
VT HEX 9.01	Williams River Trib #11	2.2	2.8	2.5	2
VT HEX 8.01	Wardsboro Bk. Trib #5	2.2	2.8	2.6	2
VT HEX 9.01	Meadow Brook	2.5	2.8	2.7	1
VT HEX 14.02	Lewis Creek	2.8	2.8	2.8	0
VT HEX 12.01	Andover Branch	2.2	3.8	3.0	5
VT HEX 8.01	Pherrins River	2.5	3.8	3.2	4
VT HEX 11.01	Ryder Brook Trib #5	2.8	3.5	3.2	2
VT HEX 6.02	Green River Trib 4	2.2	4.2	3.2	6
VT HEX 1.02	North Branch Hoosic R.	2.8	3.8	3.3	3
VT HEX 10.02	Browns River	3.5	3.2	3.4	1
VT HEX 7.01	Batten Kill	3.5	3.5	3.5	0
VT HEX 2.03	Seaver Brook	3.5	3.5	3.5	0
VT HEX 3.03	Sunny Brook	3.5	3.8	3.7	1
VT HEX 1.01	Wells Brook	3.2	3.8	3.5	2
VT HEX 8.02	No. Br. Ball Mtn. Brook	3.5	3.8	3.7	1
VT HEX 10.01	Browns River Tributary	3.2	4.2	3.7	3
VT HEX 6.01	Burgess Branch	3.5		3.5	
VT HEX 4.02	Peach Brook	2.8	4.8	3.8	6
VT HEX 3.01	Green River	3.5	4.5	4.0	3
VT HEX 9.02	Second Branch White R.	4.5	3.5	4.0	3
VT HEX 15.01	Morril Brook	3.5	4.8	4.2	4
VT HEX 15.03	Morrison Brook	3.5	4.8	4.2	4
VT HEX 11.01	Mount Tabor Brook	3.5	4.8	4.2	4
VT HEX 7.01	Lemon Fair Trib #10	4.2	3.8	4.0	1
VT HEX 14.02	Gihon River	4.2	4.5	4.4	1
VT HEX 8.03	Joe's Brook	4.5	4.2	4.4	1
VT HEX 3.02	Poultney River	4.5	4.2	4.4	1
VT HEX 5.01	South Stream	4.2	4.5	4.4	1
VT HEX 12.01	Lamoille River	4.5	4.5	4.5	0
VT HEX 8.02	Miles Stream	4.2	4.8	4.5	2
VT HEX 13.03	Mill Brook	4.2	4.8	4.5	2
VT HEX 4.01	Paran Creek	4.2	4.8	4.5	2
VT HEX 9.01	Rock River	4.5	4.5	4.5	0
VT HEX 9.02	Still Fawn Brook	4.8	4.2	4.5	2
VT HEX 5.01	Whitman Brook Trib #1	4.2	4.8	4.5	2
VT HEX 6.02	Giddings Brook	4.8	4.5	4.7	1
VT HEX 1.01	Hancock Brook	4.5	5.2	4.9	2
VT HEX 2.01	East Branch North River	5.2	5.2	5.2	0

VT HEX 4.01	Mill River (Georgia)	5.5	5.2	5.4	1
VT HEX 15.01	Mosher Meadow Brook	5.5	5.2	5.4	1
VT HEX 10.01	Mill Brook	5.5	5.5	5.5	0
VT HEX 8.02	Ompompanoosuc River	6.5	5.2	5.9	4
VT HEX 4.01	Kent Pond Outlet	6.5	5.5	6.0	3
VT HEX 14.03	Johns River	6.5	6.5	6.5	0
VT HEX 3.03	Hubbardton River				
				4.0 (2.2-6.5)	**2.0**

Appendix H. NEWS site scores (fish data) from the Vermont Mixed Water IBI, New England IBI, and the Vermont Coldwater IBI.

Hex Number	Location	MWIBI	NEIBI	CWIBI	IBI Rating	BCG
CT HEX 4.01	East Branch Salmon Brook	48	43		Excellent	2.8
CT HEX 31.02	Flat Brook	46	41		Excellent	3.0
CT HEX 11.02	Indian Meadow Brook	48	43		Excellent	3.0
CT HEX 35.05	Sawmill Brook			45	Excellent	3.0
CT HEX 46.03	Five Mile Brook			45	Excellent	3.1
CT HEX 28.01	Wood Creek			39	Very Good	3.1
CT HEX 24.02	Sawmill Brook	44	43		Excellent	3.2
CT HEX 51.02	Flat Brook	48	43		Excellent	3.2
CT HEX 45.04	Limekiln Brook	44	41		Very Good	3.4
CT HEX 13.02	Mountain Brook			39	Very Good	3.4
CT HEX 47.02	Bladdens River	36	31		Good	3.5
CT HEX 8.02	North Running Brook			39	Very Good	3.5
CT HEX 49.05	Pond Meadow Brook	42	37		Very Good	3.5
CT HEX 10.02	Hollenbeck River	36	31		Good	3.7
CT HEX 53.04	Norwalk River	36	33		Good	3.7
CT HEX 36.02	Pomperaug River	46	41		Excellent	3.7
CT HEX 7.06	Stickney Hill Brook	40	35		Very Good	3.7
CT HEX 38.01	Mill River	46	43		Excellent	3.8
CT HEX 3.01	Sandy Brook	48	43		Excellent	3.8
CT HEX 34.02	Crooked Brook	34	33		Good	3.9
CT HEX 48.01	Farm River	32	29		Fair	4.0
CT HEX 41.05	Latimer Brook	40	35		Very Good	4.0
CT HEX 40.01	Clark Creek	36	31		Good	4.1
CT HEX 17.08	Mashamoquet Brook	30	29		Fair-Poor	4.1
CT HEX 50.02	Eight Mile River	40	35		Very Good	4.2
CT HEX 42.03	Seth Williams Brook	48	43		Excellent	4.2
CT HEX 16.01	Wappoquia Brook	48	43		Excellent	4.2
CT HEX 19.02	Lake Waramaug Brook	44	39		Excellent	4.2
CT HEX 43.01	Shunock River	46	41		Excellent	4.2
CT HEX 44.01	Titicus River	36	35		Good	4.2
CT HEX 27.02	Bull Mountain Brook	44	39		Excellent	4.4
EPA HEX 41.05 CT	Eight Mile River	46	41		Excellent	4.5
CT HEX 23.01	Hop River	28	27		Fair- Poor	4.5
EPA HEX 41.04 CT	Pease Brook	40	37		Very Good	4.5
CT HEX 54.02	West Br. Saugatuck River	32	27		Fair- Poor	4.5
CT HEX 22.03	Hockanum River	30	29		Fair- Poor	4.6
CT HEX 32.01	Cabin Brook	46	41		Excellent	4.6
CT HEX 21.02	Farmington River	44	39		Excellent	4.6
CT HEX 20.02	Bantam River	34	29		Good	4.6
CT HEX 26.04	Moosup River	38	33		Good	4.6
CT HEX 5.02	Muddy Brook	40	35		Very Good	4.6
CT HEX 56.08	Farm River	24	21		Poor	4.7
CT HEX 57.04	Neck River	40	35		Good	4.7

Hex Number	Location	MWIBI	NEIBI	CWIBI	IBI Rating	BCG
CT HEX 37.01	Naugatuck River	40	37		Very Good	4.8
CT HEX 25.03	Ekonk River	42	37		Very Good	4.8
CT HEX 14.04	Freshwater Brook	32	27		Fair- Poor	4.8
CT HEX 18.01	Housatonic River	40	39		Very Good	4.8
CT HEX 30.03	Mattabesset River	28	27		Fair- Poor	4.9
CT HEX 2.05	Blackberry River	34	29		Good	5.0
CT HEX 55.01	Pumpkin Ground Brook	38	35		Good	5.0
EPA HEX 39.10 CT	Comstock Brook	38	33		Good	5.2
EPA HEX 36.03 CT	Willamantic River	34	31		Good	5.2
CT HEX 59.01	East Branch Byram River	26	21		Poor	5.4
CT HEX 15.02	Skungamaug River	28	27		Fair- Poor	5.4
EPA HEX 35.04 CT	Mill Brook	28	23		Fair- Poor	5.5
EPA HEX 39.01 CT	Saugatuck River	40	37		Very Good	5.5
EPA HEX 35.05 CT	West Branch Shepaug	44	39		Excellent	5.5
CT HEX 6.06	Still Brook	28	27		Fair- Poor	5.7
EPA HEX 40.03 CT	Steele Brook	34	31		Good	5.8
Mean		**38.6**	**34.7**	**41.4**		**4.2**
MAINE						
EPA HEX 1.01 ME	Aegan Brook			45	Excellent	2.5
ME HEX 22.05	Katahdin Stream			45	Excellent	2.5
ME HEX 26.03	Henderson Brook			45	Excellent	2.7
ME HEX 24.01	West Branch Gulf Stream			42	Excellent	2.7
ME HEX 51.08	Pleasant Stream	48	43		Excellent	2.7
ME HEX 50.02	Tunk Stream	42	37		Very Good	2.7
ME HEX 14.03	Spruce Brook	44	39		Excellent	2.9
ME HEX 19.01	Dole Brook	44	39		Excellent	3.0
EPA HEX 19.03 ME	Bear River	48	43		Excellent	3.2
ME HEX 27.08	West Branch Pleasant River	38	33		Good	3.4
EPA HEX 2.03 ME	Halfway Brook			42	Excellent	3.5
EPA HEX 1.08 ME	Petite Brook			45	Excellent	3.5
EPA HEX 5.02 ME	River DeChute			45	Excellent	3.5
ME HEX 31.01	Sag Brook			42	Excellent	3.5
ME HEX 33.06	Wilson Stream	32	27		Fair- Poor	3.5
EPA HEX 7.03 ME	Logan Brook			42	Excellent	3.8
EPA HEX 5.02 ME	Young Brook	50	45		Excellent	3.8
EPA HEX 7.07 ME	Musquash Brook			33	Good	4.2
ME HEX 55.02	Mill Brook	36	31		Good	4.4
ME HEX 57.01	Mill Stream	36	31		Good	4.4
EPA HEX 8.06 ME	Greenleaf Brook			42	Excellent	4.5
ME HEX 16.04	Kimball Brook	30	25		Fair- Poor	4.5
ME HEX 3.03	Little River	38	33		Good	4.5
EPA HEX 21.10 ME	Naraguagus River	40	35		Very Good	4.5
ME HEX 60.02	Neddick River	32	27		Fair- Poor	4.5
EPA HEX 22.09 ME	New Brook	42	37		Very Good	4.5
EPA HEX 4.01 ME	Snare Brook			33	Good	4.5

Hex Number	Location	MWIBI	NEIBI	CWIBI	IBI Rating	BCG
ME HEX 56.04	Little Ossipee Stream	28	25		Fair- Poor	4.7
ME HEX 42.03	Birch Stream	34	29		Good	4.7
ME HEX 41.05	Crooked Stream	30	25		Fair- Poor	4.7
ME HEX 58.04	Little River	36	31		Good	4.7
ME HEX 35.03	Main Stream	26	22		Poor	4.7
ME HEX 29.10	Tolman Brook	30	25		Fair- Poor	4.7
EPA HEX 3.04 ME	Chemquasabamticook Bk.	38	33		Good	4.8
EPA HEX 19.01 ME	Hardy Stream			24	Poor	4.8
EPA HEX 31.02 ME	Kennebunk River	*30*	*25*		Fair- Poor	4.8
EPA HEX 25.03 ME	Teddy's Brook	36	31		Good	4.8
ME HEX 49.03	Flood Stream	34	29		Good	4.9
ME HEX 48.05	Halfmoon Stream	32	27		Fair- Poor	4.9
EPA HEX 3.05 ME	Depot Stream	36	31		Good	5.2
EPA HEX 5.07 ME	No.Br. Meduxnekeag River	38	33		Good	5.2
EPA HEX 13.02 ME	Schoodic Brook	28	25		Fair- Poor	5.2
EPA HEX 26.11 ME	Togus Stream	32	31		Fair- Poor	5.2
ME HEX 47.09	Martin Stream	22	21		Poor	5.4
EPA HEX 11.05 ME	Horseshoe Stream			27	Fair- Poor	5.5
EPA HEX 27.01 ME	Medomak River	34	29		Good	5.5
EPA HEX 20.02 ME	Higgins Brook	28	23		Fair- Poor	6.5
Mean		***35.5***	***30.9***	**39.4**		**4.4**
New Hampshire						
NH HEX 15.01	Appleby Brook			45	Excellent	2.5
NH HEX 9.05	Newell Brook		41		Excellent	2.5
NH HEX 4.04	North Branch River			45	Excellent	2.5
NH HEX 24.02	Paugus Brook			45	Excellent	2.7
NH HEX 12.02	Peabody Brook			*		2.7
NH HEX 11.01	Bumpus Brook			45	Excellent	2.9
NH HEX 18.01	Eastman Brook			45	Excellent	2.9
NH HEX 23.01	Johnson Brook			42	Excellent	2.9
NH HEX 5.04	Simms Stream	42	37		Very Good	3.4
NH HEX 61.04	Beaver Brook	32	31		Fair- Poor	3.5
NH HEX 6.01	Unnamed Brook	32	29		Fair- Poor	3.5
NH HEX 2.05	Indian Stream	40	35		Very Good	3.7
NH HEX 45.04	Piscataquog River	34	29		Good	3.7
NH HEX 59.03	Souhegan River	36	31		Good	3.7
NH HEX 21.05	Grant Brook			36	Good	3.7
NH HEX 57.03	Ash Swamp Brook	34	31		Good	3.8
EPA HEX 11.06 NH	Cedar Stream	42	39		Very Good	3.8
EPA HEX 24.01 NH	Indian River	46	41		Excellent	3.8
NH HEX 28.03	Ames Brook	44	39		Excellent	3.9
NH HEX 14.02	Ammonoosuc River	42	37		Very Good	3.9
NH HEX 52.01	Contoocook River	32	29		Fair- Poor	3.9
NH HEX 16.02	East Branch Saco River	44	39		Excellent	3.9
NH HEX 8.02	Roaring Brook	42	37		Very Good	3.9

