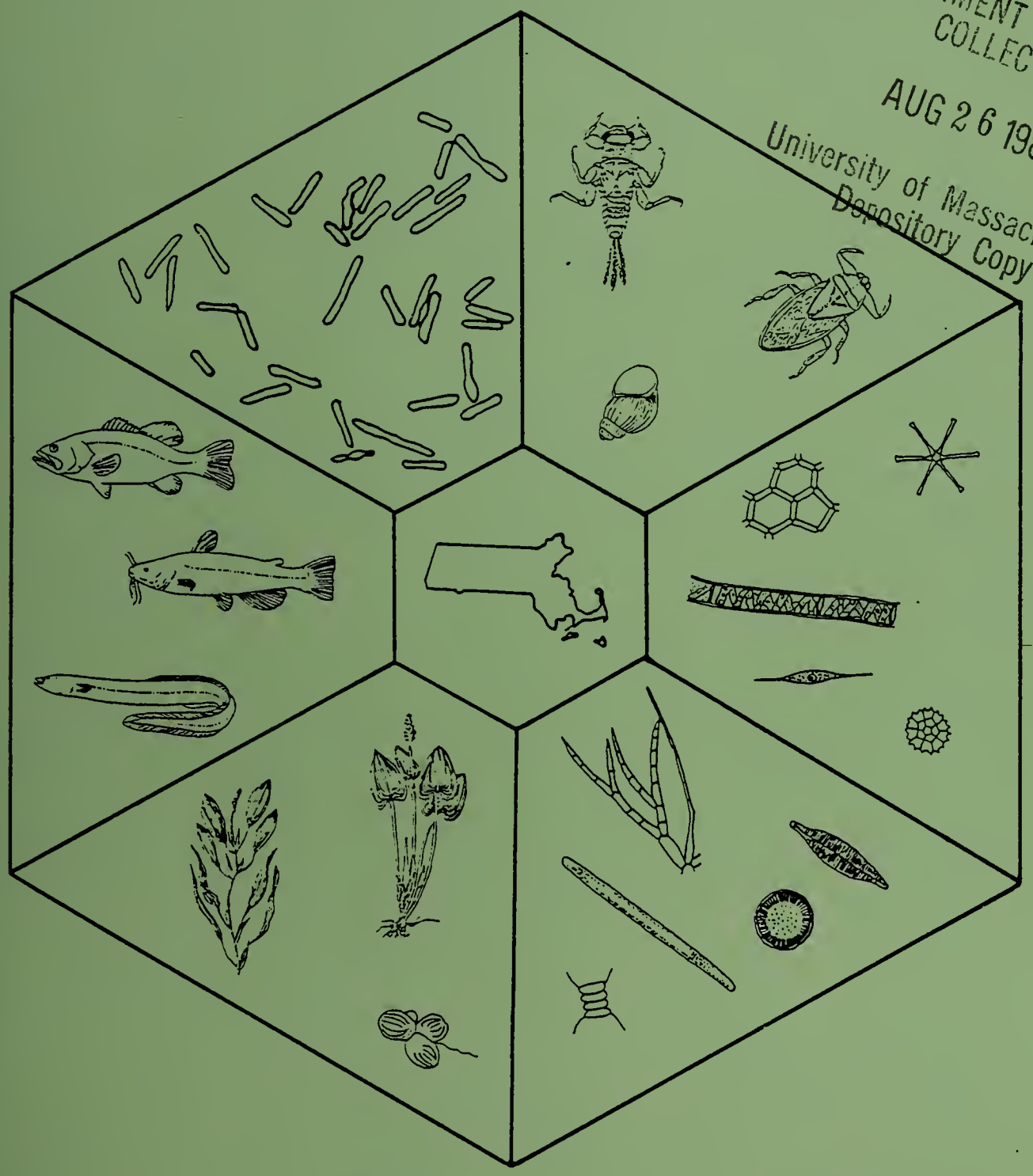
STANDARD OPERATING PROCEDURES



BIOMONITORING PROGRAM

Massachusetts Department of Environmental Quality Engineering
DIVISION of WATER POLLUTION CONTROL
Thomas C. McMahon, Director

BIOMONITORING PROGRAM

STANDARD OPERATING PROCEDURES

1987

Technical Services Branch
Massachusetts Division of Water Pollution Control
Department of Environmental Quality Engineering
Westborough

Executive Office of Environmental Affairs
James S. Hoyte, Secretary

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4.0 BIOMONITORING PROGRAM

1.0 INTRODUCTION AND PURPOSE

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It is the goal of the Federal Clean Water Act (PL 95-217) to restore and maintain the biological integrity of the nation's waters. Biological monitoring provides the most reliable measure of the attainment of this goal, i.e., water quality that provides for the protection and propagation of fish, shellfish and wildlife.

Sampling and analyzing aquatic life provides information on water quality that can easily escape standard physico-chemical sampling. The organisms themselves are efficient in-stream monitors, for their lives reflect the cumulative impact of pollution on the waterbody. They are valuable in revealing transient pollution episodes such as oil spills and brief dissolved oxygen sags. For the same reason they are the best means of measuring long term trends in a waterbody. In addition, the presence of specific indicator organisms may infer the presence of particular chemicals not included in routine analysis or in quantities below detection limits of chemical testing.

Aquatic biota are usually collected and analyzed by community. These communities include plankton, periphyton, macrophyton, macroinvertebrates and fish. The communities are used alone or in combinations to assess specific water quality problems such as thermal pollution, toxics, and eutrophication. The analysis of the samples includes taxonomic identification for diversity indices, water quality indices, trophic level and indicator organism analysis. Plant pigments are extracted for chlorophyll analysis and animal tissues are tested for bioconcentration of chemicals. The overall health and appearance of the organisms is used to detect chronic toxicity and genotoxic effects (carcinogens, mutagens and tetratogens). Standard laboratory organisms are also used *in situ* and *in vitro* to measure toxicity. Bacteria, algae, macroinvertebrates and fish are all commonly used for this purpose.

Biological monitoring can be more cost effective than chemical screening, more reliable at measuring total pollutant loads, more sensitive to extreme conditions and more faithful to the goal of the Act, than other forms of monitoring. However, the relationship between the biota and the environment is subtle and complex and by no means completely understood. Results of biological investigations are often qualitative, and even quantitative studies are open to interpretation. Therefore biological monitoring data are used to complement physico-chemical data and not replace them.

The methods of monitoring and analysis are evolving and may differ among investigators. At best, procedures used by the Division of Water Pollution Control are fully documented in this Standard Operating Procedures document, so that those attempting interpretation will be fully informed, and temper their conclusions accordingly.

2.0 BIOMONITORING SURVEY PROGRAM ELEMENTS

2.1 STREAM CLASSIFICATION

2.1.1 INTRODUCTION AND PURPOSE

This program has been developed to systematically sample and classify the Commonwealth's rivers and streams. Each survey qualitatively provides documentation of a specific watercourse's physical and chemical characteristics and predominant biological components. These data can be used - on a stream or site-specific basis - to determine water-use classifications in accordance with Massachusetts Surface Water Quality Standards.

2.1.2 OBJECTIVES

1. To identify, demonstrate, and standardize methods and procedures for the collection and analyses of stream habitat data;

2. to characterize rivers, streams, and related aquatic habitats (e.g., river impoundments) hydrophysically and chemically;

3. to qualitatively document the dominant floral and faunal components - or communities - of streams and stream-side habitats;

4. to segment and classify rivers and streams into major habitats for the purpose of water-use designation;

5. to provide supplementary information to other programs to aid in regulatory and enforcement actions, and evaluating special problems; and

6. to collect and reference plant and animal specimens for future study, and determine their state-wide distribution.

2.1.3 APPROACH

Preliminary planning and analysis first divides the river or stream into longitudinal zones - or subsystems, i.e., tidal, lower perennial, upper perennial, intermittent, and others (e.g., canals, ditches) - according to morphometric and hydrologic characteristics derived from USGS topographic maps. Physico-chemical and biological field collections are made, in most instances, at locations - or sites - determined after initial evaluation and field reconnaissance (see: "Data Record Sheets"). Specific sampling locations are arranged to cover significant and representative lotic-water and other related macrohabitats. Field dates, particularly for biological sampling, are generally during the period April to October, in order to take advantage of plant and animal availability. All field sampling is qualitative in nature, unless special needs dictate otherwise. Data collected are recorded for each community on individual standard field sheets (see: "Biological Field and Laboratory Methods").

2.1.4 PARAMETRIC COVERAGE

Physical and chemical data are collected, including: stream reach width and depth; stream reach and floodplain substrate character; stream temperature; water transparency; and water chemistry. Sampling of phytoplankton and periphyton, aquatic vascular plants, streamside and riparian vegetation, and aquatic macroinvertebrates is performed at each

2.1.5 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM CLASSIFICATION FIELD RECONNAISSANCE DATA RECORD

RIVER:

DRAINAGE:

MUNICIPALITY:

SITE:

COLLECTOR(S):

DATE:

TOPOGRAPHIC MAP(S):

WEATHER/ATMOSPHERIC CONDITIONS:

Air Temperature -

Wind Force/Direction -

Cloud Cover -

Precipitation -

PHYSICO-CHEMICAL

Flow Regime -

Substrate Character -

Light -

Other -

PHYTOPLANKTON

Channel -

Riffle -

Pool -

Other -

PERIPHYTON

Channel -

Riffle -

Pool -

Other -

AQUATIC VEGETATION

Channel -

Riffle -

Pool -

Other -

RIPARIAN VEGETATION

Trees -

Shrubs -

Herbaceous -

Grasses -

Other -

AQUATIC INVERTEBRATES

Channel -

Riffle -

Pool -

Other -

GENERALIZED SITE MAP

OTHER REMARKS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM CLASSIFICATION FIELD DATA RECORD

RIVER:	SITE:
DRAINAGE:	COLLECTOR(S):
MUNICIPALITY:	DATE:
TOPOGRAPHIC MAP(S):	TIME SAMPLING DURATION:

WEATHER/ATMOSPHERIC CONDITIONS:

AIR TEMPERATURE

WIND

Force -
Direction -

CLOUD CLASSIFICATION

Low
Middle
High
Percent Sky Coverage

PRECIPITATION (including previous day's)

DATA COLLECTIONS:

PHYSICAL	HYDROLOGIC
WATER	BIOLOGICAL

OTHER OBSERVATIONS:	INSTRUMENT CALIBRATION

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM CLASSIFICATION PHYSICO-CHEMICAL FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
STATION(S):	SAMPLE NUMBER(S):

FLOW REGIME
- Intermittent
- Upper Perennial
- Lower Perennial
- Tidal

WATER TEMPERATURE

SECCHI DISK DEPTH

SOLAR RADIATION

CHANNEL MORPHOLOGY
- Slope
- Vegetation
- Cover

SUBSTRATE (% AREAL COVER)

Inorganic	Organic
Bed Rock	Detritus
Boulders	Fibrous Peat
Rubble	Pulpy Peat
Gravel	Muck
Sand	
Clay	
Marl	

CHEMICAL

D.O.
pH
Alk
Spec. cond.
NH_3 NO_3 ON
TP OP
BOD COD TOC
TS TSS TDS
Metals
Other

WIDTH

VELOCITY
- 0.2
- 0.6
- 0.8

DEPTH

SAMPLE EQUIPMENT/METHODS:

SITE MAP:

D < 2.5 ft, V 0.6	D > 2.5 ft, V 0.2 + V 0.8	D x V $\geq$ 9 Not Wadeable

2.1.6 REFERENCES

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2.2 AQUATIC MACROINVERTEBRATE RAPID BIOASSESSMENT

2.2.1 INTRODUCTION AND PURPOSE

Macroinvertebrate rapid bioassessment (MRB) surveys involve the use of qualitative and semiquantitative sampling methods designed to minimize laboratory time requirements for taxonomic identification and enumeration of aquatic macroinvertebrate organisms.

