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A Review of Biological Assessment Tools and Biocriteria for Rivers and Streams in New England States

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A Review of Biological Assessment Tools and Biocriteria for Streams and Rivers in New England States

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TABLE OF CONTENTS

LIST OF FIGURES AND TABLES

FIGURES

TABLES

ACRONYMS AND COMMON TERMS

305(b) Report: Clean Water Act (CWA) section 305(b) requires each state to submit an assessment report biennially to U.S. EPA on the quality of surface and ground water resources. The EPA then compiles the data from state 305(b) reports and submits a National Water Quality Report to Congress

303(d) List: The section of the Clean Water Act that requires each state to identify waters that are impaired according to water quality standards. Placement of water bodies on this list requires the preparation of Total Maximum Daily Loads (TMDLs) that will aid in the cleanup of the impacted waters.

7Q10: The lowest consecutive 7-day mean stream flow that occurs during a 10-year period

30Q5: The lowest consecutive 30-day mean stream flow that occurs during a 5-year period.

ABN: Ambient Biomonitoring Network [Vermont]

AFDM: Ash Free Dry Mass

ALU: Aquatic Life Use

ALUS: Aquatic Life Use Support

BASS: Biomonitoring and Aquatic Studies Section [Vermont]

B-IBI: Benthic Index of Biotic Integrity. [New Hampshire]

BPJ: Best Professional Judgment

CALM: Consolidated Assessment and Listing Methodology

CT DEP: Connecticut Department of Environmental Protection

CWA: Clean Water Act

CWIBI: Cold Water Index of Biotic Integrity [Vermont]

DO: Dissolved oxygen

EDAS: Ecological Data Assessment System: A database system developed by Tetra Tech, Inc. that uploads ecological data to STORET for archival and has the capability to analyze, manage and store data and calculate metrics.

EMAP: Environmental Monitoring and Assessment Program

EPT: Insect orders of Ephemeroptera, Plecoptera, Trichoptera, considered sensitive benthic orders.

FBI: Family Biotic Index

GIS: Geographic Information Systems

GWHI: Ground Water Hazard Inventory

HBI: Hilsenhoff Biotic Index

HDG: Human Disturbance Gradient [New Hampshire]

HUC: Hydrologic Unit Code

IBI: Index of Biotic Integrity

MDEP: Maine Department of Environmental Protection

NA: non-attainment

MA DWM: Massachusetts Division of Watershed Management

MA DEP: Massachusetts Department of Environmental Protection

MHG: Medium High Gradient Streams [Vermont]

NH DES: New Hampshire Department of Environmental Services

MWIBI: Mixed Water Index of Biotic Integrity [Vermont]

NEWS: New England Wadeable Streams Project

NHLC: New Hampshire Land Cover

NPDES: National Pollutant Discharge Elimination System

ONRW: Outstanding National Resource Waters

QAPP: Quality Assurance Project Plan

RCRA: Resource Conservation Recovery Act

RBP: Rapid Bioassessment Protocol

RI DEM: Rhode Island Department of Environmental Management

RPS: Rapid Periphyton Survey (RBP)

SHG: Small High Gradient Streams [Vermont]

SOP: Standardized Operating Procedure

TMDL: Total Maximum Daily Load

WBS: Water Body System- Database developed by USEPA to store information to be used in 305(b) reporting and 303(d) listing for all assessed water bodies within a region

WPCA: Water Pollution Control Act

WQS: Water Quality Standards

WWMG: Warm water medium grade streams and rivers [Vermont]

VT DEC: Vermont Department of Environmental Conservation

1 INTRODUCTION

1.1 Purpose of the Document

The primary purpose of this document is to serve as a detailed description of the biological assessment programs for wadeable streams and rivers within U.S. Environmental Protection Agency (U.S. EPA) Region 1 states (i.e., Connecticut, Maine, Massachusetts, New Hampshire, Rhode Island, and Vermont). Specifically, this report concentrates on the target assemblages (e.g., benthic macroinvertebrates, periphyton, and/or fish) and the specific methods used by each state to determine whether biocriteria set for aquatic life use (ALU) are met in wadeable streams and rivers. The information contained in this report is critical to the eventual use of state data in assessing water resources on a national scale because it provides the necessary level of detail on New England state bioassessment methodologies in a single document. In addition, this report serves as a valuable resource for other states, tribes, and municipalities, both those developing bioassessment tools and those with existing programs.

Although every attempt has been made to represent the methods and protocols used by each state accurately, this document is not intended to be used as a replacement for those protocols and Standard Operating Procedures (SOPs) that are used and approved by the state agencies. Thanks to the cooperation of state scientists, all protocols and procedures were obtained through personal communication and via state and federal published and unpublished documents that are referenced within this report. Each state reviewed its respective chapter for technical accuracy and was given the opportunity to provide comments and changes prior to completion of this report. However, we recommend referring directly to state protocols before implementation of the described methods to ensure that the most updated and complete versions of protocols are used. Contact details for each of the state bioassessment programs discussed are provided in Table 1-1.

1.2 Rational for Bioassessment Programs

The modern Clean Water Act (CWA) is derived from the 1948 Federal Water Pollution Control Act (WPCA). After the passage of the 1972 amendments, the act became commonly known as the CWA and its goal was to "restore and maintain the chemical, physical and biological integrity of the nation's waters so that they can 'support the protection and propagation of fish, shellfish, and wildlife and recreation in and on the water'" (http://www.epa.gov/watertrain/cwa/). This act federally recognized the aquatic inhabitants of water bodies and began to set water quality standards to protect these organisms. The CWA amendments through 1987 outlined the guidelines by which states and tribes must use bioassessment programs and develop biocriteria to ensure the adherence to water quality standards. Specifically, Section 303(c) of the CWA requires states to have water quality standards (WQS) that consist of three components: 1) designated uses, 2) water quality criteria to protect those uses, and 3) an anti-degradation policy. States are required to review their standards every three years and revise them as needed to achieve the purposes of the CWA, including the ecological integrity objective.

Table 1-1. Contact information for bioassessment programs in New England states.

State	Program Contact	Web Site
Connecticut	**Ernest Pizzuto, Jr.** Supervising Environmental Analyst Connecticut Department of Environmental Protection **Address:** 79 Elm St. Hartford, CT 06106-5127 **Phone:** 860-424-3715 **Email:** ernest.pizzuto@po.state.ct.us	http://dep.state.ct.us/
Maine	**Susan P. Davies** Program Manager, Biologist III Maine Department of Environmental Protection **Address:** SHS 17 Augusta, ME 04333 **Phone:** 207-287-7778 **Email:** susan.p.davies@maine.gov	http://www.maine.gov/dep/
Massachusetts	**Arthur S. Johnson** Environmental Monitoring Coordinator Massachusetts Department of Environmental Protection **Address:** 627 Main Street Worcester, MA 01608 **Phone:** 508-767-2873 **Email:** arthur.johnson@state.ma.us	http://www.state.ma.us/dep/
New Hampshire	**David Neils** Biomonitoring Program Coordinator New Hampshire Department of Environmental Services **Address:** 6 Hazen Drive Concord, NH 03302-0095 **Phone:** 603-271-8865 **Email:** dneils@des.state.nh.us	www.des.state.nh.us/

State	Program Contact	Web Site
Rhode Island	**Connie Carey** Environmental Scientist Rhode Island Department of Environmental Management **Address:** 235 Promenade Street Providence, RI 02908-5767 **Phone:** 401-222-4700 x7239 **Email:** ccarey@dem.state.ri.us	http://www.state.ri.us/dem/
Vermont	**Doug Burnham** Biomonitoring and Aquatic Studies Section Chief Vermont Department of Environmental Conservation **Address:** 103 South Main Street-10N Waterbury, VT 05671 **Phone:** 802-241-3784 **Email:** doug.burnham@anr.state.vt.us	http://www.anr.state.vt.us/

1.2.1 Designated Uses

As required by CWA 40 C.F.R. § 130.10, states, territories and tribes must specify appropriate beneficial uses based on the intended use and the value of the waters, and these uses must be achieved and protected. Designated uses may be listed as general categories (e.g., drinking water source, wildlife, shellfish, aquatic life, recreational, industrial), or the uses may consist of more specific sub-categories that may target cold water versus warm water systems or contain special uses that are meant to protect unique, sensitive, or valuable aquatic habitat (U.S. EPA 1991). These designated uses are typically associated with a classification system (e.g., Class A waters, Class B waters, Class C waters) within each state's WQS that categorizes each water body according to condition.

1.2.2 Water Quality Criteria for Aquatic Life Use

Water quality criteria are narrative or numeric descriptions of those conditions that protect designated uses. In addition, the criteria need to be scientifically consistent with the intended designated use and must be accurate indicators of the designated use. Although the U.S. EPA has published guidance criteria to protect aquatic life use (U.S. EPA 2002a), individual states are not required to follow them and may develop their own criteria. Guidance for the development of numeric criteria are published in the CWA § 104(a)(1) and may be modified based on the needs of the state. Currently, only narrative descriptions of criteria for aquatic life use support are required within state WQS by the U.S. EPA. The narrative criteria are simply descriptions of the conditions necessary for a water body to attain its designated use

(U.S. EPA 2002b), and these definitions, along with organisms that can be used to assess attainment, vary from state to state in U.S. EPA Region 1.

Each state in Region 1 has established its goal for protecting waters and then defined aquatic life use (ALU). For example, New Hampshire statutes define waters achieving ALU as those waters that "provide suitable chemical and physical conditions for supporting a balanced, integrated and adaptive community of aquatic organisms (NH DES 1999). Aquatic life use support (ALUS) as defined in Rhode Island WQS is "providing suitable habitat and water quality for the protection, maintenance, and propagation of a viable community of aquatic life" (RI DEM 2000). Section 3-01 of the Vermont Water Quality Standards states the provision to, "establish and apply numeric biological indices to determine whether there is full support of aquatic biota and aquatic habitat uses" and to "establish procedures that employ standard sampling and analytical methods to characteristics of the biological integrity of the appropriate reference conditions" (State of Vermont 2000). In Massachusetts, the ALUS criteria of the standards 314 CMR 4.00 "must provide suitable habitat for sustaining a native, naturally diverse community of aquatic flora and fauna" (MA DEP 2000; 2003). Massachusetts then further designates two subclasses: Cold Water Fishery - capable of sustaining a year-round population of cold water aquatic life; and Warm Water Fishery - waters that are not capable of sustaining a year-round population of cold water aquatic life (MA DEP 2003). Connecticut WQS express that "the benthic invertebrate criteria may be utilized where appropriate for assessment of the biological integrity of surface waters. These criteria apply to the fauna of erosional or riffle habitats in lotic waters which are not subject to tidal influences" (CT DEP 2002). Connecticut defines biological integrity as the "ability of an aquatic ecosystem to support and maintain a balanced, integrated, adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of the natural habitats of a region" (CT DEP 2002). In Maine, the use of benthic organisms to determine the attainment of ALU is written directly into the standards in chapter 579 (MDEP 2003). Chapter 579 gives a detailed description of the use of benthic organisms and the methods used to make decisions about classification attainment (MDEP 2003). Furthermore, narrative standards in Maine Revised Statutes Annotate 38 Public Chapter 3 Article 4-A § 464 and § 465 define the biological narrative and numerical dissolved oxygen and bacterial standards.

1.2.3 Anti-degradation Policies

The anti-degradation policy (CWA 40 CFR §131.12) is a set of rules designed to protect high quality waters. This policy must offer a framework of decision-making if water quality changes occur. The U.S. EPA WQS require states to implement a three-tiered system for addressing anti-degradation. Tier 1 requires that water quality necessary to support existing uses is maintained and protected, Tier 2 states that in no case shall water quality decrease to a level that would interfere with the designated use, and Tier 3 maintains and protects outstanding national resource waters (ONRW), aiming to preserve those waters with exceptional recreational or ecological significance (U.S. EPA www.epa.gov/waterscience/standards/about/adeg.htm).

1.2.4 Guidance Documents

To support the assessment of attainment of beneficial uses, states are responsible for implementing a biological monitoring strategy for the design, collection and data analysis of

biological data. The U.S. EPA has published technical documents and guidance documents to offer support for the development of state biomonitoring programs for the assessment of water quality for ALUS. The most current documents include the Guidance for 2004 Assessment, Listing and Reporting Requirements Pursuant to Sections 303(d) and 305(b) of the Clean Water Act; TMDL-01-03 (U.S. EPA 2003), and The Consolidated Assessment and Listing Methodology (CALM), Toward a Compendium of Best Practices (U.S. EPA 2002b), both of which provide a framework for documenting the collection and use of water quality data for CWA Section 305(b) reporting, determining attainment of WQS, determining stream impairment for CWA Section 303(d) listing, and establishing anti-degradation policies.

This document attempts to follow the general framework provided by the CALM document to organize the information for each of the Region 1 states in a format that is conceptually easy for comparisons to be made among biomonitoring programs.

1.2.5 Biological Monitoring Programs

After beneficial uses are established, the criteria are set, and the anti-degradation policy is in place, each state then implements a monitoring program. Bioassessment programs have been employed by states to assess the water quality with established biocriteria for a range of designated uses in freshwater systems. Bioassessment is used for a number of designated uses, which may include drinking water, recreation, industry, wildlife, agriculture, and others, but it is most commonly used to evaluate aquatic life use support. In 1991, U.S. EPA policy stated the necessity of integrating biological surveys with toxicity and chemical-specific assessment methods into monitoring programs to determine the attainment or non-attainment of aquatic life use support (U.S. EPA 1991). As of 2001, 40 entities, including all of the Region 1 states, used bioassessment to determine ALUS for 305(b) reporting (U.S. EPA 2002c).

Currently, the U.S. EPA CALM guidance suggests four categories of data that may be collected and integrated to determine ALUS. These four categories are: biological, habitat, toxicological, and physical/chemical data (U.S. EPA 2002b). Although all categories of data are potentially useful depending on the rigor involved in the assessment method, only biological data provide a direct measurement of the resident aquatic organisms that integrates the abiotic conditions in the water body (U.S. EPA 2002b). The CALM document advises that states use biological data "as a core indicator for aquatic life use determinations, as they are a unique water body response measurement, providing information about a water body that no other measurement can" (U.S. EPA 2002b). The document continues to stress that the state "documentation of the adequate quality and rigor of the key elements of the state's bioassessment program" be provided so that the biological data can accurately assess water quality (U.S. EPA 2002b).

1.2.6 Bioindicator Organisms

Biological assessments of those organisms present in the aquatic system offer the most direct way to measure the condition of the biological community as a function of environmental stressors (Yoder and Rankin 1995). Community composition may be altered as a result of stresses in the system and the condition of individual organisms can show pollution impacts that may act as an early warning detection of degradation or provide a more reliable assessment of changes in the biological community over time (U.S. EPA 2002d). There are several possible

assemblages of organisms available for use in bioassessment. However, benthic macroinvertebrates, periphyton, and fish are the biological indicators suggested for use by the U.S. EPA in lotic environments (Barbour et al. 1999). All three assemblages are widely used in bioassessment, but macroinvertebrates and fish are the most common indicator organisms, with 45 entities using two to four assemblages (U.S. EPA 2002c). Although standard methods for sampling each of these assemblages are suggested by the U.S. EPA, many states alter the methods to fit into the goals of their program, adjust for ecoregional constraints, and accommodate limited budgets.

Benthic macroinvertebrates are the most commonly used assemblage. As of 2001, all 57 of the entities with a bioassessment program in place either currently used or were developing macroinvertebrate indicators (U.S. EPA 2002c). Benthic macroinvertebrates are a diverse assemblage, consist of species exhibiting a range of pollution tolerance levels, and are abundant in most streams (Plafkin et al. 1989, Barbour et al. 1999). Furthermore, they often live the majority of their lives in direct contact with both the water and sediments and their life cycles may span multiple seasons, thereby showing cumulative changes. They also serve as an important link in the food chain (Plafkin et al. 1989), maintaining the rest of the aquatic community and managing algal systems. Benthic macroinvertebrates are easy and affordable to collect, making them extremely attractive for biological monitoring.

The advantage of using periphyton as an indicator is that growth of this assemblage is directly related to nutrient eutrophication and this assemblage may show adverse effects of herbicides or other chemicals more quickly than other organisms (Barbour et al. 1999). Periphyton assemblages exhibit stressor-related changes that alter species composition rapidly, and can shift to noxious levels of overgrowth, thereby contributing to water quality degradation (Stevenson et al. 1996, Stevenson and Bahls 1999). Similar to benthic macroinvertebrates, periphyton assemblages contain species with a wide range of pollution tolerances. Furthermore, they are easy to collect and identify by experienced taxonomists (Plafkin et al. 1989, Stevenson and Bahls 1999). As of 2001, only 20 entities were using algae as an indicator, although an additional 5 entities were developing algal indicators (U.S. EPA 2002c).

Fish are another indicator of watershed health with easily identifiable species of varying trophic levels that respond differently to wide ranges of environmental stressors (Karr et al. 1986, Barbour et al. 1999). Fish are advantageous indicators because they live their entire lives in water and their large geographical ranges can indicate the effects of stressors on a greater scale than either periphyton or macroinvertebrates. Fish provide information regarding the physical, chemical, biological and habitat condition of the watershed as a whole (www.epa.gov/bioindicators/html/fish.html). As of 2001, 41 entities were using fish for biological assessments (U.S. EPA 2002c).

1.2.7 305 (b) Report and 303 (d) List

Following data collection, processing, and analysis, each state is required to submit the results in the form of a biennial 305(b) Report on the water quality conditions and provide a 303(d) List of Impaired Waters on April 1st of every even-numbered year (U.S. EPA 2003). The 305(b) report must contain all the information collected from streams and rivers located within the state's boundaries. The Integrated 305(b) Report must contain the following key components: "geographic referencing of all water resources; categorization of waters according to WQS attainment status; identification, prioritization and scheduling of waters needing Total

Maximum Daily Loads (TMDL); identification of waters where information is not sufficient to determine a water's status; and a schedule of monitoring for the next reporting cycle" (U.S. EPA 2003). The EPA requires that all of the state's assessed waters be placed into one of five categories that represent varying levels of water quality standards attainment. These five categories as stated in U.S. EPA (2003) are as follows:

Category 1: All designated uses are met;
Category 2: Some of the designated uses are met but there is insufficient data to determine if remaining designated uses are met;
Category 3: Insufficient data to determine whether any designated uses are met;
Category 4: Water is impaired or threatened but a TMDL is not needed;
Category 5: Water is impaired or threatened and a TMDL is needed.

Those impaired streams where one or more designated uses are not attained and are consequently placed in Category 5 must be listed on the 303(d) list. Once placed on the 303(d) list, a TMDL must be prioritized and established. Within the 303(d) list, Section 130.7(b)(4) requires that each state also identify the pollutants that are known to be causing the impairment (U.S. EPA 2003).

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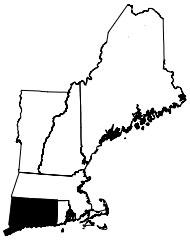
2 CONNECTICUT



This document was prepared using documents written by the State of Connecticut and via Personal Communication with the State Supervising Environmental Analyst. Any questions concerning bioassessment methods should be directed to:

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2.1 Introduction

The CT DEP Bureau of Water Management has used the benthic macroinvertebrate assemblage to assess the biological integrity of surface waters since the mid-1970's and began using fish assemblage data in 1999 in cooperation with the CT DEP Inland Fisheries Division. The benthic macroinvertebrate assemblage is assessed based on the Rapid Bioassessment Protocol (RBP) III Single Habitat method (Plafkin et al. 1989, Barbour et al. 1999), and an index modified from Plafkin et al. (1989) is used to determine the level of ALUS (i.e., Full Support, Threatened, Partial Support, Not Supporting). Connecticut WQS state that "the benthic invertebrate criteria may be utilized where appropriate for assessment of the biological integrity of surface waters. The criteria apply to the fauna of erosional or riffle habitats in lotic waters which are not subject to tidal influences" (CT DEP 2002a). In addition to the biological component, habitat, aquatic toxicity, sediment, and ambient chemical and physical data collected by the Connecticut Ambient Biological Monitoring Program are used to determine compliance with State WQS (Table 2-1) and are ultimately used to report on the ALUS under section 305(b) and 303(d) of the CWA. Furthermore, the ambient monitoring program seeks to evaluate pollution control program effectiveness, collect data for baseline characterization and identification of reference conditions, assess water quality trends, evaluate ecological damage due to emergency pollution events, identify existing and emerging pollution problems, and investigate nuisance complaints (CT DEP 1999).

2.2 Key Elements of the Biological Assessment Approach

2.2.1 Index Period and/or Temporal Conditions

Biological monitoring by CT DEP utilizes benthic macroinvertebrates as the primary aquatic assemblage for ALUS assessment purposes. Fish assemblage data have been incorporated on a limited basis since 1999. Based on differences in the biology of these indicator assemblages and logistical considerations, different index periods have been selected for their collection. Benthic macroinvertebrate data are collected in the late autumn (October 1-December 1). This time frame provides for the collection of individuals that are large enough to identify. It also

Table 2-1. Connecticut water quality standard classes.

Class	Management	Biological Standard
A	Designated as a potential drinking water supply, fish and wildlife habitat, recreation, industrial supply and other legitimate uses, including navigation.	A wide variety of macroinvertebrate taxa should normally be present and all functional feeding groups should normally be well-represented. Presence and productivity of aquatic species are not limited except by natural conditions, permitted flow regulation or irreversible cultural impacts. Water quality shall be sufficient to sustain a diverse macroinvertebrate community of indigenous species. Taxa within the orders Plecoptera (stoneflies), Ephemeroptera (mayflies), Coleoptera (beetles), and Trichoptera (caddisflies) should be well-represented.
B	Designated as a use for habitat for fish and other aquatic life and wildlife, recreation, navigation, and agricultural and industrial water supply.	Water quality shall be sufficient to sustain a diverse macroinvertebrate community of indigenous species. All functional feeding groups and a wide variety of macroinvertebrate taxa shall be present; however, one or more may be disproportionate in abundance. Waters which currently support a high quality aquatic community shall be maintained at that high quality. Presence and productivity of taxa within the orders Plecoptera, Ephemeroptera; and pollution intolerant Coleoptera and Trichoptera may be limited due to cultural activities. Macroinvertebrate communities in waters impaired by cultural activities shall be restored to the extent practical through implementation of the department's procedures for control of pollutant discharges to surface waters and through Best Management Practices (BMPs) for non-point sources of pollution.
C	Suitable for certain fish and wildlife habitat, recreational activities, industrial use, and other legitimate uses, including navigation.	Not defined in WQS
D	May be suitable for bathing or other recreational purposes, certain fish and wildlife habitat, industrial uses and navigation.	Not defined in WQS

provides the worst-case scenario for impairment of waste-receiving waters, and allows for conclusions of this impairment to be drawn based on the macroinvertebrate assemblage assessment. Fish monitoring is conducted during the summer low flow period. This is a period of high stress for fish assemblages in Connecticut streams, and low stream flow facilitates sample collection. In 2002-2003, CT DEP was funded by U.S. EPA to collect periphyton data for a pilot project. It was the intention of CT DEP to use the project for incorporating the methods developed into the ambient biological monitoring program in the future. For the pilot project, periphyton was collected in July and August, the period of stable flow and high periphyton growth rates (due to maximum ambient temperature from increased available sunlight and day length).

2.2.2 Monitoring Program Survey Approach

Water quality monitoring in Connecticut has historically employed a focused approach targeting major rivers and waste receiving waters. Consequently, many smaller streams remained unassessed. In an effort to prioritize surface water monitoring activities and increase monitoring coverage, a five-year rotating basin monitoring strategy was developed and implemented in 1997. One major drainage basin was targeted each year during the five-year cycle that ended in 2001. Within each basin, approximately fifty sites were sampled annually. Criteria used to select sites for sampling were sub-basin size, location of wastewater discharges, land use, and resource value. A subset of approximately 24 targeted sites was chosen each year to assess the fish assemblage. Additionally, an increased effort was made to incorporate data from volunteers, academics and municipalities. To work toward the goal of a comprehensive assessment, the CT DEP accepted the opportunity to participate with U.S. EPA in a two-year monitoring project following completion of the five-year rotating basin strategy in 2001. This project was conducted during 2002 and 2003 and assessed wadeable streams based on a statewide probabilistic design. Sample coverage included monitoring of macroinvertebrates at 60 sites, fish at 24 sites, and periphyton at 30 sites. Water samples were collected quarterly for chemical analyses at the 60 sites sampled for benthos. The CT DEP is currently developing a Comprehensive Monitoring and Assessment Strategy to meet CWA Section 106 requirements. This strategy will be completed by October 2004 and will cover a ten-year period. It will include elements of the previous rotating basin strategy as well as a probabilistic component.

2.2.3 Indicator Assemblages

Currently, CT DEP primarily uses benthic macroinvertebrates as the indicator assemblage for biological monitoring. The CT DEP incorporates fish assemblage data using best professional judgment to make decisions about class attainment and is currently developing a Fish Index of Biotic Integrity (IBI) based on the State of Vermont model. A pilot project for periphyton sampling was conducted in 2002-2003, and the data were used only to supplement other biological data. Methods for the use of periphyton in the monitoring program are under development.

2.2.4 Reference Condition

The CT DEP has selected reference sites to compare to test sites within each of Connecticut's major basins (Figure 2-1). Those sites designated as reference have been defined as those that are least disturbed and minimally impacted by human influences. Furthermore, reference sites are selected for comparison against test sites so that the streams are within one stream order (± 1) and drainage areas are within one order of magnitude of one another. Reference sites are also used for comparison if the stream gradient is similar to that of the test site. The natural features of a reference site should include wadeable streams with optimal habitat including a hard bottom and erosional substrate (i.e., riffle habitat with cobble or gravel substrate).

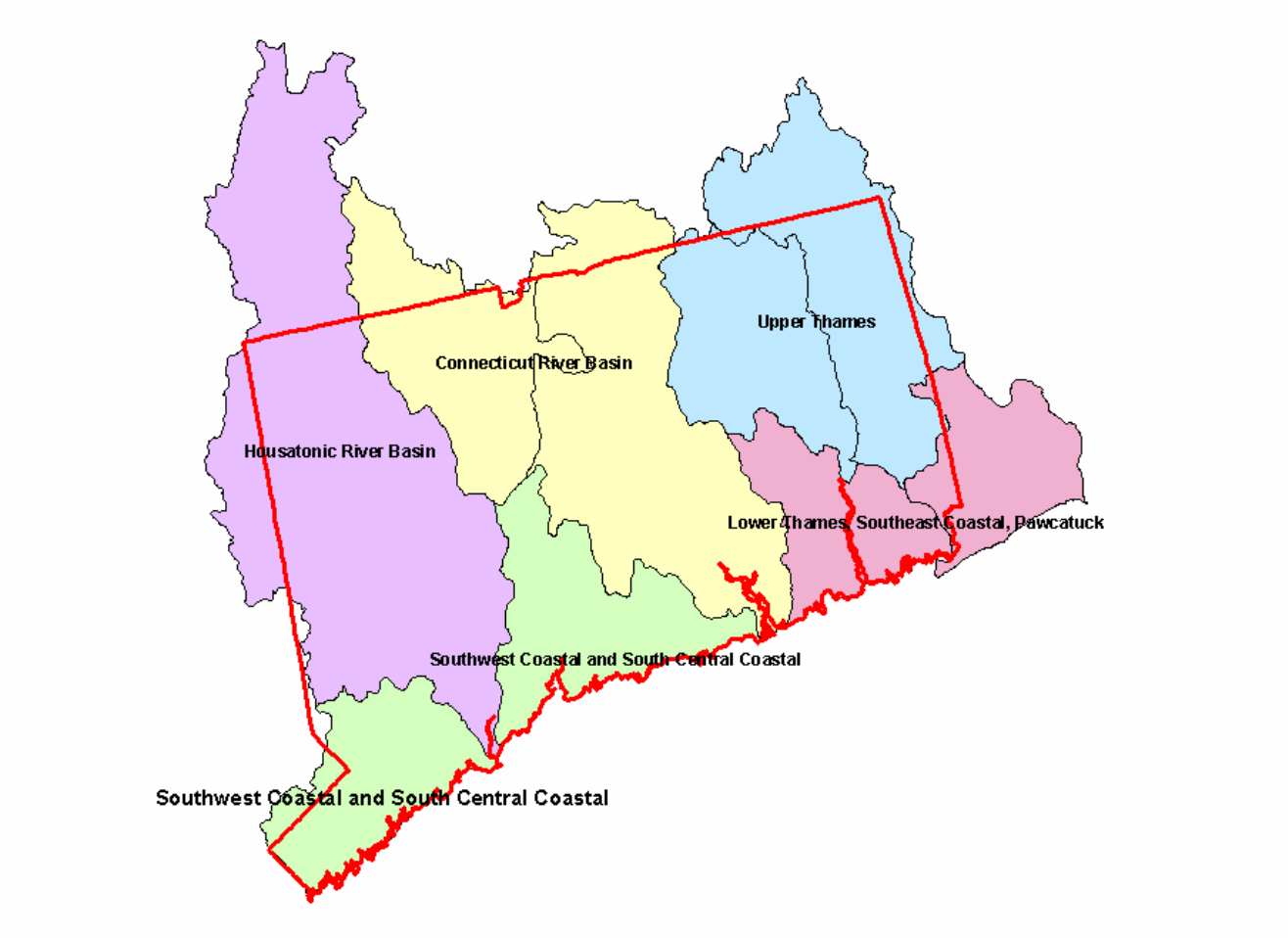

Figure 2-1. Major Connecticut basins sampled for the biological monitoring program using the rotating basin strategy.