Hex Number	Location	MWIBI	NEIBI	CWIBI	IBI Rating	BCG
NH HEX 19.01	Swift River			33	Good	3.9
NH HEX 35.01	Churchill Brook			39	Very Good	4.0
NH HEX 43.03	Cold River	36	31		Good	4.0
NH HEX 53.01	Purgatory Brook	30	25		Fair- Poor	4.2
NH HEX 1.03	Connecticut River	40	35		Very Good	4.2
NH HEX 39.01	Dolf Brook	*24*	*23*		Poor	4.2
NH HEX 46.02	Turkey River	*32*	*27*		Fair- Poor	4.2
NH HEX 51.04	Otter Brook	28	23		Fair- Poor	4.4
NH HEX 30.02	Poland Brook	*32*	*27*		Fair- Poor	4.4
NH HEX 10.02	Bog Brook	42	37		Very Good	4.5
EPA HEX 29.07 NH	Warren Brook	46	41		Excellent	4.5
NH HEX 26.05	Hewes Brook			21	Poor	4.7
NH HEX 34.03	Tioga River	34	29		Good	4.7
EPA HEX 33.03 NH	Dinsmore Stream	30	25		Fair- Poor	4.8
NH HEX 44.02	Dodge Brook	30	25		Fair- Poor	4.8
NH HEX 31.01	Taylor Brook	28	23		Fair- Poor	4.8
NH HEX 58.03	Mill Brook	28	23		Fair- Poor	4.9
NH HEX 41.04	Berry Brook	22	21		Poor	5.0
NH HEX 27.04	Mascoma River	28	23		Fair- Poor	5.0
NH HEX 38.05	Trask Brook	*26*	*21*		Poor	5.0
NH HEX 36.01	Branch River	22	21		Poor	5.2
NH HEX 17.03	Clark Brook	28	23		Fair- Poor	5.2
EPA HEX 30.04 NH	Cocheco River	*32*	*27*		Fair- Poor	5.2
NH HEX 29.03	Dan Hole River	28	23		Fair- Poor	5.2
EPA HEX 34.06 NH	Beaver Brook	36	33		Good	5.5
NH HEX 40.02	Webster Stream	20	19		Poor	5.5
EPA HEX 25.02 NH	Beech River			18	Poor	5.8
Mean		**33.6**	**29.7**	**37.6**		**4.2**
Vermont						Mean
VT HEX 13.03	Blood Brook			45	Excellent	2.5
VT HEX 8.02	Goshen Brook Trib #2			45	Excellent	2.5
VT HEX 14.02	Hubbard Brook			45	Excellent	2.5
VT HEX 15.02	Kilburn Brook			45	Excellent	2.5
VT HEX 7.03	Old City Brook			45	Excellent	2.5
VT HEX 9.01	Williams River Trib #11			45	Excellent	2.5
VT HEX 9.01	Meadow Brook			36	Very Good	2.7
VT HEX 14.02	Lewis Creek	48	43		Excellent	2.8
VT HEX 12.01	Andover Branch			45	Excellent	3.0
VT HEX 8.01	Pherrins River	44	39		Excellent	3.2
VT HEX 11.01	Ryder Brook Trib #5	46	41		Excellent	3.2
VT HEX 6.02	Green River Trib 4			45	Excellent	3.2
EPA HEX 23.02 VT	Huntington River			39	Very Good	3.2
EPA HEX 23.01 VT	Third Branch White River	46	43		Excellent	3.2
VT HEX 1.02	North Branch Hoosic River			33	Good	3.3
VT HEX 10.02	Browns River	44	39		Excellent	3.4

Hex Number	Location	MWIBI	NEIBI	CWIBI	IBI Rating	BCG
VT HEX 7.01	Batten Kill	46	41		Excellent	3.5
VT HEX 2.03	Seaver Brook	48	43		Excellent	3.5
VT HEX 1.01	Wells Brook			39	Very Good	3.5
VT HEX 8.02	No. Br. Ball Mtn. Brook	46	41		Excellent	3.7
VT HEX 3.03	Sunny Brook	40	37		Very Good	3.7
VT HEX 10.01	Browns River Tributary			33	Good	3.7
VT HEX 4.02	Peach Brook	42	37		Very Good	3.8
VT HEX 3.01	Green River	44	39		Excellent	4.0
VT HEX 7.01	Lemon Fair Trib #10	34	29		Good	4.0
VT HEX 9.02	Second Branch White River	36	33		Good	4.0
VT HEX 15.01	Morril Brook	48	45		Excellent	4.2
VT HEX 15.03	Morrison Brook			30	Good	4.2
VT HEX 11.01	Mount Tabor Brook			27	Fair- Poor	4.2
VT HEX 14.02	Gihon River	40	35		Very Good	4.4
VT HEX 8.03	Joe's Brook	42	37		Very Good	4.4
VT HEX 3.02	Poultney River	40	35		Very Good	4.4
VT HEX 5.01	South Stream	44	41		Excellent	4.4
VT HEX 12.01	Lamoille River	38	33		Good	4.5
VT HEX 8.02	Miles Stream	38	33		Good	4.5
VT HEX 13.03	Mill Brook	40	35		Very Good	4.5
EPA HEX 18.01 VT	Miller Run	36	31		Good	4.5
VT HEX 4.01	Paran Creek	36	31		Good	4.5
VT HEX 9.01	Rock River	32	27		Fair- Poor	4.5
VT HEX 9.02	Still Fawn Brook	30	25		Fair- Poor	4.5
VT HEX 5.01	Whitman Brook Trib #1			27	Fair- Poor	4.5
VT HEX 6.02	Giddings Brook	30	25		Fair- Poor	4.7
EPA HEX 28.05 VT	Mettawee River	44	43	33	Excellent	4.8
EPA HEX 9.0 VT	Trout Brook	36	33		Good	4.8
VT HEX 1.01	Hancock Brook			21	Poor	4.9
VT HEX 2.01	East Branch North River	35	31		Good	5.2
EPA HEX 10.01 VT	Willoughby River	42	39		Very Good	5.2
VT HEX 4.01	Mill River (Georgia)	28	25		Fair- Poor	5.4
VT HEX 15.01	Mosher Meadow Brook	34	29		Good	5.4
EPA HEX 9.05 VT	Black Creek	26	21		Poor	5.5
EPA HEX 29.03 VT	Black River	48	43		Excellent	5.5
VT HEX 10.01	Mill Brook	28	23		Fair- Poor	5.5
EPA HEX 17.02 VT	Winooski River (east)	28	23		Fair- Poor	5.5
VT HEX 8.02	Ompompanoosuc River	26	21		Poor	5.9
VT HEX 4.01	Kent Pond Outlet			21	Fair- Poor	6.0
VT HEX 14.03	Johns River	9	9		Poor	6.5
EPA HEX 16.07 VT	Winooski River					
Mean		**37.9**	**34.0**	**36.8**		**4.1**

Data and Assessment Comparability Among Stream Bioassessment Methods: EPA-NEWS Methods and New England State Methods

Prepared for:

USEPA Office of Water, Office of Watersheds, Oceans, and Wetlands
Washington, DC

USEPA Office of Science and Technology
Washington, DC

USEPA New England Regional Lab
Chelmsford, MA

New England Interstate Water Pollution Control Commission
Lowell, MA

Work Assignment Manager:
Susan Holdsworth
USEPA Office of Water, Office of Watersheds, Oceans, and Wetlands

Susan Jackson
USEPA Office of Water, Office of Science and Technology

Prepared by:

Ben Jessup
Jeroen Gerritsen

Tetra Tech, Inc.
15 State Street, Suite 301
Montpelier, Vermont 05602

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1.0 Introduction

Throughout New England, macroinvertebrate data have been collected by individual states for water resources assessments. Each state conducts their assessments somewhat differently from their neighbors and reports assessment results in a somewhat uniform format, such as the water quality inventory report required under the Clean Water Act section 305(b). The uniform reporting format may lead some to interpret water resource assessments similarly throughout the region. However, in New England and throughout the nation comprehensive assessment information on a regional level has been problematic due to unknown comparability among programs and methods (ITFM 1995a, NWQMC 2001, Heinz 2002, GAO 2004, U.S. EPA 2003). The differences in assessment program design, sampling methods, and analytical techniques among New England states are great enough that data comparability should be examined more closely.

In 2000, the United Sates Environmental Protection Agency (EPA) in the New England region implemented a stream monitoring project across the six New England states in an effort to uniformly assess the ecological condition of wadeable streams across the region. The New England Wadeable Streams (NEWS) project was a collaborative effort between the U.S. EPA Region I, the EPA's Atlantic Ecology Division in Narragansett, Rhode Island, EPA's Office of Research and Development in Corvallis, Oregon, the New England Interstate Water Pollution Control Commission, the six New England states environmental agencies, and key members of academia within the region.

The principal goal of the NEWS project was to provide regional unbiased assessments of ecological condition in wadeable streams across the New England landscape that would represent all streams of this type in the region. To support this goal, EPA provided assistance to the participating states in developing a method for comprehensive monitoring and reporting at the state geographic scale. The comprehensive assessment was accomplished using a probability based sampling design and consistent sampling and analytical techniques throughout New England.

Those states that chose to participate in the comparability analysis also collected macroinvertebrate samples from the NEWS sites within their respective states using the protocols typically used in the state biomonitoring program. The NEWS samples were standards to which states were able to compare their own samples in terms of representativeness, sample content, and assessment results. In so doing, states could determine whether the state-collected data could be pooled in regional assessments and whether state reports of water quality conditions used similar measures.

An important objective of the NEWS program was determining if existing state monitoring data could be pooled through an assessment model so that despite possible differences in sample representativeness or content, identical assessment outcomes from a waterbody could be derived from the various state methods. By developing such a tool, state environmental agencies would have the ability to make assessments of watersheds and ecoregions that span one or more geopolitical boundaries. The framework used to develop the assessment model was the Biological Condition Gradient (BCG).

The Biological Condition Gradient (BCG) offers an interpretive framework for communicating technical findings about biological condition, in relation to human disturbance. The BCG model is designed to place sites along a gradient of human disturbance, utilizing key ecological and biological attributes that are known indicators of impairment. The model consists of 6-tiers of progressively deteriorating conditions. It provides a means for standardizing communication about how much biological change has occurred in a sampled habitat (Davies and Jackson, In Press; U.S. EPA 2005). The definitions of the tiers were closely examined by the entire workgroup of New England biologists. They then selected macroinvertebrate samples to represent each of the tiers. Through this process, the biologists were able to calibrate their knowledge of macroinvertebrate sample content with standardized or expected levels of degradation. After initial calibration exercises, biologists assigned tiers to each sample of the comparable pairs to allow comparison of assessment results.

Data sets in Connecticut, Vermont, and New Hampshire were well suited for comparative analysis because there was sufficient sampling using both protocols. In Maine, only 3 sites were sampled using both NEWS and state protocols. No samples were collected using state methods in Massachusetts or Rhode Island. Replicate samples using the same protocol were not collected throughout the project, limiting the potential for estimating sampling error within a single method. Therefore, pairwise comparisons were made among samples collected with different protocols, though from the same site.