2.2.2 OBJECTIVES

1. To provide standardized methods and procedures for assessing the impacts of toxic and conventional organic pollution on aquatic macroinvertebrates;

2. to obtain reliable biological water quality information to supplement the collection of standard physico-chemical water quality data; and

3. to provide the basis for making relative comparisons pertaining to water quality conditions between sampling stations and/or to document long-term trends at fixed sites.

2.2.3 APPROACH

While rapid bioassessments make use of the qualitative analysis of periphyton, aquatic and wetland vegetation, and fish communities, specific semi-quantitative sampling and analytical methods have been developed for use in assessing the macroinvertebrate community.

An upstream-downstream sampling regime is employed whereby known or suspected sources of pollution are bracketed by sampling stations. Selected aquatic communities are assessed and compared with unimpacted control (or reference) communities. Conclusions relative to water quality condition are drawn from a knowledge of the environmental requirements and pollution ecology of the individual taxa or assemblages encountered.

For macroinvertebrate rapid bioassessment the components of a 100 organism subset are identified to genus or species level whenever possible. The taxonomic data are then compiled to determine the status of the various criteria used to rank water quality. These criteria include:

1. Species richness;

2. distribution "balance";

3. the EPT value;

4. percent contribution, pollution tolerances, and feeding habits of the five numerically dominant species;

5. Hilsenhoff Biotic Index (HBI).

Field observations were also considered, as they often reveal important factors contributing to the quality of the benthic community.

Species richness, the number of different kinds of organisms present, will tend to decrease in response to pollution while the distribution of individuals becomes uneven, or unbalanced. That is to say, under the influence of pollution benthic macroinvertebrate communities become less diverse, with the majority of individuals falling into fewer taxa (Tarzwell and Gaufin 1953, Bartsch and Ingram 1959, Weber 1973, Hawkes 1979, and Welch 1980). By examining the relative contribution of the five numerically dominant taxa the evenness of the distribution can be judged.

The pollution tolerances of the dominant community members can be revealing as to the degree of pollution impacting a stream. Likewise, the number of species present from the orders Ephemeroptera, Plecoptera, and Trichoptera can be tabulated to formulate the "EPT value." These orders are composed of species that are regarded as intolerant or facultative in response to enrichment with conventional pollutants--Plecoptera are all intolerant, Ephemeroptera and Trichoptera have both intolerant and facultative members (Weber 1973, Hilsenhoff 1982). Also of importance are the feeding habits of the dominant taxa, as these will reflect community shifts to exploit the food source available, e.g., a filter feeding community downstream of an effluent high in suspended solids.

Hilsenhoff (1982) developed an index (HBI) based on the tolerances of aquatic macroinvertebrates to pollution with conventional organics. While his sampling protocol was similar to the one used here, he restricted his analysis to aquatic arthropods dependent on dissolved oxygen. The MRB, on the other hand, makes use of aquatic annelids and mollusks for the information they may contribute in attempts to evaluate the impacts of various types of pollution. Consequently, if the HBI is to be used as part of the MRB it becomes necessary to assign tolerance values to organisms excluded by Hilsenhoff as well as any regionally unique aquatic arthropod taxa that otherwise would have been included by Hilsenhoff. Since Hilsenhoff's tolerance values range from zero (intolerant) to five (tolerant) and most literature provides information on pollution tolerances as tolerant, facultative, and intolerant, assigning new values was difficult. Lacking any better information the assigned values then became: intolerant=1, facultative=2.5, and tolerant=4. These modifications surely weaken the reliability of the HBI, if not by using dubious tolerance values, then at least by virtue of eliminating the sensitivity to the extremes. Nonetheless, with these considerations in mind the HBI is retained in the MRB because if the index value falls at one of the extremes it indicates either very little DO stress (HBI<2) or very serious DO stress (HBI>4).

The MRB guidelines identify the range of characteristics indicative of different levels of pollution as follows:

1. Non-Impacted - Diverse fauna, at least 30 species in riffle habitats. Biotic index about 2.00. Mayflies, stoneflies, and caddisflies are well-represented, EPT value greater than 10. Dominant species are intolerant or facultative; no species comprises more

than 25% of the individuals; oligochaete worms comprise less than 20% of the individuals.

2. Slightly Impacted - Species richness usually 20-30. Biotic index 2.00-3.00. Mayflies and stoneflies may be restricted, EPT value 6-10. Dominant species are mostly facultative. Fauna often not so well balanced, often with one species comprising more than 25% of the individuals; oligochaete worms may comprise more than 20% of the individuals.

3. Moderately Impacted - Species richness 10-20. Biotic index 3.00-4.00. Mayflies and stoneflies rare or absent, caddisflies often restricted, EPT value 2-5. Dominant species are facultative or tolerant. Oligochaetes often comprise at least 20% of the individuals.

4. Severely Impacted - Species richness less than 10. Biotic index greater than 4.00. Mayflies, stoneflies, and caddisflies rare or absent, EPT value 0-1. Fauna often restricted to midges and worms. Dominant species are almost all tolerant. Fauna usually greatly imbalanced, with dominant species comprising more than 35% of the individuals.

These are generalizations about complex ecosystems and may not always result in complete agreement of all parameters. In such cases it is necessary to select a category based on a consensus of the majority of indicators. It is also necessary to consider the integrity of each component so that those possibly influenced by factors other than pollution can be de-emphasized, or if appropriate, eliminated from the assessment. For instance, a data set may contain 21 species, no species representing more than 25% of the community, oligochaetes comprising 21%, an EPT value of three, an HBI of 3.25, with four of the five dominant species being facultative, and the fifth being tolerant. Knowing that the data set includes significant numbers of aquatic annelids and mollusks, the HBI should not weigh heavily in the analysis. A review of the other criteria would tend toward a rating of "slightly impacted" for this hypothetical community.

2.2.4 PARAMETRIC COVERAGE

Rapid assessment surveys include, at a minimum, semi-quantitative aquatic macroinvertebrate sampling and water temperature determinations. However, qualitative analyses of the algae, macrophyte, and fish communities may also be conducted. Often, flow measurements, substrate characterization, and water chemistry sampling are conducted to supplement the results of biological sampling.

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2.3 SITE ASSESSMENT

2.3.1 INTRODUCTION AND PURPOSE

While site assessments make use of a number of qualitative and semi-quantitative methods borrowed from stream classification and/or rapid assessment protocols, they may also be expanded to include quantitative sampling and analytical procedures. In fact, site assessment surveys may range in scope from a qualitative assessment of the impact of a single wastewater discharge on a single aquatic community to intensive quantitative assessments of one or more communities. The latter are labor and resource intensive and are limited to those situations where the need exists for statistically derived statements of confidence in the results.

2.3.2 OBJECTIVES

1. To provide an adequate data base for making quantitative determinations of standing crop, biomass, or measures of community structure such as species diversity and richness;

2. to provide sufficient data for testing for significant differences between communities using appropriate statistical methods;

3. to provide standard methods for assessing the impacts of pollution on aquatic biota and water uses; and

4. to supplement physico-chemical water quality data with biological information.

2.3.3 APPROACH

Whenever possible, sampling stations are located upstream and downstream from known or suspected sources of pollution or other factors that might impact water quality conditions. The underlying assumption is made that, if all other environmental factors remain constant, a change in water chemistry will alter downstream community structure or biomass. Therefore, impact assessment is carried out by making community structural comparisons between upstream or nearby reference communities and downstream communities.

Measures of community structure to be employed are selected on a case-by-case basis according to the requirements of individual site assessments. Parameters include 1) abundance; 2) taxonomic richness; 3) evenness; and 4) diversity (e.g., Shannon Weaver H^1). Comparisons of communities between sites are made using the above measures and standard significance tests such as t-tests.

Less intensive site assessments involving the use of qualitative or semi-quantitative techniques are conducted according to the methods presented in previous sections for stream classification and rapid assessment surveys.

2.3.4 PARAMETRIC COVERAGE

Site assessments may involve the use of qualitative, semi-quantitative, or quantitative analyses of one or more of the following communities: phytoplankton; periphyton; macrophyton; macroinvertebrates; or fish. Biological stream sampling is supplemented, as deemed appropriate, by hydrological and physico-chemical assessments such as the determination of stream width, depth, flow, water temperature, substrate characterization, and chemical analyses.

2.3.5 QUANTITATIVE DATA ANALYSES

Definitions of some of the more commonly used indices of community structure are presented below.

Abundance

Two abundance measures are often used: (1) the sum total of individuals found in all taxonomic groups in a particular data set (termed "total numbers"); and (2) the relative proportion of individuals found in different taxonomic categories (termed "relative abundance").

If a relationship between productivity and numbers of individuals can be established, increases from control to test sites in the total number of organisms found may be a result of increased nutrient availability. Decreases in this measure may be related to changes in nutrients and/or the influence of toxic substances. Changes in the relative abundance of major taxonomic groups may be related to habitat alterations between sites. When changes in the relative abundance of major groups are accompanied by a decrease in richness (see below) they may be due to either changes in nutrient availability and/or to toxic stress.

Taxonomic Richness

This term refers to the number of different taxonomic groups in a particular sample. Comparisons of richness are based on the assumption that physiological stress (defined as those instances under which environmental conditions such as temperature, oxygen concentration, pH, etc., exceed the tolerance limits of an individual) due to a toxic discharge can reduce the number of taxa originally inhabiting a certain area.

Richness of a sample collection is positively correlated with sampling effort. As area sampled, time spent sampling, and/or number of organisms collected are increased, the number of different taxa encountered also increases. For these reasons, comparisons should only be made between data sets for which sampling efforts are similar or nearly so.

Evenness

This is a measure of the distribution of individual organisms over different taxonomic categories. Most evenness indices range from a value of zero to 1.0, with a completely uniform distribution yielding a value of 1.0.

Diversity indices (see below) compress richness and evenness into a single number. However, information is lost in this process. In an attempt to regain some of this information, ecologists have used evenness or equitability ratios that are usually of the form: measured diversity/ standard diversity, where the latter term is the maximum diversity of a community given a certain richness value. A basic problem with this approach is that the value or the ratio is dependent upon the particular characteristics of the diversity index. Thus, biases inherent to the index are incorporated into, and perhaps magnified by, the evenness ratio.