2.3 Field and Laboratory Protocols

2.3.1 Macroinvertebrate Protocols

2.3.1.1 Field Methods

The CT DEP uses a modified RBP Single Habitat Approach to collect macroinvertebrate samples (Plafkin et al. 1989, Barbour et al. 1999). After twelve riffle sampling points ("stops") are chosen in a sampling reach, a rectangular kick net (9 in x 18 in) with 800-900 µm mesh, modified from the RBP recommended 500 µm, is placed at the bottom of each riffle with the opening facing upstream. An approximately 2-m^2 area of substrate upstream of the net is disturbed at each riffle using a kicking and stomping motion and the loosened macroinvertebrates are then trapped in the net. The sample is removed, the net is repositioned at different riffles or within the same riffle, and the process is repeated until all twelve samples are collected. The twelve samples are then composited into one jar, labeled and preserved with 70% ethanol.

2.3.1.2 Laboratory Methods

Benthic macroinvertebrate samples are rinsed through a #30 sieve (600 µm) to remove preservative and any large debris. The entire sample is then spread over a gridded tray containing 56 squares. Enough water is added to moisten organisms and spread the sample evenly. Random squares are selected to sort completely until 200 organisms (± 10%) are counted. After the sample is subsampled in this way, any large, rare or representative taxa are removed from the remaining debris. The sample is preserved with 70% ethanol. The sample is then identified by a qualified taxonomist using dissecting microscopes (10x-64x) to the lowest possible taxonomic classification using various keys and Peckarsky et al. (1990). Chironomids are placed in 15% KOH overnight, mounted on slides using glycerine, and then identified using a compound microscope. The CT DEP maintains a reference collection of taxa and any taxa unable to be identified by CT DEP taxonomists are verified by regional taxonomists.

2.3.2 Periphyton Protocols (CT DEP 2003)

2.3.2.1 Field Methods

Two methods were used to assess benthic algae during 2002-2003. Samples were collected using a modified RBP Single Habitat sampling protocol (Barbour et al. 1999) for natural substrates to assess algal biomass and taxonomic composition. In addition, the field-based Rapid Periphyton Survey (RPS) (Barbour et al. 1999) was conducted.

2.3.2.1.1 Quantitative Periphyton Sampling

At each site, a 150-m reach is selected for assessment and fifteen pieces of substrate (ideally rocks) with sizes of 6.4-25.6 cm diameter are collected from throughout the reach. If rocks are not present, then logs and large sticks are used. The fifteen pieces of substrate are carried to the bank, and attached algae are scraped from a 1-in diameter area using a flat spatula

and a toothbrush. A rinse bottle filled with deionized water is used to wash the algal material into a Nalgene® collection bottle. The algae from all 15 pieces of substrate are composited into a single sample bottle. The bottle is placed on ice and returned to the lab where 5 ml of sample is removed for chlorophyll *a* analysis. The remaining sample is then preserved with 2% formalin and analyzed to determine the identification and biomass of the sampled periphyton. A duplicate sample is collected for quality control at 10% of sites.

2.3.2.1.2 Rapid Periphyton Survey (Barbour et al. 1999, CT DEP 2003)

At each site, the width of the stream is estimated to establish the number of transects that will be laid out (5-25). Then, transects are divided into observation points ("stops") to be sampled, so that 2-10 stops are sampled at each transect. A viewing bucket with a 50 dot grid is immersed in the stream and at each stop the observer visually estimates and records the number of grid points covered by moss, macroalgae and microalgae. The observer also records the distance from the left bank, depth, dominant substrate, canopy cover, current velocity, size of the average rock/substrate within 1 ft of the observation point, and the presence or absence of vascular plants. All data are recorded on the CT RPS Data Sheet (CT DEP 2003).

2.3.2.2 Laboratory Methods (CT DEP 2003)

2.3.2.2.1 Chlorophyll *a* (APHA 1999)

After arriving at the lab, the 5 ml subsample removed from the composite periphyton sample is filtered through a 47 mm GF/F filter with a nominal pore size of 0.7 mm. The filter is then stored in an aluminum foil packet and frozen at -15ºC until transfer to a laboratory for processing (Environmental Research Institute at the University of Connecticut) within three weeks of filtration. The pigment is extracted from the filter using an aqueous acetone $MGCO_3$ solution. Chlorophyll *a* concentration is determined using the U.S. EPA Method AERP-12 fluorometric method. A Turner 450 Flourometer with a 1-cm light path length is used. The fluorometer is calibrated by using a chlorophyll *a* standard of known value, which is also run by spectrophotometric method. The fluorescence of the extract is determined and the chlorophyll *a* concentration is calculated using the following equation:

Chl*a* mg/m^2 = (C_a)(Extract volume in L)/(Substrate area in m^2 represented by filter)

where:

C_a = *Concentration of Chla in mg / L* =

$$Fluorometer\ reading\ x\ (instrument\ calibration\ \ factor) = \frac{\mu g\ Chla}{1000L} = \frac{mg\ Chla}{L}$$

Extract Volume in L = always equals 0.02 L

$$Substrate\ area\ represented\ by\ \ filter = \left(\frac{5\,ml\ \ filtered\ volume}{total\ volume\ in\ ml}\right)(total\ substrate\ area\ \ 0.0076035\,m^2)$$

2.3.2.2.2 Algal Identification and Density (contracted to Dr. R. Jan Stevenson at Michigan State University)

A Palmer Counting Cell is used to count at least 300 cells and identify soft algae to species or to subspecies/variety (rarely to genus), and count live diatoms using the Environmental Monitoring and Assessment Program (EMAP) protocol (Lazorchek et al. 2000, Charles et al. 2002). Next, diatom valves are acid cleaned and mounted in NAPHRAX. Then, 600 diatom valves are identified to species or lower level in a second count. Algal densities per unit area of substratum and relative abundances of algal taxa are calculated as advised in the RBPs for algae (Barbour et al. 1999).

2.3.2.2.3 Biomass and Biovolume Determination

Biomass, or Ash Free Dry Mass (AFDM), is determined using Standard Method 10300C (APHA 1999). Biovolumes of algae are determined by measuring at least 15 cells of each taxon that occurs with a relative abundance greater than 5% in any sample. For rarer taxa, fewer cells are measured to determine their biovolume. For taxa for which all measures cannot be made or taxa with less than a 1% average relative abundance among all samples, literature values or database values may be used to determine species biovolumes. For each sample, the relative biovolumes of taxonomic and functional groups (as defined by algal class and growth form: centric diatom, pennate diatom, filamentous cyanobacteria, filamentous green algae, etc.) are calculated.

2.3.3 Fish Protocol (Plafkin et al. 1989, Hagstrom et al. 1995, CT DEP 2002b)

Fish assemblage sampling is conducted in cooperation with the CT DEP Inland Fisheries Division. At each site, a 150-m reach is selected for assessing the fish assemblage. The upper and lower boundaries of the reach are determined by natural barriers to fish movement. The CT DEP varies the electrofishing equipment depending on the width of the stream to be sampled. A single backpack is used in the smallest streams, two backpacks are used in medium-sized streams, one generator towed in a canoe is used in large streams, and two or three generators are towed in multiple canoes for the very largest streams. In all cases, the elecrofishing crew (minimally consisting of three people), begin at the downstream barrier and move upstream collecting all species affected after one pass. All fish that are greater than 3 cm in length are measured, identified, and the condition and any anomalies are recorded in the field before they are returned to the stream. Any fish that cannot be identified in the field are sent to the laboratory (alive or preserved in ethanol) to be identified by a senior field biologist using Whitworth (1996).

2.4 Data Management/Quality

A Microsoft Access database is used to track all sample collection, analyses, and resultant metadata**.** All data are linked to sampling trips by unique sample identifiers and each location is stored as a unique point and geo-referenced. All metadata are entered in the correct sequence to keep sample results linked with sample metadata; electronic transfer of results is used whenever possible to reduce transcription error. The database is linked to ArcView GIS

software to enable the mapping and graphic analysis of data. Ultimately, all data are stored in the U.S. EPA Storage and Retrieval database (STORET), a repository for water quality, biological, and physical data.

2.5 Analysis of Biological Data

2.5.1 Macroinvertebrate Data

For the macroinvertebrate assemblage, RBP III thresholds as described in Table 2.2 are applied to seven metrics (Plafkin et al.1989) to determine metric scores. The sum of metric scores is then represented as a percent of the reference total score, and the test stream is placed into one of four impact categories: Not Impaired, Slightly Impaired, Moderately Impaired, or Severely Impaired (Table 2-2). The CT DEP recognizes any test stream score of less than 54% as not fully supporting ALU, with a gray area lying between 50-54%. The streams in the gray area are placed in categories on a case-by-case basis weighted on the evidence and available data.

Table 2-2. Metrics and scoring ranges used in RBP III determinations of the level of biological impact based on benthic macroinvertebrates (based on Plafkin et al. (1989)).

Metric	Method	Scoring Ranges			
		6	4	2	0
Taxa Richness [(a)]	The total number of distinct taxa in a sample	>80%	60-80%	40-59%	<40%
EPT Index [(a)]	The number of taxa within the orders of Ephemeroptera, Plecoptera, and Trichoptera	>90%	80-90%	70-79%	<70%
EPT/ Chironomidae (abundance ratio) [(a)]	(Abundance of EPT organisms)/ (Abundance of EPT + Chironomidae)	>75%	50-75%	25-49%	<25%
HBI (modified) [(b)]	(Number of individuals in each taxon multiplied by its assigned tolerance value)/(Total number of organisms in sample)	>85%	70-85%	50-69%	<50%
Scraper/Filtering Collector Ratio [(a)]	(Number of scrapers)/(Number of filtering organisms)	>50%	35-50%	20-34%	<20%
% Contribution of Dominant Taxon [(c)]	(Number of individuals in most common taxon)/(Total number of organisms) x 100	<20%	20-29%	30-40%	>40%

Metric	Method	Scoring Ranges			
		6	4	2	0
Community Loss Index [(d)]	A measure of the dissimilarity between a test site and a reference site (Plafkin et al. 1989). Metric values increase as biological impairment increases. Values have no limits. CLI = a - c / b where: a = number of genera in reference sample, b = number of genera in test sample, c = number of genera common to both samples	<0.5	0.5-1.5	1.6-4.0	>4.0
% Total Observed Score compared to Reference Condition					
> 83% Not Impaired 54-79% Slightly Impaired 21-50% Moderately Impaired <17% Severely Impaired					
(a) Value is converted to ratio of test to reference site *100 (b) Value is converted to ratio of reference to test site *100 (c) Actual percent contribution used in scoring, not ratio to reference (d) Uses range of values actually obtained					

2.5.2 Periphyton Data

The CT DEP is under the process of developing algae as an indicator using probabilistic sampling in their 2002-2003 pilot study. The data collected were used to supplement other biological data collected while the methods are under development. Literature values were used to calculate metrics during the first year of study. However, autoecological information for species within Connecticut populations can be generated with data from the pilot study and then tested using data from the second year of the study. Metrics calculated are derived from RBP periphyton protocols (Barbour et al. 1999). Data that are generated from the RPS and data from Chlorophyll *a*, biomass, and species composition and abundance will be evaluated to determine the components that will be used in the routine ambient monitoring program.

2.5.3 Fish Data

Although CT DEP does not currently have a fish index, they are developing a Fish Index of Biotic Integrity (IBI) based on the State of Vermont model. Until the IBI is developed the CT DEP incorporates the results of the fish assemblage data using best professional judgment to make decisions about class attainment. The data collected from fish assessments are species composition, trophic structure and age class distribution and these measurements taken from sampled streams are compared to those measurements in unimpaired and impaired streams to make inferences about the condition of the fish assemblage.

2.5.4 Summary: Determining ALU Support

Connecticut narrative criteria in WQS, the macroinvertebrate quantitative index, fish data (while metrics are under development), and any other supplemental physical, chemical, and other biological data are used to make the ALU assessment (listed in Table 2-3). These assignments are then outlined in the 305(b) report and any streams not in attainment are placed on the Connecticut 303(d) list. Table 2-3 outlines the guidelines CT DEP biologists use in conjunction with BPJ to determine ALUS at sites.

Table 2-3. Aquatic life use support categories and the criteria used for making decisions (taken from Table 2 *in* CT DEP 2002a).

ALUS	Criteria/Indicators
Fully Supporting	▪ Benthic assemblage: bioassessment indicates assemblage is non-impaired or slightly impaired (Plafkin et al. 1989), and meets narrative criteria in CT WQS; RBP III Community Score ≥ 54% of reference condition. ▪ Fish assemblage: species composition, trophic structure, and age class distribution as expected for a non-impacted stream of similar size. ▪ Conventional physical/chemical criteria not exceeded. ▪ Measured toxicants do not exceed chronic toxicity criteria. ▪ No record of catastrophic events (e.g., chemical spills, fish kills) ▪ No evidence of flow diversion
Threatened	▪ Benthic assemblage: non-impaired or slightly impaired, but still meets narrative criteria in CT WQS; RBP III Community Score ≥54% reference condition. ▪ Fish assemblage as above, but documented trend is downward or conditions exist that may impact the assemblage in the future. ▪ Slight exceedences of either conventional or toxicant criteria in < 10% of samples; exceedences difficult to discern from expected analytical variability or error. ▪ Discharge effluent constitutes >20% of stream flow. ▪ Land use conditions exist that may cause impairment. ▪ Flow reductions due to diversions have been observed.
Partially Supporting	▪ Benthic assemblage: bioassessment indicates assemblage is moderately impaired; RBP III Community Score 21-50% of reference condition. ▪ Fish assemblage: species composition, trophic structure and age class distribution significantly less than expected for non-impacted stream of similar size: diversity and abundance of intolerant species reduced; top carnivores rare; trophic structure skewed toward omnivory. ▪ Either fish or benthic assemblage meets above conditions, but the other assemblage is fully supporting. ▪ Conventional physical/chemical criteria exceeded in > 10% but <

ALUS	Criteria/Indicators
	25% of samples. ▪ Measured toxicants exceed chronic criteria < 10% of samples. ▪ Flow is reduced significantly during drought conditions.
Not Supporting	▪ Benthic assemblage: bioassessment indicates assemblage is severely impaired: RBP III Community Score < 17% of reference condition. ▪ Fish assemblage: species composition, age class distribution and trophic structure greatly impaired in comparison to a non-impacted or minimally impacted stream of similar size; assemblage dominated by highly tolerant species, omnivores and habitat generalists; in extreme cases, few species present and/or diseased fish common. ▪ Conventional physical/chemical criteria exceeded in > 25% of samples ▪ Measured toxicants exceed chronic criteria >10% of samples ▪ Stream known to dry completely for significant periods. ▪ Documented catastrophic event (e.g., chemical spill, fish kill)
Not Attainable	Stream completely enclosed in conduit or cleared concrete trough. Stream is dewatered most of the time due to an upstream impoundment or diversion.

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2.7 Resources

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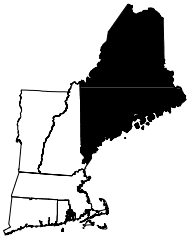
3 MAINE



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3.1 Introduction

Maine Department of Environmental Protection (MDEP) has developed a biological monitoring and biocriteria program to assess water quality and ensure the adherence to ALU designations defined in Maine's WQS. In 1983, MDEP began its standardized benthic macroinvertebrate sampling program. This program began building a database to aid in the development of numeric biocriteria using a discriminant model approach. The numeric biocriteria developed were refined on a regional scale to increase the accuracy of measuring the adherence of streams to aquatic life use standards. The biocriteria program was written into law in April 1986 with the passing of M.R.S.A. 38 Public Law Chapter 698: The Classification System for Maine Waters (State of Maine 1985). This law required the State to "restore and maintain the chemical and biological integrity" of Maine waters. This law also established a classification system, and narrative biological and habitat criteria were described for each of these classes. Furthermore, the statute details specific numerical standards to which each class must adhere for dissolved oxygen and bacterial concentrations (Table 3-1). The water quality classes are AA and A, B, and C (Table 3-1). Water quality below Class C is given Non-Attainment status.

Once water bodies were assigned a discrete water quality classification (i.e., A, B, C, NA) and narrative aquatic life standards were established, MDEP began testing whether empirical and ecological data collected from Maine's streams would show the gradients of environmental quality reflected in the narrative standards (Figure 3-1). They were able to conclude that the four established categories of biological condition did fit well with the State's four-tiered standards for dissolved oxygen, bacteria and habitat (Davies et al. Draft). Multiple exploratory multivariate analyses were performed, including k-means clustering, multidimensional scaling, principal coordinate analysis, principal components analysis, multiple regression analysis, two-way indicator species analysis, log-linear modeling, logistic regression, detrended correspondence analysis, and variance component analysis, but MDEP ultimately chose discriminant analysis to determine the probability of a stream's membership to an established class (Davies et al. Draft).

Table 3-1. Water quality classification system for rivers and streams in Maine. (M.R.S.A. Title 38 Article 4-A § 464-465).

Class	Management	Narrative Habitat and Aquatic Life Standards	Dissolved Oxygen Numeric Criteria	Bacteria (*E. coli*) Numeric Criteria
AA	Highest quality water for recreation and ecological interests. Minimal human influence. No discharges or impoundments permitted.	Habitat natural and free flowing. Aquatic life as naturally occurs.	As naturally occurs	As naturally occurs
A	High quality water with limited human interference. Discharges restricted to non-contact process water or highly treated wastewater equal to or better than the receiving water. Impoundments allowed.	Habitat natural. Aquatic life as naturally occurs.	7 ppm; 75% saturation	As naturally occurs
B	Good quality water. Discharge of well-treated effluent with ample dilution permitted. Impoundments allowed.	Habitat unimpaired. Ambient water quality sufficient to support life stages of all indigenous aquatic species. Only non-detrimental changes in community composition allowed.	7 ppm; 75% saturation	64/100 ml (geometric mean) or 427/100 ml (instantaneous level)
C	Acceptable water quality. Maintains the interim goals of the Federal Water Quality Act (Fishable/swimmable). Discharge of well-treated effluent permitted. Impoundments allowed.	Habitat for fish and other aquatic life. Ambient water quality sufficient to support life stages of all indigenous aquatic species. Change in community composition may occur but structure and function of the community must be maintained.	5 ppm; 60% saturation	142/100 ml (geometric mean) or 949/100 ml (instantaneous level)

** Classes AA and A are indistinguishable in the discriminant model because narrative criteria are both described "as naturally occurs".*

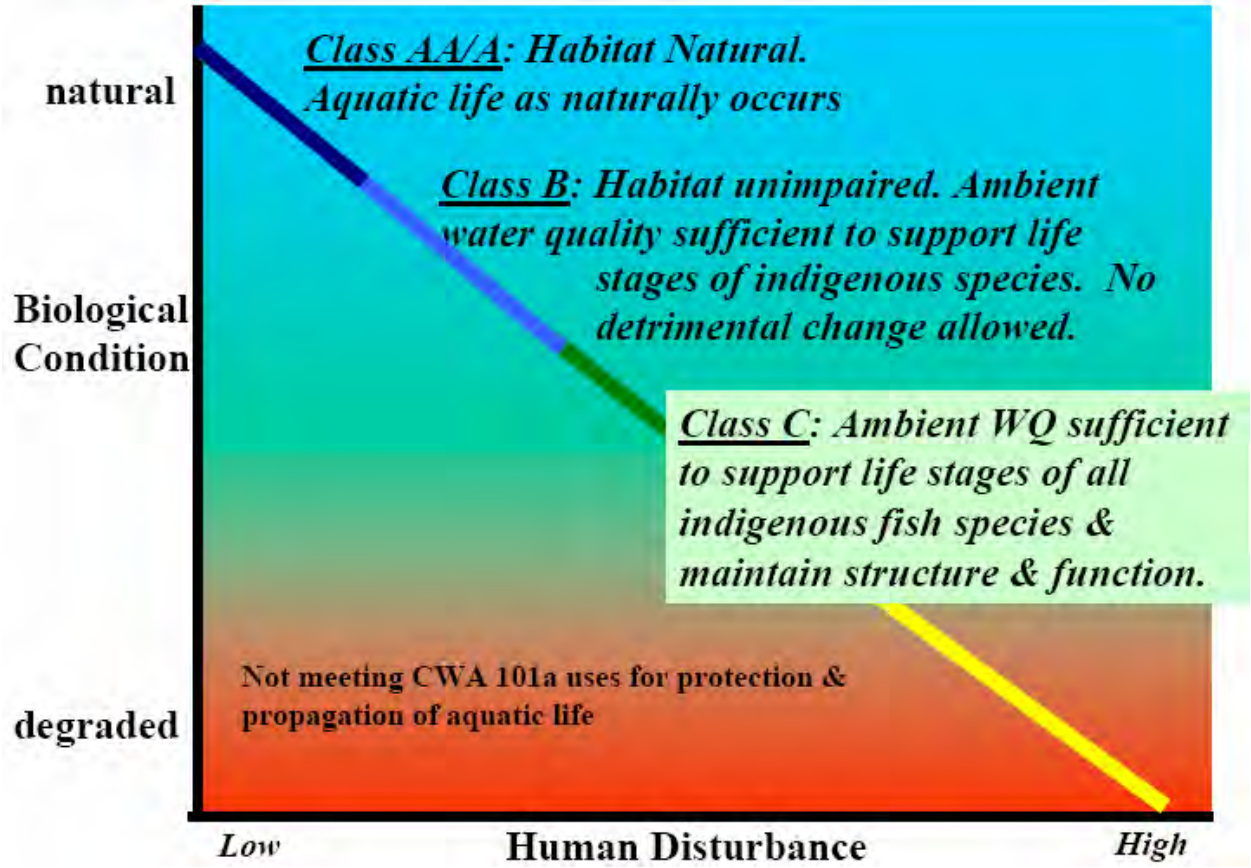

Figure 3-1. Maine's narrative aquatic life standards with the human disturbance and biological condition gradients (Taken from Courtemanch 2003).

The MDEP must report the specific class attainment of each stream under Section 305(b) of the CWA. Those sites that are found in non-attainment must be listed on the state's 303(d) list. For those streams placed on the 303(d) list, the MDEP is then expected to develop and implement a total maximum daily load (TMDL) for each stressor that is preventing the stream from reaching attainment status.

3.2 Key Elements of the Biological Assessment Approach

3.2.1 Index Period and/or Temporal Conditions

It is important to select a sampling season that is indicative of the conditions needed to collect the most suitable data to answer the objectives for the intended study. Thus, the MDEP has chosen the index sampling period to be July 1 - September 30th, the low flow period for streams in Maine. All baseline data from streams were collected during this period because late summer represents the time of the year when organisms may be exposed to maximum stressful conditions (Davies and Tsomides 2002). During this period, water levels tend to drop, which may increase the concentration of soluble contaminants or nutrients in the stream, and water temperatures tend to be at a maximum level.

3.2.2 Monitoring Program Survey Approach/Natural Classification of Water Bodies

Maine has divided the state into 5 main watersheds that are sampled every five years on a rotational basis. These basins are the Androscoggin River; Kennebec and Mid-Coast Basin; Penobscot, St. Croix, and North Coastal Rivers; Piscataqua, Saco, and Southern Coast; and the St. John and Presumpscot Basins (Figure 3-2). Although the water bodies have been divided into five main watersheds for monitoring purposes, exploratory data analysis concluded that it was not necessary to stratify the modeling approach climatically or geographically or to create separate southern Maine and northern Maine models (Davies et al. Draft).

Approximately 50-60 sites are sampled each year within a single basin. Sites are chosen based on a "targeted approach" that incorporates a variety of factors that document the degradation or improvement of each stream. These factors include: 1) a prior knowledge of existing activities that may degrade the water body and impact the biological community; 2) a continued effort to monitor the effect a potential threat may have on a water body; 3) the requirement (or scientific endeavor) to monitor remediation activities or water quality management changes; and 4) to increase documentation of natural variability by including previously unmonitored sites (MDEP 2002). Furthermore, the rotation schedule provides assessment information for scheduled wastewater renewals.

3.2.3 Indicator Assemblage

Benthic macroinvertebrates were chosen as the biological endpoint because "they have limited mobility; as a group, they include species representing a wide range of pollution tolerances, including those found in extremely polluted sites; they are diverse and have relatively long, complex life cycles; they are a food source for fish; methods of sample collection and analysis are well-established for this assemblage; and they are a cost-effective group to sample" (MDEP 1995, Davies et al. Draft).

3.2.4 Reference Condition (Establishing *a priori* Groups)

The MDEP used linear discriminant analysis for model construction, which requires that *a priori* groups be established. For this reason, reference condition is more appropriately addressed as a discussion of *a priori* groups. The MDEP used best professional judgment to set these *a priori* groups (please see Section 3.5 Analysis of Biological Data and Appendix A for an in-depth description of *a priori* group assignment).

Furthermore, MDEP does evaluate upstream and downstream of disturbed sites in order to collect information about the biological condition before and after a disturbance. This information is evaluated and used when determining the use attainment of a site (Davies et al. Draft).

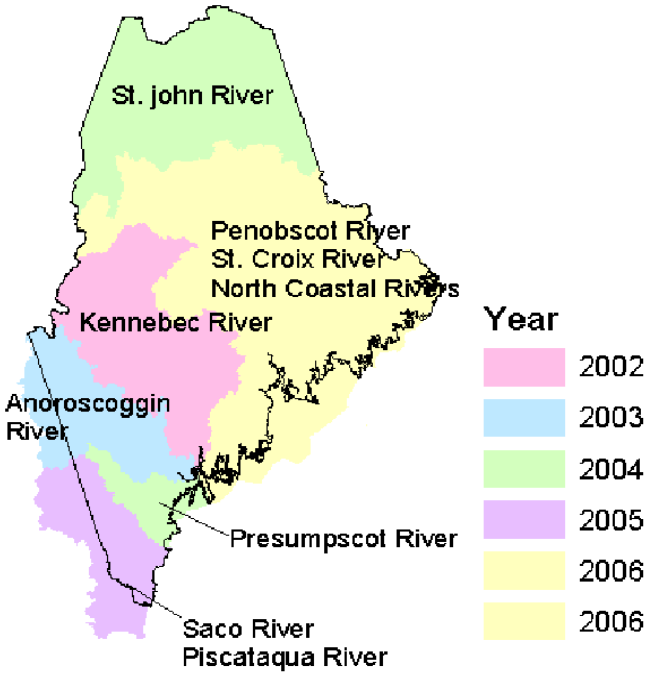

Figure 3-2. Map of basins sampled by MDEP (2002).