2.0 Methods

Samples collected using NEWS methods were compared to those collected using state methods in several ways. First, samples were compared in terms of the purpose of the sampling protocol, or what the sample is intended to represent. Second, the sample contents were compared using statistical analyses of metrics based on taxa attributes. Finally, the assessment results were compared to determine whether biologists would assign similar biological condition ratings to samples collected from the same sites with different methods.

Representativeness

Field sampling techniques and laboratory sample processing procedures were first compared in a qualitative manner. Before looking at the resulting samples, we considered potential differences in the intent of the sampling protocols, especially in terms of the substrate, habitat, and streambed area sampled. If samples collected using each sampling protocol were intended to represent similar aspects of the benthic assemblage and used similar sampling efforts, there would be greater confidence in findings of similar sample content or similar assessment results. Determination of the similarity of sample representativeness was qualitative.

Sample Content

The similarity of samples collected using different protocols was determined both by examining presence and abundance of individual taxa and by examining community metrics (measures of taxonomically or functionally similar groups of taxa). Quantitative measures and illustrations of bias were generated to determine if systematic differences in the types and numbers of organisms could be detected among protocols. Taxa occurrence in the samples was tabulated so that taxa that were uniquely captured by one protocol could be identified. Metric plots by protocol were used to show bias and a Chi square test was used to identify significance of the perceived bias. Correlations and measures of precision show the degree to which patterns in sample content differences are repeatable.

Assessment Comparisons

The biologists were able to associate tiers of the BCG with samples based solely on taxa lists, collection methods, and minimal knowledge of the sites. Thus, the tier assignments were assessments of the biological conditions of the sites. These assessments relied on the biologists' recognition of degrees of degradation evident from taxa presence, absence, and abundance in the samples. Because they were familiar with all the sampling protocols, it was assumed that tier assignments would be consistent across sampling methods, even if sample content varied.

2.1 Study Design

NEWS Sampling Design

The NEWS study incorporated the design and implementation of a randomized probability based approach for selecting sites that belonged to second order or higher stream systems. The design provided uniform spatial coverage of streams across the region using a hexagonal overlay as a framework from which to select stream segments. Within each hexagonal cell, all stream segments were identified. Random numbers were generated to select stream segments and locations along the stream segment for sampling. Additional hexagonal overlays were used within individual states to allow for random selection of spatially distributed sites within the states.

At each sampling location, data were collected on macroinvertebrates, fish, water chemistry, and habitat condition within a 150 meter reach. This study focuses on the macroinvertebrate data. Fish collection methods were relatively equal throughout the states. Water chemistry and habitat data were used with landscape level data in determining relative levels of site degradation.

NEWS Sampling Methods

For NEWS samples, macroinvertebrate collections methods resembled those used in the Mid-Atlantic Highland REMAP project (adapted from Barbour et al. 1999). The method was intended to provide a taxa inventory across the region and to be applicable in both low gradient and high gradient stream systems. To satisfy the need for a taxa inventory, multiple stream habitats were sampled, in proportion to the habitat types prevalent throughout the sampling reach. Proportionate sampling by habitat type was applicable in all stream systems.

The NEWS field sampling method used a one-fifth square meter metal square (quadrat) that was randomly tossed within a specific habitat type of the stream reach. Macroinvertebrates were collected from twenty quadrats throughout the stream reach, resulting in a composite sample covering four square meters. If the reach consisted of half riffle and half pool habitat, then ten quadrats would be randomly tossed in pools and ten in riffles. Collections in each quadrat were timed for one minute during which all substrate was rubbed and the bottom fines disturbed to a depth of approximately three centimeters. Organisms were captured in D-nets with 500 μm mesh held immediately downstream of the disturbed substrate.

In the laboratory, samples were evenly distributed over a gridded tray and grids were randomly selected for a subsample. Organisms were picked from the subsample residue with the aid of magnification. The target subsample size was 200 organisms. NEWS samples were processed by contracted laboratories. Collections were mostly made in September.

At a subset of sites where samples were collected with NEWS protocols, additional samples were collected using field collection methods particular to each state. In Connecticut, state field biologists collected NEWS and state samples on the same day. Samples were then processed and identified by the same laboratory. In Vermont, different field crews generally collected state samples later in the year than the NEWS samples. The Vermont laboratory performed sample processing and identifications for the samples collected using state methods while NEWS samples were processed and identified by a contracted laboratory. In New Hampshire and Maine, artificial substrates used by the states were deployed when the NEWS sample was collected. Samples from the artificial substrates were retrieved 4 – 8 weeks later and were processed using protocols particular to each state. Macroinvertebrate collection techniques used by the states are summarized below and in Table 1.

Connecticut Sampling Methods

The Connecticut Department of Environmental Protection (CTDEP) collection method follows EPA Rapid Bioassessment Protocols (RBP) for Streams and Rivers (Plafkin et al. 1989). The RBP targets the richest habitat by collecting 12 kick samples (stops) throughout riffles at sampling sites using a rectangular net (18”x10”) with 800-900 μm mesh. The 12 stops cover a sampling area of approximately two square meters. The sampling goal is to obtain the best coverage (laterally and longitudinally) within the designated riffle. Exact locations for these kicks are determined based on stream flow, depth and substrate characteristics. The resulting sample is meant to represent the community as a whole within the riffle.

Table 1. Major elements of the protocols used in the NEWS and state benthic macroinvertebrate sample collection programs.

Program	Equipment	Habitat	Field Method	Processing
NEWS	Kick net with 500 µm mesh, 0.20 m^2 quadrat.	All habitats sampled through random placement of sampling quadrats throughout the sampling reach. Area: 4 m^2	Substrates are scrubbed for 1 minute per quadrat. Collections from 20 quadrats composited.	Lab processing and identification by contracted lab. Subsample target size: 200 organisms using Caton grid.
Connecticut	Rectangular net with 800-900 µm mesh	Riffles targeted. Area: 2 m^2	12 kick samples composited.	Lab processing and identification by contracted lab. Subsample target size: 200 organisms using Caton grid.
Vermont	D-frame net with a 500 µm mesh	Riffles targeted. Area: 1 m^2	Four 18"x18" kick samples within one riffle composited.	Lab processing and identification by the State laboratory. Subsample target size: Minimum one quarter of the sample and 300 organisms.
New Hampshire	Rock Baskets, sieve bucket with 600 µm mesh	Riffles or hard bottom substrate targeted.	Three rock baskets are placed together and allowed to colonize for 6 – 8 weeks. Basket and rocks rinsed in sieve bucket. Retained separate samples, unless materials from each are minimal, then composited.	Enumeration and identification completed by contracted lab. Subsample is minimum of ¼ of sample with minimum 100 organism count. If ¼ / 100 organism is not met then entire sample is processed.
Maine	Rock Baskets, sieve bucket with 600 µm mesh	Riffles	Three rock baskets are placed in three locations in the reach, allowed to colonize for 28 +/- 4 days, retrieved, rinsed in sieve bucket, and processed separately.	Entire sample processed for each of three replicates.

Maine Sampling Methods

Maine Department of Environmental Protection (ME DEP) collects samples form artificial substrates. Wire baskets filled with river-run stone are placed in a stream for a period of approximately 28 days. Rock baskets are usually deployed in triplicate and each is positioned in locations of similar habitat. After the colonization period, an aquatic net or drift net (mesh size 600 microns) is positioned against the substrate immediately downstream of the basket which is then quickly lifted into the net. The contents of the basket and all net washings are emptied into a sieve bucket (600 microns); basket wires and then rocks are hand washed and the contents placed in sample jars. ME DEP processes samples from each rock basket separately, picking and identifying all organisms in the sample.

New Hampshire Sampling Methods

New Hampshire Department of Environmental Services (NH DES) uses rock baskets similar to those used in ME. Rock baskets include a 6.5 inch diameter cylindrical plastic coated wire basket 11 inches in length that holds regionally indigenous bank run gravel diameters ranging from 1.5 - 3.0 inch diameter. Baskets are placed in riffle habitats or at the base of riffles at depths that cover the artificial substrate by at least 5 inches. Each biomonitoring station uses three baskets that are anchored to the streambed by sinking ½ inch steel reinforcing rod and then attaching the baskets downstream in an array pattern with a loop of nylon coated steel cable. Substrates are left undisturbed at the site for a period of six to eight weeks in order for adequate colonization to take place. During collection, rock baskets are placed in 600-µm 3-gallon sieve buckets and scrubbed thoroughly.

Rock basket samples are composited in the field if the amount of material will all fit into one jar. Otherwise, samples from each basket are processed separately. Organisms are picked from a randomly selected quarter of the sample. If less than 100 organisms are picked from the quarter-sample, then the entire sample is picked. NH DES contracts a laboratory for sample processing.

Vermont Sampling Methods

Vermont Department of Environment Conservation (VT DEC) samples are collected using a protocol that is comparable to the riffle-run sampling techniques described in the Rapid Bioassessment Protocol III (RBPIII; Plafkin et al. 1989). An 18” wide x 12” high D-frame net with 500 µm mesh is placed in riffle habitat and an 18” x 18” square area immediately upstream of the net is thoroughly disturbed by hand, ensuring that all pieces of substrate are moved and rubbed clean of attached organisms. Each specific location is actively sampled until all the substrate has been disturbed, approximately 30 seconds, and active sampling is terminated at the end of two minutes. This is repeated moving upstream at 4 different locations within the riffle, covering approximately one square meter and representing the range of velocities and substrate types characteristic of that riffle. The samples are intended to represent the macroinvertebrate community of a single riffle that is characteristic of all riffles within the entire stream reach.

Organisms are picked from a randomly selected quarter of the sample. If less than 300 organisms are picked from the quarter-sample, then subsampling continuous until the target of 300 organisms is reached, or the entire sample has been picked. VT DEC processes samples in a state laboratory. Collections are made during an index period of September – October.

2.2 Community Metrics

Metrics included taxa richness and percent composition of individuals in the sample for taxa groups that are recognized as general indicators of water resource quality. The taxa groups include the mayflies, stoneflies, caddisflies, net-spinning caddisflies, and midges. These groups are also known by the scientific names Ephemeroptera, Plecoptera, Trichoptera, Hydropsychidae, and Chironomidae. Metrics of the whole sample include total number of taxa, percent dominance of the most abundant taxon, and the Shannon-Wiener index of community diversity. For this data analysis, all identifications were standardized to genus level taxonomy.

Additional metrics included those that were calculated based on the taxa attributes used in conjunction with the Biological Condition Gradient (BCG). These attributes relate to the rarity and pollution sensitivity of each taxon (Table 2). Attribute numbers were assigned through both group consensus and indicator analyses. Metrics describe both richness and percent composition of taxa with attributes 2 through 5.

Table 2. Taxa attributes and descriptions

Group II: Highly sensitive taxa. These taxa may be common to uncommon, but typically only occur in good to very good sites, and occur only rarely in moderate to poor sites. Many occur in low abundances only, so occurrence (presence) is more informative than abundance. Examples: *Lepidostoma*, *Protoptila*, *Leuctra*, *Drunella*, Blephariceridae.
Group III. Sensitive taxa. These taxa may occur in all sites, but they occur more frequently and are more abundant in high quality sites. Thus, they are somewhat tolerant of poor conditions, but prefer unpolluted and good quality habitat. Examples: *Psephenus*, *Eurylophella*, *Acroneuria*, *Glossosoma*, *Rhyacophila*.
Group IV: Broadly tolerant (indifferent) taxa: These taxa may occur in any conditions and at any abundance. They appear to have no detectable preference for water quality or habitat, except for some decline in the most polluted sites (Tier 6). In general, they do not provide useful information. Examples: *Stenelmis*, *Baetis*, *Orthocladius*, *Hydroptila*, *Perlesta*.
Group V. Tolerant taxa: These taxa occur more frequently and especially at increased relative abundance in poor water quality and poor habitat. Many also occur in good quality conditions, but abundances are reduced. Examples: *Gammarus*, *Cricotopus*, *Hydropsyche*.

Bias

Metrics resulting from differing protocols were examined for evidence of bias using both graphic displays and statistical analysis. Graphs include bi-plots of metric values resulting from two sampling protocols. In these plots, the unity (1:1) line was illustrated and data points would reveal consistent bias when a large percentage of points were on one side of the line. These plots are used to illustrate bias apparently caused by methods.