Diversity Indices

Most diversity indices attempt to interdigitate and refine two components of community structure: richness and evenness.

The Shannon Weaver H^1 is commonly used for two reasons: (1) it is simple in form; and (2) it has a known variance structure. Due to the latter attribute, a t-test for differences in H^1 between two data sets can be run. The form of the index and its variance structure are taken from Poole (1974) and are presented below.

$$H = \left(- \sum^{S} pi.\ln pi \right) - \frac{S-1}{2N}$$

$$\text{Var. } H' = \frac{\sum_{i=1}^{S} pi.\ln^2 pi - \left(\sum_{i=1}^{S} pi.\ln pi \right)^2}{N} + \frac{S-1}{2N^2}$$

where S = number of taxa
pi = the proportion of the total number of individuals consisting of the i^{th} taxon
N = total number of individuals

Another diversity index commonly used is Simpson's Index which can be defined as: $D = 1 - C$

$$\text{where } C = \sum_{i=1}^{S} \frac{ni(ni-1)}{N(N-1)}$$

and S = as above
ni = the number of individuals in the i^{th} species
N = as above

The term C is an approximation of the probability that two individuals drawn at random from a population of N individuals will belong to the same taxon. The higher this probability, the lower the "diversity" (as measured by this index) of the collection; hence D (equal to 1-C) is used as the index since this parameter will increase with the "diversity" of the sample.

The two indices cited above differ in their sensitivity to changes in richness and evenness. Whereas the Shannon Weaver Index is more an expression of the overall evenness of the community, the Simpson's Index expresses the relative degree of dominance of a few taxa in the community.

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3.0 BIOLOGICAL FIELD AND LABORATORY METHODS

3.1 PHYTOPLANKTON

3.1.1 DEFINITION:

Phytoplankton are the algae of lakes and large rivers that live suspended in the water. They are chlorophyll-bearing, unicellular organisms which have no true roots, stems, or leaves. They occur in free-living, colonial, frond-like or filamentous forms and vary in size from unicells 0.5 microns in diameter to the macroscopic seaweeds. Algae are generally grouped into the Divisions (and classes) Euglenophyta (Euglenophyceae); Chlorophyta (Chlorophyceae, Charophyceae); Rhodophyta (Rhodophyceae); Cyanophyta (Myxophyceae); Pyrrophyta (Desmokantae, Dinophyceae); Chrysophyta (Xanthophyceae, Chrysophyceae, Bacillariophyceae); Phaeophyta (Phaeophyceae); and Cryptophyta (Cryptophyceae).

3.1.2 OBJECTIVES

1. To document the existing phytoplankton community and determine long-term (yearly) and short-term (seasonal) trends;

2. to evaluate direct effects on water composition including dissolved oxygen, pH, hardness, and optical properties;

3. to assess conditions affecting the general condition of water quality including noxious and toxic conditions, offensive tastes and odors;

4. to identify indicators of trophic status, organic enrichment and specific chemical contamination; and

5. to quantify autotrophic biomass and make inferences concerning productivity.

3.1.3 FIELD SAMPLING

Samples for phytoplankton analyses are collected in clean one-liter bottles made of plastic or glass, that have been rinsed with sample water. Approximately one-half liter of sample water is collected.

In rivers that are mixed vertically and horizontally, samples are collected midstream 0.5 to 1.0 meters (m) below the surface. In lakes and impoundments, samples are collected at the "deep-hole" station. If the lake is thermally unstratified the sample is collected 0.5-1.0 m below the surface. If the lake is thermally stratified, an integrated column sample is collected by lowering a one centimeter (approximately) ID plastic tube (with a weight attached) to the thermocline zone, pinched below the miniscus and raised into the boat. The sample is then drained into a clean and rinsed collection bottle. This procedure is repeated until one-half liter of water is collected. All samples are cooled to 4°C and placed in the dark following collection.

For special studies in riverine and lacustrine habitats, samples are collected from major depth zones or water masses. Sampling depths at each site are determined by specific conditions. In shallow areas (2-3 m), subsurface sampling is generally conducted. In deeper areas samples are collected at regular intervals at depths throughout the euphotic zone.

Pertinent information collected and recorded in the field includes meteorological data (cloud cover, wind speed and direction, air temperature); surface water conditions; water color, turbidity, odors; total depth at station; and other descriptive information.

The frequency of sampling is dependent on the intent of the study as well as the range of seasonal fluctuations, the immediate meteorological conditions, adequacy of equipment, and availability of personnel. In tidally-influenced habitats, phytoplankton samples are collected at all tide stages, particularly at the end and the beginning of both the flood and ebb tides.

3.1.4 LABORATORY ANALYSES

Sample Preservation

Phytoplankton samples collected in the field are cooled to 4°C and kept in the dark in transit to the laboratory. Upon arrival at the laboratory, they are placed in a refrigerator until further processing. Samples are generally analyzed on the day of collection. Samples not analyzed on the day of collection are stored in a refrigerator overnight with the caps loosened to allow gas exchange. Samples stored for more than 48 hours are fixed by the following methods and preservatives:

1. Lugol's solution: For short-term storage, 0.3 ml Lugol's solution is added per 100 ml of sample aliquot and stored in the dark. For long-term storage, 0.7 ml Lugol's solution is added per 100 ml of sample. [Lugol's solution is prepared by dissolving 20 grams (g) potassium iodide (KI) and 10 g iodine crystals in 200 ml distilled water containing 20 ml glacial acetic acid].

2. Formalin: To preserve samples, 40 ml buffered formalin is added to one liter of sample.

3. M^3 Fixative: For preservation, 20 ml M^3 fixative is added to one liter of sample and stored in the dark. [M^3 is prepared by dissolving 5 g KI, 10 g iodine, 50 ml glacial acetic acid, and 250 ml formalin in one liter of distilled water].

Color -

Cupric sulfate solution is added to the sample to preserve color [Cupric solution is prepared by dissolving 21 g cupric sulfate in 100 ml distilled water].

Clumping -

To prevent clumping, a detergent solution is added to the sample [20 ml liquid detergent is added to 100 ml distilled water].

Phytoplankton Examination

Log-In Procedure -

1) Each sample is assigned a number and logged in as it is brought into the laboratory. The numbers are in consecutive order and are recorded both on the sample tag and in a notebook (log book).

2) Next to the number in the log book are also recorded the station number and location, date collected, date analyzed, initials of collector, type of samples, sample depth, and analyses requested, i.e., chlorophyll and/or algal identifications.

Phytoplankton Examination Equipment List -

1) Microscope - capable of 200x power with working distance greater than 1 mm.

2) Sedgwick-Rafter (S-R) counting cells

3) Whipple micrometer reticule

4) Stage micrometer

5) Pipettes

6) Bench sheets

7) Lens paper

Procedure for Filling the Sedgwick-Rafter Cell:

1) Place the cover glass diagonally across the cell.

2) Use large-bore 1 ml pipette to fill the S-R cell.

3) Place tip of the pipette in the corner of the S-R cell and slowly release the pressure of your finger on the end of the pipette. The cover slip will then rotate and cover the sample.

4) To reduce error:

   a. Do not overfill the cell which would yield a depth greater than 1 mm.

   b. Do not allow large air bubbles to form. To prevent the formation of these air spaces, a drop of distilled water is placed on the edge of the cover glass occasionally during the microscopic examination.

Procedure for Phytoplankton Examination:

1) Shake the sample bottle to mix well.

2) Rinse 1 ml pipette with distilled water (inside and out) and three times with sample water.

3) Fill counting cell with 1 ml of sample water (see: "Procedure for Filling the Sedgwick-Rafter Cell").

4) Allow sample to settle for 15 minutes (the settling rate for algae is 4 mm/hr; since the depth of the counting cell is 1 mm, a 15 minute settling time is used.

5) While sample is settling, prepare a microscopic slide or Palmer cell which will allow you to view the sample at a higher power. List the algal genera identified.

6) Use the keys to determine unknown organisms; particularly dominant ones.

7) Scan the Sedgwick-Rafter counting cell at 4x and determine need for concentration or dilution.

8) At 200x find the edge of the counting cell and focus on the top of the cell. Continue turning the coarse focusing knob on the microscope until the bottom of the cell comes into focus.

9) At least two strips in the S-R counting cell must be counted.

10) Counts are done on both the bottom of the cell and the top or underside of the cover slip.

11) Identify and count all the algae that are located in the Whipple grid. Algae which are half in and half out of the top of the grid should be included in the count. Algae which are half in and half out of the bottom of the grid are not included in the count.

12) If the algal density appears to be high then fields can be counted instead of strips. A field is represented by a Whipple grid. Ten fields on two slides are counted and then averaged.

13) A strip is represented by the width of Whipple grid and the length of a Sedgwick-Rafter cell.

Explanation of the Phytoplankton Examination Sheet:

(Refer to: "Phytoplankton Examination" Form)

1) Line 1 - station location, station number, date of collection

2) Line 2 - initials of analyst, milliliters of sample, which will be either 1 ml or the total concentrated, type of count, i.e., fields or strips and the date of analysis.

3) Lab number - the number assigned the sample by the investigating laboratory (see: "Log-In Procedures").

4) Bottom two lines - chlorophyll in mg/m^3, total live algae (cells/ml), multiplication factor (S-R) for the particular microscope and power used, microscope manufacturer and type, the microscope power used (10x, 20x, etc.), type of preservative used, and a box for the initials of the person who does the quality control check of the multiplication and addition on the examination sheet.

5) Center of the phytoplankton examination sheet - seven algal classes and eight types are delineated. Identifications are recorded under the organism column, running counts are recorded under counts. The running counts are tallied and multiplied by the S-R factor to obtain totals in cells/ml. A total is given for each class and type as well as for the sample.

Determination of the S-R Factor:

When strip counts or field counts are done on a Sedgwick-Rafter counting cell, only a portion of the 1 ml sample is examined. Therefore, a calibration of "S-R" factor must be determined. The following formula is used in this calibration:

$$\text{S-R factor (strip count)} = \frac{1000 \text{ mm}^3}{\text{LxWxDxS}}$$

where: L = length of a strip (mm)
S-R cell is 500 mm long

W = width of a strip which is the Whipple grid image width (determined by using a stage micrometer)

D = depth of chamber (1 mm)

S = number of strips counted

The S-R (strip count) times C, the number of organisms counted (tally) equals the number of algae per milliliter.

$$\text{units/ml} = \text{S-R (strip count)} \times C$$

The S-R factor (field count) is calculated by using the following formula:

$$\text{S-R factor (field count)} = \frac{1000 \text{ mm}^3}{\text{AxDxF}}$$

where: A = area of a field, which is the Whipple grid image area

D = depth of chamber (1 mm)

F = number of field counts

The number of algae per ml equals the S-R (field count) times C, the number of organisms counted (tally).

Units/ml = S-R (field count) x C

Procedure for Phytoplankton Counts:

In the unit (or clump) count each cell or colonial group of cells receives one unit.