3.3 Field and Laboratory Protocols

3.3.1 Macroinvertebrate Protocols (from Davies et al. 1999, Davies and Tsomides 2002)

3.3.1.1 Field Methods

The MDEP follows a highly standardized and quantitative "Classification Attainment Evaluation" protocol to collect data (see Davies et al. 1999, Davies and Tsomides 2002). Depending on water depth encountered, the MDEP uses rock baskets, riffle bags, or rock-filled cones to collect macroinvertebrates. Rock-filled mesh bags are used in small streams with depths of at least 5 cm, and rock-filled cones are used in non-wadeable rivers that must be accessed by boats for placement and retrieval. Rock baskets are used to sample wadeable streams deep enough to allow the baskets to be fully submerged. Rock baskets consist of a cylindrical wire barbecue basket filled with substrate. Each basket is constructed according to U.S. EPA methods and has at least 1.5 cm spaces between wires, with a hinged opening and a secure closure (Klemm et al. 1990). The substrate material is a clean cobble, relatively uniform in diameter ranging from 3.8-7.6 cm. Each basket contains approximately 7.25 ± 0.5 kg of

substrate. Rock baskets are placed in the riffle/run portion of a stream reach at a depth to ensure that they will remain fully submerged. The apparatus is positioned in a portion of the stream that reflects the overall characteristic of the stream sampled, located in the middle 50% of bank-to-bank width or in an area that depicts the overall flow of the channel. At each site, a minimum of three baskets are used, and baskets are placed in the stream so that the long axis is parallel to stream flow. Samplers remain in the water for a period of 28 days (± 4 days) within the sampling season (typically late August/early September). However, baskets may be required to remain in the stream for 56 days (± 4 days) if the stream has a low velocity or is impounded. Baskets are placed so that influences including bridges, culverts, channelized areas, slack water areas, and eddies are avoided.

During removal of the rock baskets, they are approached from downstream to minimize the kicking up of sediments and subsequent addition of organisms to the basket. Macrophytes, algae, and debris are carefully removed from the surface of the basket. A 600-µm mesh net is then placed against the substrate downstream of the basket and the basket is then quickly lifted into the net. All contents of the net and the basket are then processed through a 600-µm sieve. The basket and each rock are inspected to ensure the complete removal of all macroinvertebrates. All of the sieved materials from each basket are then placed into a separate sample jar and preserved with 95% ethanol and stream water to a final concentration of approximately 70% ethanol in the field.

3.3.1.2 Laboratory Methods

The MDEP requires that all samples be handled by qualified personnel working under the supervision of a professional aquatic biologist and that all taxonomy be performed by a person who has experience in freshwater macroinvertebrate taxonomy. Valid samples must yield at least 50 organisms each. The entire sample is sorted in small portions (1-2 tablespoons at a time) until no more organisms can be found. Sorting is considered complete when no organisms are found after 45 seconds of searching. While sorting, the sample is kept wet using water but does not remain unpreserved for more than eight hours. All sorted organisms are then placed in a vial containing a 70% alcohol and 5% glycerin solution. The detritus is returned to the sample jar and preserved with 70% alcohol. Any samples used for regulatory purposes are kept for five years. A qualified Biologist evaluates 10% of samples for sorting completeness.

3.3.1.2.1 Subsampling and Identification

If the mean number of organisms in a sampler is greater than 500, then subsampling may be performed to yield at least 100 organisms per sampler. The MDEP does note that subsampling will reduce sample richness by an amount that may affect the outcome when performing linear discriminant analysis. This subsampling effect is taken into account by biologists when making the final determination of classification attainment. If subsampling is required, MDEP follows the methods outlined by Wrona et al (1982). First, sorted macroinvertebrates are gently agitated in an Imhoff settling cone fitted with an aquarium bubbler stone for two to five minutes. Then 25% of the sample is removed from the settling cone in five aliquots using a settling cone and combined into one sample vial, insuring that the required 100 organisms have been obtained. It is important that the individual performing the subsampling randomly dips from the cone and does not target specific organisms. Once the five aliquots are

combined, the sample vial is then labeled indicating the fraction that the subsample represents. An important precaution taken is assuring that large, dense organisms are not included in the subsample because they are too heavy to be suspended for capture in the subsampler cone. These organisms (e.g., crayfish, molluscs or caddisflies with stone cases) are counted separately. The MDEP has tested the randomness of the sample distribution to conclude that five aliquots can be combined into one sample (Elliott, 1977). After sorting, samples are identified by a qualified taxonomist. The MDEP recommends that all macroinvertebrates be identified to species; however, all numbers are adjusted to genus for use in metric calculations. In cases where taxonomic expertise is lacking or when the specimen is too small or in poor condition, the organism is identified to the lowest taxonomic level possible. Each taxonomist submitting data to the MDEP is required to submit a reference collection of identified taxa and a list of references used to identify samples. The reference collections are checked by a MDEP taxonomist using the MDEP's master collection.

3.3.1.2.2 Chironomidae Identification and Subsampling

Chironomid midges are identified using slide mounts of the cleared head capsule and body parts. For identification purposes, Euparol or Berlese mounting medium is preferred; however, for permanent slide mounting, MDEP recommends that CMCP-9 be used (Wiederholm 1983).

For any sample containing less than 100 midges, all midges are identified to the lowest possible taxonomic level. In samples containing 100-199 midges, a subsample containing 50% of randomly selected specimens for identification, and in samples containing 200-499 midges, 25% of the specimens are randomly subsampled. The subsamples are identified to genus/species level, and then the unsampled midges are examined for unusual or rare specimens. Those rare or unusual specimens are also identified to genus/species level and kept separate from the subsample. If a sample contains 500 or more midges, midges are grouped by genus, and a random subsample of 100 organisms is selected from the remaining ungrouped midges. These midges are identified to species level. If any rare or unusual specimens are found after examination, they are identified to species level and kept separate from the subsample.

3.4 Data Management/Quality

The data generated (i.e., identified organisms) are entered into the database management system ORACLE. ORACLE stores the taxonomic code table and all sampling event data, computes analytical variables, and computes and reports the results of linear discriminant models (Davies et al. 1999). All data are checked and verified following rigorous data entry and data editing protocols. After entry into the database, all data are adjusted to the same taxonomic resolution (genus) for comparison (Davies et al. 1995). All site data in ORACLE are also georeferenced in ARCINFO so that spatial relationships can be studied.

3.5 Analysis of Biological Data (*Information compiled from*: Davies et al. 1995, Davies et al. 1999, Davies and Tsomides 2002, MDEP 2003)

The MDEP uses linear discriminant analysis to assess the attainment of aquatic life standards. A series of discriminant models based on macroinvertebrate metrics is used to divide

observations among two or more predetermined classes (i.e., A, B, and C in Maine WQS). The M.R.S.A. 38 Public Law Chapter 698: The Classification System for Maine Waters (State of Maine 1985) outlines the system for assuring both the attainment of aquatic life and that the objectives of maintaining the chemical, physical and biological integrity of waters are upheld by the state. Minimum standards for dissolved oxygen, bacteria and aquatic life for each class, as well as a detailed explanation of the classification system (Table 3-1), are described in this document. The aquatic life standards are narratively established for attainment of each class and are derived from measurable parameters. The results of the discriminant analysis are ultimately used to assign each site to a class and determine ALUS.

The MDEP originally developed the linear discriminant models based on 145 rock basket samples collected from across the state and representing a range of water quality during 1983-1989. They recalibrated the models in 1998 using a much larger macroinvertebrate database with a total of 376 sampling events (Davies et al. 1999). Quantifiable measures for each class (A, B, C, and NA) were determined (Figure 3-3). The final step involved assigning each of the 376 sites in the database to one of four *a priori* groups using the quantifiable measures.

Linear discriminant analysis requires that *a priori* groups be established. *A priori* groups consist of samples of "known" classification from which a predictive model can be developed to characterize streams with unknown classifications (Davies et al. Draft). Based on Maine's Water Quality Laws, MDEP chose four groups to which streams could be assigned: AA/A, B, C and non-attainment (Figure 3-1). After testing multiple statistical modeling techniques (e.g., k-means clustering, Two-Way Indicator Species Analysis, multivariate ordination), they decided that the use of best professional judgment of expert aquatic biologists would be the most ideal way to assign *a priori* groups. An explanation of the criteria biologists followed to establish the *a priori* groups can be found in Appendix A. Once these groups were determined subjectively and independently by three biologists, univariate and multivariate analysis of variance (ANOVA and MANOVA, respectively) confirmed that the assigned groups were statistically distinct. To determine variability in expert judgment assignments, a subset of data was assigned to *a priori* groups by two non-MDEP biologists, yielding an average concurrence with MDEP biologists' assignments of 80%. Furthermore, to check the model, MDEP chose 27 minimally disturbed sites that were not originally used to build the model to determine the success of the model and to assign them to Class A conditions. These sites had no known point sources and land uses comprised 97% forested (3% logged), 2% crop and 1% residential/industrial/commercial.

Next, 25 biological community variables were ultimately identified from a list of 400 variables using stepwise discriminant analysis and iterative backward selection procedures to best predict membership of an unknown stream sample to one of the four water quality classes (A, B, C, and non-attainment). The 25 variables and the methods used to calculate them are found in Table 3-2.

Maine Tiered Uses Based on Measurable Ecological Values

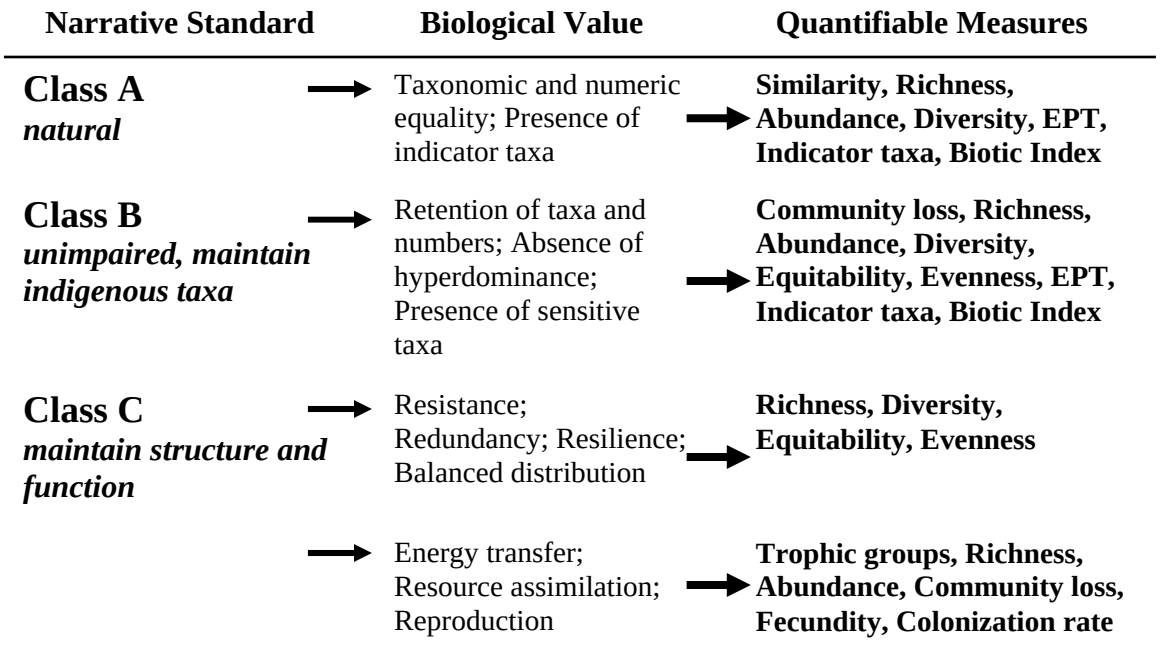

Figure 3-3. Maine tiered uses based on measurable ecological values (modified from Courtemanch 2003).

Linear discriminant functions were developed from the 25 quantitative macroinvertebrate variables (Table 3-2). The discriminant functions determine the probability that a site belongs to a given water quality class. Using a linear optimization algorithm to calculate the discriminant function coefficients, multivariate space distance was minimized between sites within a class, while the distance between classes was maximized. The linear discriminant model consists of functions to compute an association value in the following form (MDEP 2003):

$Z = C + W_1X_1 + W_2X_2 + ...W_nX_n.$
Z = discriminant score
C = constant
W_i = the coefficients or weights
X_i = the predictor variable (metric) values

For each site, 25 quantitative metrics are calculated with the data from the three replicate samples combined. Then, the discriminant function is calculated using one four-way model and three two-way models. First, using only nine variables and calculated coefficients (Table 3-3), the four-way model calculates the probability (range 0.0 - 1.0) that a site fits into each of the three attainment classes (AA/A, B, or C), and the non-attainment (NA) class. The resultant probabilities are then transformed and used as variables in the three two-way models (Table 3-4).

Table 3-2. Methods for the calculation of variables and measures of community structure used in linear discriminant models (*from* Davies and Tsomides, 2002).

	Variable	Calculation Method
1	**Total Mean Abundance**	Count all individuals in all replicate samples from one site and divide by the number of replicates to yield the mean number of individuals per sample.
2	**Generic Richness**	Count the number of different genera found in all replicates from one site. Counting rules for Generic Richness: a) All population counts at the species level are aggregated to the generic level. b) A family level identification that includes no more than one taxon identified to the generic level is counted as a separate taxon in generic richness counts. c) A family level identification with more than one taxon identified to generic level is not counted towards generic richness. Counts are divided proportionately among the genera that are present. d) Higher level taxonomic identifications (Phylum, Class, Order) are not counted toward generic richness unless they are the only representative. e) Pupae are ignored in all calculations.
3	**Plecoptera Mean Abundance**	Count all individuals from the order Plecoptera in all replicate samplers from one site and divide by the number of replicates to yield mean number of Plecoptera individuals per basket.
4	**Ephemeroptera Mean Abundance**	Count all individuals from the order Ephemeroptera in all replicate samplers from one site and divide by the number of replicates to yield mean number of Ephemeroptera individuals per basket.
5	**Shannon-Wiener Generic Diversity (Shannon and Weaver, 1963)**	After adjusting all counts to genus following counting rules in Variable 2: $\bar{d} = \frac{c}{N}(N \log_{10} - \sum n_i \log_{10} n_i)$ Where: C= 3.321928 (converts base 10 log to base 2) N= Total abundance of individuals n_i= Total abundance of individuals in the i^{th} taxon
6	**Hilsenhoff Biotic Index (Hilsenhoff, 1987)**	$HBI = \sum \frac{n_i a_i}{N}$ Where: HBI= Hilsenhoff Biotic Index n_i= number of individuals in the i^{th} taxon a_i = tolerance value assigned to taxon i N= total number of individuals in sample with tolerance values.
7	**Relative Chironomidae Abundance**	Calculate the mean number of individuals in the family Chironomidae, following counting rules in Variable 4, and divide by total mean abundance (Variable 1).

	Variable	Calculation Method	
8	**Relative Diptera Richness**	Count the number of different genera from the order Diptera, following counting rules in Variable 2, and divide by generic richness (Variable 2).	
9	***Hydropsyche* Mean Abundance**	Count all individuals from the genus *Hydropsyche* in all replicate samplers from one site, and divide by the number of replicates to yield mean number of *Hydropsyche* individuals per basket.	
10	**Probability (A + B + C) from First Stage Model**	Sum of probabilities for Classes A, B, and C from first stage model.	
11	***Cheumatopsyche* Mean Abundance**	Count all individuals from the genus *Cheumatopsyche* in all replicate samplers from one site and divide by the number of replicates to yield mean number of *Cheumatopsyche* individuals per basket.	
12	**EPT- Diptera Richness Ratio**	EPT Generic Richness (Variable 19) divided by the number of genera from the order Diptera, following counting rules in Variable 2. If the number of genera of Diptera in the sample is 0, a value of 1 is assigned to the denominator.	
13	**Relative Oligochaeta Abundance**	Calculate the mean abundance in the class Oligochaeta, following counting rules in Variable 4, and divide by total mean abundance (Variable 1).	
14	**Probability (A+B) from First Stage Model**	Sum of probabilities for Classes A and B from the First Stage Model.	
15	**Perlidae Mean Abundance (Family Functional Group)**	Count all individuals from the family Perlidae in all replicate samplers from one site. Divide by the number of replicates to yield mean number of Perlidae per basket.	**Perlidae Functional Group** *Acroneuria* *Agnetina* *Attaneuria* *Beloneuria* *Eccoptura* *Neoperla* *Paragnetina* *Perlesta* *Perlinella*
16	**Tanypodinae Mean Abundance (Family Functional Group)**	Count all individuals from the subfamily Tanypodinae in all replicate samplers from one site and divide by the number of replicates to yield mean number of Tanypodinae per basket.	**Tanypodinae Functional Group** *Ablabesmyia* *Clinotanypus* *Coelotanypus* *Conchapelopia* *Djalmabatista* *Guttipelopia* *Husonimyia* *Labrundinia* *Larsia* *Meropelopia* *Natarsia* *Niltanypus* *Paramerina* *Pentaneura* *Procladius* *Psectrotanypus* *Rheopelopia* *Tanypus* *Telopelopia* *Thienemannimyia* *Trissopelopia* *Zavrelimyia*

	Variable	Calculation Method	
17	**Chironomini Mean Abundance (Family Functional Group)**	Count all individuals in the tribe Chironomini in all replicate samplers from one site and divide by the number of replicates to yield the mean number of Chironomini per basket.	**Chironomini Functional Group** *Axarus* *Chironomus* *Cryptochironomus* *Cladopelma* *Cryptotendipes* *Demicriptochirono-mus* *Dicrotendipes* *Endochironomus* *Einfeldia* *Gyptotendipes* *Harnishchia* *Kiefferulus* *Lauterborniella* *Microchironomus* *Microtendipes* *Nlothauma* *Parachironomus* *Paracladopelma* *Paralauterborniella* *Paratendipes* *Paenopsectra* *Polypedilum* *Pseudochironomus* *Pagastiella* *Robackia* *Stelochomyia* *Stenochironomus* *Stictochironomus* *Tribelos* *Xenochironomus*
18	**Relative Ephemeroptera Abundance**	Variable 4 divided by Variable 1.	
19	**EPT Generic Richness**	Count the number of different genera from the orders Ephemeroptera, Plecoptera, and Trichoptera in all replicate samplers, according to counting rules in Variable 2, generic richness.	
20	**Variable Reserved***		
21	**Sum of Mean Abundances of: *Dicrotendipes, Microspectra, Parachironomus* and *Helobdella***	Sum the abundances of the 4 genera and divide by the number of replicates (as performed in Variable 4).	
22	**Probability of Class A from First Stage Model**	Probability of Class A from First Stage Model	
23	**Relative Plecoptera Richness**	Count number of genera of Order Plecoptera, following counting rules in variable 2, and divide by generic richness (Variable 2).	
24	**Variable Reserved***		

	Variable	Calculation Method	
25	**Sum of Mean Abundances of *Cheumatopshyche, Cricotopus, Tanytarsus* and *Ablabesmyia***	Sum of the number of individuals in each genus in all replicate baskets and divide by the number of replicates (as performed in Variable 4).	
26	**Sum of Mean Abundances of *Acroneuria* and *Stenonema.***	Sum the number of individuals in each genus in all replicate baskets and divide by the number of replicates (as performed in Variable 4).	
27	**Variable Reserved***		
28	**Ratio of EP Generic Richness**	Count the number of different genera from the orders Ephemeroptera (E), and Plecoptera (P) in all replicate baskets, following counting rules in Variable 2, and divide by 14 (maximum expected for Class A).	
29	**Variable Reserved***		
30	**Ratio of Class A Indicator Taxa**	Count the number of Class A indicator taxa that are present in the sample and divide by 7 (total possible number).	Indicator Taxa: Class A *Brachycentrus* (Trichoptera Brachycentridae) *Serratella* (Ephemeroptera: Ephemerellidae) *Leucrocuta* (Ephemeroptera: Heptageniidae) *Glossosoma* (Trichoptera: Glossosomatidae) *Paragnetina* (Plecoptera: Perlidae) *Eurylophella* (Ephemeroptera: Ephemerellidae) *Psilotreta* (Trichoptera: Ondontoceridae)

*These variable numbers are not used in discriminant models.

Table 3-3. Coefficients for the First Stage Model (*from* MDEP 2003).

Variable #	Coefficients				
	Transformation	Class A	Class B	Class C	Nonattainment
Constant		-99.95508	-105.70948	-112.67581	-107.74283
1	ln (value + 0.001)	10.77061	11.46981	11.80888	11.26793
2		-0.38619	-0.43340	-0.50051	-0.48822
3	ln (value + 0.001)	0.23940	0.03946	-0.60923	-0.95480
4	ln (value + 0.001)	-0.59970	-0.55500	-0.67722	-1.79032
5		21.22732	20.91256	21.07602	19.46547
6		8.01620	9.12163	10.31492	10.72746
7	ln (value + 0.001)	-11.70298	-11.52650	-11.49414	-11.66371
8		70.77937	71.09637	72.46514	70.22517
9		-0.00535	-0.00398	-0.00152	0.00007

Table 3-4. Coefficients for the Final Classification Models (AA/A, B, and C) (MDEP 2003).

Class C or Better Model			
Variable #	**Coefficients**		
	Transformation	**Class A-B-C**	**Nonattainment**
Constant		-25.70020	-8.55844
10	Arcsin	19.98470	3.36032
11	ln (value + 0.001)	-0.26001	-0.43781
12	Square Root	5.57672	5.92732
13	ln (value + 0.001)	-2.33229	-1.89945
Class B or Better Model			
Variable #	**Coefficients**		
	Transformation	**Class A-B**	**Nonattainment**
Constant		-17.81016	-6.93836
14	Arcsin	12.04826	3.63707
15	ln (value + 0.001)	-1.11091	-1.03934
16	ln (value + 0.001)	-0.10582	0.01978
17	ln (value + 0.001)	0.17813	0.10825
18		4.03202	-1.14508
19		0.87400	0.63310
21	ln (value + 0.001)	-0.69360	-0.53194

Class A Model			
Variable #	**Coefficients**		
	Transformation	**Class A-B-C**	**Nonattainment**
Constant		-9.59254	-4.08552
22	Arcsin	8.34341	1.52080
23		3.78999	4.27447
25	ln (value + 0.001)	0.53110	0.77851
26	ln (value + 0.001)	-0.55838	-0.51448
28		12.32529	9.81592
30		6.94828	-0.67475

The next step is to use three two-way models to test the probability of a site belonging to class AA/A, B, or C. These models distinguish between a given class plus any higher classes as a group and any lower classes as a group (i.e., Classes AA/A + B + C vs NA; Classes AA/A + B vs Class C + NA; Class AA/A vs Classes B + C + NA). The three models are applied using the equations in Table 3-4 (MDEP 2003, Davies and Tsomides 2002).

The discriminant scores (Z) are known as the Mahalanobis Distances (MDEP 2003) where:

$$\textbf{Mahalanobis Distance} = Z_t\,(\text{sample } x) = g_1\,(x,t) + g_2\,(t)$$

And where:

Z_t = discriminant score for sample *x*, class *t*

$g_1\,(x,t) = (x - m_t)\,S^{-1}\,(s - m_t)$

$g_2\,(t) = -2\log_e\,(q_t) = 0$ (if prior probabilities are equal)

x = a vector containing all the values of all the variables for a given linear discriminant function, for a given sample, of class *t*

m_t = a vector, as for *x*, but containing the means of all predictor variables in the given linear discriminant function, for the given sample, of class *t*

S = pooled covariance matrix (the variance of the multivariate observation)

q_t = value of the prior probability that a given sample is Class A, B, C, or NA

The probability (association value) of a sample *x* belonging to a particular class *t* is:

$$P_t(x) = \frac{e^{[-0.5Z_t(x)]}}{\sum_{A}^{NA}[e^{[-0.5Z_{A,B,C,NA}(x)]}]}$$

Where:

$P_t(x)$ = the probability that sample *x* belongs to class *t* (for Classes AA/A, B, C, NA)

e = the exponential function

-0.5 = a standardized constant from the normal distribution

Z_t = the discriminant score or Mahalanobis Distance for class *t* (Classes AA/A, B, C, NA)

Once the probability that a site belongs to a certain class is calculated, the MDEP follows a process to determine whether the site attains at least that particular class (Davies and Tsomides 2002, MDEP 2003). In order to determine whether a site attains at least Class C or is in non-attainment, the association value (Z_C) calculated using the 'C or better model' needs to be used. If the association value is greater than 0.6, then the sample attains to Class C or higher, but if it is less than 0.4 then the site is in non-attainment. If a site falls within 0.4 - 0.6, then best professional judgment is used to determine if the site belongs in Class C, is in non-attainment, or is indeterminate of Class C. For any site found to be indeterminate, additional monitoring may be needed in order to make a decision.

Those samples that do attain to at least Class C are then tested for Class B attainment using the association values (Z_B) from the 'B or better model'. If the association value is greater than 0.6 then the sites are at the minimum attaining to Class B status. Those values below 0.4 are now considered to be sites that attain to Class C. If a value falls between 0.4 - 0.6 then it is indeterminate of Class B and may require additional monitoring to determine to which attainment class the site belongs.

When the association value for a site is 0.6 or greater using the Class B or better model, it is then tested using the 'A or better model'. If the association value (Z_A) is 0.6 or greater, then the class attains to A. If the value is 0.4 or less, then the class attains to Class B. If the value is between 0.4 - 0.6, the finding is indeterminate of Class A and additional sampling may be required. Figure 3-4 graphically shows the process for calculating model variables and association values using linear discriminant models and Figure 3-5 shows how the attainment classes are determined using association values (MDEP 2003).

After a site is placed into an aquatic use attainment class, a provision in MDEP regulation Chapter 579 (MDEP 2003) allows for professional judgment to make an adjustment to the evaluation. Any adjustment may be made using analytical, biological, and habitat data. Professional judgment also may be employed when the condition of the stream does not allow for the accurate use of the linear discriminant models. Such factors may include habitat influences (e.g., lake outlets, impounded waters, substrate characteristics, tidal waters), sampling issues (e.g., disturbed samples, unusual taxa assemblages, human error in sampling), or analytical and sample processing issues (e.g., subsample vs. whole sample analysis or human error in processing) (Davies and Tsomides 2002).

If a water body falls into a lower class than its assigned statutory class after a MDEP biologist determines that adjustment is not needed, then the site is determined to be in non-attainment of its statutory class. When a site is found to be in non-attainment of its legally

defined class, then certain actions must be taken that include: 1) the notification of other programs (e.g., Licensing or Land Use Regulation); 2) the listing of the stream in question on the 303(d) list of impaired water bodies; and 3) the development of a total maximum daily load (TDML) for pollutants.

If a water body falls into a higher class than its assigned statutory class, then the higher aquatic life conditions must be maintained if the finding confirmed under critical water quality conditions. This finding also requires certain actions to ensure the findings were accurate and include: 1) the confirmation of the finding by resampling; 2) the confirmation that higher aquatic life quality exists at the maximal pollution loads; and 3) MDEP can propose that the water body be upgraded if dissolved oxygen, bacteria and habitat standards also adhere to the next higher class (MDEP 2002). Furthermore, data collected from two or more sampling events over different seasons must be evaluated to determine if a site has improved in quality and moved into the next higher statutory class.