Statistical evidence of significance in bias was derived from a Chi-square test on the points above and below the unity line (Manley 2001). Points that differed by no more than 20% of the mean metric value were down-weighted in the Chi-square test, assuming that such small differences are within the margin of error for either sampling protocol individually.

Precision

Precision is the closeness of two measures of the same entity, in this case the reproducibility of metrics of the benthic macroinvertebrate community. Two samples were collected at some sites, using different methods to collect each sample. There were not sufficient replicate samples collected with the same method at the same site to define the margin of error for any individual method. Therefore, the measures of precision in this analysis are best compared among state programs, allowing a relative evaluation of precision.

Precision was measured by estimating the variance among multiple samples at sites. For the sake of convenience, we used an analysis of variance (ANOVA) to estimate the mean square error term (MSE), from which the root mean square error (RMSE) can be calculated. RMSE values are lower when measures are more precise. The coefficient of variation (CV) was then calculated, which standardizes variability on the mean of measures (CV = RMSE/mean). The CV thus allows comparison of relative precision among metrics and among protocol comparisons (Diamond et al. 1996).

The confidence interval (CI) is the minimum difference between means that will be significant at the chosen significance (alpha) level. For this analysis the significance level of the CI was set at 90%, thus describing the range within which the true mean is likely to fall in 90% of the cases. CI is calculated from the RMSE using the equation:

$$\mathrm{CI} = \pm \mathrm{RMSE} \times t_\alpha$$

where t_α is the 90% CI value (i.e., $p = 0.10$) from a standard t table (Zar 1999), which in this analysis equals 1.64. A smaller CI for a method indicates more precise data.

Correlations

Correlation is way of determining agreement between two sets of values. The correlation analyses compared metric values generated from NEWS samples to those generated from state samples. High correlation coefficients (Pearson product-moment r) indicate general agreement between values, though not necessarily agreements that are close to the 1:1 line. Thus, correlation helps to describe precision among the measures, but not bias.

2.3 Biological Condition Gradient Tiers

Tier Assignments

The workgroup of state biologists discussed the conceptual model of the BCG for New England, developed preliminary definitions of tiers 2 - 6 of the gradient, and reviewed sample taxa lists that exemplified each of the tiers. The biologists were familiar with the streams, macroinvertebrate assemblage, and sampling protocols. They were given the sample taxa lists with counts of organisms, taxa attributes, and sampling protocols. They were also given the state in which the sample was collected, overall organism density, and stream gradient category (high, moderate, low). They were not informed of the stream name or location within the state. From the taxa lists, they were asked to work independently to assign the sample to a tier of the BCG.

BCG tiers are categorical, 1 through 6, from best biological condition to worst. The biologists used a more continuous scale when assigning tiers, allowing ratings that were squarely in the tier (e.g., 3.5), a little better than normal for the tier (e.g., 3.2), or a little worse than normal for the tier (e.g., 3.8). These decimal categories were not further divided and all ratings fell into X.2, X.5, or X.8 sub-categories.

Reviewer Variability

Because there were multiple reviewers assigning tiers for each sample, precision of tier assignment could be assessed. ANOVA was used as described above, using the site and protocol as the grouping variables and calculating RMSE based on reviewer variability. Further comparisons were made among the precision attained with NEWS or state sample collection protocols.

Protocol Variability

During the review process, in which biologists assigned BCG tiers based on sample taxa lists, the sample collection protocol was known and the biologists were aware of the general biases in sample contents due to protocol (as presented in discussions of metric comparability). The biologists were thus encouraged to calibrate their tier assignments based on sampling protocol. The following analyses were performed to determine the degree to which the biologists could interpret the effects of sample protocol on sample contents, and thus, tier assignment. Sensitivity of tier assignment to sampling protocol was measured as the difference among tier assignments of different protocols as a factor of reviewer precision.

Sensitivity to Pressures

While the relationships between BCG tier assignments and stressor conditions was not the focus of the comparative study, it was of interest to determine whether tier assignments were more or less responsive to stressors as a factor of sampling protocol. Stressor categories were therefore defined based on land use, habitat, and water quality criteria. Four categories were defined, with the highest category (1) meeting physical criteria for BCG tier 1 status. These criteria were developed through group consensus and matched generally accepted stressor conditions associated with unimpacted, slightly impacted, moderately impacted and severely impacted conditions (Table 3). The field biologists that were familiar with the sites decided final stressor categories for sites, altering the assignment by at most 1 category from the criteria-indicated category. It should be noted that this part of the comparability study was exploratory and was part of an effort to calibrate tier assignments to the best and worst environmental conditions. BCG tier assignments were plotted by stressor category and protocol.

Table 3. General criteria for categorizing sites by degrees of environmental stress. After application of these criteria, categorical adjustments were made based on the number of criterion failures and biologists' familiar knowledge of the sites.

	1: Best	2	3	4: Worst
Land Use	>95% natural	85 – 95% nat.	50 – 85% nat	<50% natural
Habitat Score	>160			<120
Total Nitrogen	<1.0 mg/L			>1.0 mg/L
Total Phosphorus	<0.02 mg/L			> 0.02 mg/L
Conductivity	<150 µS/cm			>200 µS/cm
Metals*				

*U.S. EPA hardness adjusted criteria for:
Cadmium, Chromium, Copper, Lead,
Mercury, Nickel, Silver, and Zinc

3.0 Results

3.1 Sample Representativeness

The state sampling protocols consistently differed from the NEWS protocols in one way: the habitat targeted for sampling. The NEWS protocols require that the sample be collected from all habitat types that are encountered within the stream reach. Samples could therefore include organisms that dwell in riffles, runs, pools, banks, vegetation, or snags. On the contrary, all state sampling protocols target riffle habitats and largely ignore other habitats.

Other notable differences include sampling equipment and subsampling effort (Table 1). NEWS, VT, and CT protocols specify use of a D-frame net and active disturbance of existing substrates. In ME and NH, artificial substrates (rock baskets) are placed on the streambed, colonized over a 4 – 8 week period, and retrieved with the residing organisms at the end of the colonization period. Subsampling effort ranges from 200 organisms for NEWS and CT to whole sample identification in ME. In NH and VT, at least ¼ of the sample is identified. If the ¼ sample does not yield 100 (NH) and 300 (VT) organisms, then the entire sample is identified in NH, while in VT additional grids are picked until the target has been reached.

These differences among field and laboratory protocols may result in samples that are not directly comparable in terms of sample content or assessment results. NEWS samples represent those benthic organisms that reside in multiple habitats as randomly encountered in the stream reach, sampled actively with a D-frame net, and subsampled to 200 organisms. CT protocols use the same subsampling effort as NEWS, but riffles only are sampled by CT. VT protocols are similar in sampling equipment, but differ in habitats and subsampling effort. ME and NH protocols differ from NEWS protocols in terms of targeted habitat, sampling equipment, and subsampling effort.

3.2 Sample Content

Metric Bias

Fifteen (15) metrics based on taxa groups were calculated and tested for bias due to sampling protocol when comparing NEWS samples to those collected with state methods in CT, VT, and NH. In CT and NH, six metrics showed significant bias as did 10 metrics in VT (Table 4, Appendix A). NEWS protocols resulted in samples with greater numbers of taxa in CT, but not in VT or NH. Percent EPT and percent Trichoptera were significantly biased in all three states, where state protocols resulted in higher percentages. Midges (Diptera: Chironomidae) were collected in higher percentages in the NEWS samples from CT and VT.

Table 4. Chi square values and significance of bias among protocols, by state. Values in bold type highlight metric comparisons with significant bias ($p<0.05$) and show the sample protocol resulting in higher metric values (N = NEWS, S = state).

	X^2		
	CT	VT	NH
Total taxa	**9.0 (N)**	1.1	0.4
Percent dominant	3.2	0.4	2.0
Shannon-Wiener Index	1.4	0.0	0.0
EPT taxa	0.0	**7.7 (S)**	**4.2 (S)**
EPT percent	**16.0 (S)**	**16.4 (S)**	**10.7 (S)**
Mayfly taxa	2.6	0.0	0.4
Mayfly percent	0.0	**7.7 (S)**	**6.0 (S)**
Stone taxa	0.4	**20.0 (S)**	**6.0 (S)**
Stonefly percent	3.2	**11.6 (S)**	0.4
Caddisfly taxa	0.4	1.6	**7.0 (S)**
Caddisfly percent	**14.4 (S)**	**10.2 (S)**	**4.2 (S)**
Hydropsychidae taxa	1.0	**5.5 (S)**	0.7
Hydropsychidae percent	**17.6 (S)**	**18.2 (S)**	0.0
Midge taxa	**19.4 (N)**	**9.6 (N)**	2.0
Midge percent	**16.0 (N)**	**18.2 (N)**	2.7
Attribute 2 taxa	0.4	**5.5 (N)**	0.7
Attribute 2 percent	0.4	3.7	0.7
Attribute 3 taxa	0.2	**11.6 (S)**	**6.0 (S)**
Attribute 3 percent	2.6	**20.0 (S)**	**9.4 (S)**
Attribute 4 taxa	**6.8 (N)**	0.4	0.7
Attribute 4 percent	1.0	**5.5 (N)**	3.4
Attribute 5 taxa	**10.2 (N)**	**14.7 (N)**	1.0
Attribute 5 percent	**14.4 (N)**	**6.5 (N)**	2.0

The bias towards greater numbers of taxa in CT NEWS samples may be due to the sampling of multiple habitats, as opposed to just riffles in the state method. Other biases may likewise be attributed to habitats sampled or sampling effort. We would expect to find more midges in non-riffle habitat and more EPT taxa in riffle habitat. The NEWS samples apparently captured more midges and less EPT individuals because the non-riffle habitat was sampled.

Of the metrics based on taxa attributes, bias was again most noticeable in comparisons of NEWS samples to VT samples. VT samples contained more attribute 3 (sensitive and common) taxa and individuals, fewer attribute 2 taxa (sensitive and uncommon), and fewer attribute 5 (tolerant) taxa and individuals. In CT, tolerant taxa were more diverse and abundant in the NEWS samples. In NH, sensitive common taxa were more diverse and abundant in NEWS samples.

Metric Precision with Protocol

Metric variability was measured as the CV of repeated measures using NEWS and state methods (Table 5). This statistic indicates the variability of the metric due to sampling method as a function of the mean of metric values. Standardization on the mean allows comparison of variability across metrics and across states. Most metrics measuring counts of taxa had CVs less than 50%. Exceptions to this rule include the stoneflies (Plecoptera) and attribute 5 taxa. The higher CVs in these metrics may be due to scant and patchy distributions of the organisms in the streambed. The composition metrics had CVs near or greater than 50%, except for % EPT and % attribute 4. In general, CVs were lower for metric comparisons in CT and VT than in NH, suggesting that on a relative scale, CT and VT protocols yield samples that are more comparable to NEWS samples than NH protocols.

The CI for metrics indicates the range around an observation in which the true value would be expected to occur in 90% of the cases, given the variability associated with two sampling protocols. These statistics represent real metric units and are therefore easy for biologists to interpret. For EPT taxa, the 90% CI is 4.9, 5.2, and 7.6 in CT, VT, and NH, respectively (Table 5). The greatest CIs are seen in the composition metrics, especially percent midge individuals. Attribute based metrics had relatively low CIs in CT.

Metric Correlations

Correlations indicate the degree to which sets of values are related, without regard to bias. A high correlation coefficient may be associated with metric pairs that are not on the 1:1 line. In the correlations calculated between metrics generated from NEWS samples and those generated from state samples it appears that the highest correlations are between CT and NEWS samples and the lowest correlations are between NH and NEWS samples (Table 6).

3.3 BCG Tier Assignments

Reviewer Variability

Multiple reviewers were asked to independently assign samples into BCG tiers. This allowed an assessment of the precision of tier assignment in light of different biologist's interpretations of the taxa lists. The CVs and CIs for NEWS sites in VT were lower than other comparisons, showing that the different reviewers essentially agreed on tier assignments when reviewing the same sample (Table 7). The highest CV and CI, indicating the most disagreement among reviewers, were for NEWS samples in NH. The CIs show that in most cases, reviewers agree about tier assignments to within one tier level. Agreement is better for state collected samples in CT and NH and better for NEWS collected samples in VT and ME. When considering all samples, there was more agreement among reviewers for state samples than there was for NEWS samples. Degrees of variability did not appear to change over the range of tiers (Figure 1). Average state and NEWS tier assignments are given in Appendix B.

Table 5. Coefficient of variation (CV) and 90% confidence interval (CI) calculated from repeated measures using NEWS and state protocols.