Examples:

1. Anacystis - one unit per clump

2. Anabaena - one unit per chain

3. "Filamentous green" - one unit per filament

4. Scenedesmus - one unit each (4, 8, 16 etc., celled organism.)

5. Fragilaria and Melosira - count each cell (may be best to average the area for a single cell and divide into total area.)

6. Asterionella - each "arm" one unit

7. Dinobryon - each colony one unit.

An attempt is made to identify all organisms to generic level. If this can not be accomplished then an effort is made to assign the organism to the proper class and type. Unidentified organisms are described as "UI" on the phytoplankton examination sheet. Subscripts are assigned, i.e., "UI1", "UI2", "UI3", etc., if more than one kind of unidentified organism are present within a particular class and type.

Counts below 500 cells/ml are generally unreliable. In general, an attempt is made to observe at least 20 organisms while making tallies in strip counts. Any manipulation of the sample (concentration or dilution) adds error. Therefore, on samples with high concentrations, a field count rather than concentrate is performed. On samples with low counts, more strips are counted. Precision is achieved in field counts by determining the coefficient of variation for counts in the number of fields counted and adjusting the number of fields counted to meet an $\pm$ 10% error, as outlined in precision calculations (see: "Precision Data").

Precision Data:

1. $S = \sqrt{\frac{\sum(x-m)^2}{n-1}}$

   Where:

   S = Standard deviation
   M = Mean (average)
   X = Count
   n = Number of fields

2. $C_v = \frac{s}{m}$ or $\sqrt{\frac{\frac{\sum x^2}{M^2} - n}{n-1}}$

   Where C_v = Coefficient of variation

3. P = % standard deviation of mean = $\frac{100c_v}{\sqrt{n}}$

4. C_v must be 0.317 or less if results in a 10-field count are to be $\pm$ 10% within a 2/3 probability and a practical certainty (95%) of $\pm$ 20% precision error.

5. Using past data it was found that if ten fields are counted:

   C_v = 1.0 or $\pm$ 31.7% error was found in 90% of samples
   0.7 or $\pm$ 22% error was found in 75% of samples
   0.45 or $\pm$ 14% error was found in 50% of samples
   0.317 or $\pm$ 10% error was found in 33% of samples

   On the average, a third of random samples were within $\pm$10% error when 10 fields were counted, and half were within $\pm$14%.

6. P = The Standard error of count (percent) and is found in the log-log plot of C_v versus n.

3.1.5 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat, motor, gas can (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Collecting and Sampling Gear

- ☐ secchi disk
- ☐ pocket thermometer
- ☐ photometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ plastic tubing with weight attached
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ sample preservative, fixative

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.1.6 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

PHYTOPLANKTON FIELD DATA RECORD

RIVER: SITE: STATION(S):	COLLECTOR(S): DATE: SAMPLE NUMBER(S): LABORATORY NUMBER(S):

TYPE OF SAMPLE: Sample Depth(S): Water Temperature Light Attenuation: Secchi Disk Illuminance Meter Surface Foot-Candles Foot-Candles	SAMPLE EQUIPMENT/METHODS: REMARKS:

SITE MAP	HABITAT DESCRIPTION

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

PHYTOPLANKTON EXAMINATION SHEET

River/Lake		Station	Date Collected	Lab No.
Analysis by:	Mi	Count	Date Analyzed	

Class	Type	Organism	Count	Tally	Cells/ml	Total
Bacillariophyceae (Diatoms)	Centric					
	Pennate					
Cyanophyceae (Blue-Greens)	Coccaid					
	Filamentous					
Chlorophyceae (Greens)	Coccaid					
	Desmids					
	Filamentous					
	Flagellates					
Chrysophyceae (Golden-Browns)						
Cryptophyceae (Cryptamonads)						
Dinophyceae (Dinoflagellates)						
Euglenophyceae (Euglenids)						

Chlorophyll a/ in mg/m^3 Tot. live algae (c/ml) SR =

Microscope _____ Power _____ Preserved _____ Quality Control

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3.2 PERIPHYTON

3.2.1 DEFINITION:

Periphyton as used here shall mean the attached algal community. Any associated bacteria, fungi, mosses or epiphytic animals are identified to a rudimentary level only. Occasionally, planktonic algae are collected during periphyton sampling in lotic waters. These identifications are reported under the heading periphyton, although, strictly speaking, they are plankton.

3.2.2 OBJECTIVES

1. To document the existing periphyton component in lotic and lentic environments and determine dominant types; and

2. to evaluate water quality conditions by the use of indicator species.

3.2.3 FIELD SAMPLING

Prior to disturbing the streambed or lakebed, stations (or reaches) selected for qualitative investigation are visually inspected for algal growth. Representative samples are collected from each macrohabitat: pools, riffles, channel, streambank, backwater, open-water; and all substrates: rocks, sand, vegetation, twigs and other debris. Each type of alga encountered is collected using forceps, pipette, knife or by hand. Specimens are placed in labeled glass (or plastic) vials with water from the sampling site, and deposited into a cooler on ice for transportation to the laboratory. Information concerning growth habit and relative abundance of the representative algae are duly noted on field sheets. Photographic documentation of site conditions may also be conducted.

3.2.4 LABORATORY ANALYSES

Log-In Procedure

Each sample is recorded in the algae log book along with the phytoplankton samples. The sample is assigned a number followed by the letter P indicating periphyton. This is done in order to distinguish it from phytoplankton samples. Also recorded in the log book are the station number and location, the date collected, initials of the collector, and date analyzed.

Microscopic Analysis

Samples collected in the field are stored in the refrigerator until they are viewed. Specimens are identified within one to two days following collection while they are still alive and healthy. This facilitates identification since preservatives tend to alter the color and - in some cases - the structure of the algae. Identifications are made from wet mounts using a compound microscope equipped with 10x, 20x, 40x, and 100x objectives. Identifications are made using various taxonomic keys to the lowest level possible and recorded on a Periphyton Lab Bench Sheet (see Section 3.2.6). Certain specimens are photographed as a means of documentation and for use in presentations or as a teaching tool. Taxonomic lists of the results are compiled for each survey and published in appropriate reports.

Periphyton Examination Laboratory Equipment List

1. Microscope with 10x, 20x, 40x, 100x objectives
2. Microscope slides and coverslips
3. Pipettes
4. Forceps, probes
5. Lens paper
6. Bench sheets

3.2.5 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat motor, gas can (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Collecting and Sampling Gear

- ☐ secchi disk
- ☐ pocket thermometer
- ☐ photometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ sample preservative, fixative

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.2.6 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

PERIPHYTON FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
STATION:	SAMPLE NUMBER(S):

HABITAT	LIGHT	WATER TEMPERATURE	SAMPLE EQUIPMENT/METHODS:
Pool Riffle Backwater Impoundment Other	Open Total Shade Partial Shade Comments:		

Sample #	Substrate Type	Areal Cover(%)	Growth Habit	Color	Texture	Smell

Site Map:

Other Remarks:

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TECHNICAL SERVICES BRANCH

PERIPHYTON LAB BENCH SHEET

BASIN: _____ BASIN NUMBER: _____

RIVER: _____ STREAM INVENTORY NUMBER: _____ TOWN: _____

STATION: _____ COMMENTS: _____

Date Collected: _____ Date Analyzed: _____

Collector(s): _____ Analysis by: _____

_____ Microscope: _____ Power: _____

Number of Samples: _____ Photo: _____

Sample #:	Habitat:	Substrate:	Relative Abundance:

Identification:

Code(s):

Sample #:	Habitat:	Substrate:	Relative Abundance:

Identification:

Code(s):

Sample #:	Habitat:	Substrate:	Relative Abundance:

Identification:

Code(s):

Relative Abundance: Most Abundant, Abundant, Common, Sparse
Habitat: Pool, Riffle, Backwater, Impoundment, Spillway, etc.
Substrate: Rock, Mud, Sand, Wood, Bottle, etc.

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3.3 AQUATIC AND WETLAND VEGETATION

3.3.1 DEFINITION

DEFINITION: Aquatic flora as used here pertains to several taxonomic groups including the Characeae (stoneworts and muskgrass); Musci, Hepaticae, and Ricciaceae (mosses, leafy liverworts, thallose liverworts); Osmundaceae (flowering ferns); Equisetaceae (horsetail, scouring rush); Isoetaceae (Quillwort); and the Angiospermae (the seed plants).

3.3.2 OBJECTIVES

1. To identify and test reliable methods and procedures for the collection, identification and enumeration of aquatic and wetland vegetation;

2. to document existing aquatic plant species and communities; and

3. to determine areal coverage and dominant plant types.

3.3.3 FIELD SAMPLING

For riverine habitats, aquatic and wetland vegetation are located and qualitatively mapped by visually examining the streambed, streamside, and immediate riparian areas by walking or wading. A reach of stream approximately 10-meters in length is generally investigated. Each macrohabitat is sampled and the predominant vegetation noted and recorded on standard type field data sheets. A schematic map is prepared for each site. Photographic documentation is sometimes made. Vegetation is generally identified on-site.

The aquatic and wetland plant community in lacustrine habitats is located and mapped by examining the limnetic, shoreline, and littoral areas by boat or waders. Occasional samples are collected at regular intervals on imaginary transects run across open-water areas of the lake or impoundment. All habitats are sampled and the relative abundance of each plant type noted and mapped on prepared outline maps. Representative macrophytes are collected by hand and, in deeper water, by dragging a simple grappling hook with a weight attached to the shaft. An Ekman or Ponar dredge is sometimes used to collect deeply-submerged vegetation. Identifications of most plant specimens are made in the field.

3.3.4 LABORATORY ANALYSES

Vegetation not identified in the field is collected and returned to the laboratory for further analysis using a stereoscopic microscope or hand lens and various taxonomic keys. Representative plant specimens collected from each site are pressed and dried in preparation for permanent mounting. Plant specimens are deposited in the Botanical Reference Library of the Technical Services Branch.

3.3.5 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat motor, gas can, (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Field Apparel

- ☐ rain gear, (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Collecting and Sampling Gear

- ☐ secchi disk
- ☐ pocket thermometer
- ☐ photometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ plastic bags (and ties)
- ☐ sample preservative, fixative
- ☐ rake
- ☐ grappling hook, rope
- ☐ Ekman, Ponar dredges
- ☐ white enamel trays
- ☐ trowel
- ☐ plant press and vasculum

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.3.6 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

AQUATIC VASCULAR PLANT FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
STATION(S):	SAMPLE NUMBER(S):

TAXA	DEPTH	AREAL COVER(%)	REMARKS	SITE MAP

SAMPLING EQUIPMENT/METHODS

OTHER REMARKS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

STREAM-SIDE AND RIPARIAN HABITAT FIELD DATA RECORD

RIVER:

SITE:

STATION(S):

COLLECTOR(S):

DATE:

SAMPLE NUMBER(S):

STREAM-BANK/SHORELINE CHARACTER:

REMARKS:

FLOODPLAIN

Structural	Areal Cover(%)	Composition/Type
1. Trees		
2. Shrubs		
3. Herbaceous		
4. Grasses		
5. Others		
6. Open		

SITE MAP

UPLAND

SAMPLE EQUIPMENT/METHODS

OTHER REMARKS:

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3.4 AQUATIC MACROINVERTEBRATES

3.4.1 DEFINITION: The aquatic macroinvertebrate community is defined as the assemblage of invertebrate organisms which can be seen by the unaided eye and retained by a U.S. Standard No. 30 sieve (i.e., 28 meshes per inch; 0.595 mm apertures). All or some life-cycle stages of these animals occur either attached to plants, other animals, debris, or inorganic substrates, or they float or swim in the water column of lentic and lotic waterbodies. Representative members of this community include - but are not limited to - sponges, bryozoa, flat worms, segmented worms, arthropods (water mites, crustaceans, insects), and mollusks.