All results and classification attainment decisions made by MDEP must be reported in the State of Maine Water Quality Assessment Report that is required under Section 305(b) of the CWA. If a water body is found to be in non-attainment of its statutory classification, then it is placed on Maine's list of impaired waters as required in Section 303(d) of the CWA.

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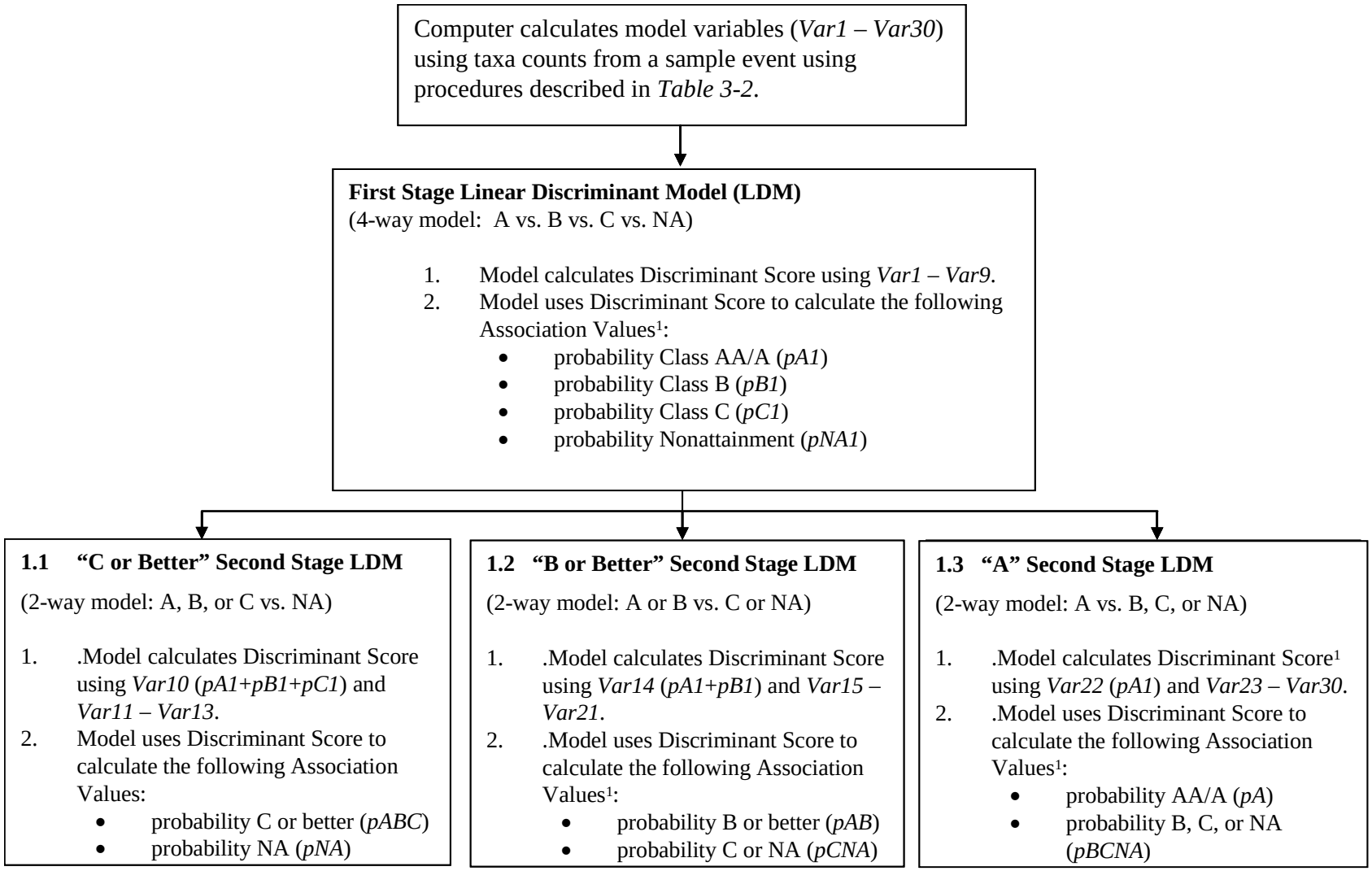

Figure 3-4. Process of calculating model variables and association values using linear discriminant models (taken from MDEP 2003).

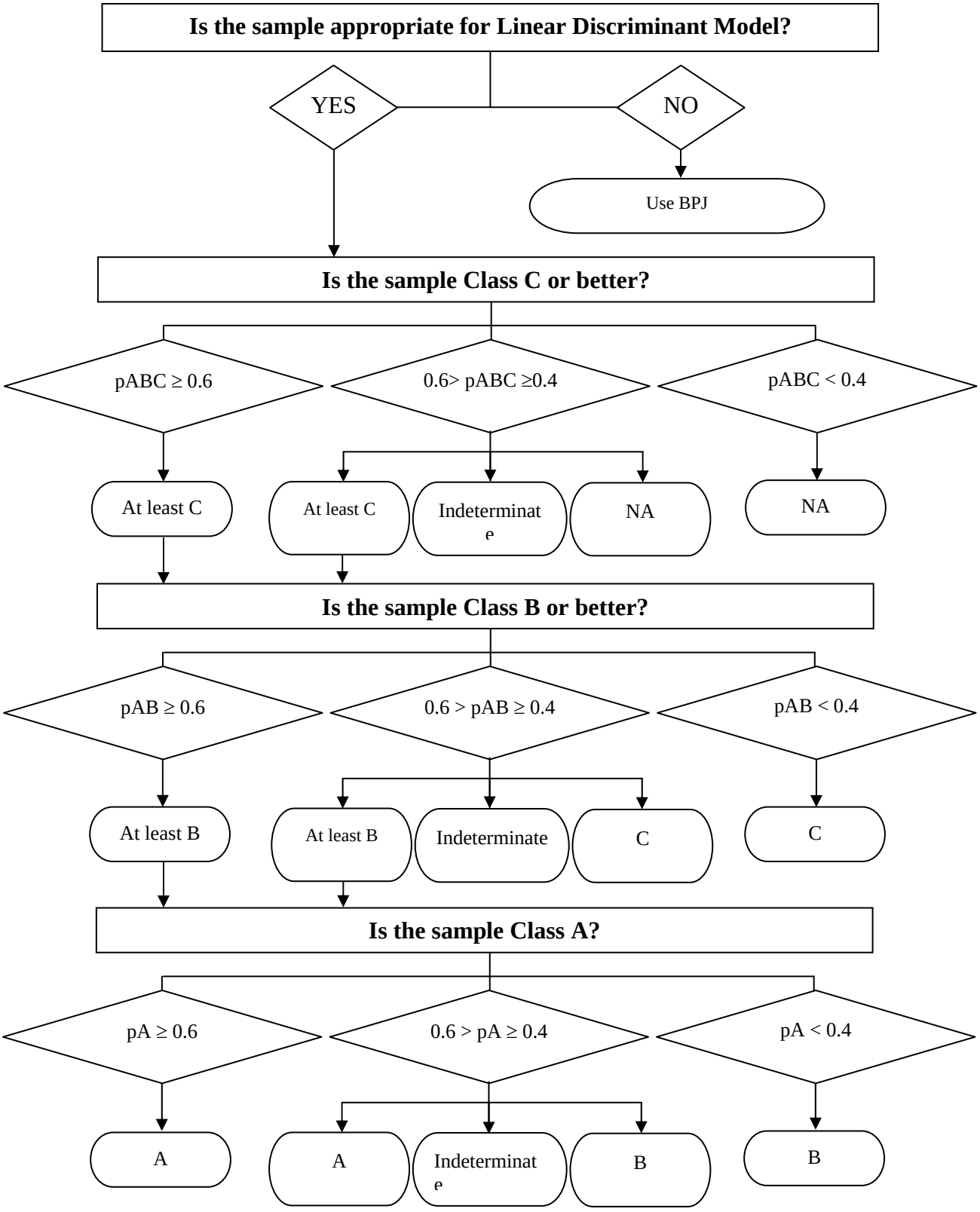

Figure 3-5. Process for determining attainment class using association values (modified from MDEP 2003).

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3.7 Resources

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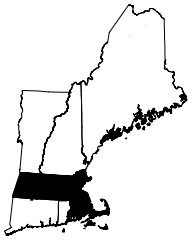
4 MASSACHUSETTS



This document was prepared using documents written by the Massachusetts Department of Environmental Protection. Any questions concerning bioassessment methods should be directed to:

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4.1 Introduction

As required by the CWA, MA DEP submits a biennial 305(b) report that describes the status of the state's water resources with respect to the classes defined by the Massachusetts Surface Water Quality Standards. The Massachusetts Surface Water Quality Standards assign support classes according to intended use, which include aquatic life, fish and shellfish consumption, drinking water supply, primary recreational contact, and secondary recreational contact (314 CMR 4.00 2000, Table 4-1). These standards were set to account for the most severe hydrologic conditions. In rivers, the standards must apply to waters at or above the seven-day ten-year flow statistic (7Q10). In regulated water systems, the standards must apply to the lowest discharge that meets or exceeds criteria 99% of the time on a yearly basis and any alternatives must be approved by the MA DEP Commissioner or the entity controlling flow (MA DEP 2003).

The ALUS criteria of the standards require that a suitable habitat be provided, "to sustain a native, naturally diverse community of aquatic flora and fauna" (MA DEP 2003). For assessment purposes each attainment class is composed of two sub-classes, the cold water fishery (capable of sustaining a year-round population of cold water aquatic organisms) and the warm water fishery (not capable of sustaining a year-round population of cold water organisms). Each stream that is assessed is designated as fully supporting, partially supporting, or non-supporting for its ALU class. Sites that are designated as "fully supporting" for a particular attainment class also may be given a threatened status if the stream is in danger of becoming polluted within a two-year period. Sites with too little or no data are not assessed.

The MA DEP uses biocriteria to aid in the assessment of the ALUS of surface waters. Macroinvertebrate, periphyton, and fish data are used by Massachusetts State Biologists to aid in determining ALUS attainment. Furthermore, MA DEP combines the biological data with habitat evaluations, toxicological and chemical data, and other environmental variables in order to make a final ALUS decision.

Table 4-1. Massachusetts attainment classes with management strategy and narrative biological and habitat criteria as stated in 314 CMR 4.00 (2000).

Class	Management	Biological and Habitat Narrative Criteria
A	These waters are designated as a source of public water supply. To the extent compatible with this use they shall be an excellent habitat for fish, other aquatic life and wildlife, and suitable for primary and secondary contact recreation. These waters shall have excellent aesthetic value. These waters are designated for protection as Outstanding Resource Waters under 314 CMR 4.04(3).	Excellent habitat for fish, other aquatic life and wildlife, supporting normal species diversity, successful migration, reproductive functions or growth of aquatic organisms.
B	These waters are designated as a habitat for fish, other aquatic life, and wildlife, and for primary and secondary contact recreation. Where designated they shall be suitable as a source of public water supply with appropriate treatment. They shall be suitable for irrigation and other agricultural uses and for compatible industrial cooling and process uses. These waters shall have consistently good aesthetic value.	
C	These waters are designated as a habitat for fish, other aquatic life and wildlife, and for secondary contact recreation. These waters shall be suitable for the irrigation of crops used for consumption after cooking and for compatible industrial cooling and process uses. These waters shall have good aesthetic value.	These waters are designated as a habitat for fish, other aquatic life and wildlife, protect normal species diversity, successful migration, reproductive functions or growth of aquatic organisms.

4.2 Key Elements of the Biological Assessment Approach

4.2.1 Index Period and/or Temporal Conditions

Originally, in order to represent the "worst-case" scenario, all sampling events were performed during low-flow, dry-weather, high-stress conditions. However, when MA DEP began to consider the impact of non-point source pollution, they altered the schedule to include a larger range of weather conditions. Typically, the sampling season is July through September and this sampling schedule is consistent from year to year so that data collected from surveys can be compared to make historical inferences. This time frame also coincides with streams returning to base flow conditions and occurs after the spawning season for most fish species present within the state.

4.2.2 Monitoring Program Survey Approach

The protocol of the MA DEP biomonitoring program divides the state into 27 major watersheds and coastal drainage areas that are sampled using a five-year basin rotation strategy

(MA DEP 2003, Figure 4-1). Within each basin, the first year of the program involves reconnaissance of basins and research to identify available data and/or information gaps. During the second year, streams are sampled and data are collected according to a MA DEP Quality Assurance Project Plan (QAPP). These sites are targeted based on known or suspected water quality degradation. In the third year, the stream assessments are made and published in State documents and/or 305(b) reports. Over the next two years, other watershed management issues are targeted (e.g., TMDL calculations, permit issuance, targeting of problem watersheds, outreach program implementation). In order to determine ALUS, MA DEP samples approximately 75 streams per year.

4.2.3 Natural Classification of Water Bodies

Massachusetts contains three Level III Ecoregions: the Northeastern Highlands, the Northeastern Coastal Zone, and the Atlantic Coastal Pine Barrens (Griffith et al. 1994). The Northeastern Highlands contains seven Level IV ecoregions (Taconic Mountains, Western New England Marble Valleys, Green Mountain/Berkshire Highlands, Lower Berkshire Hills, Berkshire Transition, Vermont Piedmont, and Worcester/Monadnock Plateau). The Northeastern Coastal Zone contains five Level IV ecoregions (Connecticut Valley, Lower Worcester Plateau/Eastern Connecticut Upland, Southern New England Coastal Plains and Hills, Boston Basin, and Narraganset/Bristol Lowland). The Atlantic Coastal Pine Barrens contains one Level IV ecoregion (Cape Cod/Long Island). For sampling purposes, the state is assessed on a basin-level scale (Figure 4-1), which may incorporate more than one Level IV ecoregion (Figure 4-2). Cold water and warm water streams are assessed as separate categories.

4.2.4 Indicator Assemblages

Biological, toxicological and chemical data are collected and used to make ALUS decisions based on a "weight of evidence" approach. Biological indicators include assessments of the macroinvertebrate, fish, and algal communities. An index based on the RBP II and III (Plafkin et al. 1989) is used to assess the macroinvertebrate assemblage. The overall structure and condition of the fish assemblage is assessed using some measurements from the RBP V (Plafkin et al. 1989). Water quality condition is also assessed using algal measurements (i.e., Chlorophyll *a* concentration, percent cover of green algae, and biomass).

4.2.5 Reference Condition (Arthur Johnson, Personal Communication)

Reference sites are identified by examining criteria and by using the best professional judgment of MA DEP Biologists. Sites that are described as "least impacted" have minimal or no potential to receive point or non-point source pollution and lack land use patterns that would degrade water or habitat quality. Maps and field reconnaissance are both used to locate unique reference sites when RPBs are used to assess a site. Multiple reference sites often exist for a study and occasionally reference sites of adjoining watersheds of the study site may be used. Sites are only considered reference for streams of similar elevation and drainage area.

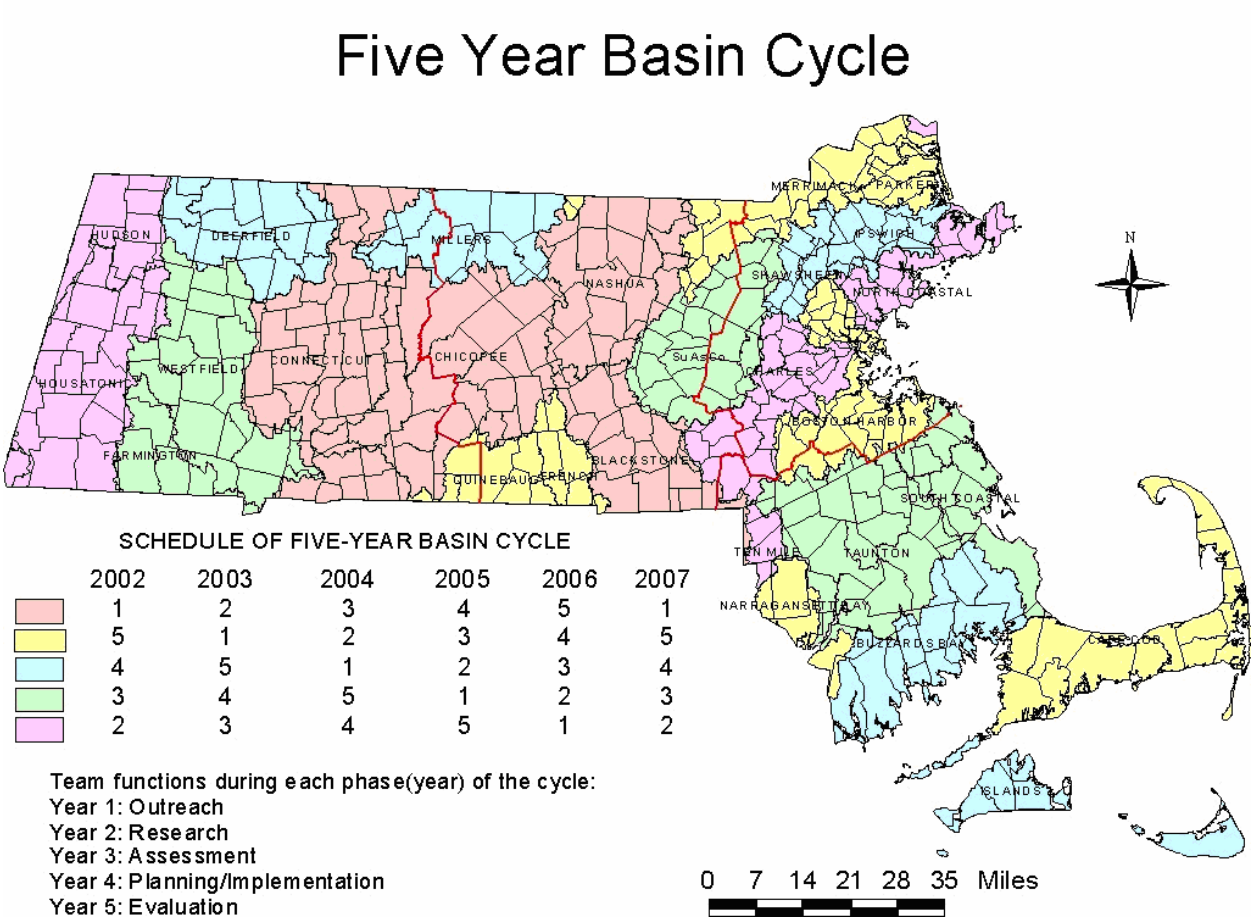

Figure 4-1. Massachusetts 5-Year Basin Rotation Strategy *(taken from the Massachusetts Department of Environmental Protection website www.mass.gov/dep/brp/wm/files/cyclemap6.jpg).*

4.3 Field and Laboratory Protocols

4.3.1 Macroinvertebrate Protocols (Taken from Nuzzo 2003)

4.3.1.1 Field Methods

The MA DEP uses one of three different methods to collect macroinvertebrates, depending on the depth and substrate of the stream. Kick sampling is the most commonly used method and is designed for use in wadeable streams that have coarse substrates, rock baskets are used in streams that are not wadeable or have fine substrates and for studies that require quantitative measurements, and Hester-Dendy multi-plate samplers are used in deep rivers. Each of the methods requires a habitat assessment to accompany the macroinvertebrate data to identify problematic areas and habitat destruction or loss. The MA DEP uses a visual-based rapid habitat assessment method (as described in Barbour et al. 1999) that includes ten habitat categories that are rated from 0 (lowest) to 20 (optimal).

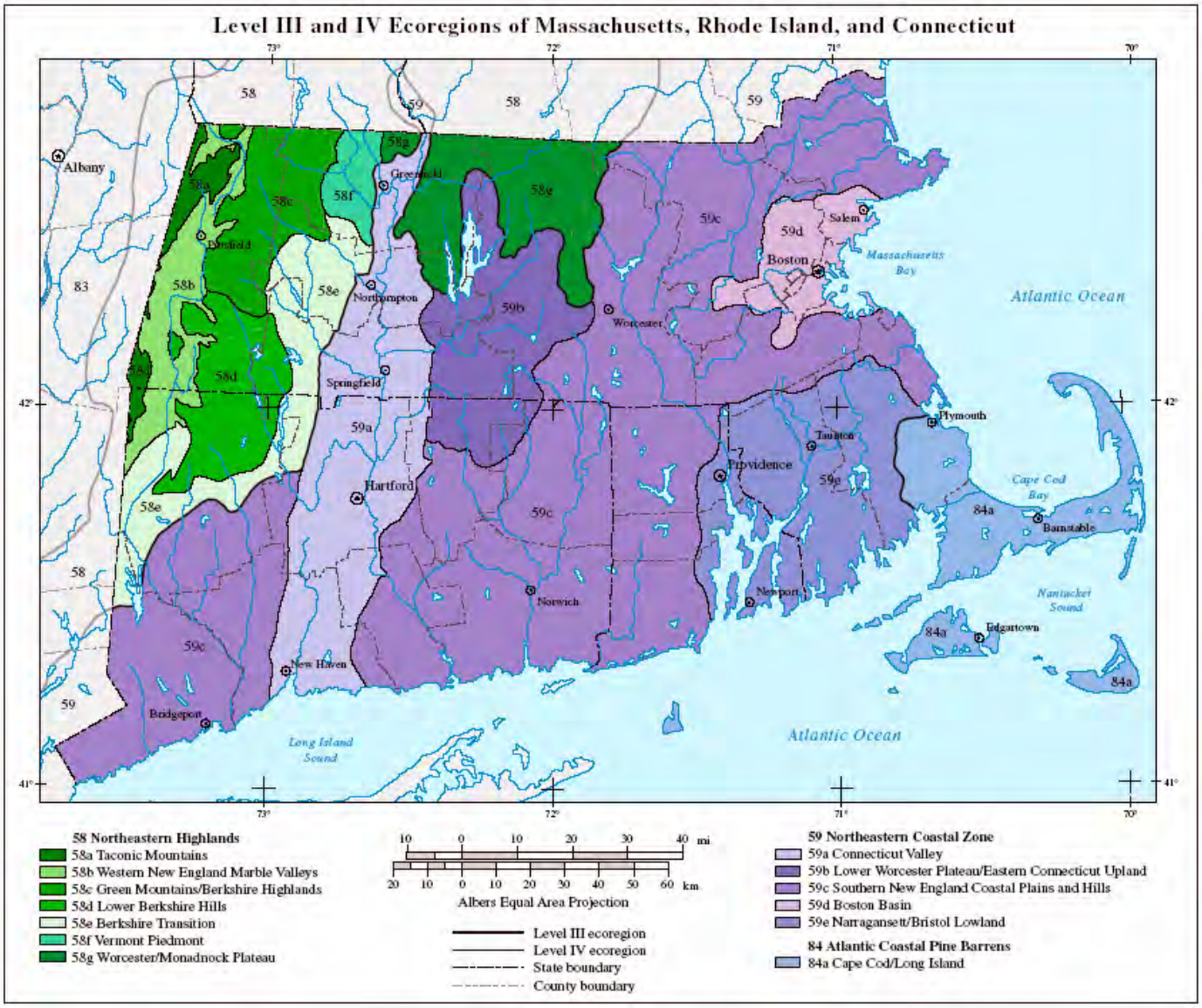

Figure 4-2. Level III and Level IV Ecoregions of Massachusetts *(taken from Griffith et al. 1994, http://www.epa.gov/wed/pages/ecoregions/mactri_eco.htm).*

4.3.1.1.1 Kick Sampling

Kick sampling is used for routine biomonitoring in wadeable streams with coarse substrates and riffle habitats. A 100-meter reach is chosen to represent the best habitat for the particular sample area. Then, a 0.46-m x 0.46-m, 500-µm mesh kick net is placed into the riffle and pressed firmly against the bottom so that the net is facing upstream. A biologist then kicks upstream in an area approximately the same size as the kick net, allowing material to be caught in the net. Following a kick (minimally 30 seconds), the net is examined, any macroinvertebrates residing on large debris are rinsed off into the net, and the excess debris is then placed back into the stream. In streams where the riffles within the reach are inadequate to allow for a 2-m^2 composite, other productive habitats are sampled by jabs into snags and/or by rubbing substrates. The contents of the net are then emptied into a 2-L wide-mouth leak-proof Nalgene bottle. Ten kick samples (approximately equaling 2-m^2) are collected at a site and composited into one bottle. The contents are preserved with enough denatured 100% reagent alcohol (5% methanol, 5% isopropanol, 90% ethanol) to cover the residue. If preservative is not added to the sample, it must be placed on ice and processed within 48 hours of collection.

4.3.1.1.2 Rock Basket Sampling

When kick sampling is deemed inappropriate for stream biomonitoring, rock baskets are used to collect benthic macroinvertebrates. Situations where rock baskets may be employed include planned statistical treatments of benthos data requiring quantitative collection methods, water depth too great for kick sampling, and substrate which is too fine for kick sampling at one or more sites used for comparison studies. The MA DEP uses rock baskets that are filled with roofing stone. Each basket is approximately the same weight and uses 2.5 -7.5 cm sized rocks. Three baskets are placed separately at the bottom of the stream in a riffle for six to eight weeks (in a current velocity of 15 - 76 cm/s). Upon retrieval, a kick-net is pressed tightly against the stream bed along the edge of the basket's downstream edge. The basket is then lifted onto the net. If the removal is difficult due to water depth, a cover is draped over the basket (Courtemanch 1984). After removal, each basket is placed into a separate large bucket or tub of water and then opened. All of the contents are emptied into the bucket and each rock is rinsed and set aside. After all of the rocks have been rinsed, the contents of each bucket are run through a #30 mesh (600 µm) and then placed into a sample container and preserved with denatured 100% reagent alcohol. If the sample is to be processed within 48 hours, the sample is put on ice without preservative.

4.3.1.1.3 Hester-Dendy Multi-plate Sampling

Hester-Dendy multi-plate samplers are used in deep rivers or where kick sampling is inappropriate and rock baskets are impractical. The MA DEP uses round-plate samplers as specified by U.S. EPA (Klemm et al. 1990). The samplers are placed in deep waters and tethered to an anchored float so that the sampler is suspended 1 m below the surface. Samplers placed in shallow water are mounted to a patio block or 4-in cinder blocks. In order for comparable data to be collected, each sampler is placed in a riffle of a similar velocity (within the range of 15 and 76 cm/s). Three Hester-Dendy multi-plate samplers are independently deployed in a stream at the same time for six to eight weeks. When they are retrieved, samplers

are completely surrounded with a 500-µm mesh net, a plastic bag, or a 2-L wide-mouth jar and pulled from the water. Enough water is placed in the 2-L wide-mouth jar to completely cover each sampler and then it is tightly capped and placed on ice and labeled. All multi-plate samplers are removed, placed in separate bottles in a refrigerator, and processed within 48 hours. After processing, samples are preserved with 100% reagent ethanol.

4.3.1.2 Laboratory Methods (Nuzzo 2003)

4.3.1.2.1 Processing of Kick Net and Rock Basket Samples

The MA DEP uses RBPs to process the macroinvertebrate data collected using kick nets or rock baskets (RBP II and RBP III; Plafkin et al. 1989) to obtain 100 organisms in the subsample. Each sample to be sorted is first run through a 600-µm mesh sieve and held over a waste collection vessel to collect the decanted alcohol preservative, then rinsed three times with enough water so that the sample is washed free of any preservative. Following rinsing, the sample is left to drain in the sieve for one minute and then the sample is spread evenly across a gridded pan that contains 25 squares 6-cm x 6-cm. Multiple pans are used to distribute the material evenly and to reduce the density of organisms per grid to a workable number (e.g., pan A contains grids 1-25, and pan B contains grids 26 - 50). Sufficient water is then added to distribute the material evenly across the pans. Next, the sorter chooses random numbers within the range of the sum of total squares used to hold the sample. Once a random grid square is chosen, only the material from that square is removed and it is placed into a petri dish that has been divided into quarters. One of the quadrants inside the petri dish is randomly selected and all organisms are sorted from that portion. The remaining debris in the petri dish is discarded into the waste collection vessel. The process is then repeated, so that complete quadrants are sorted until at least 100 organisms are obtained in the sample or until sorting yields no fewer than 90 organisms and the spread between the highest and lowest count among subsamples being compared is not greater than ±10%. If a problem arises from the original sample because the primary data are questioned, an additional 100-200 organisms are sorted, stored separately and labeled as “subsample extras”. Furthermore, if any specimens in good condition are encountered, they may be kept separately from the subsample and stored in separate vials as voucher specimens.