	CT		VT		NH	
Metric	CV	90%CI	CV	90%CI	CV	90%CI
Total taxa	23.4	±14.7	13.1	±10.1	15.8	±7.8
Shannon-Wiener Index	10.8	±0.8	6.2	±0.5	59.5	±33.1
Percent dominant	35.2	±11.9	34.1	±10.2	17.5	±1.0
EPT taxa	21.0	±4.9	18.5	±5.2	31.8	±7.6
EPT percent	38.7	±29.7	45.2	±36.3	62.7	±40.6
Mayfly taxa	25.5	±1.5	17.2	±1.6	30.4	±2.4
Mayfly percent	50.2	±9.1	50.6	±16.1	100.9	±29.7
Stone taxa	38.6	±2.1	50.6	±3.8	72.8	±3.1
Stonefly percent	48.9	±5.6	89.6	±12.1	106.5	±5.8
Caddisfly taxa	27.4	±3.2	18.1	±2.1	33.8	±4.0
Caddisfly percent	57.0	±26.9	49.6	±17.3	87.8	±26.2
Hydropsychidae taxa	27.7	±0.9	39.6	±1.0	41.9	±1.1
Hydropsychidae percent	76.7	±21.8	87.2	±13.7	138.7	±13.6
Midge taxa	46.8	±7.4	31.8	±6.8	18.2	±0.9
Midge percent	64.0	±24.3	77.3	±41.6	67.2	±43.0
Attribute 2 taxa	45.5	±1.9	46.5	±2.8	49.4	±2.1
Attribute 2 percent	54.9	±3.5	98.6	±10.2	173.7	±14.2
Attribute 3 taxa	18.6	±3.7	32.0	±8.2	35.0	±5.5
Attribute 3 percent	21.8	±11.5	63.8	±35.0	87.4	±31.4
Attribute 4 taxa	25.9	±6.8	21.0	±6.5	24.3	±5.5
Attribute 4 percent	21.4	±16.9	31.1	±25.1	38.7	±35.3
Attribute 5 taxa	68.4	±5.4	63.5	±4.2	47.2	±3.4
Attribute 5 percent	92.3	±17.0	86.0	±10.0	80.2	±15.9

Protocol Variability

BCG tier assignment variability due to protocol differences was calculated using the average of multiple reviewers' tier assignments for the samples. The CVs and CIs based on protocol differences are similar to those for reviewer differences (compare Table 8 to Table 7). For ME and NH, the 90% CI is near one tier level, indicating that the average tier assignment is generally within one tier level difference between sample collection protocols. In VT and CT, the CI is somewhat less. Based on the CV, CI and correlation coefficients of the comparison, it appears that biologists in CT were most successful at assessing samples collected using the two protocols similarly. In all states, the average tier assignments are lower (perceived better biological condition) for samples collected using state protocols compared to those collected using NEWS protocols. VT biologists were most consistent in assigning state collected samples to a lower tier. These differences existed despite the information biologists were given regarding differences in sample content due to collection protocol and their awareness of sampling protocol while assigning samples to tiers.

While all state biologists assigned NEWS samples to poorer tiers on average, the difference was only significant (Chi-square test, $p<0.05$) in VT (Figure 2). In all cases where the tier assigned to the VT sample was less than 3, the tier assigned to the NEWS sample was poorer (Figure 3). This suggests that the biologists were less likely to recognize the best biological conditions when the NEWS protocol was used and may be influenced by sampling effort, habitat sampled, and index period.

Table 6. Correlation coefficients (Pearson R) among metrics calculated from samples collected using NEWS versus state protocols, by state. For attribute metrics, correlations are shown for attributes assigned by indicator analysis, which are higher than correlations based on group consensus.

Metric	CT	VT	NH
Total taxa	0.63	0.57	0.16
Percent dominant	0.66	0.29	-0.47
Shannon-Wiener Index	0.80	0.55	0.28
EPT taxa	0.72	0.84	0.57
EPT percent	0.66	0.54	0.23
Mayfly taxa	0.79	0.76	0.56
Mayfly percent	0.73	0.69	-0.09
Stone taxa	0.68	0.69	0.47
Stonefly percent	0.78	0.37	0.29
Caddisfly taxa	0.54	0.68	0.59
Caddisfly percent	0.70	0.23	-0.14
Hydropsychidae taxa	0.38	0.45	0.44
Hydropsychidae percent	0.88	0.16	0.07
Midge taxa	0.31	0.29	-0.18
Midge percent	0.65	0.17	-0.48
Attribute 2 taxa	0.66	0.39	0.64
Attribute 2 percent	0.80	0.73	-0.02
Attribute 3 taxa	0.71	0.56	0.69
Attribute 3 percent	0.84	0.76	0.43
Attribute 4 taxa	0.48	0.29	0.49
Attribute 4 percent	0.69	0.63	-0.18
Attribute 5 taxa	0.43	0.51	0.65
Attribute 5 percent	0.84	0.53	0.51

Table 7. CV and 90% CI for BCG tier assignments of multiple reviewers, by data subset and field collection method. Regional samples were not used in the comparison analyses.

Method	Subset	CV	90%CI
NEWS	All Samples	15.2	0.97
	Maine	10.7	0.60
	New Hampshire	19.6	1.22
	Vermont	7.3	0.45
	Connecticut	15.7	1.04
State	All Samples	11.3	0.67
	Maine	21.4	1.09
	New Hampshire	11.9	0.72
	Vermont	10.5	0.55
	Connecticut	10.4	0.65

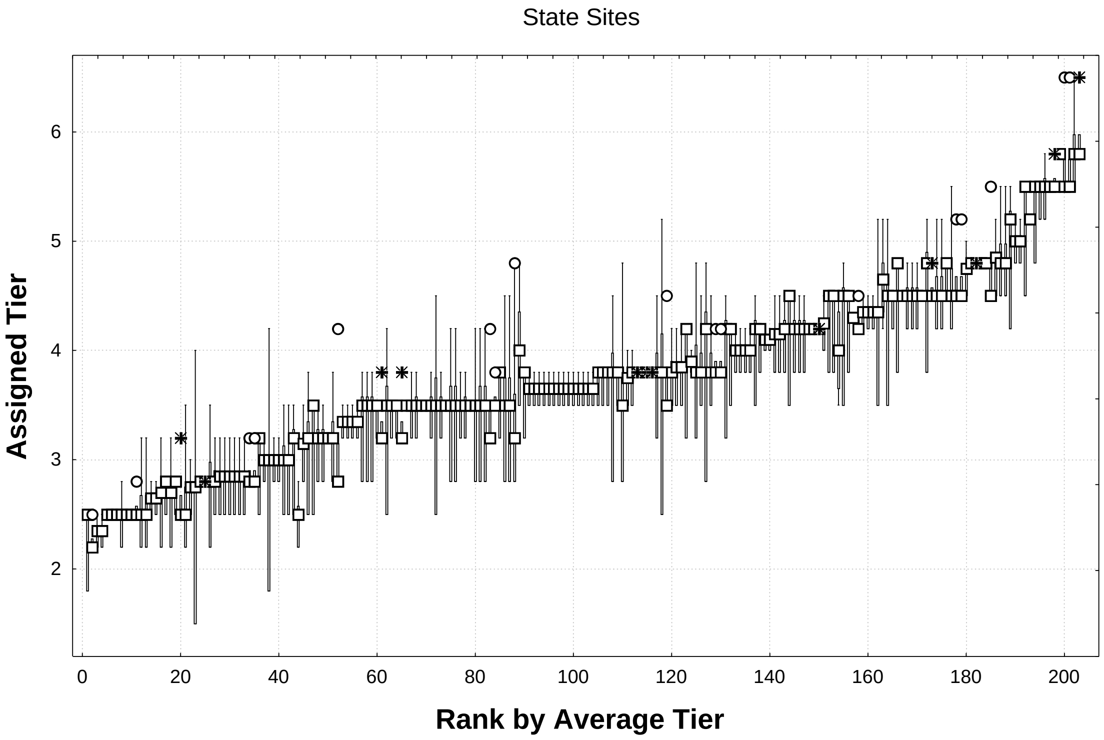

Figure 1. Variability among reviewers illustrated as ranges of BCG tier assignments made for individual samples.

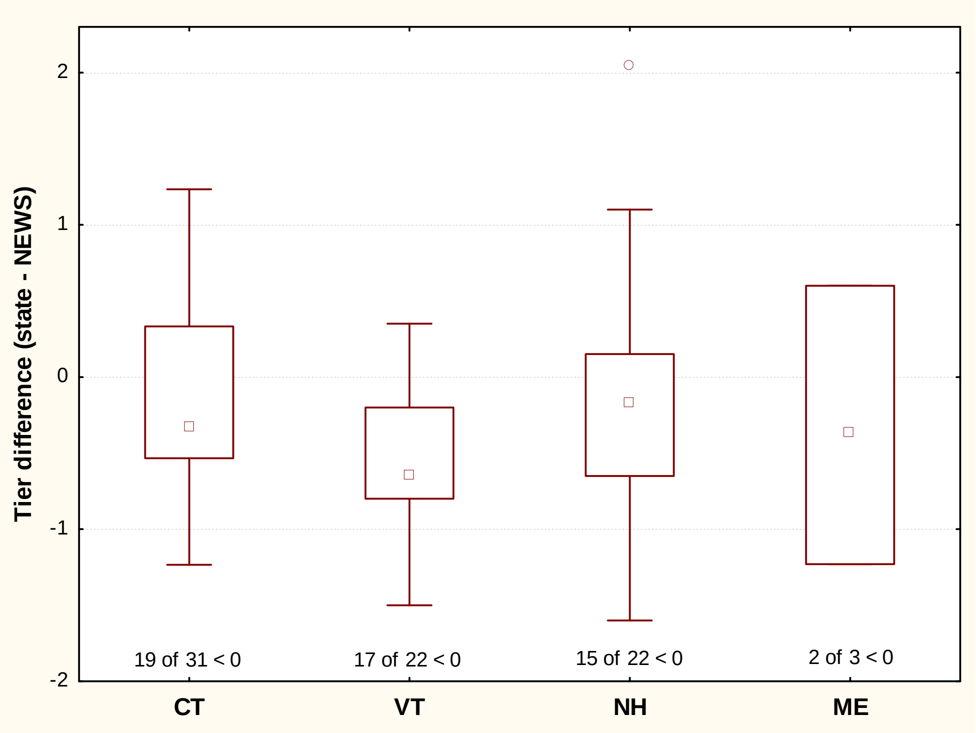

Figure 2. Differences in average tier assignments among samples collected with state and NEWS protocols, by state. Only VT shows statistical bias (Chi-square test, p<0.05).

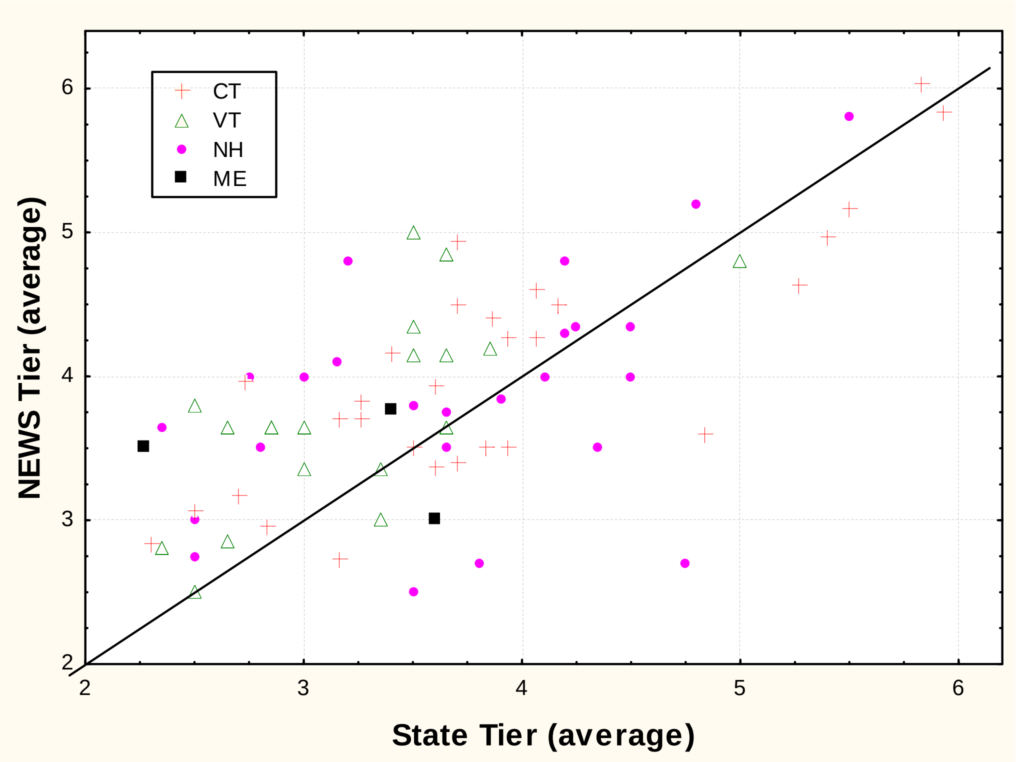

Figure 3. Average BCG tier assignments, comparing tiers assigned to state samples with those assigned to NEWS samples at individual sites. The unity line (1:1) is shown to illustrate apparent bias.