3.4.2 OBJECTIVES

1. To provide information for stream classification, assessment of water quality conditions and trends, and direct impact assessment;

2. to interpret data using knowledge of the pollution ecology of component taxa (e.g., indicator schemes; biotic indices), or by observing changes in invertebrate community structure (e.g., richness; diversity); and

3. to determine the severity of water pollution problems by comparing unimpacted control or reference communities with potentially impacted communities.

3.4.3 FIELD SAMPLING

Qualitative

Qualitative macroinvertebrate sampling for stream classifications or special site assessments involves the use of a variety of sampling devices to collect samples from all available habitats encountered within a sampling site. Generally, D-frame nets are used to sweep aquatic vegetation, collect under cut stream banks, and agitate substrates to dislodge benthic organisms. Depending upon the taxonomic level desired, organisms are identified in the field to family level or placed in jars with 70% ethanol (95% if sample contains sediment materials or debris) for transport to the laboratory where further analyses are conducted.

Rapid Assessment

To obtain a sample for the Rapid Assessment Methodology, a D-frame net is pressed against the substrates, and substrate material just upstream and in front of the net is agitated by kicking. This procedure is continued for five minutes while gradually moving upstream. Sampling is executed in areas of comparable substrate and current velocity (usually riffle areas within the central one third of the channel).

At the end of the five minutes of kick-sampling the contents of the net are emptied into a white enamel pan. Organisms clinging to the net are removed, using forceps, and placed in the sample container, as are

organisms on substrate materials too large to fit into the sample container. Once the organisms have been removed, these larger materials are returned to the stream. The remainder of the sample is added to the container and preserved with 95% ethanol, containing 130 mg/l Rose Bengal stain. Completed labels are placed inside each container and attached to the outside. Field notes record the major taxonomic groups encountered during field processing.

Quantitative

When quantitative sampling is required, the following routine is employed:

1. Depending on depth, flow, and substrate conditions sampling gear is selected from among Ekman, Petersen and Ponar grab samplers or Surber and Hess substrate samplers. One set of four replicate samples is obtained following a random transect whereby both banks and two quarter points are sampled.

2. The substrate obtained is characterized according to particle size and composition, placed into a basin, and mixed thoroughly. When the sample consists of heavily organic or sand-silt type substrate, one-quarter of the sample is randomly selected and retained after mixing. The remaining material is qualitatively examined and discarded. Subsampling is often necessary due to the time required for sorting a large quantity of substrate.

3. The sample portion is passed through a standard U.S. No. 30 brass sieve (0.595 mm apertures). Organisms and substrate left behind are placed into labeled plastic or glass wide-mouth containers (approx. 1 liter) and returned alive or preserved with 95% ethanol to the laboratory for further analysis.

3.4.4 LABORATORY ANALYSES

All samples are recorded in a log book upon arrival at the laboratory. Preserved samples are drained on a U.S. Standard No. 30 mesh screen and rinsed with tap water. Live and preserved samples are placed in individual white enamel pans for sorting. Samples for quantitative analyses are preferably sorted alive by removing all benthic organisms manually from the substrate and separating them by taxonomic order into glass vials containing 70% ethanol. For the Rapid Assessment Methodology, the contents of the enamel pan are subdivided by scooping material successively (one after the other) into four to eight glass petri dishes until all the material is distributed among the dishes. The number of dishes used depends on the volume of substrate and debris in the sample.

Before picking out organisms, the petri dishes are assigned a number (one to four, if four are used). Numbers are then drawn at random to determine the order of processing. The dish with the number corresponding to the first number drawn is placed on the stage of a stereomicroscope by deliberate orientation (first random field). All organisms within the field of view at low power are picked and placed in labeled vials with 70% ethanol. When all organisms in the field of view have been removed

the dish is moved to another random field for removal of additional organisms. This procedure is repeated until 100 organisms have been selected, moving to the next randomly selected petri dish as required. The remaining sample materials are again sieved on a #30 mesh screen, labeled, and archived in 95% ethanol.

Macroinvertebrate specimens other than chironomids and oligochaetes are identified through examinations using a Wild M5A stereomicroscope equipped with fiber optics lighting. Oligochaetes, chironomid larvae, and chironomid pupae must be mounted on microscope slides before examination with an Olympus BH-2 compound microscope equipped with Nomarski optics. Semi-permanent slide mounts are made by placing the specimens on a 25 x 75 mm microscope slide in CMC-10. The oligochaetes and chironomid larvae are mounted in the CMC-10 without prior clearing. The heads of the chironomid larvae are excised and positioned above the bodies, usually with three specimens under each of two 18 x 18 mm square coverslips per slide. Chironomid pupae are first cleared in 10% KOH (potassium hydroxide) before mounting in CMC-10 with one specimen per 18 x 18 mm slide. The heads of the pupae are also separated from the body once mounted on the slide.

3.4.5 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ roof racks
- ☐ boat trailer
- ☐ pram, oars (and locks)
- ☐ canoe, paddles
- ☐ boat motor, gas can (and line)
- ☐ anchor, rope
- ☐ life jackets, seat pads

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Collecting and Sampling Gear

- ☐ pocket thermometer
- ☐ tape measure
- ☐ range finder
- ☐ Ekman, Peterson, Ponar dredges
- ☐ Surber samplers
- ☐ Hess sampler
- ☐ metal holding tub
- ☐ white enamel trays
- ☐ sieves (of various sizes)
- ☐ plastic bucket, rope
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ ethanol, formalin
- ☐ killing jar, killing agent
- ☐ aerial net, D-frame net

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice

3.4.6 DATA RECORD SHEETS

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

AQUATIC MACROINVERTEBRATE FIELD DATA RECORD

RIVER:	COLLECTOR(S):
SITE:	DATE:
SAMPLE NUMBER(S):	SAMPLING DURATION:

HABITAT:					
BOTTOM TYPE:					
BOTTOM VEGETATION:					
SAMPLE DEPTH:					
DOMINANT ORGANISM/ASSEMBLAGE:					
SAMPLE EQUIPMENT/METHODS:					

SITE MAP:	OTHER REMARKS:

60
09

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

AQUATIC MACROINVERTEBRATE LAB BENCH SHEET

Name of Water Body_____ Station No._____ Location_____
Date Collected_____ Collector_____ Sorted By_____ Code_____

ORGANISM	# /LS/TV/TI	ORGANISM	# /LS/TV/TI
Nematoda		Plecoptera	
Annelida		.....	
Oligochaeta		Hemiptera	
Hirudinea		.....	
		Megaloptera	
Isopoda			
		.....	
Amphipoda		.....	
		Trichoptera	
Decapoda		.....	
		Coleoptera	
Hydracarina			
		.....	
		Diptera	
Collembola			
.....			
Ephemeroptera		Gastropoda	
		Pelecypoda	
.....			
Odonata		Others	

Total No. of Organisms_____
Total No. of Kinds_____

\# = Number of individuals tallied
TV = Biotic Index Tolerance Value
TI = Taxonomist's initials
LS = Life stage: I = Immature
P = Pupa
A = Adult

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

SLIDE INVENTORY CATALOG SHEET

Page_____ of_____

SURVEY NAME:

SURVEY CODE:

SLIDE BOX____OF____

SLOT/STATION	CS	TAXA	COMMENTS	SLOT/STATION	CS	TAXA	COMMENTS
______/______	A	________		______/______	A	________	
		________				________	
		________				________	
	B	________			B	________	
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______/______	A	________		______/______	A	________	
		________				________	
		________				________	
	B	________			B	________	
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	B	________			B	________	
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______/______	A	________		______/______	A	________	
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	B	________			B	________	
		________				________	
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______/______	A	________		______/______	A	________	
		________				________	
		________				________	
	B	________			B	________	
		________				________	
		________				________	

CS = cover slip

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3.5 FISH

3.5.1 DEFINITION: For the purpose of this standard operating procedure, fish shall include those vertebrate species belonging to the classes Agnatha (jawless fishes), Chondrichthyes (cartilaginous fishes), and Osteichthyes (bony fishes).

3.5.2 OBJECTIVES

1. To provide data for surface water quality standards evaluation and the National Pollutant Discharge Elimination System (NPDES) permit program;

2. to provide data to assess human health concerns with special regard to fish consumption; and

3. to provide complementary data for assessing water quality impacts to aquatic and semi-aquatic biota.

3.5.3 FIELD SAMPLING

The collection of fish samples and field data pertaining to the objectives stated above are conducted in cooperation with the Massachusetts Division of Fisheries and Wildlife (MDFW). The MDFW supplies one full-time biologist and equipment when necessary. Fish are collected under guidelines included in a "Scientific Collecting Permit for Fish" issued to the Division of Water Pollution Control by the Division of Fisheries and Wildlife. This permit is renewed annually.

Physical Measurements

When assessing water quality impacts as stated in objective 3.5.2(3) data concerning stream reach length, width, and average depth are recorded. Substrate characteristics are visually inspected and noted. Water temperature is also recorded. Also under objective 3.5.2(3) all fish are identified, weighed, and measured. Scales or spines are sampled and used for aging. All fish are then released if they show minimal stress. Under objectives 3.5.2 (1) and (2), only targeted species of appropriate size are collected, identified, weighed, and measured. These fish are brought back to the laboratory for processing. In lakes and ponds, collection areas are marked on prepared maps, and amount of effort (time) is recorded. When electrofishing is performed conductivity is recorded along with voltage used and relative success.

Gill Netting

Gill nets are entanglement gear best described as vertical walls of netting. The typical net used by this Division is of an experimental design. The nets are 38 meters in length and two meters in depth stretched. They usually include a 1.27 cm polypropylene float line and a 23 kg lead line. The net itself is composed of five 7.6 meter monofilament panels. Mesh sizes are: 2.54 cm; 3.175 cm; 3.81 cm; 4.445 cm; and 5.08 cm.

Gill nets are set overnight for approximately 16-20 hours. The Division is experimenting with two-hour sets to minimize the number of unwanted fish collected. Nets are usually set in at least 2.5 m of water and are marked by a buoy on each end. An additional buoy is attached near the center of the net in water less than 3.0 m in depth to warn boaters and/or fishermen of the obstruction.