4.3.1.2.2 Processing of Hester-Dendy Multi-plate Samples

Hester-Dendy multi-plate sampling of the macroinvertebrate assemblage uses a different protocol because all processing must take place within 48 hours of collection. After removal from the refrigerator, the entire multi-plate sampler and the water in which it is stored are transferred into a large pan. The sample container is then rinsed into the pan three times with small volumes of water to ensure that no organisms remain attached to the inside of the container. The multi-plate is then disassembled and all pieces are rinsed. The sample is then poured through a #30 mesh sieve, and the resultant debris is placed into a jar and preserved with denatured 70% ethanol or sorted immediately as required by the study design. Normally all macroinvertebrates recovered are identified; however, if the macroinvertebrate density is high, only a subsample is identified.

4.3.1.2.3 Taxonomic Identification

A taxonomist identifies macroinvertebrates to genus or species level if the RBP III protocol is used. Identification may be to the family or order level if the RBP II protocol is being followed, or if the organism is in poor condition, immature, or not identified in an available key. MA DEP uses a number of keys to identify macroinvertebrates (Klemm 1985; Kathman and Brinkhurst 1998; Jokinen 1983; Smith 1986; 1987; 1988; Merritt and Cummins 1996; and Peckarsky et al. 1990). After identification, specimens are placed in labeled glass vials and preserved with denatured 70% ethanol.

4.3.1.2.4 Oligochaeta and Chironomidae Identification

Chironomid midges and oligochaetes are identified using slide mounts under a compound microscope using permanent or semi-permanent mounting medium. A 3-in x 1-in microscope slide is situated with the label end facing left. Three worms or midges are then placed side-by-side with heads towards the top. A sufficient amount of CMC-10 is then added to the specimens to cover them and surround them. Worms are uncoiled and placed on their sides using forceps. The heads of the midges are separated from the body using an insect pin and forceps and then they are oriented so that their bodies are on their side and the heads are placed with the ventral side up. A #1 cover slip is then added and gentle pressure is applied to ensure that all air bubbles have been removed and so that the mandibles of midges are opened. CMC-10 is added to the edge of the cover slips and the slides are dried for at least one week horizontally before placement into a storage box. Identification by taxonomists are made using the keys by Kathman and Brinkhurst (1998), Bode (1990), Coffman and Ferrington (1996), and Wiederholm (1983, 1986).

4.3.2 Periphyton Protocols (Beskenis 2002, Draft 2003; Barbour et al. 1999)

4.3.2.1 Field Methods

MA DEP biologists evaluate stream condition using assessments of the periphyton assemblage above and below point and non-point sources or compared to a reference stream to look for toxicity issues, nutrient impacts and habitat alterations. The data from the periphyton sampling is used to evaluate if either the aesthetics or ALU is affected. MA DEP identifies both diatoms and soft algae to the genus level, and they incorporate other algal measurements: biomass (AFDM), chlorophyll *a* determination, and percent coverage.

4.3.2.1.1 Algal Abundance and Identification

Within the same 100-m reach used to collect macroinvertebrate samples (see section 4.3.1), benthic algal samples are collected using the RBP Single Habitat Approach from riffles with a current of 10-50 cm/s. All samples are scraped from either natural or artificial substrates following U.S. EPA's RBPs (Barbour et al. 1999). Substrate (preferably cobble) within the stream reach is scraped with a knife and the detached algae are then washed into a labeled glass vial using stream water. All vials collected from a site are tightly closed and placed into a large plastic jar containing stream water to ensure a regular temperature is maintained. Then the

samples are transported back to the laboratory to be stored in the refrigerator until identified and counted. If a sample is held for an extended time before analysis the sample is preserved with M3 fixative.

4.3.2.1.2 Biomass

MA DEP uses artificial substrates to collect algae data that determine periphyton biomass and chlorophyll *a* measurements (APHA 1992). The artificial substrate apparatus is constructed from a wooden tray attached to a cinder block with nylon twine. Slides are placed vertically in the wooden tray and the apparatus is placed into the water at a depth of at least 0.25 m and less than 1 m for a period of approximately three weeks. Any duplicate apparatuses are placed at comparable light and flow regimes.

4.3.2.1.3 Chlorophyll *a*

Chlorophyll *a* samples are typically measured using two slides from trays that have been deployed into a stream for three weeks to measure biomass. The two slides removed from the tray are then placed on ice, brought back to the lab and either filtered and ground or frozen until analysis (Beskenis Draft 2003). An alternative method to collect periphyton chlorophyll *a* is to scrape a known area of rocks clean. Then, the material is rinsed into a bottle with water and a subsample is removed for chlorophyll *a* analysis. Samples are typically processed immediately upon arrival and must be analyzed within 21 days of first filtering or freezing.

4.3.2.1.4 Percent coverage calculation (Beskenis 2002)

Percent algal cover is determined in the riffle zones and is usually determined visually by the biologist conducting the on-site survey. The surveyor will also note whether the algal composition is microalgae or macroalgae. If a stream consists of greater than 50% macroalgae, it may be considered to be threatened based upon review of other data including, if available, DO measurements and impacts on the macroinvertebrate assemblage.

MA DEP also uses the point-intercept method to determine percent algal cover. For this method, a bottom viewer constructed by cutting a hole in the bottom of a bucket greater than 0.5 m wide) and attaching a piece of clear acrylic material to the bottom with silicon caulk is used to assess the algal cover. The viewing bucket has 50 evenly spaced dots forming a grid on the clear acrylic bottom. Three transects are then laid out across areas where benthic algae are observed, insuring that the left and right banks and the middle are covered. The viewer is then placed into the stream and the number of dots covering the macroalgae are counted and recorded. A subsample of the macroalgae is collected for identification. Then, the process is repeated to count the number of rocks that contain substrata with microalgae on them. The microalgal composition is assessed to determine if it is composed of diatomaceous, green algae, or blue-green algae and a subsample is collected for identification. If an algal mat exists, the thickness is determined in the field using a ruler and a score is given to describe the mat as a function of thickness:

Score	
0	Substrate is rough with no visible evidence of microalgae
0.5	Substrate is slimy and no visible accumulation of microalgae is present
1	A thin layer of microalgae is present
2	Accumulation of a microalgae mat from 0.5 - 1 mm thick is present
3	The microalgae mat is 1- 5 mm thick
4	The mat is 5 mm - 2 cm thick
5	The mat is > 2 cm

Abundance is calculated by counting the number of organisms of each genus in each field view. If less than one cell per field is counted, the genus is considered rare (R). If at least one cell of a genus (but fewer than five cells) is counted per field, the genus is common (C). If between five and 25 cells are counted per field then the genus is very common (VC). A genus is abundant (A) if greater than 25 cells per field are present and very abundant (VA) if the cells are too abundant to count.

4.3.2.1.5 Biomass Determination

When the artificial substrate sampling device is retrieved, the wooden trays and slides are removed from the cinder blocks and placed in plastic bags on ice. The two outside slides are discarded and a random number generator is used to choose two slides to be analyzed for chlorophyll *a* (See chlorophyll *a* determination). The remaining four slides are used to measure biomass as AFDM. After removal from the tray the slides are first air-dried. China crucibles are cleaned and dried at 105ºC and then cooled in a desiccator and weighed. Crucibles are returned to the oven for one hour, cooled and re-weighed until there is less than a 1% change in weight for each of the crucibles. After the slides are removed from the muffle furnace, one slide is retained for the archive and three of the slides are used as replicates and scraped into the pre-cleaned and pre-weighed china crucibles. A few drops of water are added to the crucibles and then they are placed into the combustion oven at 500ºC for one hour (Thermolyne Sybron furnatrol 11, type 13300). After the oven cools to 200ºC, the samples are removed and placed in a dessicator before water is added for re-hydration. After the sample is re-hydrated, it is dried to a constant weight at 105ºC and then the final weight is recorded. To calculate productivity, the following equation is used:

$$P = \frac{(mg\ of\ \ AFDM\ \ per\ slide)}{t * A}$$

P= net productivity (mg ash-free weight/m^2/day)
t= exposure time (days)
A= area of slide (m^2)

The mean weight from the slides is calculated and reported as dry weight and ash-free dry weight/m^2 using the formula:

$$g / m^2 = \frac{Mean\ AFDM\ in\ g}{0.00375\, m^2}$$

To calculate the Autotrophic Index (Biggs 1996):

$$AI = \frac{Biomass\,(ash\ \ free\ weight\ of\ \ organic\ matter\ \ in\ \ mg\ /\ m)^2}{Chlorophyll\ a\ in\ mg\ /\ m^2}$$

AI values range from 50 - 200. Larger values indicate a greater number of heterotrophic (fungi, bacteria) than autotrophic (algae) organisms.

4.3.2.1.6 Chlorophyll *a* Determination (Beskenis Draft 2003)

MA DEP has modified the U.S. EPA Fluorometric Method 445.0 to measure chlorophyll *a* samples using a Turner Design Fluorometer TD-700. The modified method used does not require acidification of the sample since background compounds are eliminated with the use of a filter and blue (mercury) lamp for analysis. Before any samples are run on the fluorometer, it is calibrated using chlorophyll *a* standards, and samples are analyzed according to Massachusetts Division of Watershed Management (MA DWM) methods (Beskenis 2003 Draft). All chlorophyll *a* concentrations are reported as mg/m^3 and used in conjunction with abundance, biomass, and percent coverage data to make water quality condition inferences.

4.3.3 Fish Protocols (from Maietta and Decesare 2001)

MA DEP evaluates fish populations using a method based on U.S. EPA RBP V (Plafkin et al. 1989) to aid in the determination of ALUS classifications in streams. While monitoring streams for fish, biologists also assess the habitat and characterize the physical and chemical water quality within the sample reach. A representative 100-m reach is selected and measured such that the primary physical habitat characteristics of the stream are included within the reach (i.e., riffle, run and pool habitats, when available). MA DEP selects sample reaches away from the influences of major tributaries and major bridge and road crossings. Fish are collected using a pulsed direct current (DC) backpack electrofisher. A crew of at least 3 people begins at a shallow riffle or other physical barrier at the downstream limit of the stream reach and moves upstream sweeping from bank to bank until an upstream barrier is reached. If a natural barrier is not present, block nets are set prior to sampling. All fish caught are added to a bucket or live well. All wadeable habitats within the reach are sampled with one pass. However a second pass is required at sites where estimated capture efficiency of observed fish is less than 75%. Fish are identified to species or subspecies on-site by a qualified/trained fish taxonomist, familiar with Massachusetts ichthyofauna. Fish are also examined for any deformities, eroded fins, lesions, and tumors. Any young-of-the-year fishes less than 20 mm in length are not identified or counted and are released back to the water. A subsample (maximum of 25 specimens) of each species is measured to the nearest mm in length and weighed to the nearest g. Any unknown specimens are preserved in 10% formalin solution and transported to the lab for identification by a second qualified fish taxonomist.

4.4 Data Management/Quality

All assessment data collected by the MA DEP are entered into the U.S. EPA's Water Body System (WBS) electronic database. This system aids in the determination of use support and compiles the data collected throughout the state. The MA DEP also uses a database called MABEN to analyze macroinvertebrate samples and to determine the condition of the community at a site using a multimetric approach (Nuzzo 2002). Within the database, taxa richness, HBI (or FBI), EPT index, ratio of EPT abundances to Chironomidae abundance, ratio of scrapers to filtering collectors, % contribution of dominant family, and similarity are calculated (Table 4-2).

4.5 Analysis of Biological Data

4.5.1 Macroinvertebrate Data (Nuzzo 2003)

MA DEP uses modifications of the RBPs (II and III) to calculate metrics for the benthic macroinvertebrate assemblage (Plafkin et al. 1989). The RBP II is designed for family level taxonomic identification of macroinvertebrates, while the RBP III protocol separates streams based on generic- and species-level identifications. The metrics are nearly identical between the two with the exception that the FBI is used for the RBP II (Table 4-2), and the HBI is used for the RBP III analysis (Table 4-3). Each metric is calculated and then assigned a metric score (6, 3, or 0 for the RBP II; 6, 4, 2, or 0 for the RBP III) based on a comparison with values from reference sites. Details of metric calculation and scoring are provided in Tables 4-2 and 4-3. The scores given to each of the individual metrics are then summed to yield a single index value. Based on the overall index value, the RBP II has the capability to discern between three different impact categories: Not Impacted, Moderately Impacted, and Severely Impacted. The RBP III discerns between four different impact categories: Not Impacted, Slightly Impacted, Moderately Impacted, and Severely Impacted. The overall impact category is determined by comparing the index score of the test stream to that of the reference site(s), and then determining if the score lies within the threshold value assigned to the expected level of impairment (Tables 4-2 and 4-3).

Table 4-2. Methods for the calculations of metrics and scoring ranges used in RBP II determinations of level of biological impact (Plafkin 1989; Nuzzo 2003).

Metric	Method	Scoring Ranges		
		6	3	0
Taxa Richness [(a)]	The total number of distinct taxa in a sample	>80%	40-80%	<40%
EPT[(a)]	The number of taxa within the orders of Ephemeroptera, Plecoptera, and Trichoptera	>90%	70-90%	<70%
EPT/Chironomidae (abundance ratio) [(a)]	(Abundance of EPT organisms)/(Abundance of EPT + Chironomidae)	>75%	25-75%	<25%
FBI (modified)[(b)]	(Number of individuals in family *i*) x (Family *i* tolerance value)/(Total number organisms in sample)	>85%	50-85%	<50%

Metric		Method	Scoring Ranges		
			6	3	0
Scraper/Filtering Collector Ratio[a]		(Number of scrapers)/(Number of filtering organisms)	>50%	25-50%	<25%
% Contribution of Dominant Taxon [c]		(Number of individuals in most common taxon)/ (Total number of organisms) x 100	<30%	30-50%	>50%
Community Similarity Index	Community Loss [d]	A measure of the dissimilarity between a test site and a reference site (Plafkin et al. 1989). Metric values increase as biological impairment increase. Values have no limits. CLI = a - c / b where: a = number of families in reference sample, b = number of families in test sample, c = number of families common to both samples	<0.5	0.5-4.0	>4.0
	% Similarity [d]	$\%\ Similarity = \sum_i \min(a_i, b_i)$ Where a_i is the percentage of taxon *i* in sample *a* and b_i is the percentage of taxon *i* in sample *b*. Higher values indicate a healthier site.	>70%	30-70%	<30%
	% Reference Affinity[d]	A test site compared to the reference site for seven faunal groups (Ephemeroptera, Trichoptera, Plecoptera, Chironomidae, Oligochaetes, Coleoptera, and Other. Calculated similarly to % Similarity but only percentages for the aggregate groups are used (Novak and Bode 1992).	>64%	35-64%	<35%

% Compared to Reference and Impact Category
> 79% Not Impaired 29-72% Moderate Impaired 21% Severe Impaired

a) Value is converted to ratio of test to reference site *100

b) Value is converted to ratio of reference to test site *100

c) Actual percent contribution used in scoring, not ratio to reference

d) Uses range of values actually obtained

Table 4-3. Methods for the calculations of metrics and scoring ranges used in RBP III determinations of level of biological impact (Plafkin 1989; Nuzzo 2003).

Metric		Method	Scoring Ranges			
			6	4	2	0
Taxa Richness[a]		The total number of distinct taxa in a sample	>80%	60-80%	40-59%	<40%
EPT[a]		The number of taxa within the orders of Ephemeroptera, Plecoptera, and Trichoptera	>90%	80-90%	70-79%	<70%
EPT/ Chironomidae (abundance ratio)[a]		(Abundance of EPT organisms)/(Abundance of EPT + Chironomidae)	>75%	50-75%	25-49%	<25%
HBI (modified)[b]		(Number of individuals in taxon *i*)x(tolerance value of taxon *i*)/(Total number of organisms in sample)	>85%	70-85%	50-69%	<50%
Scraper/Filtering Collector Ratio[a]		Number of scrapers/filtering organisms.	>50%	35-50%	20-34%	<20%
% Contribution of Dominant Taxon[c]		Number of individuals in most common taxon/ total number of organisms x 100.	<20%	20-29%	30-40%	>40%
Community Similarity Index	Community Loss[d]	A measure of the dissimilarity between a test site and a reference site (Plafkin et al. 1989). Metric values increase as biological impairment increase. Values have no limits. CLI = a - c / b where: a = number of genera in reference sample, b = number of genera in test sample, c = number of genera common to both samples	<0.5	0.5-1.5	1.6-4.0	>4.0
	% Similarity[d]	$\%\ Similarity = \sum_i \min(a_i, b_i)$ Where a_i is the percentage of taxon *i* in sample *a* and b_i is the percentage of taxon *i* in sample *b*.	>70%	50-70%	30-49%	<30%
	% Reference Affinity[d]	A test site compared to the reference site for seven faunal groups (Ephemeroptera, Trichoptera, Plecoptera, Chironomidae, Oligochaeta, Coleoptera, and Other). Calculated similarly to % Similarity but only percentages for the aggregate groups are used (Novak and Bode 1992).	>64%	50-64%	35-49%	<35%

% Compared to Reference Sites
> 83% Not Impaired 54-79% Slight Impaired 21-50% Moderate Impaired <17% Severe Impaired

Metric	Method	Scoring Ranges			
		6	4	2	0
a) Value is converted to ratio of test to reference site *100 b) Value is converted to ratio of reference to test site *100 c) Actual percent contribution used in scoring, not ratio to reference d) Uses range of values actually obtained					

4.5.2 Algal Data (Arthur Johnson, Personal Communication)

Algal data are used in aquatic use attainment as an indicator of nutrient enrichment of a water body. MA DEP has not developed metrics for algae but does use chlorophyll *a*, percent cover of green algae, and biomass to determine if the levels of algae are high enough to disrupt the biological value of the stream. Aquatic life support is considered fully supporting if no algal blooms are detected, while persistent algal blooms may represent impairment of ALU support.

4.5.3 Fish Data

MA DEP uses a modification of the RBP V (Plafkin et al. 1989) to assess some aspects of the fish assemblage in streams. A suite of measurements is used to assess the overall structure and condition of the fish assemblage. These calculations require the taxonomic identification of fish to the species level and include: number of fish species, number of fluvial specialists/dependents, number of intolerant species and number of salmonid species. Although MA DEP does not use an IBI, they do use the fish assemblage data with macroinvertebrate metrics qualitatively to help determine biological attainment. Furthermore, a detailed habitat assessment (Barbour et al. 1999) is made to accompany the data.

4.5.4 Summary: Determining ALU Support

The MA DEP uses a "weight of evidence" approach to assign ALU designations and attainment status for monitored streams. Data are collected from the biological (biotic and habitat), toxicological and chemical components and referenced against criteria (narrative or numerical) to make a final decision on the ALUS (Table 4-4).

The biological component used to make the decision takes into consideration the outcome of the macroinvertebrate index (i.e., impairment designation: Tables 4-2, 4-3 and 4-4), the structure and condition of the fish assemblage, the habitat and flow regime of the stream, and algal presence as measured by chlorophyll *a*, biomass, and percent coverage. Once the available evidence is analyzed and weighed, a site is placed into an attainment category (e.g., Supported or Impaired) for the water quality class to which it was originally assigned (e.g., A, B or C). These assignments are then outlined in the 305(b) report and any streams not in attainment are placed on the Massachusetts 303(d) list.

Table 4-4. Biological, toxicological, and chemical parameters that are used collectively to determine ALUS. Attainment is assigned based on a "weight of evidence" evaluation. (MA DEP 2003) (Numerical criteria for dissolved oxygen, pH, and temperature can be found in 314 CMR 4.00 (MA DEP 2000). MA DEP uses the recommended limits published by EPA pursuant to Section 304(a) of the Federal Act for Toxic Pollutant Criteria).

Variable	***Support*** – Data available clearly indicates support or minor modification of the biological community. Excursions from chemical criteria not frequent or prolonged and may be tolerated if the biosurvey results demonstrate support.	***Impaired*** – There are frequent or severe violations of chemical criteria, presence of acute toxicity, or a moderate or severe modification of the biological community.
BIOLOGY		
Macroinvertebrates: Rapid Bioassessment Protocol (RBP) III*	Non/Slightly impacted	Moderately or Severely Impacted
Fish Assemblage	Best Professional Judgment (BPJ)	BPJ
Habitat and Flow	BPJ	Dewatered streambed due to artificial regulation or channel alteration, BPJ
Eelgrass Bed Habitat	No/minimal loss, BPJ	Moderate/severe loss, BPJ
Macrophytes	BPJ	Exotic species present, BPJ
Plankton/ Periphyton	No/infrequent algal blooms	Frequent and/or prolonged algal blooms
TOXICITY TESTS**		
Water Column/Ambient	≥75% survival either 48 hr or 7-day exposure	<75% survival either 48 hr or 7-day exposure
Sediment	≥75% survival	<75% survival
CHEMISTRY-WATER**		
Dissolved oxygen/percent saturation (MA DEP 1996, U.S. EPA 1997)	Infrequent excursion from criteria, BPJ (minimum of three samples representing critical period)	Frequent and/or prolonged excursion from criteria [river and shallow lakes: exceedances >10% of measurements; deep lakes (with hypolimnion): exceedances in the hypolimnetic area >10% of the surface area].
pH (MA DEP 1996, U.S. EPA 1999)	Infrequent excursion from criteria	Criteria exceeded >10% of measurements.
Temperature (MA DEP 1996, U.S. EPA 1997)	Infrequent excursion from criteria[1]	Criteria exceeded >10% of measurements.
Toxic Pollutants (MA DEP 1996, U.S. EPA 1999) Ammonia-N (MA DEP 1996, U.S. EPA 1999) Chlorine (MA DEP 1996, U.S. EPA 1999)	Infrequent excursion from criteria Ammonia is pH and temperature dependent[2] 0.011 mg/L (freshwater) or 0.0075 mg/L (saltwater) total residual chlorine (TRC)[3]	Frequent and/or prolonged excursion from criteria (exceeded >10% of measurements).

Variable	***Support*** – Data available clearly indicates support or minor modification of the biological community. Excursions from chemical criteria not frequent or prolonged and may be tolerated if the biosurvey results demonstrate support.	***Impaired*** – There are frequent or severe violations of chemical criteria, presence of acute toxicity, or a moderate or severe modification of the biological community.
CHEMISTRY-SEDIMENT**		
Toxic Pollutants (Persaud et al. 1993)	Concentrations ≤ Low Effect Level (L-EL), BPJ	Concentrations ≥ Severe Effect Level (S-EL)[4], BPJ
CHEMISTRY-TISSUE		
PCB – whole fish (Coles 1998)	≤500 µg/kg wet weight	BPJ
DDT (Environment Canada 1999)	≤14.0 µg/kg wet weight	BPJ
PCB in aquatic tissue (Environment Canada 1999)	≤0.79 ng TEQ/kg wet weight	BPJ

*RBP II analysis may be considered for assessment decision on a case-by-case basis.
**For identification of impairment, one or more of the following variables may be used to identify possible causes/sources of impairment: NPDES facility compliance with whole effluent toxicity test and other limits, turbidity and suspended solids data, nutrient (nitrogen and phosphorus) data for water column/sediments. [1]Maximum daily mean T in a month (minimum six measurements evenly distributed over 24-hours) less than criterion. [2] Saltwater is temperature dependent only. [3] The minimum quantification level for TRC is 0.05 mg/L. [4]For the purpose of this report, the S-EL for total polychlorinated biphenyl compounds (PCB) in sediment (which varies with Total Organic Carbon (TOC) content) with 1% TOC is 5.3 ppm while a sediment sample with 10% TOC is 53 ppm.

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4.6.1 Resources

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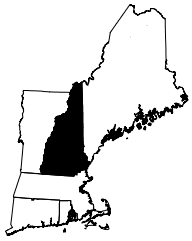
5 NEW HAMPSHIRE



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5.1 Introduction

The State of New Hampshire Water Quality Standards (NH WQS), as defined by the NH DES, recognizes six designated uses in surface freshwaters: aquatic life, drinking water supply, fish consumption, primary contact recreation, secondary contact recreation, and wildlife. The narrative standards for water use classifications are stated as: "(a) State surface waters shall be divided into class A and class B, pursuant to RSA 485-A:8, I, II and III. Each class shall identify the most sensitive use which it is intended to protect; (b) All surface waters shall be restored to meet the water quality criteria for their designated classification including existing and designated uses, and to maintain the chemical, physical, and biological integrity of surface waters; (c) All surface waters shall provide, wherever attainable, for the protection and propagation of fish, shellfish and wildlife, and for recreation in and on the surface waters; (d) Unless the flows are caused by naturally occurring conditions, surface water quantity shall be maintained at levels adequate to protect existing and designated uses" (NH DES 1999).

Furthermore, NH DES standards define biological and aquatic community integrity such that "a) The surface waters shall support and maintain a balanced, integrated, and adaptive community of organisms having a species composition, diversity, and functional organization comparable to that of similar natural habitats of a region" and where "b) Differences from naturally occurring conditions shall be limited to non-detrimental differences in community structure and function" (NH DES 1999). For rivers and streams and associated impoundments (4^{th} order or less), ALUS is determined based on ten indicators, with the macroinvertebrate assemblage as the core indicator of biological condition (NH DES 2004a). The other indicators are: dissolved oxygen, pH, habitat, water quality criteria for toxic substances, toxicity tests of ambient water, sediment quality, exotic macrophytes, flow, and benthic deposits. In all other surface waters, ALUS is determined based on biological assemblage data or a minimum of DO and pH. If these constituents are within allowable standards or if there is documentation from a trained biologist that there is no other obvious impairment to the biological community, then the water body can be listed as fully supporting.

Table 5-1. NH DES water quality classes and the defined designated uses for each class. Dissolved oxygen exceedance values for aquatic life criteria are also listed (NH DES 1999).

Classification	Designated Uses	Exceedances of the water quality criteria (aquatic life) for dissolved oxygen (Applies to any depth in free flowing rivers)
Class A	Generally of the highest quality and considered potentially usable for water supply after adequate treatment. Discharge of sewage or wastes prohibited to waters of this classification.	Daily Average Measurement: < 75% saturation; Instantaneous Measurement: <6 mg/l **<u>In cold water naturally producing fisheries</u>** Daily Average Measurement: From October 1 to May 14, a 7 day mean DO based on the daily average of < 9.5 mg/L; Instantaneous Measurement: From October 1 to May 14 DO < 8 mg/L
Class B	Of the second highest quality, considered acceptable for fishing, swimming and other recreational purposes, and, after adequate treatment, for use as water supplies.	Daily Average Measurement:<75% saturation; Instantaneous Measurement: < 5mg/l **<u>In cold water naturally producing fisheries</u>** Daily Average Measurement: From October 1 to May 14, a 7 day mean DO based on the daily average of < 9.5 mg/L; Instantaneous Measurement: From October 1 to May 14 DO < 8 mg/L

The NH DES uses a weight of evidence approach when taking in consideration biological, RBP habitat, *in situ* chemistry, physical (e.g., landuse coverages and point sources) and toxicological data to support narrative criteria that determine ALUS (NH DES 2004a). Biological and physical habitat data are given the highest weight as "they are a direct measurement of the aquatic life and detect the cumulative impact on the aquatic community including new or previously undetected stressors over time" (NH DES 2004a). NH DES collects fish and macroinvertebrate data in their biomonitoring program to assess the condition of streams for 305(b) reporting and 303(d) listing. A benthic macroinvertebrate multimetric index has been developed recently, and a fish index is currently under development through the modification of the Vermont Cold Water Index of Biotic Integrity (CWIBI) and Mixed Water Index of Biotic Integrity (MWIBI) (see chapter 7 in this document, as well as VT DEC 2004). All other data collected (chemical, physical habitat and toxicological) provide complementary information and assist the biologists in determining ALUS.