Tier Assignments with the Stressor Gradient

Tier assignments were in general agreement with environmentally defined stressor gradients for samples collected by both NEWS and state protocols (Figure 4). Tiers assigned to state samples were less than those assigned to NEWS samples over all four categories.

Table 8. Measures of variability due to protocol differences for BCG tier assignments for all samples and by state. Average values were used in deriving these statistics, except where noted that one random tier assignment was used to compare among methods.

Subset	CV	90%CI	PearsonR	AvgDiff	SD of Diff
All samples (average)	13.5	0.83	0.67	-0.28	0.66
All (random sample)	17.2	1.04			
Maine	17.8	0.95	-0.31	-0.33	0.92
New Hampshire	15.5	0.97	0.48	-0.15	0.84
Vermont	15.1	0.86	0.70	-0.57	0.49
Connecticut	10.1	0.66	0.81	-0.17	0.55

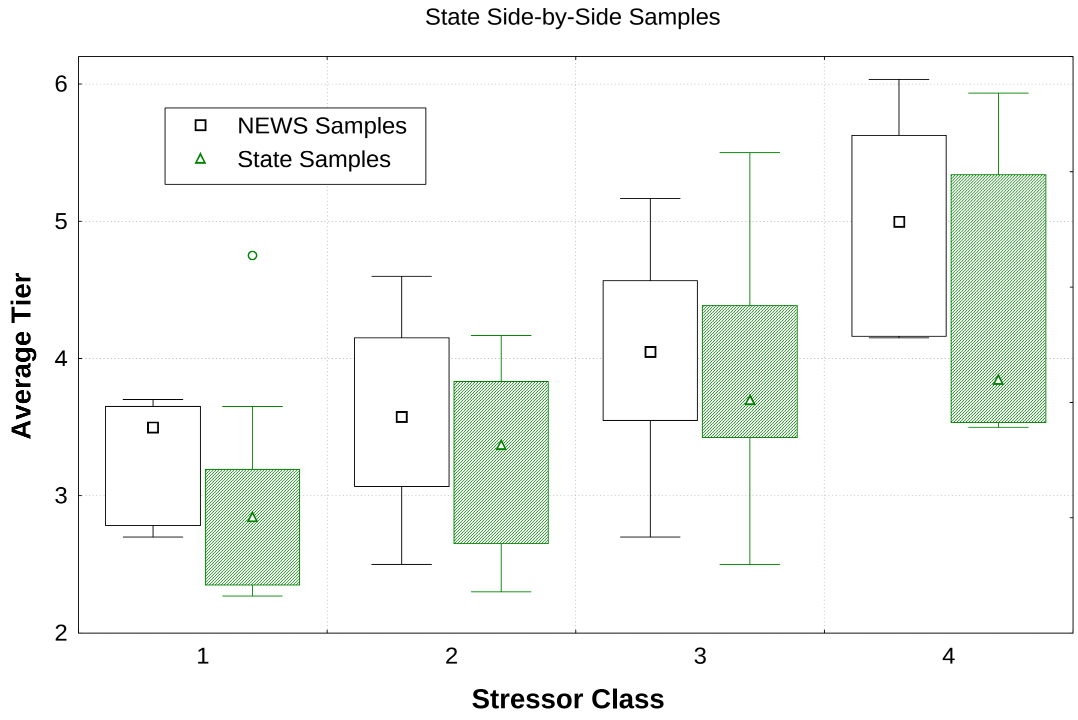

Figure 4. Distributions of average BCG tier assignments by stressor class and protocol. Stressor class 1 has the least environmental pressures.

4.0 Conclusions

The protocols used in the NEWS project and those typically used in the New England states result in benthic macroinvertebrate samples that contain somewhat different types and numbers of organisms and that are interpreted somewhat differently on the BCG scale. This may be due to different sampling target habitats, different subsampling target sizes, or different sampling equipment, among other variables. In general, the multihabitat NEWS method results in samples with greater numbers of midges and fewer mayflies, stoneflies, and caddisflies, in comparison to the riffle targeted state samples.

State biologists in all states perceived samples collected with their own methods to have a somewhat better biological condition than samples collected from the same site using NEWS methods. In CT this difference was consistently low, in NH it was low but variable, and in VT the difference was consistently greater, but still less than one tier. The NEWS methods were consistently applied as a standard for comparison in all states; therefore, there is reason to believe that state biologists have similar conceptions of the characteristics of their own macroinvertebrate samples that represent the tiers of the BCG. This suggests that assessments prepared by the individual states are based in a common conception of biological integrity. Further development of assessment tools may eventually lead to similar assessment endpoints region-wide, even across varied sampling protocols.

Assessment variability among reviewers for individual samples was similar to the variability of averages among protocols, with 90% confidence intervals of ±1 tier or less. It is clear that tier assignments were lower for state collected samples, but it is not clear that reviewer variability was consistently biased by the individual reviewer.

Connecticut

In terms of representativeness, the CT protocols are more similar to the NEWS protocols than are other states. CT targets riffle habitats and NEWS targets multiple habitats. Nine of 23 metrics showed significant bias due to protocol, including total taxa, which was higher in the NEWS samples. However, there was relatively high correlation among metrics produced by both protocols and BCG tier assignments were not significantly biased. The average tier assignment was higher in NEWS samples, compared to state samples, by about one fifth of one tier. The CT data set is more comparable to the NEWS data set than the other states.

Vermont

VT protocols differ from NEWS protocols in the habitat sampled and the subsample target size and index period. Metrics describing sample contents show significant bias in 16 of 23 metrics. Bias persisted in the assessments, where BCG tier assignments were higher in NEWS samples despite efforts to calibrate to known differences in sample content. Tier assignments were correlated among protocols, showing that the bias in assessments was a consistent shift in the BCG scale. Because the shift was consistent, additional analysis may provide a method for predicting tier assignments across methods.

New Hampshire

In NH, samples represent different habitats, are collected with different equipment, and are subsampled to different target levels. With all these possible sources of variability it is not surprising to find that both sample contents and BCG tier assignments differed due to the protocol used to collect the sample. While relatively few metrics showed significant bias with protocol (8 of 23), there was also low correlation of metrics between sampling protocols. This combination of indicators suggests that there is little pattern of difference in the sample contents due to protocol. It may be due to the somewhat random difference in sample content that there was also low correlation between BCG tier assignments for samples resulting from the two protocols. On average, tier assignments were lower for state collected samples, but the bias was not significant.

Maine

Sampling protocols in Maine specify use of artificial substrates (rock baskets), as in New Hampshire. Only three comparable samples were collected using state protocols in Maine and quantitative analyses were not conducted because of this small sample size.

Massachusetts and Rhode Island

Comparable samples were not collected using state methods in MA or RI. Therefore, the assessment of comparability did not include these states.

Recommendations

None of the analyzed state data were comparable to the NEWS data to a degree that would allow analyses on data sets with samples collected using multiple protocols. Analyses should be performed using data collected with NEWS protocols for regional assessment. State data should be used for analysis or model development within each state; extrapolation of model predictions to data sets outside of the state or collected with other protocols is not recommended.

The assessment comparison results suggest a consistent bias to rate state collected samples as having better biological conditions than NEWS, multihabitat samples. This is despite a training period in which sample expectations were presented to the biologist reviewers. A preliminary assessment model has been developed based on tier assignments and sample contents in CT. It is likely that decision rules in the model could be adjusted to account for the differences in sample content that are due to protocols, especially in VT, where the bias seems so consistent.

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Table J-1. Average BCG tier assignments for samples collected with NEWS and state protocols.

Station ID	Stream Name	NEWS	State
Connecticut			
CT HEX 39.01	Beaver Meadow Brook	4.2	3.4
CT HEX 33.04	Bentley Brook	4.5	4.2
CT HEX 47.02	Bladdens Brook	4.3	4.1
CT HEX 27.02	Bull Mountain Brook	4.6	4.1
CT HEX 59.01	East Branch Byram River	4.5	4.2
CT HEX 25.03	Ekonk River	3.9	3.6
CT HEX 21.02	Farmington River	3.4	3.7
CT HEX 31.02	Flat Brook	3.5	3.5
CT HEX 51.02	Flat Brook	3.6	4.8
CT HEX 11.02	Indian Meadow Brook	3.1	2.5
CT HEX 19.02	Lake Waramaug Brook	3.5	3.8
CT HEX 41.05	Latimer Brook	4.3	3.9
CT HEX 45.04	Limekiln Brook	5.0	5.4
CT HEX 17.08	Mashamoquet Brook	3.8	3.3
CT HEX 13.02	Mountain Brook	3.2	2.7
CT HEX 20-LG	Natchaug River	4.0	2.7
CT HEX 29.03	Naugatuck River	6.0	5.8
CT HEX 37.01	Naugatuck River	5.8	5.9
CT HEX 57.04	Neck River	4.9	3.7
CT HEX 53.04	Norwalk River	4.6	5.3
CT HEX 49.05	Pond Meadow Brook	4.4	3.9
CT HEX 55.01	Pumpkin Ground Brook	5.2	5.5
CT HEX 9.02	Rocky Brook	3.5	3.8
CT HEX 1.08	Sages Ravine Brook	3.7	3.3
CT HEX 3.01	Sandy Brook (sampled 2002)	2.8	2.3
CT HEX 3.01	Sandy Brook (sampled 2003)	3.0	2.8
CT HEX 35.05	Sawmill Brook	2.7	3.2
CT HEX 43.01	Shunock River	4.5	3.7
CT HEX 52.07	Shunock River	3.4	3.6
CT HEX 7.06	Stickney Hill Brook	3.7	3.2
CT HEX 44.01	Titicus Brook	3.5	3.9
Vermont			
VT HEX 12.01	Andover Branch	2.9	2.7
VT HEX 7.01	Batten Kill River	4.4	3.5
VT HEX 13.03	Blood Brook	3.4	3.0
VT HEX 2.01	East Branch North River	3.7	3.0
VT HEX 14.02 (RD)	Gihon River	3.4	3.4
VT HEX 8.02 (2006)	Goshen Brook Trib #2	2.8	2.4
VT HEX 1.01 (RD)	Hancock Brook	4.2	3.5
VT HEX 14.02	Hubbard Brook	3.0	3.4
VT HEX 4.01 (RD)	Kent Pond Outlet	4.8	5.0
VT HEX 8.02 (2005)	Miles Stream	3.7	2.9
VT HEX 13.03 (RD) (2004)	Mill Brook (Fairfax VT)	4.2	3.7
VT HEX 10.01 (RD) (2004)	Mill Brook (Rupert VT)	4.9	3.7
VT HEX 4.01 (2004)	Mill River (Georgia VT)	5.0	3.5
VT HEX 15.01 (2005)	Morril Brook	3.7	2.7

Table J-1. Continued.