Electrofishing

Electrofishing using alternating current (a.c.) or direct current (d.c.) is conducted in streams and in shallow water habitats in lakes, ponds and impoundments. In lotic environments sampling begins and continues until a satisfactory sample is attained at the lower end of a reach of approximately 50-200 meters in length. Electrically-activated electrodes are swept together along and under stream banks and around rocks, logs and other obstructions. Stunned fish are collected with a dip-net and placed in a tub of stream-water for later processing. Fish sampling in lakes, impoundments and deep rivers is performed using a boat driven slowly forward through shallow areas. In both types of habitats, an estimate of the fish species and numbers missed is noted.

Trapping

Wooden cylindrical catfish traps are used to collect catfish and bullheads (Ictaluridae). These are baited, set in suitable locations, and periodically checked. The trap has an opening on one end with a cone-shaped entrance. The fish enter through the cone and cannot find the entrance once in the box end of the trap.

Processing

Fish collected from each station are identified, weighed, measured, and labeled, accordingly. Selected specimens are placed in plastic bags and stored on ice in a cooler. Scales from representative fish are collected and placed in "scale envelopes" for further analysis.

3.5.4 LABORATORY ANALYSIS

Processing

Fish collected for objectives 3.5.2 (1) and (2) are used for bioaccumulation data analysis which is incorporated into public health determinations or National Pollutant Discharge Elimination System permit reviews. Each fish is weighed whole with entrails intact. Length is measured from the tip of snout with mouth closed to the longest part of the caudal fin slightly compressed. This is expressed as total length.

Each fish is rinsed with deionized water and filleted. A clean, sharp fillet knife is run along each side of the backbone and then just to the outside of the rib cage. This removes a boneless fillet from each side of the fish. The fillet is then placed, skin down, on the filleting board. A knife is used to separate the flesh from the skin. The skin is discarded. One fillet, depending on the study, is either wrapped

individually, or composited with fillets from other fish of the same species and size. The opposite fillet is wrapped individually, tagged with a three or four letter code and number, and archived for future use. Samples for metals analysis are wrapped in plastic (e.g. Saran) wrap. Samples to be tested for PCB's are wrapped in household grade aluminum foil. Fillets to be analyzed for dioxin are wrapped in aluminum foil which has been rinsed with methanol and methylene chloride. The filleting board and knife are rinsed thoroughly after each fish is filleted. Processed fish are kept frozen until they are transported to the analytical laboratory for analysis.

Fish are analyzed for metals and/or organics depending on the individual study being performed. All results are reported as mg/kg. Quality control and assurance data are recorded with each run of samples by the analytical laboratory.

Aging

All fish collected are aged by use of scales or spines. Scales are taken from various areas of a fish depending on the species being sampled. Scales are dried in scale envelopes. The impressions are made on butyrate slides, with a scale press. The impressions can then be read off a scale reader or microfilm reader. Pectoral spines are collected from Ictalurids. These spines are dried and cleaned of excess skins and flesh. They are soaked in Axion detergent, which helps loosen the skin and flesh which results in easier removal. Spines are cross-sectioned at the basal recess on a low speed diamond bladed saw. Cross-sections of .10-.20 mm. can then be read through a compound microscope. Ages are expressed as years^{+}, for example 1^{+}, 2^{+}, 3^{+}.

3.5.5 DATA MANAGEMENT

Reporting of Results

In most cases involving objectives 3.5.2 (1) and (2) results are put into tabular form and a technical memorandum is written detailing the nature of the study, methods used, and any applicable recommendations. The memorandum is distributed to interested parties including the Massachusetts Department of Public Health and the DEQE Office of Research and Standards.

Computer Files

All fish data are entered into one of 4 DBase files. The files include station identification information (STAID), a record of samples (SAMPREC), the results of analyses for metals (FISHMET), and the results for organics (FISHORG). These files are linked in such a manner that data can be retrieved by species, waterbody, analyses type, concentration of contaminant, year, size, and other metrics. Data from these files are the beginning of a statewide data base.

3.5.6 FIELD EQUIPMENT AND SUPPLY LIST

Vehicles, Boats and Accessories

- ☐ state vehicle, clipboard
- ☐ electrofishing boat
- ☐ boat motor, gas can (and line)
- ☐ generator and gas can
- ☐ generator tote barge
- ☐ anchor, rope
- ☐ life jackets, seat pads
- ☐ fire extinguisher
- ☐ boat lights

Collecting and Sampling Gear

- ☐ backpack electrofishing gear
- ☐ pocket thermometer
- ☐ tape measure
- ☐ range finder
- ☐ plastic bucket, rope
- ☐ plastic bags (and ties)
- ☐ glass and/or plastic vials
- ☐ glass and/or plastic jars, bottles
- ☐ formalin
- ☐ dip-nets
- ☐ gill nets
- ☐ fish measuring board
- ☐ pan balance

Field Apparel

- ☐ rain gear (jacket, pants, hat)
- ☐ hip boots and/or chest waders
- ☐ rubber gloves

Miscellaneous Items

- ☐ USGS topographic maps
- ☐ clipboard
- ☐ field data sheets, maps
- ☐ length-weight, length-frequency forms
- ☐ tags and labels (with elastics or string)
- ☐ pencils, pens
- ☐ field identification manuals, keys
- ☐ dissecting kit, hand lens
- ☐ aluminum foil and plastic wrap
- ☐ camera, film
- ☐ first-aid kit
- ☐ field glasses
- ☐ insect repellent
- ☐ tool kit
- ☐ cooler(s), ice
- ☐ paper towels
- ☐ flashlights
- ☐ ear protectors

3.5.7 DATA RECORD SHEETS AND FRESHWATER AND ANADROMOUS FISHES CODING LIST

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EXAMPLE OF SCALE (ENVELOPE)

WATERS	TOWN	
TAG NO.	SP.	NO.
TL. IN.	SL. MM.	WGT.
SEX	M	G
STOM.		D
Mass. F. & W. Fish Scale Record		

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TECHNICAL SERVICES BRANCH

FISH LENGTH - WEIGHT DATA SHEET

Collection Method:			Collector:			Date/Time:		
Weather:			Water:			Station:		
Species	Length	Weight	Species	Length	Weight	Species	Length	Weight

MASSACHUSETTS DIVISION OF WATER POLLUTION CONTROL
TECHNICAL SERVICES BRANCH

FISH LENGTH - FREQUENCY DISTRIBUTION SHEET

Collection Method:		Collector:	Date/Time:	
Weather:		Water:	Station:	
Milliliters	Tally		Milliliters	Tally
30 - 39			450 - 459	
40 - 49			460 - 469	
50 - 59			470 - 479	
60 - 69			480 - 489	
70 - 79			490 - 499	
80 - 89			500 - 509	
90 - 99			510 - 519	
100 - 109			520 - 529	
110 - 119			530 - 539	
120 - 129			540 - 549	
130 - 139			550 - 559	
140 - 149			560 - 569	
150 - 159			570 - 579	
160 - 169			580 - 589	
170 - 179			590 - 599	
180 - 189			600 - 609	
190 - 199			610 - 619	
200 - 209			620 - 629	
210 - 219			630 - 639	
220 - 229			640 - 649	
230 - 239			650 - 659	
240 - 249			660 - 669	
250 - 259			670 - 679	
260 - 269			680 - 689	
270 - 279			690 - 699	
280 - 289			700 - 709	
290 - 299			710 - 719	
300 - 309			720 - 729	
310 - 319			730 - 739	
320 - 329			740 - 749	
330 - 339			750 - 759	
340 - 349			760 - 769	
350 - 359			770 - 779	
360 - 369			780 - 789	
370 - 379			790 - 799	
380 - 389			800 - 809	
390 - 399			810 - 819	
400 - 409			820 - 829	
410 - 419			830 - 839	
420 - 429			840 - 849	
430 - 439			850 - 859	
440 - 449			860 - 869	
Total Number				
Total Weight (Kg)				

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FRESHWATER AND ANADROMOUS FISHES CODING LIST

CODE	COMMON NAME[1]	LATIN NAME	ABBREVIATION
	PETROMYZONTIDAE - Lampreys		
01	American brook lamprey	(Lampetra appendix)	BL
	ACIPENSERIDAE - Sturgeons		
02	Shortnose sturgeon	(Acipenser brevirostrum)	SST
03	Atlantic sturgeon	(Acipenser oxyrhynchus)	AST
	ANGUILLIDAE - Freshwater eels		
04	American eel	(Anguilla rostrata)	E
	CLUPEIDAE - Herrings		
05	Blueback herring	(Alosa aestivalis)	H
06	Alewife	(Alosa pseudoharengus)	A
07	American shad	(Alosa sapidissima)	S
	SALMONIDAE - Trouts		
08	Coho salmon	(Oncorhynchus kisutch)	CO
09	Kokanee salmon	(Oncorhynchus nerka)	KO
10	Rainbow trout	(Salmo gairdneri)	RT
11	Atlantic salmon	(Salmo salar)	AS
12	Brown trout	(Salmo trutta)	BT
13	Brook trout	(Salvelinus fontinalis)	EBT
14	Lake trout	(Salvelinus namaycush)	LT
	OSMERIDAE - Smelts		
15	Rainbow smelt	(Osmerus mordax)	RS

[1] Listed phylogenetically by family

FRESHWATER AND ANADROMOUS FISHES CODING LIST (CONTINUED)

CODE	COMMON NAME	LATIN NAME	ABBREVIATION
	UMBRIDAE - Mudminnows		
16	Eastern mudminnow	(Umbra pygmaea)	CM
	ESOCIDAE - Pikes		
17	Redfin pickerel	(Esox americanus americanus)	RP
18	Northern pike	(Esox lucius)	NP
19	Chain pickerel	(Esox niger)	CP
	CYPRINIDAE - Carps and Minnows		
20	Goldfish	(Carassius auratus)	G
21	Lake chub	(Couesius plumbeus)	LC
22	Common carp	(Cyprinus carpio)	C
23	Eastern silvery minnow	(Hybognathus regius)	SM
24	Golden shiner	(Notemigonus crysoleucas)	GS
25	Emerald shiner	(Notropis atherinoides)	ES
26	Bridled shiner	(Notropis bifrenatus)	BM
27	Common shiner	(Notropis cornutus)	CS
28	Spottail shiner	(Notropis hudsonius)	SS
29	Mimic shiner	(Notropis volucellus)	MS
30	Northern redbelly dace	(Phoxinus eos)	NRD
31	Bluntnose minnow	(Pimephales notatus)	BNM
32	Fathead minnow	(Pimephales promelas)	FM
33	Blacknose dace	(Rhinichthys atratulus)	BND
34	Longnose dace	(Rhinichthys cataractae)	LND
35	Creek chub	(Semotilus atromaculatus)	CC
36	Fallfish	(Semotilus corporalis)	F
	CATOSTOMIDAE - Suckers		
37	Longnose sucker	(Catostomus catostomus)	LS
38	White sucker	(Catostomus commersoni)	WS
39	Creek chubsucker	(Erimyzon oblongus)	CCS