5.2 Key Elements of the Biological Assessment Approach

5.2.1 Index Period and/or Temporal Conditions

The NH DES biomonitoring program collects both macroinvertebrates and fish during the mid-summer to early fall index period. This period represents the lowest flows and most stressful hydrologic conditions for instream biota. During this period, the larvae of the macroinvertebrate assemblages are easily identifiable because the late instars are larger. Furthermore, the fish assemblage is more stable and resident species are more likely to remain in a localized area when flow conditions are not fluctuating dramatically. The sampling index period is consistent from year to year in order to accurately compare monitoring data through time.

5.2.2 Monitoring Program Survey Approach

When developing the biocriteria program, an effort was made by NH DES to initially sample least impacted sites from a variety of habitats. Since then the program has collected samples from moderate and high impact sites in order to develop a multimetric index that best represents a response of the biological community to human disturbance. Sites have also been chosen for macroinvertebrate and fish monitoring using a targeted approach for 305(b) reporting and for identifying sites that cover geographical data gaps. Approximately 25 – 30 sites are sampled statewide per year.

5.2.3 Natural Classification of Water Bodies

New Hampshire consists of five major river basins: Androscoggin, Connecticut, Saco, Merrimack, and Piscataqua (Figure 5-1). For macroinvertebrate biomonitoring purposes, the state is separated into bioregions defined by distinct biological community types. The boundaries for the "Northern" and "Southern" bioregions represent similar Ecological Drainage Units as defined by The Nature Conservancy (Figure 5-1). For fish, streams are divided into two categories: cold and warm water.

5.2.4 Indicator Assemblages

Currently (for the 2004 305 (b) report), NH DES is using the macroinvertebrate assemblage to determine ALUS. A Benthic Index of Biotic Integrity (B-IBI) is used to assess the macroinvertebrate assemblages (with separate biocriteria for northern and southern bioregions). A fish Index of Biotic Integrity (IBI) is currently being developed to assess the fish assemblages of both cold and mixed waters. NH DES is basing the fish IBI on Vermont's CWIBI and MWIBI because of the presumed similarity in fish assemblage composition, structure, and function.

5.2.5 Reference Condition

NH DES defines reference condition as the least disturbed sites. Reference sites were chosen based on a state-wide scoring system that takes into account both local and watershed

scale variables using GIS coverages of land use and point sources, as well as an immediate in-stream measure of human influence using the U.S. EPA's RBP habitat assessment (Barbour et al. 1999). On a watershed scale, land use was portioned into percentages developed, undeveloped and agricultural lands categories using the New Hampshire Land Cover (NHLC) dataset.

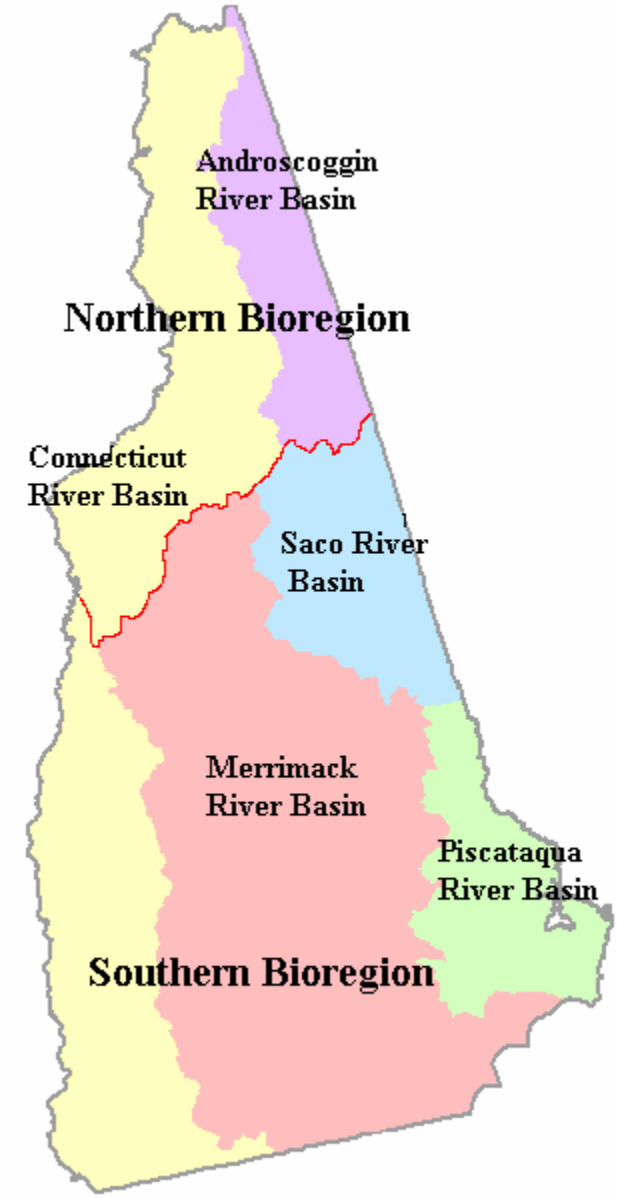

Figure 5-1. Major New Hampshire basins and the northern and southern bioregion boundaries used for macroinvertebrate sampling (indicated by the red line).

Percent water impounded was also calculated at the watershed scale. Local scale variables were estimated for a 300-ft buffer on either side of all hydrologic features within the watershed and within a 1-mile radius of the site. Densities of Ground Water Hazard Inventory (GWHI) sites, Resource Conservation and Recovery Act (RCRA) sites, junkyards, dams, water withdrawals, National Pollutant Discharge Elimination System (NPDES) sites, and roads were calculated at this local scale for each site. Each variable was scored on a 0-3 scale, with 0 representing

minimal disturbance for that variable and 3 representing maximum disturbance, based upon overall distributions across all sites sampled between 1997 and 2001. Scores were summed for both the local and watershed scale disturbance scores, as well as across all variables to yield a total disturbance score (the human disturbance gradient (HDG) score). The total score was divided into four disturbance levels with equal scoring ranges: 0-4 (Best), 5-9 (Good), 10-14 (Fair), and >14 (Worst). The entire range observed across all sites was 0 to 19. Those sites scoring in the best range were identified as the least disturbed sites. This HDG resulted in the identification of 46 reference condition sites within New Hampshire, 26 in the northern bioregion and 20 in the southern region.

5.3 Field and Laboratory Protocols

5.3.1 Macroinvertebrates Protocols

5.3.1.1 Field Methods (NH DES 2004b)

NH DES uses rock baskets to collect macroinvertebrates for B-IBI calculations. Rock baskets consist of an 11-in cylindrical plastic coated wire barbecue basket with 1-in^2 mesh openings. The bottom of the basket has a hinged opening. The basket is filled with regionally indigenous bank run gravel ranging in diameter from 1.5-3.0 in. The hinge is then secured with a plastic tie wrap. Baskets are placed in groups of three replicates attached to a ½-in steel rod anchor. Baskets are submerged to depths of greater than 5 in. Each of the attached baskets is arranged so that the bottom is facing downstream.

The baskets are left in the stream for eight weeks to allow for colonization. At removal, the baskets are approached from downstream and a 3-gal bucket containing a 600-µm sieve bottom is placed downstream of the rock baskets. The plastic cable ties that secure the three baskets to the steel rod are cut and the baskets are quickly placed into the bucket. Any extra debris or algae clinging to the outside of the rock baskets are removed and discarded. The bucket containing the baskets is then transported to the stream side for sample retrieval. Using a knife, the plastic tie wraps are cut and the rocks are emptied into the sieve bucket. Then, the empty rock basket is added to a 5-gal bucket containing 3-4 gal of water. The basket is scraped free of organisms using a soft bristle brush. The 5-gal bucket is then emptied into the sieve bucket. Next, 2-3 gal of water are added to the 5-gal bucket and the sieve bucket is nested inside. Using a soft bristle brush, the rocks inside the sieve bucket are scrubbed free of attached organisms, and each scrubbed rock is then returned to the rock basket. After all organisms and detritus have been removed from the rocks, the sieve bucket is lifted from the 5-gallon bucket, capturing the targeted sample in the sieve. The contents of the sieve bucket are then placed into a one-quart, wide-mouthed jar and preserved with a solution of 1/3 water and 2/3 ethanol. This process is repeated for each of the replicate baskets. The scraped rocks from the baskets are allowed to dry before baskets are reused.

5.3.1.2 Laboratory Methods (NH DES 2004b)

NH DES processes macroinvertebrate samples collected in rock baskets using the U.S. EPA Caton Method (Caton 1991). A tray is fitted with a gridded screen with 16 uniform squares. Enough water is added to the tray so that the entire sample can be dispersed evenly over

the screen. The screen is then removed so that the organisms are settled on the screen. Random numbers are then selected to choose the grids from which to sort organisms. A metal square is used to delineate a grid square and the organisms are removed using a scoop. Those organisms that occupy more than one grid are counted in the grid that contains the head, unless the head is missing. Then, the grid with the largest portion of the organism is counted. At least 25% of the squares are counted yielding a minimum sample of 100 organisms. In the event that the 100-organism target is not achieved, the entire sample is sorted. After sorting, benthic organisms are identified to the lowest possible taxonomic level. For data analysis, however, all Chironomidae were aggregated to the family level.

5.3.2 Fish Protocol (NH DES 2004b)

NH DES follows U.S. EPA's RBP V (Barbour et al. 1999) to collect fish from wadeable streams using backpack electrofishing units. For wadeable streams, a minimum reach length of 150 m is selected in close proximity to the location where the macroinvertebrate rock baskets were placed. NH DES has established a minimum 150-m reach as "the reasonable limit to prevent oversampling, while optimizing efficiency and representation of the resident species" (NH DES 2004b). A field sampling crew comprised of one shocker, at least two netters, and one person to carry an aerated bucket, begins at the most downstream portion of the reach and moves upstream. The crew collects a representative fish sample along the available instream habitat using a single-pass method. All fish identifications are made streamside. The identities, number of fish and external anomalies are recorded, and the fish are then released. For important cold water game fish, young-of-the-year (YOY) are recorded in order to document natural spawning activity. Fish that cannot be easily identified are taken back to the laboratory for identification by a trained biologist or using the fishes of New Hampshire key (Scarola 1973).

5.4 Data Management/Quality

NH DES has adapted the Ecological Data Assessment System (EDAS) to manage the chemical, physical and biological data collected in New Hampshire (EDAS 1999). EDAS is a Microsoft Access-based data storage and retrieval warehouse that contains station information, basic water chemistry data, fish data, habitat assessments, macroinvertebrate data and flow data. Updated GIS coverages of sample locations and upstream watersheds are also maintained using ARCVIEW software. The GIS coverages include data pertaining to watershed size, station elevation, latitude, longitude, stream order, Human Disturbance Gradient (HDG) status, bioregion, and hydrologic unit code (HUC). The NH DES uses the Assessment Database (ADB) developed by the U.S. EPA to submit electronic reports. Since 2002, the new Oracle-based version has been used by NH DES to submit surface water assessments.

5.5 Analysis of Biological Data

5.5.1 Macroinvertebrate Data (Neils and Blocksom 2004)

The following seven metrics comprise the New Hampshire Benthic Index of Biotic Integrity (B-IBI): Total number of taxa, number of Plecoptera taxa, % Chironomidae, % non-insects, % clingers, number of tolerant taxa, and % individuals in intolerant taxa. NH DES used

the 95th percentile across all sites as a threshold for metrics that decrease in response to disturbance (i.e., positive metrics) and the 5th percentile across all sites for metrics that increase in response to disturbance (i.e., negative metrics) (Table 5-2). Scoring for each metric is on a continuous scale ranging from 0 to 100. Positive metrics are scored using the following equation:

$$SCORE = \frac{observed}{threshold} * 100$$

Negative metric scores are calculated using the following equation:

$$SCORE = \frac{(\max - observed)}{(\max - threshold)} * 100$$

The maximum is either the maximum possible (for percentage metrics) or the maximum observed in the calibration data set (for all other metrics). The metric scores for each site are calculated and averaged across all metrics, and the score is compared to the bioregional threshold value.

Within each bioregion, the distribution of reference sites was used to set the biocriterion for the New Hampshire B-IBI. NH DES does not regard the reference sites used as truly unimpaired conditions for the state and has set the thresholds for attainment of ALU standards to take into consideration the possibility of incomplete information about sites. The 25th percentile of the reference distribution within each bioregion was used as the biocriterion. In the Northern Region, the threshold score is 77, and in the Southern Region, the threshold score is 66.4. Sites with B-IBI scores at or above the threshold in each bioregion are considered to be in attainment of ALUS.

5.5.2 Fish Data (personal communication, David Neils, NH DES; VT DEC 2004)

Currently, NH DES is modifying the Vermont Cold Water Index of Biotic Integrity (CWIBI) and the Mixed Water Index of Biotic Integrity (MWIBI) for use in New Hampshire streams. Detail of the Vermont indices can be found in Chapter 7. Vermont uses the CWIBI in streams that contain two to four species, and the MWIBI in streams that contain greater than four species (VT DEC 2004). NH DES used native fish species richness to adapt the method to use the MWIBI for any stream containing five or more taxa and used the CWIBI for other streams containing less than five taxa. Best professional judgment was used to ensure a stream was placed into the correct category. After this initial categorization, sites identified as belonging in MWIBI category were further refined as cold or warm water fish communities utilizing Eastern Brook Trout and Slimy Sculpin as indicator species. Site elevations were also used to classify MWIBI sites into cold and warm fish communities. Within the MWIBI, the differentiation between cold and warm water fish assemblages was applied in scoring individual metrics. Although the use of the CWIBI and MWIBI is currently under development, NH DES found the indices to be promising because the method showed significant differences between reference and non-reference sites. Further refinement of the CWIBI and MWIBI will be completed in the near future to calibrate final index scores and establish attainment cutoffs specific to New

Hampshire fish communities. Although the CWIBI or MWIBI appeared to work well for many sites, as in VT, neither index is applicable in slow, winding sites.

Table 5-2. Metrics and scoring for the New Hampshire B-IBI.

Metric	Definition	Expected Response to Disturbance	Scoring Equation
Total taxa	Number of distinct macroinvertebrate taxa.	*Decrease*	*Total taxa*/21.5*100
Plecoptera taxa	Number of taxa in the order Plecoptera (genus or species level).	*Decrease*	*Plecoptera taxa*/4.4*100
% Chironomidae	% individuals in the family Chironomidae.	*Increase*	(100 - *% Chironomidae*)/100-0)*100
% Non-insects	% individuals in a sample that are not in the class Insecta.	*Increase*	(100- *% Non-insects*)/100-0)*100
% Clingers	% insects having fixed retreats or adaptations for attachment to surfaces in flowing water.	*Decrease*	*% Clingers*/94.6*100
% Intolerant	% of individuals considered to be sensitive to various types of pollution.	*Decrease*	*% Intolerant*/76.1*100
Tolerant taxa	Taxa richness of those organisms considered to be tolerant to increased disturbance.	*Increase*	(6.2-*Tolerant taxa*)/(6.2-0)*100

5.6 Summary: Determining ALU Support

NH DES follows the guidelines listed in the Comprehensive Assessment and Listing Methodology (CALM) to assess streams for ALUS (NH DES 2004a). This document lists the assessment criteria necessary for making the decision for specific segments (Assessment Units, AUs) of wadeable streams. Non-support in an ALU segment can be determined based on the B-IBI score or the failure to meet chemical criteria (e.g., DO or pH). However, Full Support status cannot be given to a segment without a B-IBI evaluation within that segment. This assessment must also include data from the most recent calendar year. Other data, such as RBP habitat

assessments, fish assessments, benthic deposits, flow, macrophyte composition, sediment and ambient water toxicity tests are also used to aid in the decision-making process.

5.7 Literature Cited

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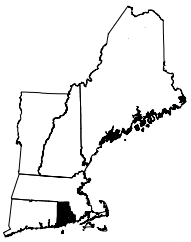
5.8 Resources

New Hampshire Department of Environmental Services, Watershed Management Bureau, Biomonitoring Program Web Page: http://www.des.state.nh.us/wmb/biomonitoring/

U.S. EPA. Biological Indicators of Watershed Health, New Hampshire Webpage: http://www.epa.gov/bioindicators/html/state/nh-bio.html

U.S. EPA. 2002. Summary of Biological Assessment Programs and Biocriteria Development for States, Tribes, Territories, and Interstate Commissions: Streams and Wadeable Rivers. EPA-822-R-02-048. U.S. Environmental Protection Agency. http://www.epa.gov/bioindicators/html/program_summary.html

6 RHODE ISLAND



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6.1 Introduction

Rhode Island WQS define water quality goals for the state's waters by designating uses and setting criteria necessary to protect those uses. Rhode Island WQS provide for the protection of the waters from pollutants so that the waters shall, where attainable, be available for all designated uses (i.e., drinking water supply, shell fish consumption, fish consumption, swimming, aquatic life) and thus assure protection for the public health, welfare, and the environment (RI DEM 2000). Rhode Island WQS define ALU as "providing suitable habitat and water quality for the protection, maintenance, and propagation of a viable community of aquatic life". In accordance with Section 305(b) of the CWA, states are required to evaluate water quality of all water body types (i.e., rivers/streams, lakes/ponds, estuarine waters) for attainment of their designated uses. The water quality criteria and assessment methodology used for the evaluation is detailed in the Integrated Water Quality Monitoring and Assessment Report. Any water bodies that are not attaining their designated uses are placed on the 303(d) List of Impaired Waters and a TMDL must be developed for each exceedance that results in non-attainment status.

The Rhode Island Department of Environmental Management (RI DEM) has used biological assessments to supplement physical and chemical water quality monitoring data for evaluation of ALU attainment in rivers and streams. The Rhode Island WQS currently only contain narrative biological criteria to utilize in evaluating biological assessment data. RI DEM implements a reference site approach for evaluation of the macroinvertebrate assemblage, as described below. The data collected from the bioassessment program is compared to corresponding reference sites and scored relative to conditions observed at the reference station. Scoring information from multiple years is used to determine a site's ALU attainment status for 305(b) reporting and 303(d) listing purposes.

6.2 Key Elements of the Biological Assessment Approach

6.2.1 Index Period and/or Temporal Conditions

The RI DEM conducts its biological monitoring program during the summer and fall seasons. Sites are sampled annually during this index period and any data collected and evaluated during drought conditions are noted. A long-term database allows for current and historical comparisons of data using these seasons.

6.2.2 Monitoring Program Survey Approach

Currently, RI DEM samples 45 fixed-river stations located throughout the state. The sampling sites are located within one of two subecoregions in Rhode Island: the New England Coastal Plains and Hills, or the Narragansett/Bristol Lowland. The stations were originally located on rivers/streams that were considered unassessed for ALU. They generally consist of smaller, wadeable rivers that do not have point-source discharges located on them, and range from first order to fifth order in size.

6.2.3 Natural Classification of Water Bodies

Rhode Island has two Level IV Omernik subecoregions represented within the Northeastern Coastal Zone ecoregion: the New England Coastal Plains and Hills subecoregion and the Narragansett/ Bristol Lowland subecoregion. Each subecoregion is represented by a reference site that is used to compare to test sites within the subecoregion.

6.2.4 Indicator Assemblages

RI DEM currently uses only the macroinvertebrate assemblage as a biological indicator. Macroinvertebrates are typically identified to the lowest practical taxonomic level. Rhode Island follows the U.S. EPA's RBP for evaluation of benthic macroinvertebrate communities (Plafkin et al. 1989).

6.2.5 Reference Condition

The RI DEM currently uses two reference sites in their bioassessment program, one representing each of the two Level IV Omernik subecoregions present in the state (Figure 6-1). One reference site, the Wood River, is located in the New England Coastal Plains and Hills subecoregion, while Adamsville Brook serves as the reference site for the Narragansett/Bristol Lowland subecoregion. Currently, no specific reference criteria exist, but each reference site represents minimally disturbed, high quality, historically natural sites within its subecoregion. The Wood River site is located on a fourth order, minimally disturbed portion of the river within the Pawcatuck River Basin. This reference site is located almost completely within the boundaries of a state park, and, therefore, is not expected to undergo the degradation that would remove it from the reference category. The Adamsville Brook reference station is located on a second order portion of the brook in the Cape Cod Basin. This area consists of very low density residential and 82% rural land use.

Figure 6-1. Level IV Omernik subecoregions and reference streams used in RI DEM's biological monitoring program.

6.3 Field and Laboratory Protocols

6.3.1 Macroinvertebrate Protocols

6.3.1.1 Field Methods (RI DEM 2002a, RI DEM 2003)

RI DEM uses the U.S. EPA's RBP III Single Habitat Approach (Barbour et al. 1999) to assess the macroinvertebrate assemblage of wadeable streams. This method has been in use since 1991 at the 45 fixed-station riffle stream sites. A physical evaluation is conducted at each of the sites that includes: surrounding land use; subsystem classification; documentation of dams, erosion and non-point source pollution; width, depth and flow measurements; inorganic and organic substrate types; and presence of odors, oils and deposits. Along with a physical evaluation, water quality measurements are taken (DO, pH, specific conductance, turbidity, and water temperature), and a habitat assessment is conducted. The habitat assessment and biological data are scored and compared against the reference station's data. The information is

then integrated with water quality, physical, rainfall, and historical trends data, to determine the ALU attainment status relative to the reference site from the same subecoregion.

RI DEM uses the RBP III Single Habitat Approach to collect representative samples of the stream reach. A 100-m reach is chosen for sampling, assuring that the reach is located at least 50 m upstream of bridges or road crossings to minimize their effect on stream velocity, depth and overall habitat quality. An effort is also made to not sample immediately downstream of stream confluences. The reach is approached from the downstream to upstream direction collecting a composite sample of macroinvertebrate kick samples from riffles (multiple if present) with a dominant cobble substrate. A 0.3-m wide D-Frame net with 500-µm mesh is placed into the bottom of the riffle, and the substrate is disturbed for three minutes. Larger substrate particles and debris are rubbed to remove attached organisms. Collected material is rinsed using stream water before being transferred to a sample container. If multiple riffles are sampled, all contents of the net from each riffle are placed into a single jar. The sample is preserved with 70% ethanol.

6.3.1.2 Laboratory Methods (RI DEM 2002a)

Samples preserved in 70% ethanol are rinsed thoroughly with tap water through a 500-µm mesh sieve to remove fine sediment and preservative. Any large organic material is discarded and the sample is then distributed evenly in an 18-in x 13-in x 1-in gridded tray with eight equally-sized squares. The entire sample is scanned, and any rare or large organisms are removed, identified and used only to report as supplemental data. Then, one section is randomly selected and all of the material in that section is removed using a 6-cm flat scoop and transferred to a separate container. Any overhanging debris is cut using scissors. Macroinvertebrates are sorted under a dissecting microscope on a clean Petri dish and placed into one of the three following groups in glass vials containing 70% ethanol for identification: 1) Oligochaetes and Chironomids, 2) Crustaceans and Mollusks, and 3) other organisms. Additional, randomly selected sections are completely sorted until 100 organisms are sorted or until the entire sample has been inspected for macroinvertebrates. Random quality checks are performed to assure that there is less than a 10% discrepancy between the sorter and the quality assurance check.

After sorting, macroinvertebrates are identified to the lowest practical taxonomic level. Vials containing chironomids and oligochaetes are sent to a sub-contractor, ARC, where they are mounted on labeled slides using an appropriate medium (e.g., Eupcral, CMC-9) and identified. All organisms are identified using a compound microscope or a dissecting microscope (up to 45X magnification), a fiber optic lamp, standard dissecting tools, and taxonomic keys.

6.4 Data Management/Quality

RI DEM has recently developed a Microsoft Access database to maintain the data collected by the biological monitoring program. Portions of the historic biological data have been entered into the database. Because the Rhode Island Office of Water Resources, where the biological monitoring program resides, has been without staff for over a year, the more recent data are maintained only in hard copy format. The Access database has been developed to calculate RBP metrics for macroinvertebrate data and will assist in the evaluation of developing a biological condition matrix for the state.

6.5 Analysis of Biological Data

6.5.1 Macroinvertebrate Data (RI DEM 2002a, RI DEM 2002b)

RI DEM uses the RBP III (Plafkin et al. 1989) metric-based method to give a bioassessment score relative to the reference site. Eight RBP metrics and three supplemental metrics (i.e., EPT abundance, Shannon Weaver Diversity Index, and % Hydropsychidae of total Trichoptera) are calculated (Table 6-1). Each metric is given a score (6, 4, 2, or 0), primarily based on the percentage of the reference site value observed at a site. The overall score is the sum of metric scores. Then, the overall score is compared to the reference site score by calculating the percentage of the reference score achieved. Thresholds of percentages relative to the reference site score places a stream into one of four bioassessment categories: Non-impaired, Slightly Impaired, Moderately Impaired, or Severely Impaired (Table 6-2). Although Table 6-2 lists the threshold percentages for assignment to a particular bioassessment category, the actual threshold may be adjusted slightly based on best professional judgment of the assessor, as well as analysis and comparison to historical data and trends.

Table 6-1. Metrics used by the Rhode Island Biomonitoring program and the methods for the calculation of metrics and their scoring ranges based on the RBP III (Plafkin et al. 1989, RI DEM 2002a, RI DEM 2002b).

Metric	Method	Scoring Ranges			
		6	4	2	0
Total Taxa Richness [(a)]	The total number of distinct taxa in the sample	>80%	60-80%	40-60%	<40%
EPT Taxa Richness [(a)]	The number of taxa within the orders of Ephemeroptera, Plecoptera, and Trichoptera.	>90%	80-90%	70-80%	<70%
EPT Abundance	All Ephemeroptera, Plecoptera, and Trichoptera individuals are added together.	Only used as supplementary data, not used in the RBP approach.			
Hilsenhoff Biotic Index [(b)]	(Number of individuals in taxon *i*)x(tolerance value of taxon *i*)/(Sum of individuals with tolerance values in sample)	>85%	70-85%	50-70%	<50%
Shannon Weaver Diversity Index	Number in each species is counted and index is calculated as: $\Sigma (p_i \log_2 p_i)$ Where p_i = the proportion of individuals in the i^{th} species.	Used as a measure of aquatic environmental health, but not used for RBP III.			
% Contribution of Dominant Taxon [(d)]	((a/b) x 100) Where: a = the number of individuals in the dominant taxon, b = the total number of individuals recorded at the stream segment.	<20%	20-30%	30-40%	>40%
EPT/Chironomidae (abundance ratio)[(a)]	(No. of EPT individuals)/(No. Chironomidae individuals)	>75%	50-75%	25-50%	<25%

Metric	Method	Scoring Ranges			
		6	4	2	0
Percent Hydropsychidae of Total Trichoptera	(No. Hydropsychidae individuals)/(No. all other Trichoptera) x 100	Used only as additional potential tolerance/intolerance measure metric (RI DEM 2003; modified from Barbour et al. 1999)			
Ratio of Shredders to Total Number of Macroinvertebrates [(a)]	(No. shredder individuals)/(Total no. of other individuals) (RI DEM 2003)	>50 %	35-50%	20-35%	<20%
Ratio of Scrapers to Filterers[(a)]	(No. of Scraper individuals)/(No. of filterer individuals)	>50 %	35-50%	20-35%	<20%
Community Loss Index[(d)]	A measure of the dissimilarity between a test site and a reference site (Plafkin et al. 1989). Metric values increase as biological impairment increase. Values have no limits. CLI = (a – b) / c where: a = no. genera in reference sample, b = no. genera in test sample, c = no. genera common to both samples	<0.5	0.5-1.5	1.5-4.0	>4.0

a) Value is converted to ratio of test to reference site *100

b) Value is converted to ratio of reference to test site *100

c) Actual percent contribution used in scoring, not ratio to reference

d) Uses range of values actually obtained

6.6 Summary: Determining ALU Support

RI DEM uses a combination of biological, habitat, chemical, and physical data to assign ALUS (Table 6-3). RI WQS contain some numeric criteria for dissolved oxygen, pH, temperature and priority pollutants, but other criteria are assessed based on a narrative description of water quality condition.