Station ID	Stream Name	NEWS	State
VT HEX 15.01 (2004)	Mosher Meadow Brook	3.7	2.9
VT HEX 11.01	Mount Tabor Brook	3.7	2.9
VT HEX 8.02	North Branch Ball Mtn. Brook	3.7	2.9
VT HEX 8.01 (2004)	Pherrins River	3.7	2.9
VT HEX 2.03	Seaver Brook	4.2	3.9
VT HEX 6.02	Trib To Green River	3.8	2.5
VT HEX 8.01	Wardsboro Brook Trib #5	2.5	2.5
VT HEX 5.01 (RD)	Whitman Brook Trib # 1	3.7	3.7
New Hampshire			
NH HEX 28.03	Ames Brook	3.8	3.5
NH HEX 57.03	Ash Swamp Brook	4.4	4.5
NH HEX 61.04	Beaver Brook	4.0	4.1
NH HEX 10.02	Bog Brook	3.0	2.5
NH HEX 11.01	Bumpus Brook	2.7	4.8
NH HEX 35.01	Churchill Brook	4.4	4.3
NH HEX 17.03	Clark Brook	4.1	3.2
NH HEX 52.01	Contoocook River	3.8	3.7
NH HEX 44.02	Dodge Brook	4.3	4.2
NH HEX 16.02	East Branch Saco River	3.5	2.8
NH HEX 18.01	Eastman Brook	2.5	3.5
NH HEX 21.05	Grant Brook	4.0	2.8
NH HEX 26.05	Hewes Brook	2.7	3.8
NH HEX 2.05	Indian Stream	4.8	4.2
NH HEX 23.01	Johnson Brook	3.7	2.4
NH HEX 58.03	Mill Brook	4.8	3.2
NH HEX 9.05	Newell Brook	2.8	2.5
NH HEX 51.04	Otter Brook	4.0	3.0
NH HEX 45.04	Piscataquog River	3.9	3.9
NH HEX 30.02	Poland Brook	3.5	4.4
NH HEX 53.01	Purgatory Brook	3.5	3.7
NH HEX 54.01	Riddle Brook	5.8	5.5
NH HEX 59.03	Souhegan River	4.0	4.5
NH HEX 48.04	Winnicut River	5.2	4.8
Maine			
ME HEX 30.01	Cupsuptic River	3.5	2.3
ME HEX 48.05	Halfmoon Stream	3.8	3.4
ME HEX 55.02	Mill Brook	3.0	3.6

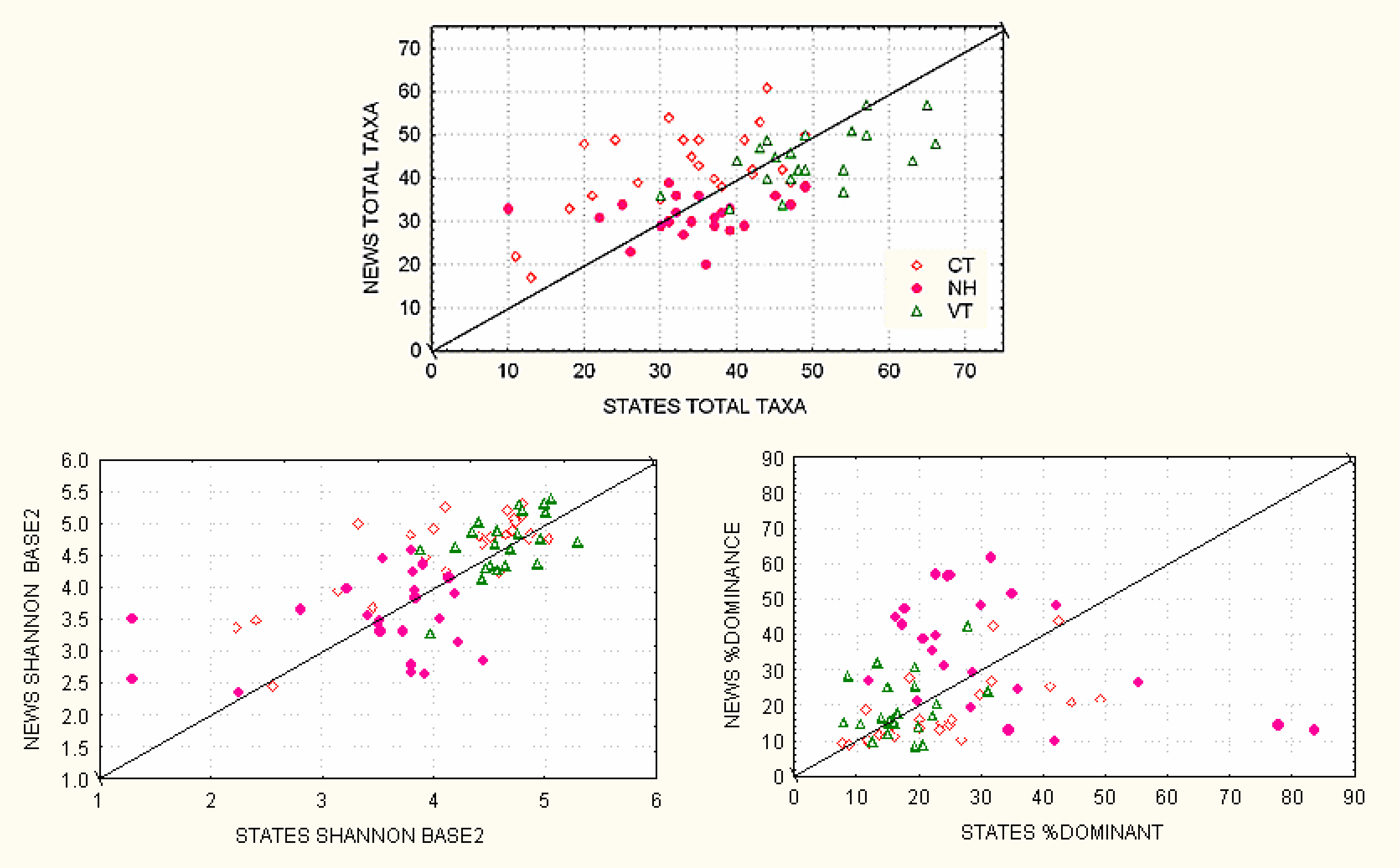

Figure K-1. Comparisons of richness metrics among state (x-axis) and NEWS (y-axis) collected samples.

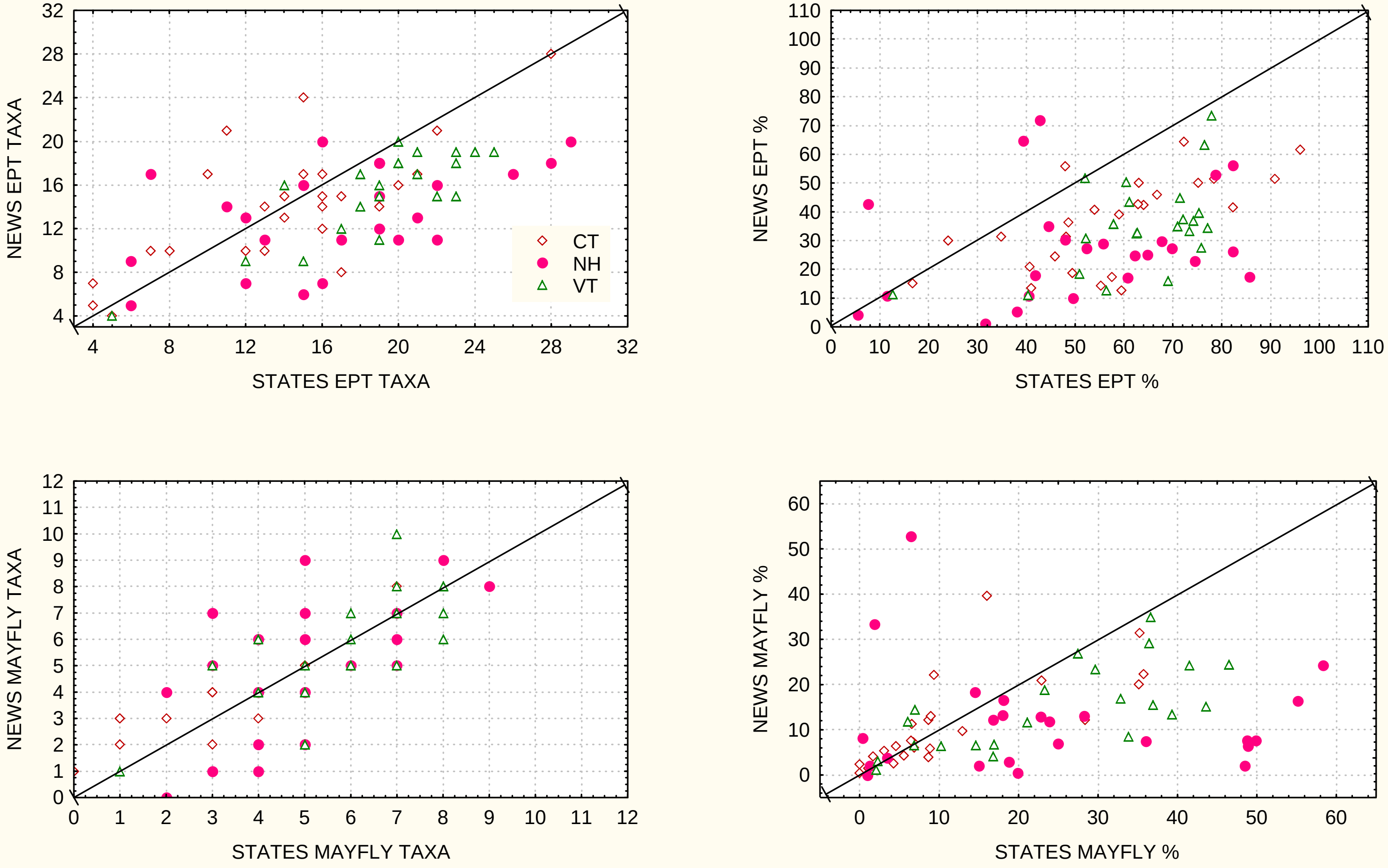

Figure K-1. Comparisons of richness and composition metrics among state (x-axis) and NEWS (y-axis) collected samples.

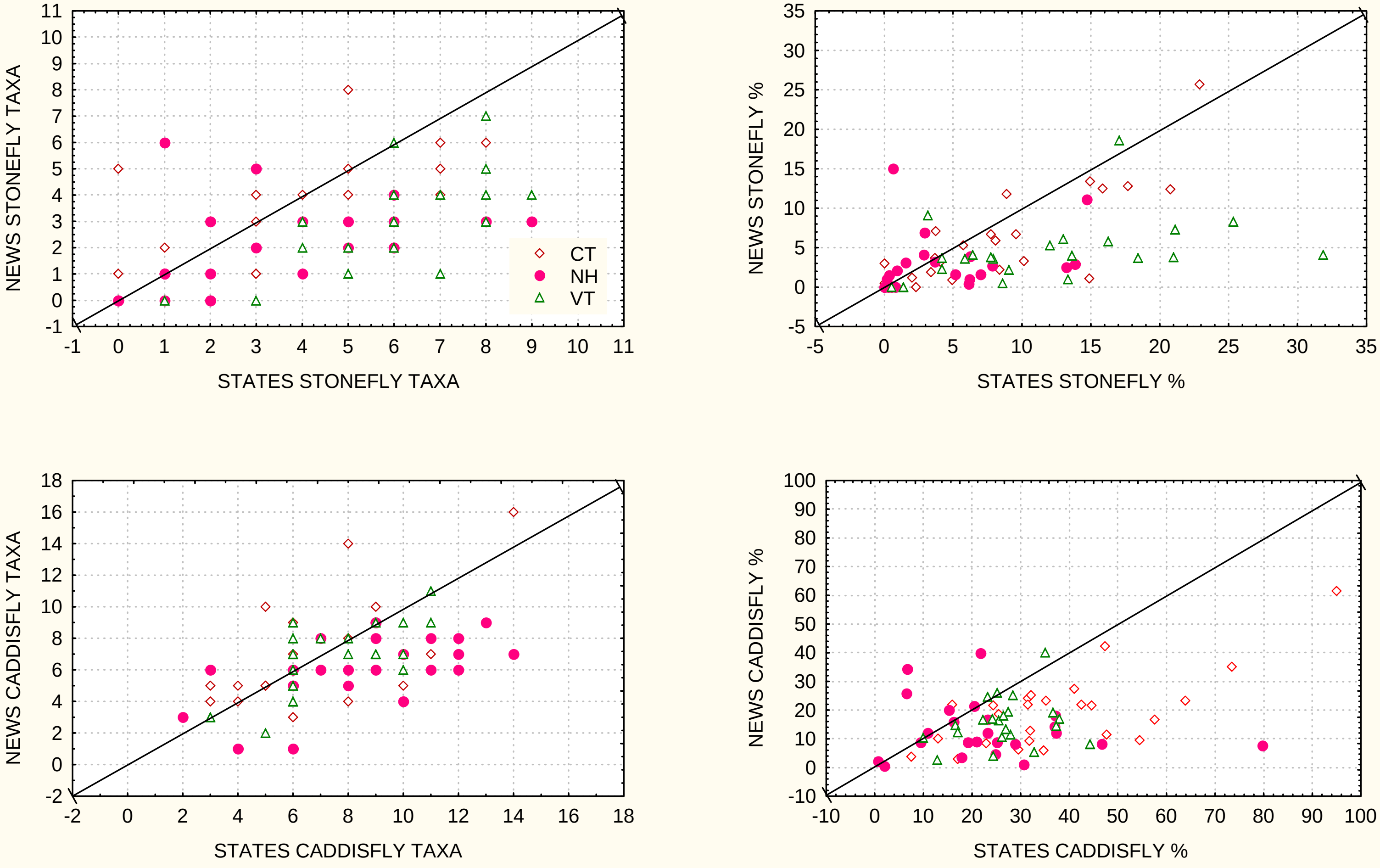

Figure K-1. Comparisons of richness and composition metrics among state (x-axis) and NEWS (y-axis) collected samples.