FRESHWATER AND ANADROMOUS FISHES CODING LIST (CONTINUED)

CODE	COMMON NAME	LATIN NAME	ABBREVIATION
	ICTALURIDAE - Bullhead catfishes		
40	White catfish	(Ictalurus catus)	WB
41	Yellow bullhead	(Ictalurus natalis)	YB
42	Brown bullhead	(Ictalurus nebulosus)	BB
43	Channel catfish	(Ictalurus punctatus)	CB
44	Tadpole madtom	(Noturus gyrinus)	MT
	PERCOPSIDAE - Trout-perches		
45	Trout-perch	(Percopsis omiscomaycus)	TP
	GADIDAE - Codfishes		
46	Burbot	(Lota lota)	BBT
	CYPRINODONTIDAE - Killifishes		
47	Banded killifish	(Fundulus diaphanus)	K
48	Mummichog	(Fundulus heteroclitus)	M
49	Spotfin killifish	(Fundulus luciae)	SPK
50	Striped killifish	(Fundulus majalis)	SK
51	Rainwater killifish	(Lucania parva)	RK
	GASTEROSTEIDAE - Sticklebacks		
52	Fourspine stickleback	(Apeltes quadracus)	FSS
53	Threespine stickleback	(Gasterosteus aculeatus)	TSS
54	Blackspotted stickleback	(Gasterosteus wheatlandi)	BSS
55	Ninespine stickleback	(Pungitius pungitius)	NSS
	PERCICHTHYIDAE - Temperate basses		
56	White perch	(Morone americana)	WP
57	Striped bass	(Morone saxatilis)	SB

FRESHWATER AND ANADROMOUS FISHES CODING LIST (CONTINUED)

CODE	COMMON NAME	LATIN NAME	ABBREVIATION
	CENTRARCHIDAE - Sunfishes		
58	Rock bass	(Ambloplites rupestris)	RB
59	Banded sunfish	(Enneacanthus obesus)	BS
60	Redbreast sunfish	(Lepomis auritus)	Y
61	Green sunfish	(Lepomis cyanellus)	GRS
62	Pumpkinseed	(Lepomis gibbosus)	P
63	Bluegill	(Lepomis macrochirus)	B
64	Smallmouth bass	(Micropterus dolomieui)	SMB
65	Largemouth bass	(Micropterus salmoides)	LMB
66	White crappie	(Pomoxis annularis)	WC
67	Black crappie	(Pomoxis nigromaculatus)	BC
	PERCIDAE - Perches		
68	Swamp darter	(Etheostoma fusiforme)	SD
69	Tesselated darter	(Etheostoma olmstedi)	TD
70	Yellow perch	(Perca flavescens)	YP
71	Walleye	(Stizostedion vitreum vitreum)	W
	COTTIDAE - Sculpins		
72	Slimy sculpin	(Cottus cognatus)	SC

3.5.8 REFERENCES

1. Bagenal, T. ed. 1978. Methods for Assessment of Fish Production in Fresh Waters. IBP Handbook No. 3. Blackwell Scientific Publications, Oxford, England. xvi + 365 p.

2. Clayton, G., C. Cole, and S. Murawski. 1978. Common Marine Fishes of Coastal Massachusetts. Massachusetts Cooperative Extension Service, Amherst. x + 231 p.

3. Eddy, S., and J.C. Underhill. 1978. How to Know the Freshwater Fishes. W.C. Brown Company, Publishers. Dubuque, Iowa. viii + 215 p.

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3.6 MICROTOX™ ANALYSIS

3.6.1 DEFINITION: The Microtox™ toxicity analyzer uses a lyophilized (freeze-dried) marine bioluminescent bacterium (Photobacterium phosphoreum) which, upon reconstitution, emits a fairly constant level of light. Upon exposure to a toxicant, the level of bioluminescence is diminished in direct proportion to the toxicant concentration.

3.6.2 OBJECTIVES:

1. To assess the effectiveness of the Commonwealth's municipally-owned and industrial wastewater treatment plants in eliminating or preventing aquatic toxicity;

2. to selectively screen water and sediment samples prior to performing more expensive and time-consuming conventional toxicity tests;

3. to determine the toxicity of known toxicants using laboratory-prepared solutions of known concentrations; and

4. to compare Microtox™ test results with results from other toxicity tests.

3.6.3 FIELD SAMPLING

Qualitative

Because the Microtox™ system is designed as a quantitative test, qualitative results cannot be determined.

Quantitative

Based on the objectives of the study and an understanding of the short- and long-term operations and schedules of the discharger, either grab or composite-type samples are collected for testing. If the suspected toxicity of the source is variable, grab samples collected during peaks of toxicity provide a measure of maximum impact. The compositing technique has an averaging effect, which tends to dilute toxicity peaks, and may provide misleading results when testing for acute toxicity. Composite samples, therefore, are more appropriate for chronic toxicity tests where peak toxicity of short duration is of less concern.

Sample Container Preparation

The 450 ml borosilicate-type glass containers are prepared according to the methods described in the United States Environmental Protection Agency's Handbook for Sampling and Sample Preservation of Water and Wastewaters (See: "References"), unless the containers are previously unused.

Sample Collection and Handling

Sample containers are rinsed once with sample water prior to collection. An effort is made to fill the sample container to near capacity, with little or no air space. After the container is filled with the sample, a pH reading is taken by the collector with an Orion Model 201 field pH meter. Plastic wrap is placed under the container cap and the sample is put on ice for transport back to the Microtox™ laboratory.

Upon arrival at the Microtox™ laboratory, the sample is either (1) tested or (2) refrigerated until the following day and then tested. The maximum holding time for a sample after collection is 24 hours.

3.6.4 LABORATORY ANALYSIS

The basic procedure for the Microtox™ system employs duplicates of a non-toxic control and four serial dilutions of the sample. The mean response of the duplicate control is used to normalize the duplicate responses of the four test concentrations of sample when the test results are reduced. Detailed operating procedures for using the Microtox™ Analyzer are found in the Microtox System Operating Manual (see: "References").

Laboratory Equipment and Related Supplies

1. Beckman Microtox™ model 2055 toxicity analyzer
2. strip chart recorder, chart paper
3. Microtox™ reagent (lyophilized)
4. Microtox™ reagent diluent
5. Microtox™ reconstruction solution
6. Microtox™ osmotic adjusting solution
7. cuvettes, glass, disposable [11.75 mm x 50 mm in size]
8. recorder pen, black
9. Eppendorf 10 µl pipet, micropipette tips 1-100 µl
10. Eppendorf 500 µl pipet, micropipette tips 101-1000 µl
11. parafilm, kimwipes
12. disposable gloves

3.6.5 QUALITY ASSURANCE

Every tenth sample is tested in duplicate to check consistency and reproducability of results.

3.6.6 FIELD EQUIPMENT AND SUPPLY LIST

Collecting and Sampling Gear

- ☐ 450 ml borosilicate type glass containers with caps
- ☐ Orion model 201 field pH meter
- ☐ rubber gloves

Miscellaneous Items

- ☐ tags, labels, elastics
- ☐ pencils, pens
- ☐ plastic wrap
- ☐ first aid kit
- ☐ cooler, ice

3.6.7 INTERPRETATION AND REPORTING OF MICROTOX RESULTS

Test Description

Microtox™ is the trade name for a particular acute toxicity test. The test is used as a toxics screening tool in addition to other, more traditional, methods of analysis.

The Microtox™ analyzer uses freeze-dried luminescent bacteria as its test organisms. When re-hydrated, these bacteria emit light. To test a water sample for toxicity using Microtox™, an analyst prepares a series of dilutions of the sample and adds re-hydrated bacteria to these. The light intensity of each sample dilution is measured at preselected time intervals over a 30-minute period and compared with that of a control (bacteria only). It is assumed that changes in light intensity are due to toxicant interference with the biochemical reaction that produces light. Toxicity is then measured as the percent decrease in light intensity of each of the sample dilutions compared with that of the control.

Data Interpretations

The most commonly used result from these tests is the 30-minute EC_{50}. This is defined as the sample Concentration causing a 50% reduction in the measured Effect (light production) over a 30-minute time period. The relationship of the EC_{50} to toxicity is an inverse one; i.e., the lower the EC_{50}, the greater the toxicity of the sample.

A useful conversion of the EC_{50} is the Toxic Unit. This is simply the inverse of the EC_{50} multiplied by a factor of 100:

$$\text{Toxic Units} = \frac{100}{EC_{50}\ (\%)}$$

Toxic Units approximate the amount of dilution a sample must undergo so as not to induce a toxic response in the test organisms (the Microtox™ bacteria). As Toxic Units increase, so does the relative toxic strength of a sample. The relationship of EC_{50}'s, Toxic Units, and toxicity are demonstrated below:

EC_{50} (%)	Toxic Units	Toxicity
0.5	200	High
1.0	100	
10.0	10	
100.0	1	Low

Samples not toxic enough to produce a full 50% decrease in light over the time allotted for the test may still be toxic enough to produce a response in the test. The EC_{20} and EC_{10} (sample concentrations causing a 20% and 10% reduction in light intensity respectively) are reported in order to give the regulator an idea of incipient toxicity - sample dilutions which induce a small, but measurable response in the test.

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MICROTOX™ RESULTS REPORTING FORM

SAMPLES TESTED

LOG #					
SITE					
SAMPLE TYPE					
DATE COLLECTED					
DATE TESTED					
COLLECTOR					
FIELD pH					
LAB pH					
HARDNESS					
SPEC. COND.					

MICROTOX™ RESULTS

LOG #	5 MIN.	15 MIN.	30 MIN.	
				EC_{10}
				EC_{20}
				EC_{50}
				TOXIC UNITS (T.U.)

NOTE: RESULTS GIVEN AS % VOLUME OF SAMPLE

Results of the Microtox™ test are also reported for three different periods of exposure: 5-minute, 15-minute, and 30-minute. A decrease in the EC_{50} over time (increase in Toxic Units) usually indicates the presence of persistent toxicants (e.g., metals) in the sample. An increase in the EC_{50} over time (decrease in Toxic Units) suggests that non-persistent toxics (e.g., volatile, biodegradables, photo or hydrolyzible material) are present at time of sampling.

3.6.8 MICROTOX™ SEDIMENT TOXICITY TESTING

The Microtox™ bioassay can also be used to determine the toxicity of the water soluble fraction (WSF) of sediment samples. Detailed sample preparation procedures are found in the U.S. Environmental Protection Agency's draft Permit Guidance Manual on Hazardous Waste Low Treatment Demonstrations (See: "References").

Laboratory Equipment and Related Supplies

1. Eberbach shaker table - small tabletop model with carrying tray
2. IEC high speed centrifuge model HN
3. Mettler balance
4. Dessicator
5. Drying oven
6. Evaporating dishes
7. Fleaker beakers
8. Centrifuge tubes
9. Graduated cylinders
10. Tongs

3.6.9 REFERENCES

1. Beckman, Inc. 1980. Microtox™ Model 2055 Toxicity Analyzer System. Bulletin 6984. Beckman Instruments, Inc., Carlsbad, CA. 8 p.