Taking into consideration all of the above factors, RI DEM determines the ALU attainment status of the streams. ALU is fully supported if there are no exceedances of the water quality criteria and the biological data indicate a fully supporting community. RI DEM gives the biological component more weight than the water chemistry data for the ALU assessment. Therefore, a river/stream may be considered fully supporting ALU if the biological community demonstrates non-impairment or slight impairment, even if minor exceedances of water quality criteria exist. A stream is considered partially supporting ALU if the macroinvertebrate assemblage indicates a bioassessment category of “slightly impaired” or “moderately impaired” and/or if there is an exceedance of any chemical water quality criterion (acute or chronic) more than once in a three-year period but in fewer than 10% of the samples. A site is determined to be not supporting ALU if the biological community is “severely impaired” and/or if there are severe

Table 6-2. Percent comparability evaluation for macroinvertebrate bioassessment scores used by the State of Rhode Island.

Bioassessment Categories	Definition	Percent Comparability to Reference
Non-impaired	Comparable to the best condition expected within an ecoregion. Trophic structure is balanced and community structure is optimal for the stream size and habitat quality.	> 83 %
Slightly impaired	Community structure is less than expected. Species composition is lower as evident by the loss of some intolerant species. The percent contribution of tolerant species increases.	54 – 79 %
Moderately impaired	Fewer species are present and most intolerant species disappear.	21 -50 %
Severely impaired	Few species are present and the stream is often dominated by one or two species**.**	≤ 17 %

or frequent (>10% of samples) violations of chemical water quality criteria. States are required by the CWA to describe the water quality of their state's waters in the 305(b) report and any water bodies that are found to be not in attainment of their designated uses must be listed on the 303(d) list. In Rhode Island, any water bodies that are considered partially or not supporting any designated uses are listed on the State's 303(d) List of Impaired Waters.

Table 6-3. Biological, physical and chemical criteria used to determine aquatic life use (*modified from* RI DEM 2000).

Component	Description of Criteria
Biological	• Macroinvertebrate Index (Plafkin et al. 1989) • Non-impaired, slightly impaired, moderately impaired, and severely impaired determined for the stream based on the macroinvertebrate assemblage
Physical	• Land use evaluation • Subsystem classification • Documentation of dams, erosion, and non point source pollution • Width and depth measurement • Flow measurement • Inorganic and organic substrate types • Presence of odors, oils and deposits • RBP Habitat Assessment (Barbour et al. 1999)
Chemical	

Component	Description of Criteria
Dissolved Oxygen	• Cold water fish habitat: dissolved oxygen should be above 75% saturation based on a daily average and than the instantaneous minimum concentration shall be of at least 5 mg/l. o To protect early life stages that are intergravel during the fish spawning period of October 1st to May 14th, the 7-day mean water column dissolved oxygen concentration shall not be less than 9.5 mg/l, while the instantaneous minimum dissolved oxygen shall not be less than 8 mg/l. o To protect those early life stages that are exposed directly to the water column, the 7-day mean water column dissolved oxygen concentration shall not be less than 6.5 mg/l and the instantaneous minimum dissolved oxygen concentration shall not be less than 5.0 mg/l. • Warm water fish habitat: daily average dissolved oxygen shall be above 60% saturation and the instantaneous minimum dissolved oxygen concentration shall be at least 5.0 mg/l. The 7-day mean water column dissolved oxygen concentration shall not fall below 6 mg/l.
pH	The pH should be as naturally occurs (6.5-9.0)
Temperature	Increase should not rise above the recommended limit that would cause the growth of nuisance species nor rise above 83ºF. In cold water habitats, heated discharges must not raise the temperature above 68ºF and in not case shall the receiving water be raised more than 4ºF.
Secchi depth and chlorophyll *a*	Secchi depth and chlorophyll *a* are used to measure the impact of nuisance algal blooms that may degrade the quality of life for fish and wildlife.
Priority Pollutants *Please refer to Appendix B of the Water Quality Regulations for a list of pollutant criteria (RI DEM 2000).*	Shall not be found in concentrations or combinations that would be harmful to humans or fish and wildlife for the most sensitive and governing water class use, or unfavorably alter the biota, or which would make the water unsafe or unsuitable for fish and wildlife or their propagation, impair the palatability of same, or impair waters for any other existing or designated use. • Aquatic Life Criteria: The acute and chronic aquatic life criteria for freshwaters shall not be exceeded at or above the lowest average 7 consecutive day low flow with an average recurrence frequency of once in 10 years (7Q10). • Human Health Criteria: The freshwater human health criteria for non-carcinogens are applicable at or in excess of the lowest average 30 consecutive day low flow with an average recurrence frequency of once in 5 years (30Q5). The freshwater human health criteria for carcinogens are applicable at or in excess of the harmonic mean flow, which is a long-

Component	Description of Criteria
	term mean flow value calculated by dividing the number of daily flows analyzed by the sum of the reciprocals of those daily flows.

6.7 Literature Cited

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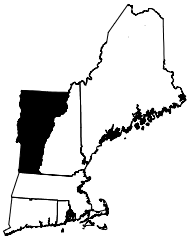
7 VERMONT



This document was prepared using documents written by the State of Vermont. Any questions concerning bioassessment methods should be directed to:

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7.1 Introduction

The Vermont Department of Environmental Conservation (VT DEC) has developed a biocriteria program for wadeable streams that uses both fish and macroinvertebrate assemblages for the calculation of indices indicative of stream conditions and biological integrity as it pertains to ALU classes for 303 (d) listed streams. The foundation of this program was set in 1982 with the creation of the Ambient Biomonitoring Network (ABN) in the Biomonitoring and Aquatic Studies Section (BASS) comprised of five biologists focusing on river and stream biological monitoring. The objectives of the ABN within BASS are to monitor long term trends over time, evaluate potential impacts from non-point and point sources, establish a reference database specific to Vermont's classification and attainment determinations, support VT DEC permitting programs that require data, and conduct special studies to assess emerging water quality and environmental management issues.

In 1985, VT DEC began using standardized methods to sample fish and macroinvertebrate communities. These methods included guidelines on evaluating physical habitat, processing samples, and analyzing and evaluating the data. Vermont now uses an IBI to measure the condition of the fish community, and multiple metrics applied individually to assess the integrity of the macroinvertebrate community. These data are then used together to determine the level of ALUS and attainment for wadeable streams and rivers in Vermont.

Section 3-01 of the VT WQS (Vermont Water Resources Board 2000) states the provisions to "establish and apply numeric biological indices to determine whether there is full support of aquatic biota and aquatic habitat uses" and to "establish procedures that employ standard sampling and analytical methods to characteristics of the biological integrity of the appropriate reference conditions." VT WQS also lists the water quality management classes and the biological standards that define the classes. Sections 3-02, 3-03, and 3-04 identify the management strategies and narrative standards for these water quality management classes. These classes range from the highest ALUS class A1 (ecological or natural conditions) to Class B Water Management Classes Type 1, 2, and 3, and Class A2 (public water supplies) (Table 7-1).

Table 7-1. Biological ALUS management classes and associated narrative biological criteria for rivers and streams in Vermont.

Class	Management	Biological Standard
A1	Ecological. Managed to achieve and maintain waters in a natural condition. Highest quality waters.	Reference condition, minimal impacts from human activity. Highest quality water that have significant ecological value.
A2	Managed for public water supplies and to achieve and maintain waters with uniformly excellent character and level of water quality.	High quality aquatic biota and habitat necessary to support their life cycle and reproductive requirements. Moderate change from the reference condition in the relative proportions of tolerant, intolerant, taxonomic, and functional components.
Class B, Water Management Type 1	Managed so that no change from the reference condition would prevent the full support of aquatic biota, wildlife or aquatic habitat uses.	Minor change from reference condition, minor changes in relative proportions of taxonomic and functional components, relative proportions of tolerant and intolerant components within range of reference conditions. Changes in the aquatic habitat shall be limited to minimal differences from the reference condition consistent with the full support of all aquatic biota and wildlife uses.
Class B, Water Managements Type 2	Managed so that no change from the reference condition would prevent the full support of aquatic biota, wildlife or aquatic habitat uses.	Moderate change in the relative proportions of tolerant, intolerant, taxonomic, and functional components. Changes in the aquatic habitats shall be limited to minor differences from the reference condition consistent with the full support of all aquatic biota and wildlife uses.
Class B, Water Managements Type 3	Managed so that no change from the reference condition would prevent the full support of aquatic biota, wildlife or aquatic habitat uses.	Moderate change in the relative proportions of tolerant, intolerant, taxonomic, and functional components. Changes in the aquatic habitats shall be limited to moderate differences form the reference condition consistent with the full support of all aquatic biota and wildlife uses. When such habitat changes are a result of hydrological modification or water level fluctuation, compliance may be determined on the basis of aquatic habitat studies.
Other Class B Waters	Managed so that no change from the reference condition would prevent the full support of aquatic biota, wildlife or aquatic habitat uses.	No change from the reference condition that would have an undue adverse effect on the composition of the aquatic biota, the physical or chemical nature of the substrate or the species composition or propagation of fishes.

7.2 Key Elements of the Biological Assessment Approach

7.2.1 Index Period and/or Temporal Conditions

The VT DEC has chosen late summer and fall (i.e., September to mid-October) for its index period to conduct both fish and macroinvertebrate reference and test site sampling. Samples must be collected during this index period to maintain consistency between the samples taken from year to year and for accurate community comparisons. This index period was selected because of the presence of stable, low-flow conditions and because the macroinvertebrate larvae and fish YOY are larger and easier to identify. Each site is visited one time during a sampling season.

7.2.2 Monitoring Program Survey Approach

The majority of the streams that are selected for sampling are targeted sites. These sites encompass specific sampling stations that have been monitored for the purpose of discharge permitting and TMDLs and can include sites that have been chosen for specific projects. Additionally sites are selected based on human land use and interest of watershed groups or the VT DEC planning section's need for data to determine the current biological condition of a stream reach. Vermont recognizes 17 watershed planning units that are a combination of smaller sub-basins. Seasonally monitored sites are sampled using a five-year rotating watershed strategy. This strategy covers three to four basins each year and approximately 125 sites are sampled per year within these basins (VT DEC 2004).

7.2.3 Natural Classification of Water Bodies

The 17 watershed planning units used by VT DEC were chosen nearly 30 years ago using the three major watersheds in the state. The three watersheds were then separated into 17 identifiable units. These classifications into 17 units were made based on local habitat features including elevation, drainage order, stream gradient and substrate composition.

7.2.4 Indicator Assemblages

VT DEC monitors both benthic macroinvertebrates and fish. Benthic macroinvertebrates are identified to genus or species in the laboratory (Class Oligochaeta is identified to family). A set of metrics to assess the macroinvertebrate assemblage has been developed using multivariate statistics. The fish assemblage is identified to species in the field and a multimetric fish index has been developed for both cold water and mixed water categories.

7.2.5 Reference condition

Reference conditions have been derived from macroinvertebrate and fish assemblage data independently and take into consideration a range of physical and chemical parameters a particular community would naturally encounter. The reference sites have been chosen and defined using the best professional judgment of VT DEC biologists, based on human activity and the potential that it may affect the stream. Specific reference sites and conditions can be found in

VT DEC (2004). There are three reference condition stream types for macroinvertebrates and two reference condition stream types for fish, totaling 150 reference sites. If a stream has unique properties and cannot be compared to one of the reference condition stream types, then a localized reference stream(s), historic data and best professional judgment are used to determine the expected natural range of metrics.

Macroinvertebrates reference sites are categorized into one of the following stream types (VT DEC 2004):

1. Small High Gradient Streams (SHG) are small, first-to-third order headwater streams with drainage areas averaging 10 km^2. These streams are located typically over 1500 ft in elevation and are highly canopied (83% average canopy cover). These streams have a high gradient and substrate dominated by gravel/cobble/boulder and approximately 3% fine sediments. Water is soft and alkalinity would typically measure less than 20 mg/l.

2. Medium Sized High Gradient Streams (MHG) are medium, third to fourth order streams with a drainage area averaging 88 km^2. These streams are found at elevations averaging 814 ft, and are covered by about 50% canopy. These streams have a high gradient and substrate dominated by gravel/cobble/boulder and approximately 6% fine sediments. Water has a moderate alkalinity, typically averaging 48 mg/l.

3. Warm Water Medium Gradient Streams and Rivers (WWMG) are larger streams, fourth to sixth order, or small streams within the Champlain Valley. Because this category contains larger streams than those in the Champlain Valley, the drainage areas vary, but the average size is 480 km^2. All streams are found at lower elevations averaging 369 ft, are less shaded with an average 30% canopy, and are warmer. These streams have a moderate gradient and substrates dominated by gravel/cobble/boulder and approximately 7% fine sediments. Water has a high alkalinity, typically averaging 70 mg/l.

VT DEC categorizes a site by comparing chemical and physical data from a test stream to those conditions found in a reference stream type. Factors that are taken into consideration when categorizing a stream are elevation, drainage area, stream order/size, stream gradient, substrate composition, pH, alkalinity, specific conductance, and other unique characteristics. If these characteristics at a site are outside the range of those found in the reference sites, then the VT DEC uses an alternative analysis to describe an appropriate reference condition for site comparison. This prescription may include using historical monitoring data from the same or adjacent or similar water body; using a regional reference site; using a site-specific reference site (e.g., from upstream sites or adjacent sites); using paleo-ecological data collected from the sediments; or using quantitative models developed from field, historical, and experimental laboratory data. Any deviation from methods is fully documented by VT DEC and determination is based on the establishment of a compelling weight-of-evidence argument derived from monitoring data and best professional judgment.

Fish reference conditions fall into two stream types for which metrics are calculated (VT DEC 2004):

1. Small cold water streams: A site will be categorized for assessment by the Coldwater Index of Biotic Integrity (CWIBI) only if the stream can naturally support two to four native, naturally reproducing species of fish. Any wadeable stream site that is located at an elevation greater than 500 ft or within the Connecticut River drainage is classified as a coldwater stream for these

purposes. For other streams that are below 500 ft in the Champlain Valley, fish composition will determine which IBI will be used. Furthermore, the site is classified as cold water if one of the following is present: one salmonid species, slimy sculpin, or longnose sucker. Those sites that would naturally meet the cold water criteria but are populated with warm water species as a result of human influence areas are considered cold water sites for attainment status purposes.

2. Warm water and cold water streams: A site will be placed in this category only if the stream can naturally support five or more native, naturally reproducing species of fish. Stream sites in this group are evaluated using the Mixed Water Index of Biotic Integrity (MWIBI). Both warm and coldwater streams fall into this category.

7.3 Field and Laboratory Protocols

7.3.1 Macroinvertebrate Protocols (taken from VT DEC 2004)

7.3.1.1 Field Methods

VT DEC uses a method to collect macroinvertebrates similar to that described in the Single Habitat RBP III (Plafkin et al. 1989). A riffle habitat is chosen within the stream sampling reach for macroinvertebrate sampling. Then, an 18-in wide x 12-in high D-frame net (500 µm mesh) is placed in the riffle and an area immediately upstream of the net (approximately 1.5-ft x 1.5-ft) is thoroughly disturbed by hand, ensuring that all pieces of substrate are moved and rubbed clean of attached organisms. Moving up-stream, this is repeated at four to five different locations within the riffle, representing a range of velocity and substrate type characteristics of that riffle. Each specific location is actively sampled until all the substrate in approximately an 18-in x 18-in square in front of the net has been disturbed. This generally takes about 30 seconds of active sampling per location, and active sampling is terminated at the end of approximately two minutes. The contents of the net are allowed to drain of excess water, placed into a quart mason jar and preserved with 75% ethanol. Then, to obtain a replicate sample for the site, this entire process is repeated, being careful to avoid areas previously disturbed. This “composite” sampling methodology effectively collects samples representative of the macroinvertebrate assemblage of that riffle. The VT DEC then measures and records the physical condition of the riffle (i.e., stream wetted and bankfull widths, depth, water velocity, water temperature, weather conditions, substrate composition, substrate embeddedness, canopy cover, stream bank condition, and immediate upstream land use) and takes a water sample for specific conductance, pH and alkalinity determination. On a site-specific basis, other water chemistry parameters are determined. The site and sampling event codes are recorded on the field sheet.

7.3.1.2 Laboratory Methods (VT DEC 2004)

Sample processing takes place in the laboratory where macroinvertebrate samples are washed of preservative through a #30 sieve. The rinsed sample is then spread evenly over a white gridded tray (minimum 24 squares) by adding a small amount of water to allow the sample to be evenly spread, but not so much as to cause the macroinvertebrates to float freely around the tray. Six squares are randomly selected by first choosing one random number and then isolating

five surrounding squares from the rest of the sample. This method ensures that one quarter of the sample collected will be picked. After all macroinvertebrates from one quarter of the tray have been sorted, additional squares are then sorted until a minimum of 300 organisms has been reached. The total number of squares sorted is recorded so that sample density or relative abundance can be calculated. Macroinvertebrates are then identified to genus or species except for the Class Oligochaeta which is identified to the family level (VT DEC 2004). All macroinvertebrates are separated into major groups and preserved with 75% ethanol. If the entire sample is not sorted, it is qualitatively examined for all EPT organisms and other larger organisms, such as crayfish or mussels, not detected in the subsample to determine species distributions only. The additional EPT sample is preserved in a separate jar. A reference collection of all identified taxa is kept to assure consistent identifications.

7.3.2 Fish Protocols (VT DEC 2004)

7.3.2.1 Field Methods

The VT DEC collects fish to determine the community condition of wadeable streams. Wadeable is defined as a stream or river that at some time during the year can be sampled by an individual wading in the thalweg of the channel. In streams less than 6 m wide, a single backpack electrofishing unit is used. In wider streams, the VT DEC uses multiple backpack units. Reach length is chosen by the overall width of the stream with a minimal reach length of 75 m for a 3-m wide stream, 100 m for a 4- to 5-m wide stream, 120 m for a 6- to 8-m wide stream, 140 m for a 9- to 11-m wide stream, 160 m for a 12- to 14-m wide stream, and 180 m for a stream with a width of 15 m. The reach should represent a subsample of the overall stream and may also be dependent on the expected density of fishes. For example, if an unproductive cold water stream is sampled, a longer reach is sampled to compensate for the low density of fish expected. Those streams that are productive cool and warm water sites may have smaller sample reaches. Furthermore, the stream section that is sampled would represent the overall habitat of the surrounding stream reach. All habitat types are sampled within the reach to maximize species richness.

When electrofishing, the crew begins in the most downstream portion of the reach and moves upstream. If a stream is being screened, only one pass is required. Two or three passes are made in those streams where the density is being evaluated as a result of a specific impact. If multiple passes are made, the VT DEC calculates a removal population estimate to ensure the accurate calculation of fish density. All fish that are stunned are collected using a net and placed into buckets of water for on-site identification to species and then released. All fish are examined for anomalies and salmonids are measured for length. Generally all identifications occur in the field. If a positive identification cannot be made, the specimen is preserved and is positively identified by a VT DEC biologist using keys (Smith 1985, Langdon et al. in preparation).

7.4 Data Management/Quality

Data are stored and managed in a Microsoft Access database, and metrics are calculated in this program. Data stored in the Access database can be moved to other spreadsheet programs such as Excel and to graphical and statistical analysis programs such as SigmaPlot, SigmaStat,

and PC-ORD. VT DEC uses SigmaStat for the statistical analysis calculations of parametric ANOVAs and nonparametric comparisons, and uses PC-ORD for multivariate analyses.

7.5 Analysis of Biological Data

7.5.1 Macroinvertebrate Data (VT DEC 2004)

VT DEC uses multivariate statistics and multiple metrics to evaluate the macroinvertebrate assemblage. Eight metrics have been developed to measure the macroinvertebrate community integrity (Table 7-2). These metrics measure specific ecological attributes of the community and include: Density, Richness, EPT index, Percent Model Affinity Orders, HBI, Percent Oligochaeta, EPT/(EPT + Chironomidae), and the Pinkham-Pearson Coefficient of Similarity-Functional Groups (Table 7-2). Using macroinvertebrate data, each metric is scored and an overall condition rating is determined for each site sampled. After metrics are calculated, the values are compared to a table of threshold values for each of the water quality classes (Table 7-3). These values represent a single point in a continuum of values reflecting changes from the reference condition. Along with best professional judgment and a weight-of-evidence decision process, VT DEC assigns a site to an attainment class. The biologist assigns a pass, fail, or indeterminate value for each of the metrics depending on the score. If five or more metrics score a pass and no metrics are below the threshold value, then the ALU is supported. However, if one or more of the metrics fail the ALU is not supported. If neither of these conditions is met for the Water Management Class of the stream being assessed (i.e., Class A1 or B), then the site is assigned an indeterminate finding and may require additional data and/or sampling to make a support or non-support decision.

Table 7-2. VT DEC macroinvertebrate metrics and methods used to calculate each of the metrics.

Variable Number	Indices/ Measures of Biological Integrity	Method
1	**Density**	The relative abundance of animals in a sample. (Number of animals in subsample)/(Proportion of sample processed)
2	**Richness**	The number of species in a sample unit. Calculated as the total number of distinct taxa identified in a sample and averaged across replicate samples. Note: immature larva identified to family or genus is not considered new taxon if genus or species identification is determined within its group.
3	**EPT Index**	The number of distinct taxa identified in a sample from the orders Ephemeroptera, Plecoptera, and Trichoptera. Calculated as for Variable 2. Note: immature larva identified to family or genus is not considered new taxa if genus or species identification is determined within its group.

Variable Number	Indices/ Measures of Biological Integrity	Method
4	**Percent Model Affinity Orders (PMA-O)**	A measure of order-level similarity to a model based on the reference streams. PMA-O is calculated by determining the percent composition for each major group (Coleoptera, Diptera, Ephemeroptera, Plecoptera, Trichoptera, and Oligochaeta) at the assessment site. Then those values are compared to the mean percent composition of each corresponding order from the reference condition (model). The sum of the minimum of the two values for each order is the PMA-O. PMA-O = Σ min (X_a or X_r) Where: X_a= the percent composition of order X from the assessment site X_r = the percent composition of order X from the appropriate reference conditions (Novak and Body 1992).
5	**Hilsenhoff Biotic Index (HBI)**	$HBI = \frac{\sum_i n_i a_i}{N}$ Where: n_i = number of individuals in taxon *i* a_i = tolerance value for taxon *i* (as assigned by VT DEC 2004, after Hilsenhoff 1987, Bode et al. 1996) N = number of individuals in the sample with a tolerance value
6	**Percent Oligochaeta**	(Abundance of Oligochaeta)/(Total number of individuals) x 100
7	**EPT/EPT + Chironomidae**	(Abundance of Ephemeroptera, Plecoptera and Trichoptera) / (Abundance of Ephemeroptera, Plecoptera, Trichoptera + Chironomidae)
8	**Pinkham-Pearson Coefficient of Similarity-Functional Groups (PPCS-F)**	A measure of functional feeding groups calculated by determining the percent composition of the six major functional groups (collector-gatherer, collector-filterer, predator, shredder-detritus, shredder-herbivore, scraper) at the site as assigned by VT DEC (2004) (based on Merrit and Cummins 1996, Bode et al. 1996). $PPCS - F = \frac{1}{K}\left(\sum_{i=1}^{K} \frac{\min(x_{ia}, x_{ib})}{\max(x_{ia}, x_{ib})}\right)$ Where: K = the number of comparisons between stations (6) x_{ia} = the number of individuals in functional group *i* in sample *a* (reference site) x_{ib} = number of individuals in functional group *i* in sample *b*

The final decision is based on a number of considerations in accordance with the VT WQS. These include the use of chemical and physical data from the sample site to determine which of the three wadeable stream macroinvertebrate categories are used to determine

attainment, as well as the evaluation of data quality. Macroinvertebrate data must be collected using standard methods and trained qualified personnel. Furthermore, any diversions from average conditions must be documented by VT DEC. These diversions could include any hydrological, meteorological or other extreme events that occurred before sampling, and any errors committed during sampling. Finally, variability of the samples from a site is analyzed and evaluated on a case-by-case basis. If variability exceeds 40%, but is less than 75% the data will be handled with caution. If the percent standard error of the mean of abundance is greater than 75%, the data are rejected. If this is the case, any other data collected from the site (e.g., physical, chemical, and/or fish data) can be used to submit a temporary ALUS classification.

VT DEC then sets threshold index values to define how the biological indices relate to the narrative class standards established in the VT WQS. The threshold values were derived from the distribution of metrics within both the reference and the impacted data sets. Thresholds are also minimally adjusted at each site on a case-by-case basis by a VT DEC biologist using BPJ.

Class A includes only those highest quality sites that exhibit minimal change from natural conditions. For those metrics that decrease in value with *impairment* from stressors (i.e., Richness, EPT, PMA-O, EPT/ EPT +C, PPCS-FG, and Density), it is reasonable to expect that the upper 75 percent of the reference sites best meet the true natural condition (threshold set at 25^{th} percentile). For the metrics that increase in value with *impairment* from a stressor (i.e., HBI and percent oligochaetes) the threshold was set at the 75^{th} percentile.

Streams in Class B, Water Management Type 1 exhibit minor changes from "natural condition". The reference streams are likely include a small percentage of lower quality reference sites; therefore, the threshold values for Class B1 were set to the 5^{th} and 95^{th} percentiles of the reference streams to ensure against the influence of outlier reference values. Since a minor change was expected for these sites, the threshold was set at the 5^{th} percentile (to include the upper 95 percent of the reference sites) for the metrics that decrease in response to stress (e.g., Richness). For metrics that increase in response to stress, the threshold was set at the 95^{th} percentile of reference sites, such that most reference sites fell below the threshold.

Class B Water Management Types 2 and 3 and Class A2 allow a moderate change from "natural condition" as a management goal. Thresholds for these classes were set based on BPJ using the range of reference values and the median, and $10^{th}/90^{th}$ percentile values of the distribution from sites known to be impacted.

7.5.2 Fish Data (VT DEC 2004)

The fish assemblage at sampling sites is assessed using either the Cold Water Index of Biotic Integrity (CWIBI) or the Mixed Water Index of Biotic Integrity (MWIBI). VT DEC derived index values consistent with narrative biological criteria in VT WQS using data from reference and impaired sites. The CWIBI consists of six metrics and is applied to small cold water stream fish communities, while the MWIBI consists of nine metrics and is applied to cold or warm water communities in Vermont. Tables 7-4 and 7-5 list the metrics, calculations, and scoring criteria for the CWIBI and MWIBI, respectively. The scoring criteria were derived for the CWIBI (7.5, 4.5, and 1.5) and the MWIBI (5, 3, and 1) to compare the cold water and mixed water sites with each other. For example, there are six CWIBI metrics and the highest overall score possible is 45 (7.5 x 6 = 45), whereas there are nine MWIBI metrics and the highest overall score possible is also 45 (9 x 5 = 45). Each metric is scored based on definitions and habitat or

basin conditions. A final summation of each of the scores is made to place a fish assemblage (i.e., site) into a condition category (i.e., Excellent, Very Good, Good, Fair, Poor). "Excellent" is assigned to those streams that achieve a score of at least 41, "Very Good" to streams with scores of at least 36, "Good" for scores of at least 33, "Fair" for scores of at least 27, and anything below 27 is assigned a condition of "Poor". The CWIBI or MWIBI score is then compared to the range of scores that correspond to each of the VT WQS Classes to determine which class could potentially best describe the fish assemblage (Table 7-6). For example, if a cold water fish assemblage scores a 36 on the CWIBI, the stream could be classified B Water Management Type 1, defined as exhibiting a minor change from the reference condition.