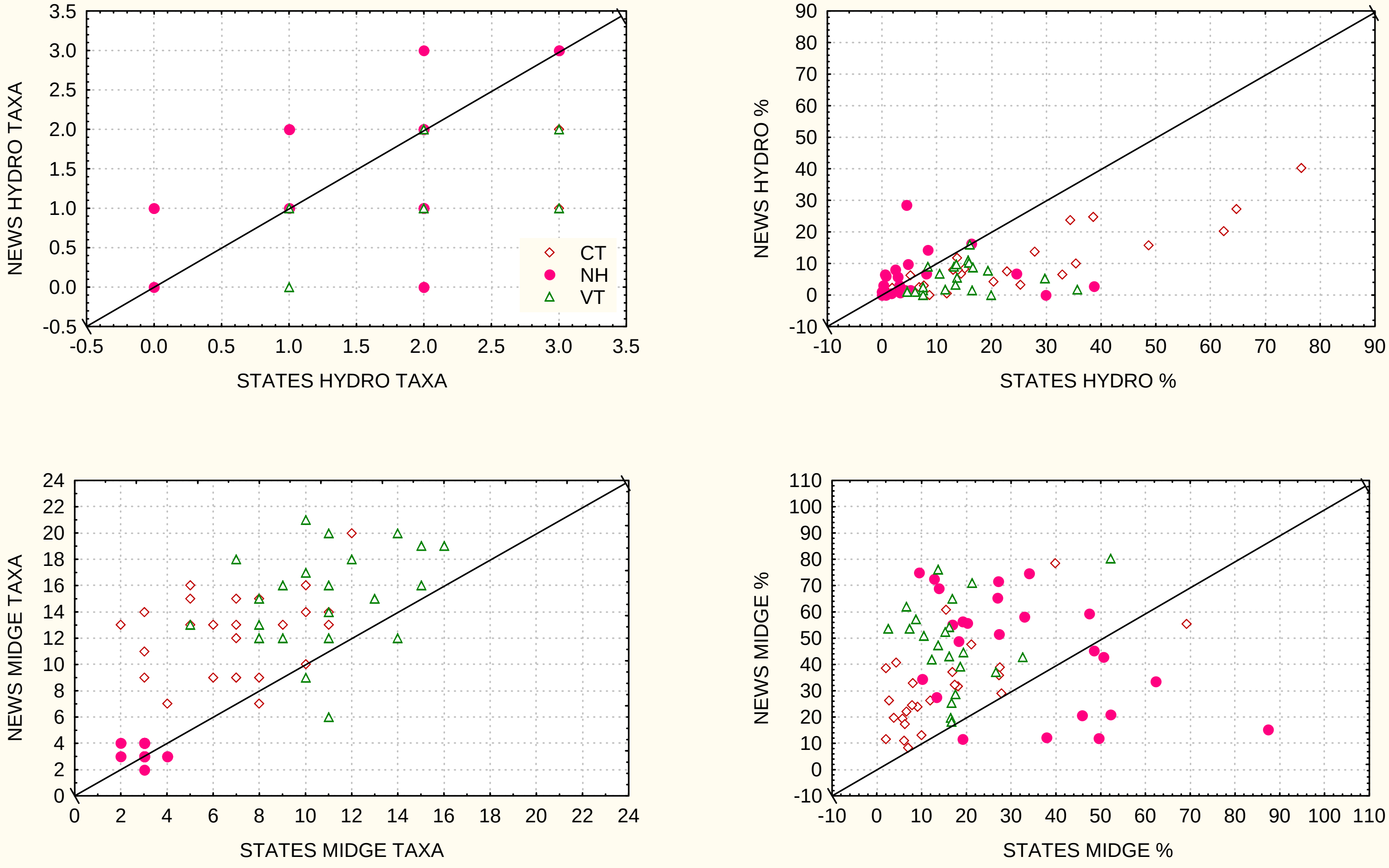

Figure K-1. Comparisons of richness and composition metrics among state (x-axis) and NEWS (y-axis) collected samples.

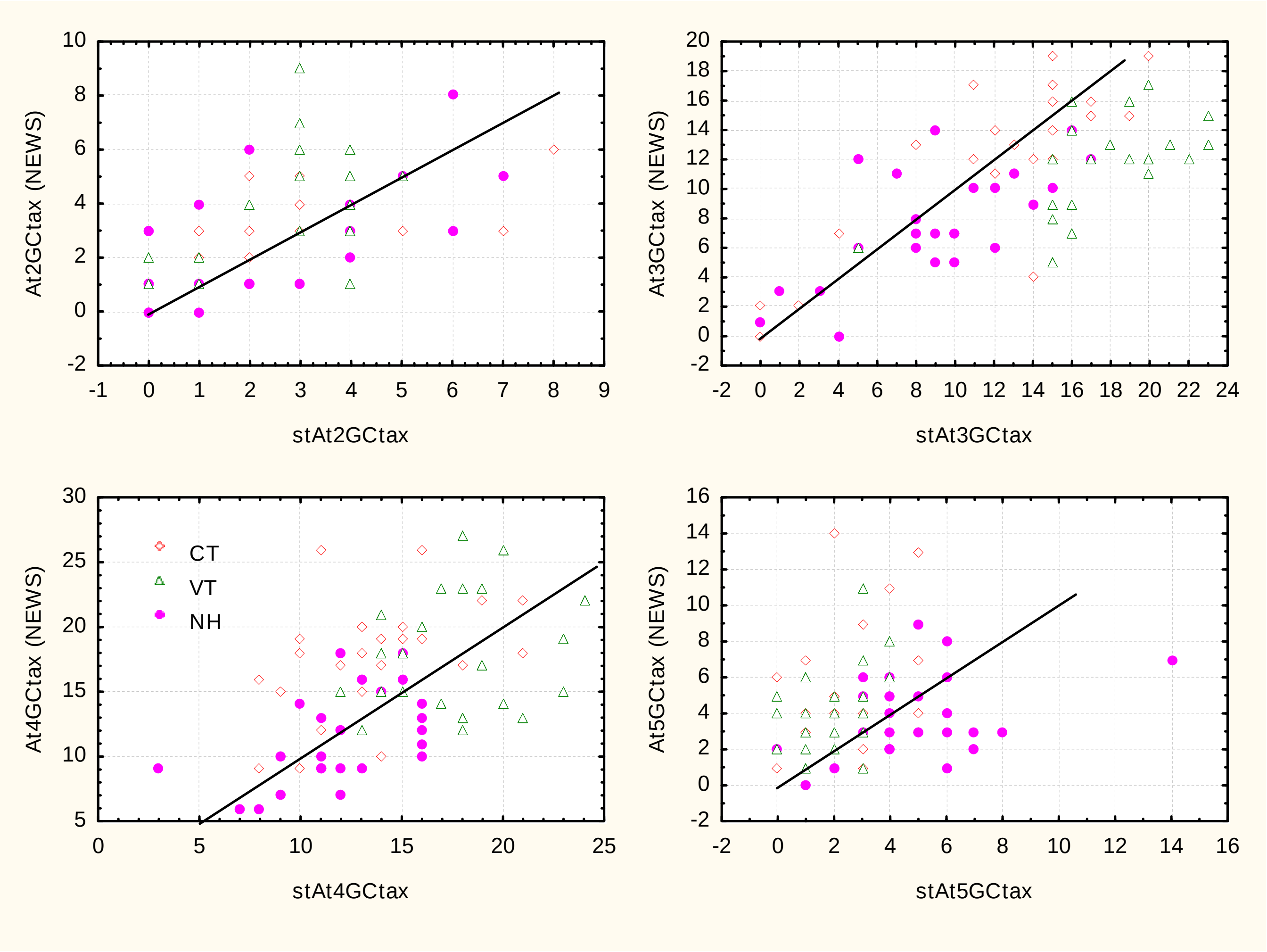

Figure K-2. Comparisons of taxa counts by attribute among state (x-axis) and NEWS (y-axis) collected samples. From upper left attributes are 2, 3, 4, and 5.

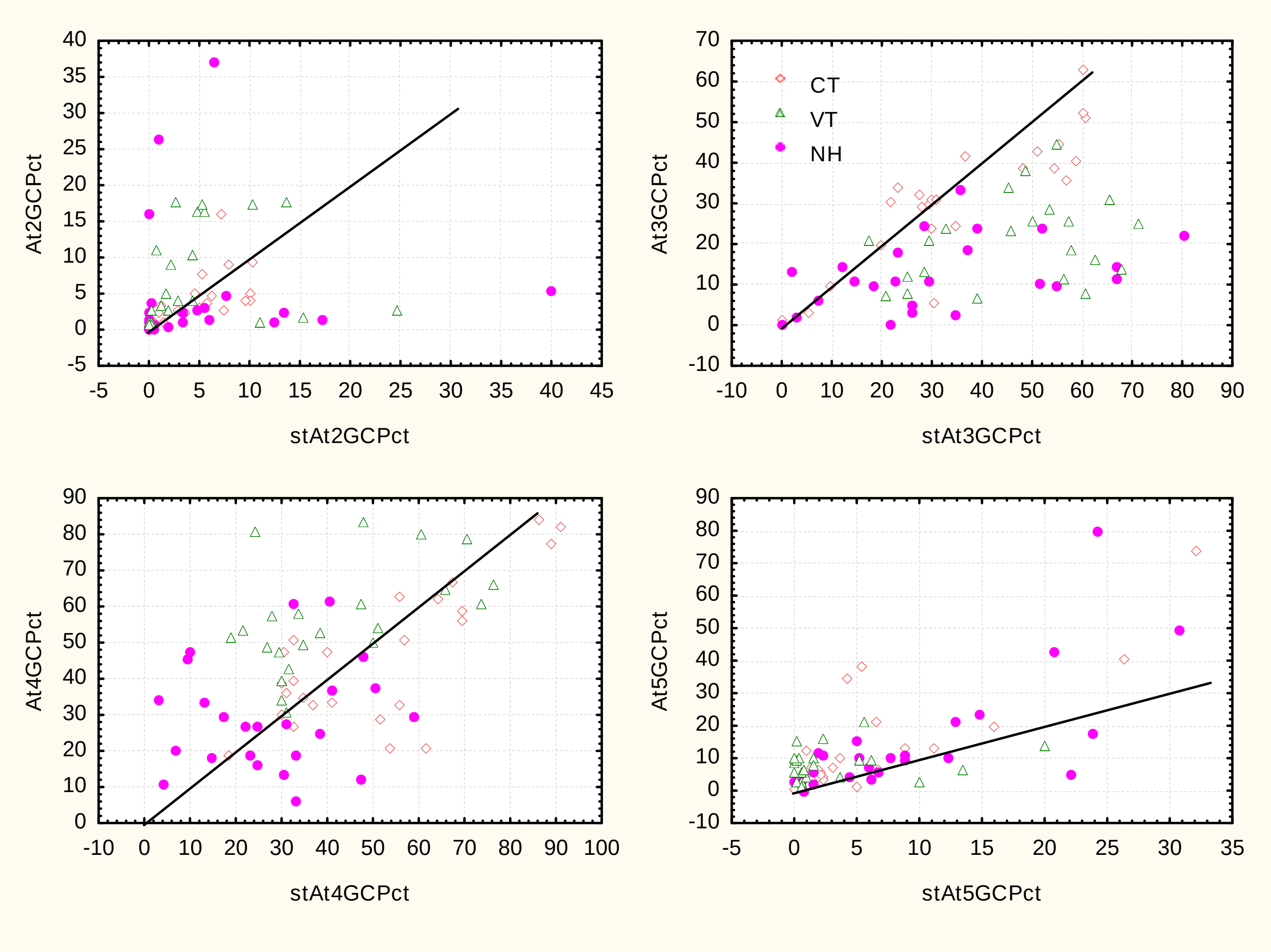

Figure K-2. Comparisons of percentage of individuals by attribute among state (x-axis) and NEWS (y-axis) collected samples. From upper left attributes are 2, 3, 4, and 5.