2. Beckman, Inc. 1982a. Microtox™ Application Notes No. M304: Toxicity Testing of Complex Effluents. Beckman Instruments, Inc., Carlsbad, CA. 2 p.

3. Beckman, Inc. 1982b. Microtox™ System Operating Manual. Beckman Instruments, Inc., Carlsbad, CA. 59 p.

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3.7 CHLOROPHYLL ANALYSIS

3.7.1 DEFINITION: Chlorophyll is a pigment found in plants that allows the organism to use radiant energy for converting carbon dioxide into organic compounds in a process called photosynthesis. Several types of chlorophylls exist and these and other pigments are used to characterize algae. One type, chlorophyll a, is measured for it is found in all algae. A knowledge of chlorophyll a concentrations provides qualitative and quantitative estimations of phytoplanktonic and periphytic biomasses for comparative assessments of geographical, spacial and temporal variations.

3.7.2 EQUIPMENT NEEDS

1. Fluorometer - either Turner 111 or the Turner Design 10-005-R field fluorometer is used. They must be equipped with blue lamp F4T5.

   Corning filter - 5-60-excitation
   Corning filter - 2-64-emission
   Photomultiplier

2. Tissue grinder and tube - Thomas Tissue Grinder

3. Side arm vacuum flask and pump

4. Millipore filter holder

5. Glass fiber filter: Reeve angel, grade 934H, 2.1 cm

6. Centrifuge (Fisher Scientific Safety Centrifuge)

7. 15 ml graduated conical end centrifuge tubes with rubber stoppers

8. 90% aqueous acetone

9. 1 N HCL

10. Saturated magnesium solution in distilled water

11. Test tube racks

12. Borosilicate cuvettes - Turner 111 - 3" cuvettes
    Turner Design - 8" cuvettes

13. Aluminum foil

14. Test tube brushes - conical end

15. Parafilm

3.7.3 LOG-IN PROCEDURE

As samples are received they are logged in and assigned a number. The samples can be frozen for further analysis, or the filter ground up for analysis the following day.

3.7.4 SAMPLE PREPARATION

Samples are generally processed as soon as they come into the laboratory, unless there are extenuating circumstances, such as faulty equipment and/or time constraints. Samples not to be analyzed within 24 hours are frozen for future analysis.

The procedure for freezing samples follows:

1) Label a 2-inch Whatman petri dish with the sample number using an indelible pen.

2) Using tweezers, take a 2.1 cm Reeve Angel, grade 934AH, glass fiber filter and place it on the Millipore filtering flask screen. Do not touch the filter. Attach the glass tube to the filter flask with the metal clamp.

3) Shake the sample well.

4) Measure out 50 mls of sample or less. If an amount other than 50 mls is used it should be recorded in the chlorophyll data book.

5) Pour the measured sample into the filter tube and turn on the vacuum. The sample should pass quickly through the glass fiber filter; therefore more of the sample should be added. If the sample is not filtering through - either because too much sediment is present or the algal concentration is too high - then less than 50 mls can be filtered. A notation is made in the chlorophyll data book which lists the amount that was filtered.

6) Unclamp the filter holder and with tweezers transfer the filter to the previously marked petri dish.

7) Cover the petri dish and wrap it in aluminum foil to keep out the light. The petri dish with the glass fiber filter is then stored in the freezer.

8) Return the sample bottle to the refrigerator if algal counts or identifications are requested.

9) Rinse the graduated cylinder and filter holder in distilled water.

3.7.5 ANALYTICAL PROCEDURE

1) Follow steps 2-6 under "Sample Preparation."

2) Filter 50 ml (or less if necessary) of sample through a glass fiber filter under vacuum.

3) Push the filter to the bottom of tissue grinding tube.

4) Add about 3 ml of 90% acetone and 0.2 ml of the $MgCO_3$ solution.

5) Grind contents for 3 minutes.

6) The contents of the grinding tube are carefully washed into a 15 ml graduated centrifuge tube.

7) Bring the sample volume to 10 ml with 90% acetone.

8) Test tubes are wrapped with aluminum foil and stored in the refrigerator for 24 hours.

9) Test tubes are taken out of the refrigerator and put into the centrifuge.

10) Test tubes are then centrifuged for 20 minutes and the supernatant decanted immediately into stoppered test tubes.

11) Tubes are allowed to come to room temperature. The temperature is recorded and the samples are poured into a cuvette (3" for Turner 111 and 8" for Turner Design).

12) The Turner 111 requires a warm-up period of at least one-half hour, while the Turner Design 10-005-R does not require a warm-up period.

13) With Turner 111, use a blank of 90% aqueous solution of acetone to zero the instrument. Open the front door of the fluorometer and put in the cuvette containing the 90% acetone and close the door. Press the start switch. The dial should move back to 0; adjustments can be made with the calibration knob. This process should be repeated as often as necessary, i.e., if the blank is not staying on zero; but no alteration should be made until a series of samples is completed.

14) The Turner Design must also be zeroed to an acetone blank. The sample holder is located at the top of the Turner Design field fluorometer and should be recovered with the black cap after the sample is put in it.

15) Readings for both the Turner 111 and the Turner Design should be within 20-80% of the scale. This can be achieved by either reducing or increasing the opening to the lamp by moving the knob on the right front of the Turner 111 fluorometer. The sensitivity levels are 1x, 3x, 10x, and 30x. The sensitivity level must be recorded in the chlorophyll data book in addition to whether the high intensity or regular door was used. After the first reading, 2 drops of 2N HCl is added to the cuvette. A piece of parafilm is used to cover the cuvette which is then inverted four times to mix the sample thoroughly. The sample is re-read and the new value recorded.

16) The procedure for the Turner Design field fluorometer is basically the same as for the Turner 111. The sample is put into the cuvette holder and the manual switch used to go from one sensitivity level to the next without opening the door. A reading of between 20-80% is still required for accuracy. Readings are taken before and after acid is added to the sample. The level of sensitivity (1x, 3x, 6x, 10x, 31.6x) must be recorded in the chlorophyll data book, as well as whether the levels were set at 1 or 100.

Calculation of Chlorophyll Concentrations

Chlorophyll concentrations are determined by using the following formulas:

$$\text{chlorophyll (ug/l)} = Fs \frac{rs}{rs-1} (Rb-RA)$$

$$\text{pheophytin (ug/l)} = Fs \frac{rs}{rs-1} (rsRa-Rb)$$

where,
Fs = conversion factor for sensitivity level "s"
rs = before and after acidification ratio of sensitivity level "s"
Rb = fluorometer reading before acidification
Ra = fluorometer reading after acidification

A computer program is used to calculate the chlorophyll concentrations for samples run on the Turner Design fluorometer. This program requires the investigator to type in the sensitivity level and the difference between the before and after acidification values.

During the summer of 1986 personnel of the Technical Services Branch (TSB) conducted a laboratory experiment with a Turner Design Fluorometer in order to determine the effect of pheophytin b on freshwater chlorophyll a readings. Pheophytin b is the degradation product of chlorophyll b which is the primary pigment of green algae. The Turner Design instrument measures the fluorescence of chlorophyll a as well as that of pheophytin a and b. Chlorophyll b is not read at the same frequency as chlorophyll a. The emission filter used at the TSB (Corning C/S 2-64) partially rejects pheophytin b (See: "References" - Turner Designs, 1981). It was found and recorded in various unpublished memoranda (See "References") that unless a sample had elevated counts of green algae the readings obtained prior to acidification and 90 seconds thereafter would give a reliable estimate of the concentration of chlorophyll a in an algal sample. In cases with elevated counts of green algae an annotation should be made alongside the chlorophyll a concentration stating that the concentration may reflect the presence of chlorophyll b and is probably lower than as recorded. As a result of this investigation, the TSB now presents chlorophyll data as chlorophyll a in mg/m^3.

3.7.6 INSTRUMENT CALIBRATION

Fluorometers are calibrated using chlorophyll samples provided by the United States Environmental Protection Agency. Calibrations are performed at the start of every field season and redone if any changes are made to the fluorometer such as changing the light bulb.

Samples for chlorophyll analysis are periodically split with another laboratory or run on two separate fluorometers.

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4.0 QUALITY ASSURANCE

4.0 QUALITY ASSURANCE

4.1 PURPOSE AND SCOPE

A quality assurance program has been put in place to validate both the reliability of field and laboratory techniques and the integrity of the biomonitoring data. An essential element of this program is the development of standardized field and laboratory methodologies as outlined in this manual of operating procedures. Standard methods allow for the determination of the accuracy, precision, and variability of biomonitoring data.

Although details pertaining to the quality assurance program have already been presented for individual biomonitoring program elements, major components of the program that are applicable to most biomonitoring activities are summarized in this section.

4.2 INTRALABORATORY QUALITY ASSURANCE

1) A staff of adequately trained aquatic biologists is maintained; each with knowledge of the taxonomy and pollution ecology of one or more freshwater communities. These include bacteria, algae, macrophyton, aquatic macroinvertebrates, and fish.

2) Collecting gear such as nets, sieves, and grab samplers are inspected and maintained frequently.

3) Field and laboratory equipment such as pH and dissolved oxygen meters, microscopes, and fluorometers are maintained and calibrated on a routine basis.

4) Field studies are carefully planned in advance to insure that appropriate sites are sampled and that the proper number of samples are obtained to meet survey goals and objectives.

5) All samples are clearly labeled at the time of collection, recorded in hard-bound log books, and tracked in a step-wise fashion throughout their processing in the laboratory.

6) A reference library is maintained which includes up-to-date identification manuals and keys and both benchmark and recent literature on all aspects of water pollution and its impact on aquatic life.

7) A reference specimen collection is maintained for confirming the proper identification of aquatic invertebrates. Similar collections for other communities (e.g., fish) are under development. In addition, many reference specimens and other organisms of interest are photographed and added to an extensive collection of slides to be used as taxonomic aids and for training purposes.

8) Aquatic macroinvertebrate, algae, chlorophyll, and Microtox™ data are input to computerized data storage and retrieval systems insofar as is allowed by time and personnel constraints. All data sets are carefully proofread and edited during this process. A similar system is proposed for the storage of data generated by the fish sampling program.

9) All reporting elements receive peer and/or supervisory review and numerical analyses are checked for mathematical errors.

4.3 INTERLABORATORY QUALITY ASSURANCE

1) Reference samples containing known chlorophyll a concentrations, predetermined phytoplankton counts, or known invertebrate taxa are routinely provided to the biomonitoring staff by the United States Environmental Protection Agency (U.S. EPA) for instrumentation calibration and evaluation of laboratory performance.

2) Occasionally biological surveys are conducted simultaneously with the USEPA or other state agencies to compare field and laboratory methods and to determine interlaboratory variability of results.

3) Specimens that present particular problems with their identification are often sent to expert taxonomists for confirmation. A separate log book is used to record the date and to whom specimens are sent, and, ultimately, the date and details pertaining to the taxonomists' responses.

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