As is the case with macroinvertebrate data, fish data must undergo a stringent evaluation. Qualified personnel must adhere to the SOPs when collecting and analyzing the data. If a sample is deemed unacceptable due to error or a unique event other than what occurs in an average year, VT DEC can sample the site again. This event is to be scheduled at least three weeks from the initial sampling date. Until new data are collected, the VT DEC can assign a temporary attainment value based on the valid biological, chemical, and physical data already collected.

7.6 Summary: Determining ALU Support

VT DEC calculates metrics for both macroinvertebrate and fish data collected from probabilistic and monitoring stations for use in determining ALU attainment. Although each of the metrics and indices were derived independently, they each provide a quantitative way to assess the biological condition in the diverse types of streams in Vermont. VT WQS provide a classification system describing the condition of these streams (Table 7-1) that include Class A1, A2 and Class B1, B2, and B3 streams. Once these classes were established with management provisions in place, indices were developed that describe the stream condition in relation to reference condition site values (e.g., CWIBI, MWIBI and macroinvertebrate metrics). The VT DEC uses the CWIBI, MWIBI and individual macroinvertebrate metrics to determine the ALUS based on the thresholds described in the tables above (Tables 7-3, 7-4, 7-5, and 7-6). The resultant product is a narrative description for each monitored site for the ALU attainment part of the 305(b) report on the condition of streams in Vermont. In addition, this information is used to list non-supporting sites on the 303(d) list for Vermont.

Table 7-3. Macroinvertebrate assemblage biocriteria thresholds for the macroinvertebrate community stream categories, and associated WQ classes of Vermont (VT DEC 2004).

		Small-size High Gradient Streams (SHG)			Medium-size High Gradient Streams (MHG)			Warm Water Medium Gradient Streams and Rivers (WWMG)		
WQ Class		**A1 Ecological**	**B-WMT1**	**B B-WMT 2-3 A2 (water supply)**	**A1 Ecological**	**B-WMT1**	**B B-WMT 2-3 A2 (water supply)**	**A1 Ecological**	**B-WMT1**	**B B-WMT 2-3 A2 (water supply)**
Metric	**Direction of metric as water quality improves**	Reference Condition	Minimal Change from Reference Condition	Moderate Change from Reference Condition (undue adverse effect)	Reference Condition	Minimal Change from Reference Condition	Moderate Change from Reference Condition (undue adverse effect)	Reference Condition	Minimal Change from Reference Condition	Moderate Change from Reference Condition (undue adverse effect)
Richness	Positive	>35	>31	>27	>43	>39	>30	>40	>35	>30
EPT	Positive	>21	>19	>16	>24	>22	>18	>21	>19	>16
PMA-O	Positive	>65	>55	>45	>65	>55	>45	>65	>55	>45
HBI	Negative	<3.00	<3.50	<4.50	<3.50	<4.00	<5.00	<4.25	<4.75	<5.40
% Oligo	Negative	<2	<5	<12	<2	<5	<12	<2	<5	<12
EPT/EPT +Chiron.	Positive	>0.65	>0.55	>0.45	>0.65	>0.55	>0.45	>0.65	>0.55	>0.45
PPCS-FG	Positive	>0.50	>0.45	>0.40	>0.50	>0.45	>0.40	>0.50	>0.45	>0.40
Density	Positive	>500	>400	>300	>500	>400	>300	>500	>400	>300

Table 7-4. The six metrics used in scoring the fish assemblage for the CWIBI. These streams must naturally support two to four native species (VT DEC 2004).

Metrics and qualifications/calculations	Scoring Criteria		
	7.5	**4.5**	**1.5**
1. Number of intolerant species (One exotic trout species may be substituted for brook trout)	2	1	0
2. Proportion of individuals as cold water stenotherms	> 75%	50-75%	< 50%
3. Proportion of individuals as generalist feeders	< 5%	5-9%	> 9%
4. Proportion of individuals as top carnivores	> 35%	25-35%	< 25%
5. Brook trout density (number/100 m^2 - 1pass)	> 4.0	2.0-4.0	< 2.0
6. Brook trout age class structure Young-of-the-year (YOY) 100 mm, adult 100mm)	YOY and adults present	YOY only	YOY absent

Index Scores		Conditions for use
Excellent	42-45	1. Only fishes over 25 mm in length should be considered.
Very Good	36	2. Only naturally reproducing salmonids are to be considered; no stocked fish are to be included. No Atlantic salmon are to be included.
Good	33	3. Only species represented by more than a single individual are entered into metrics 1 and 6.
Fair	27	4. No non-resident species shall be entered into metric calculations.
Poor	< 27	

Table 7-5. The nine metrics used in scoring cold and warm water sites for the MWIBI. These streams must naturally support more than four native fish species (VT DEC 2004).

Metric and Qualification	Stream descriptor	Scoring Criteria		
Species Richness and Composition		**5**	**3**	**1**
1. Total number of native fish species		Follows maximum species richness lines		
2. Number and identity of native, intolerant species (A non-native trout may be substituted for brook trout when absent)	Site Elevation > 400 feet	> 1	1	0
	Site Elevation < 400 feet	> 0	-	0
3. Number and identity of native benthic insectivores	Site Elevation < 400 feet with site drainage < 25 km^2	> 0	-	0
	All other sites	> 1	1	0
4. Proportion of individuals as white suckers and creek chubs.		< 11%	11-30%	> 30%
Trophic Composition				

Metric and Qualification	Stream descriptor	Scoring Criteria		
5. Proportion of individuals as generalist feeders.	Site Elevation > 500 feet	< 20%	20-45%	> 45%
	Site Elevation < 500 feet	< 30%	30-60%	> 60%
6. Proportion of individuals as water column and benthic insectivores (score a "1" if blacknose dace is >60% of total assemblage or 100% of insectivores)	Site Elevation > 500 feet	> 65%	30-65%	< 30%
	Site Elevation < 500 feet	> 55%	20-55%	< 20%
7. Proportion of individuals as top carnivores (Nonnative trout included)	Cold water assemblage	> 15%	5-15%	< 5%
	Warm water assemblage with site drainage > 25 km^2	> 10%	3-10%	< 3%
	Warm water assemblage with site drainage < 25 km^2	$\geq$ 0	-	-
Fish Abundance and Condition				
8. Proportion of individuals with deformities, fin erosion, lesions, or tumors.		< 1%	1-4%	>4%
9. Abundance in sample (One pass - # 100 m^2) (Nonnative species included) * Site automatically scores Poor	Site Elevation < 500 feet	> 20	10-20	< 10*
	Site Elevation > 500 feet Alkalinity > 9 mg/l	> 10	7-10	< 7*
	Site Elevation < 500 feet Alkalinity < 9 mg/l	> 6	3-6	<3*

Sum of Metric Scores		Conditions for Use
Excellent	41-45	1. For wadeable streams only. 2. Site should naturally support at least five native species. 3. Only individuals more than 25 mm TL are to be entered in the determination. 4. Only species with more than one individual captured are entered into metrics 2 and 3. 5. Stocked fish are not considered in determinations. 6. All sites within the Connecticut River drainage are to be scored as > 500 m elevation.
Very Good	37	
Good	33	
Fair	27	
Poor	<27	

Table 7-6. All possible scores for the CWIBI and MWIBI that correspond to the VT WQS classification scheme (VT DEC 2004).

WQS Classification	Range	Possible Scores	
		CWIBI	MWIBI
A-1	41-45	42, 45	41, 43, 45
A-1 or B-1 based on BPJ	39	39	39
B-1	36-37	36	37
B-1, A-2, or B-2, B-3 based on BPJ	35		35
A-2, B-2, B-3	33	33	33
B-2, B-3 or Non-Support based on BPJ	29-31	30	31, 29
Non-Support	< 29	27, 24, 21, 18, 15, 12, 9	27, 25, 23, 21, 19, 17, 15, 13, 11, 9

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8 SUMMARY

8.1 Comparison Across States

The U.S. EPA allows for each state to implement its own bioassessment program. This document reviews the methods used by U.S. EPA Region 1 states to monitor and assess streams for ALU attainment for 305(b) reporting and 303(d) listing as required by the CWA. Although Region 1 states share many commonalities such as the Connecticut River, which traverses four of the six states (New Hampshire, Vermont, Massachusetts, and Connecticut) and has a watershed that covers 11,000 mi^2, the entire region has diverse habitats ranging from northern broadleaf forests to southern New England forests, coastal systems, northern conifer forests and alpine regions (Alden and Cassie 2000). The diverse topography causes distinct differences in biota and, therefore, requires states to make adjustments to their bioassessment programs to account for these differences. For example, although 41 entities use the fish assemblage for bioassessment, Maine does not assess fish (U.S. EPA 2002b). One reason Maine has chosen to assess benthic macroinvertebrates and not the fish assemblage as well, is due to the great diversity of this assemblage in state waters compared to the fish assemblage (Personal Communication, Susan Davies). Meanwhile, Vermont has diverse benthic macroinvertebrate and fish fauna and they have adapted their bioassessment program to include a benthic metrics and indices to assess cold water and warm water fish assemblages. The rationale for program development can stem from a biological point of view but may be influenced by historical sampling methods, feasibility of sampling protocols, and by funding available to the program. Due to regional similarities, some states share many of the same protocols and have borrowed ideas for their own index development. Summary Table 8-1 offers a comparison and contrast of the key components of the state bioassessment programs and Table 8-2 lists all of the metrics used by the states.

States are responsible for setting narrative criteria that will protect waters and define ALU. Bioassessment programs are then designed to aid in the determination of ALU using biological, physical, chemical and habitat data. Biological data provide the most accurate information about the resident aquatic organisms (U.S. EPA 2002a); therefore, it is essential that appropriate indicator assemblages be chosen to properly assess the natural system. All U.S. EPA Region 1 states minimally have chosen to assess the benthic macroinvertebrate assemblage, but some attempt is being made by most of the states to at least include information about fish assemblages (i.e., Connecticut, Massachusetts, New Hampshire, and Vermont) and/or algae (i.e., Connecticut and Massachusetts). It is then the responsibility of the states to decide the best way to analyze the biological data in order to show biotic gradients as water quality changes.

As apparent in Table 8-1, these programs do share some commonalities such as using modified methods from the U.S. EPA RBP document, although the details of the methods may differ considerably. These differences are evident at all levels, including the strategy used for site selection, the decision making process used to select reference sites, field collection methods, laboratory sorting and taxonomic methods, and finally the selection of metrics and data analysis. These differences could be due to the necessity to compare to historical data by using similar methods, topographical or regional differences, available equipment, logistical constraints, and funding provided to the states.

Table 8-1. Comparisons of the key components of state bioassessment programs.

Component	CONNECTICUT	MAINE	MASSACHUSETTS	NEW HAMPSHIRE	RHODE ISLAND	VERMONT
Index Period	Late fall	July-September	July-September	mid summer-early fall	Summer and fall	Late summer to fall
Indicator assemblages used	Benthic Fish Periphyton	Benthic	Benthic Fish Periphyton	Benthic Fish	Benthic	Benthic Fish
Survey approach	5-yr rotating basin; Targeted. CT in the process of developing a probabilistic component to monitoring program	5-yr rotating basin; Targeted	5-yr rotating basin; Targeted	Targeted within Northern and Southern regions	Fixed stations within two Level IV Omernick ecoregions (Adamsville Brook and Wood River)	5-yr rotating basin; Targeted
Total stream miles	5,830 miles	31,672 miles	8,229 miles	10,881 miles	1,498 miles	7,099 miles
# sites sampled/year	Benthic-50 sites Fish- 24 sites	50-60 sites	75 sites	25-30 sites	45 sites	125 sites
Reference condition	Least disturbed sites comparable to test sites based on natural features such as gradient	BPJ for *a priori* placement into four pre-defined classes for linear discriminant analysis	Least impacted sites; no potential to receive point or non-point source pollution and lack land use patterns that would degrade water quality	Least impacted sites based on Human Disturbance Gradient, reference sites within each bioregion used to set attainment thresholds	2 least disturbed reference sites, one in each of the ecoregions	BPJ used to identify reference sites for 3 stream types for benthos, 2 types for fish
Macroinvertebrates						
Sampling approach	RBP III Single Habitat (Plafkin et al. 1989)	Classification Attainment Evaluation (Davies et al. 1999, Davies and Tsomides	RBP II and III Single Habitat Approach (Plafkin et al. 1989)	NH DES methods (NH DES 2004)	RBP III Single Habitat (Plafkin et al. 1989)	modification of RBP III Single Habitat (Plafkin et al. 1989)

Component	CONNECTICUT	MAINE	MASSACHUSETTS	NEW HAMPSHIRE	RHODE ISLAND	VERMONT
		2002)				
Field method(s)	Kick net 9-in x 18-in rectangle, 800 x 900 µm mesh	Rock baskets, riffle bags, rock filled cones. Deployed for 28-56 days. 600 µm mesh sieve	Kick net 0.46 m x 0.46 m, 500 µm mesh. Rock baskets deployed for 6 to 8 weeks.	Rock baskets deployed for 8 weeks, 600 µm mesh	0.3 m wide D-frame net, 500 µm mesh	18-in x 12-in D-frame net, 500 µm mesh
Lab sorting method	Entire sample over a 56-square grid	Entire sample in small quantities	RBP II and III (Plafkin et al. 1989) 25 squares 6-cm x 6-cm	Caton method (Barbour et al. 1999) using 16 square grids.	18-in x 13-in x 1-in gridded tray with 8 equal squares	24-square grid
Organisms subsample count	200	Entire sample, unless >500 organisms in sample	100	minimum of 100	100	minimum of 300
Taxonomic resolution	genus/species or lowest practical taxonomic level	genus/species or lowest practical taxonomic level	genus/species or lowest practical taxonomic level	genus/species or lowest practical taxonomic level	genus/species or lowest practical taxonomic level	Genus or species, oligochaetes to family
Analysis method	RBPIII metrics and index (Plafkin et al. 1989)	Linear discriminant analysis	RBP II and III metrics and index (Plafkin et al. 1989)	B-IBI for northern and southern regions (Neils and Blocksom 2004)	RBP III metrics and index (Plafkin et al. 1989)	Multiple metrics (VT DEC 2004)
# of metrics (See Table 8-2 for metrics)	7	25	9	7	11	8
Periphyton						
Current Use	Developing an algal indicator using probabilistic monitoring; currently used only for	Not Used	Used for the detection of algal blooms and as indicator of water quality to identify toxicity issues, nutrient	Not Used	Not Used	Not Used

Component	CONNECTICUT	MAINE	MASSACHUSETTS	NEW HAMPSHIRE	RHODE ISLAND	VERMONT
	supplementary information.		impacts, and habitat alterations.			
Field method(s)	Modified RBP Single Habitat method, field-based Rapid Periphyton Survey (viewing bucket) (Barbour et al. 1999)		RBP Single Habitat method, artificial substrates (biomass and chl *a*), and viewing bucket (% coverage) (Barbour et al. 1999)			
Measurements taken	Chlorophyll *a*, biomass, species composition and abundance		Chlorophyll *a*, biomass, and percent coverage			
Use for attainment	Literature metrics to derive conclusions		Use to evaluate if the aesthetics or Aquatic Life Use are affected			
Fish						
Field method	RBP V protocols (Plafkin et al. 1989): electrofish 150 m reach, fish identified to species in field	Not Used	RBP V protocols (Plafkin et al. 1989): electrofish 100 m reach, fish identified to species in field	RBP V protocols (Plafkin et al. 1989): electrofish 150 m reach, fish identified in field	Not Used	Electrofish a minimum reach of 75 m, reach length varies with stream width, usually identified in field
Analysis method	Currently developing an index modeled after Vermont; data collected currently used as supplementary information for ALU determination		Modification of RBP V metrics (Plafkin et al. 1989)	IBI under development and modeled after Vermont. In the process of refining CWIBI and MWIBI for NH stream fish communities		CWIBI for cold water stream fish communities, MWIBI applied to cold or warm water communities (VT DEC 2004)

Component	CONNECTICUT	MAINE	MASSACHUSETTS	NEW HAMPSHIRE	RHODE ISLAND	VERMONT
Use of fish data	Fish Species composition, trophic structure and age class distribution along with BPJ used to make assessment		Structure and function of the fish assemblage and BPJ used to determine support or impairment of aquatic life use	Currently supplements macroinvertebrate and other data for attainment decisions		Used to make attainment decisions for ALUS
ALUS determination: data used	Biological (macroinvertebrate quantitative index, supplemental fish data) physical, chemical, toxicological and habitat data also used	Heaviest weight on biological data (outcome of the benthic linear discriminant analysis), physical, chemical, bacterial, and habitat	Weight of Evidence approach using biological, habitat, chemical, and toxicological data	Heavy weight placed on biological (B-IBI) data, with fish assessments, benthic deposits, flow, habitat (RBP), macrophyte composition, sediment and ambient water toxicity tests, pH and DO	Biological (macroinvertebrate community score), physical, chemical (DO, pH, temperature), and habitat (RBP) data	Heaviest weight on biological (benthic metrics and fish CWIBI and MWIBI) data, with physical, chemical, and habitat data

Table 8-2. Comparison of the macroinvertebrate metrics used by states in the New England Region. Color shading indicates equivalent metrics across states.

CONNECTICUT	MAINE		MASSACHUSETTS	NEW HAMPSHIRE	RHODE ISLAND	VERMONT
Community Loss	*Cheumatopsyche* Mean Abundance	Ratio of Class A Indicator Taxa	Community Loss	Percent Chironomidae	Community Loss	Density
EPT Index	Chironomini Mean Abundance (Family Functional Group)	Ratio of EP Generic Richness	EPT Index	Percent Clingers	EPT Abundance	EPT Index
EPT/Chironomidae (abundance ratio)	Ephemeroptera Mean Abundance	Relative Chironomidae Abundance	EPT/Chironomidae (abundance ratio)	Percent Intolerant	EPT Taxa Richness	EPT/EPT + Chironomidae
HBI	EPT Generic Richness	Relative Diptera Richness	HBI/FBI	Percent Non-insects	EPT/Chironomidae (abundance ratio)	HBI
Percent Contribution of Dominant Taxon	EPT-Diptera Richness Ratio	Relative Ephemeroptera Abundance	Percent Contribution of Dominant Taxon	Percent Tolerant taxa	HBI	Percent Model Affinity Orders (PMA-O)
Scraper/Filtering Ratio	Generic Richness	Relative Oligochaeta Abundance	Percent Reference Affinity	Plecoptera Taxa	Percent Contribution of Dominant Taxon	Percent Oligochaeta
Taxa Richness	HBI	Relative Plecoptera Richness	Percent Similarity	Total Taxa	Percent Hydropsychidae of Total Trichoptera	Pinkham-Pearson Coefficient of Similarity-Functional Groups (PPCS-F)
	Hydropsyche Mean Abundance	Shannon-Weiner Generic Diversity	Scraper/Filtering Ratio		Ratio of Shredders to Total Number of Individuals	Richness

CONNECTICUT	MAINE		MASSACHUSETTS	NEW HAMPSHIRE	RHODE ISLAND	VERMONT
	Perlidae Mean Abundance (Family Functional Group)	Sum of Mean Abundances of: *Cheumatopsyche*, *Cricoptopus*, *Tanytarsus* and *Ablabesmyia*	Taxa Richness		Scraper/Filtering Ratio	
	Plecoptera Mean Abundance	Sum of Mean Abundances of: *Dicrotendipes*, *Microspectra*, *Parachironomus* and *Helobdella*			Shannon Weaver Diversity Index	
	Probability (A + B + C) from First State Model	Sum of Mean Abundances of: *Acroneuria* and *Stenonema*			Total Taxa Richness	
	Probability (A + B) from First Stage Model	Tanypodinae Mean Abundance (Family Functional Group)				
	Probability of Class A from First Stage Model	Total Mean Abundance				

Currently, U.S. EPA Region 1 is conducting the New England Wadeable Streams (NEWS) Project. The primary purpose of this study is to apply a random probability-based site selection strategy across New England states. As part of this study, fish, macroinvertebrate, water chemistry, physical chemistry, and habitat data are being collected at 50 sites in each participating state, either by the state itself or by Region 1. In some cases, individual states are collecting samples using both the standardized method for this study and their own method, allowing for a possible comparison of field sampling methods. Sampling was to be completed by the end of 2003, and a final report presenting the findings of this study is anticipated by late 2004 (Personal Communication, Hillary Snook, U.S. EPA Region 1).

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APPENDIX A: PROCESS AND CRITERIA FOR THE ASSIGNMENT OF BIOLOGIST'S CLASSIFICATION

DRAFT

From: Appendix H *in* Stream Biological Monitoring and Numeric Criteria Development in Maine

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Ranking Process

Each biologist independently reviewed biological information for each sampling event, as listed below, including identities and abundances of taxa occurring in the biological sample and computed index values for the biological data (e.g. diversity, richness, EPT, etc). Physical habitat information was also reviewed including water depth, velocity, substrate composition, canopy cover, etc., in order to evaluate the effects of various habitat conditions on the structure of the macroinvertebrate assemblage. Sample information was reviewed for the values of the given measures, relative to values for other samples in the data set. The actual classification assignment was determined by how closely the biological information conformed to the aquatic life classification standards, correcting for habitat effects. Numerical ranges, per se, were not established, *a priori*, for each measure. Instead, the information was reviewed for it's compatibility with the mosaic of findings expected for each Class, listed in the Relative Findings Chart in this Appendix (H-1). The biologists did not have any knowledge of the actual location of the sampled sites, nor did they have knowledge of any pollution influences. Following the

independent assignment of classes the biologists established a consensus classification, following an open exchange of justifications for each biologist's assignment.

Biologist's Classification Criteria

Each biologist reviewed the sample data for the values of a list of measures of community structure and function. Criteria used by biologists to evaluate each measure are listed in the Relative Findings Chart, Appendix A-1.

TOTAL ABUNDANCE OF INDIVIDUALS
TOTAL ABUNDANCE OF EPHEMEROPTERA
TOTAL ABUNDANCE OF PLECOPTERA
ABUNDANCE OF EPHEMEROPTERA/TOTAL ABUNDANCE
ABUNDANCE OF PLECOPTERA/TOTAL ABUNDANCE
ABUNDANCE OF HYDROPSYCHIDAE/TOTAL ABUNDANCE
ABUNDANCE OF EPHEMEROPTERA+PLECOPTERA/TOTAL ABUNDANCE
ABUNDANCE OF *GLOSSOSOMA*/TOTAL ABUNDANCE
ABUNDANCE OF *BRACHYCENTRUS*/TOTAL ABUNDANCE
ABUNDANCE OF OLIGOCHAETES/TOTAL ABUNDANCE
ABUNDANCE OF HIRUDINEA/TOTAL ABUNDANCE
ABUNDANCE OF GASTROPODA/TOTAL ABUNDANCE
ABUNDANCE OF CHIRONOMIDAE/TOTAL ABUNDANCE
ABUNDANCE *CONCHAPELOPIA+THIENNEMANNYMIA*/TOTAL ABUNDANCE
ABUNDANCE OF *TRIBELOS*/TOTAL ABUNDANCE
ABUNDANCE OF *CHIRONOMUS*/TOTAL ABUNDANCE
GENERIC RICHNESS
EPHEMEROPTERA RICHNESS
PLECOPTERA RICHNESS
EPT RICHNESS
EPHEMEROPTERA RICHNESS/GENERIC RICHNESS
PLECOPTERA RICHNESS/GENERIC RICHNESS
DIPTERA RICHNESS/GENERIC RICHNESS
EPHEMEROPTERA+PLECOPTERA RICHNESS/GENERIC RICHNESS
EPT RICHNESS/DIPTERA RICHNESS
NON-EPT OR CHIRONOMIDAE RICHNESS/GENERIC RICHNESS
PERCENT PREDATORS
% COLLECTOR FILTERERS+GATHERERS/%PREDATORS+SHREDDERS
NUMBER OF FUNCTIONAL FEEDING GROUPS REPRESENTED
SHANNON-WEINER GENERIC DIVERSITY
HILSENHOFF BIOTIC INDEX

In addition, in cases where a valid, clean-water, upstream reference station existed, the following comparative index data was also reviewed:

JACCARD TAXONOMIC SIMILARITY
TAXONOMIC SIMILARITY OF DOMINANT TAXA
COEFFICIENT OF COMMUNITY LOSS

PERCENT SIMILARITY

Results

In 64% of the cases there was unanimous agreement among the independent raters, and in an additional 34% of the samples two of the raters were in agreement and one had assigned a different classification. In 3 of the rated samples there was disagreement among all three raters (2%).

Table A-1. RELATIVE FINDINGS CHART

Measure of Community Structure	**Relative Findings**			
	A	**B**	**C**	**NA**
Total Abundance of Individuals	often low	often high	variable	variable: often very low or high
Abundance of Ephemeroptera	high	high	low	low to absent
Abundance of Plecoptera	highest	some present	low to absent	absent
Proportion of Ephemeroptera	highest	variable, depending on dominance by other groups	low	zero
Proportion of Pleocoptera	highest	variable, depending on dominance by other groups	low	zero
Proportion of Hydropsychidae	intermediate	highest	variable	low to high
Proportion of Ephemeroptera & Plecoptera	highest	variable	Low	absent
Proportion of Glossoma	highest	low to intermediate	very low to absent	absent
Proportion of Brachycentrus	highest	low to intermediate	very low to absent	absent
Proportion of Oligochaetes	low	low	low to moderate	highest
Proportion of Hirudinea	low	variable	variable	variable to highest
Proportion of Gastropoda	low	low	variable	variable to highest
Proportion of Chironomidae	lowest	variable, depending on the dominance of other groups	highest	variable

Measure of Community Structure	Relative Findings			
	A	**B**	**C**	**NA**
Proportion of Conchapelopia & Thienemannimyia	lowest	low to variable	variable	variable to highest
Proportion of Tribelos	low to absent	low to absent	low to variable	variable to highest
Proportion of Chironomus	low to absent	low to absent	low to variable	variable to highest
Generic Richness	variable	highest	variable	lowest
Ephemeroptera Richness	highest	high	low	very low to absent
Plecoptera Richness	highest	variable	low to absent	absent
EPT Richness	high	highest	variable	low
Proportion Ephemeroptera Richness	highest	variable	low	zero
Proportion Plecoptera Richness	highest	high	low	low to zero
Proportion Diptera Richness	low to variable	variable	highest	variable to high
Proportion Ephemeroptera & Plecoptera Richness	highest	high	low to variable	low to absent
EPT Richness dvided by Diptera Richness	high	highest	low to variable	lowest to zero
Proportion Non-EPT or Chronomid Richness	high	high	low	lowest
Percent Predators	low	low	high to variable	highest
Percent Collector, Filterers & Gatherers divided by Percent Predators & Shredders	high	highest	low	lowest
Number of Functional Feeding Groups	variable	highest	variable	lowest

Measure of Community Structure	**Relative Findings**			
	A	**B**	**C**	**NA**
Represented				
Shannon-Weiner Generic Diversity	low to intermediate	highest	variable to intermediate	lowest
Hilsenhoff Biotic Index	lowest	low	intermediate	highest

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