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# Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters

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# MACROINVERTEBRATE FIELD AND LABORATORY METHODS FOR EVALUATING THE BIOLOGICAL INTEGRITY OF SURFACE WATERS

by

Donald J. Klemm[1], Philip A. Lewis[1], Florence Fulk[2],

and James M. Lazorchak[1]

Aquatic Biology Branch[1] and Development and Evaluation Branch[2],
Quality Assurance Research Division,
Environmental Monitoring Systems Laboratory - Cincinnati

ENVIRONMENTAL MONITORING SYSTEMS LABORATORY - CINCINNATI
OFFICE OF MODELING, MONITORING SYSTEMS, AND QUALITY ASSURANCE
OFFICE OF RESEARCH AND DEVELOPMENT
U. S. ENVIRONMENTAL PROTECTION AGENCY
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## FOREWORD

Environmental measurements are required to determine the chemical and biological quality of drinking water, surface waters, ground waters, waste waters, sediments, sludges, and solid waste. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- Develop and evaluate methods to identify and measure the concentration of chemical pollutants.
- Identify and quantitate the occurrence of viruses, bacteria, other human pathogens and indicator organisms.
- Perform ecological assessments and measure the toxicity of pollutants to representative species of aquatic organisms and determine the effects of pollution on communities of indigenous freshwater, estuarine, and marine organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish.
- Develop and operate a quality assurance program to support achievement of data quality objectives for environmental measurements.

This manual describes guidelines and standardized procedures for the use of macroinvertebrates in evaluating the biological integrity of surface waters. It was developed to provide biomonitoring programs with the most recent benthic invertebrate methods for measuring the status and trends of environmental pollution on freshwater, estuarine, and marine macroinvertebrates in field and laboratory studies. These studies are carried out to assess water quality criteria for the recognized beneficial uses of water and to monitor surface water quality.

Thomas A. Clark
Director
Environmental Monitoring Systems
Laboratory - Cincinnati

# PREFACE

The Aquatic Biology Branch, Quality Assurance Research Division, Environmental Monitoring Systems Laboratory - Cincinnati is responsible for the development, evaluation and standardization of methods for the collection of biological field and laboratory data by EPA regional, enforcement, and research programs engaged in inland, estuarine, and marine water quality and permit compliance monitoring, and other studies of the effects of impacts on aquatic organisms, including the phytoplankton, zooplankton, periphyton, macrophyton, macroinvertebrates, and fish. The program addresses methods for sample collection; sample preparation; organism identification and enumeration; the measurement of biomass and metabolic rates; the bioaccumulation and pathology of toxic substances; bioassay; biomarkers; the computerization, analysis, and interpretation of biological data; and ecological assessments. Biological methods recommended for use in the U.S. Environmental Protection Agency are included in this manual: "Macroinvertebrate Field and Laboratory Methods for Evaluating the Biological Integrity of Surface Waters."

This document provides macroinvertebrate methods for evaluating the biological integrity of fresh, estuarine, and marine waters. The subjects covered include selection of sample sites, qualitative and quantitative sampling methods, sample processing, data analysis techniques, quality assurance and quality control procedures, safety and health recommendations, taxonomic bibliography, and the pollution tolerance of selected macroinvertebrate species.

The manual is a revision and enlargement of the chapter on macroinvertebrate methods originally published in the document, "Biological Field and Laboratory Methods for Measuring the Quality of Surface Waters and Effluents," Environmental Monitoring Series, USEPA, 1973, EPA-670/4-73-001, and was developed in the Aquatic Biology Branch, Environmental Monitoring Systems Laboratory - Cincinnati to provide biomonitoring programs with current methods for assessing point and non-point sources of impacts, status and trends water quality monitoring.

## ABSTRACT

This manual describes guidelines and standardized procedures for using benthic macroinvertebrates in evaluating the biological integrity of surface waters. Included are sections on quality assurance and quality control procedures, safety and health recommendations, selection of sampling stations, sampling methods, sample processing, data evaluation, and an extensive taxonomic bibliography of the benthic macroinvertebrate groups. Supplementary information on the pollution tolerance of selected species, examples of macroinvertebrate bench sheets and macroinvertebrate data summary sheets, and a list of equipment and supplies for conducting biomonitoring studies are provided in the Appendices.

CONTENTS

Foreword . . . . . iii

Preface . . . . . iv

Abstract . . . . . v

Figures . . . . . ix

Tables . . . . . x

Acknowledgment . . . . . xii

1. Introduction . . . . . 1
   Literature Cited . . . . . 4

2. Quality Assurance and Quality Control . . . . . 7
   Introduction . . . . . 7
   Data Quality Objectives . . . . . 8
   Facilities and Equipment . . . . . 9
   Calibration Documentation and Record Keeping . . . . . 9
   Qualifications and Training . . . . . 10
   Standard Operating Procedures . . . . . 11
   Literature Cited 11

3. Safety and Health . . . . . 13
   Introduction . . . . . 13
   General Precautions . . . . . 13
   Safety Equipment and Facilities . . . . . 14
   Field and Laboratory Operations . . . . . 14
   Disease Prevention . . . . . 15
   Literature Cited . . . . . 15

4. Selection of Sampling Stations . . . . . 16
   Introduction . . . . . 16
   Location of Sampling Stations . . . . . 17
   Selecting Control Stations . . . . . 18
   Study Design . . . . . 19
   Considerations of Abiotic Factors . . . . . 26
   Literature Cited . . . . . 30

5. Sampling Methods . . . . . 32
   Introduction . . . . . 32
   Qualitative Sampling . . . . . 32
   Semi-quantitative Sampling . . . . . 33
   Quantitative Sampling . . . . . 33
   Sampling Devices . . . . . 35
   Commonly Used Grabs . . . . . 40
   Stream Net Samplers . . . . . 48
   Drift Nets . . . . . 53

Artificial Substrate Samplers . . . . . 59
Coring Devices. . . . . 66
Frames. . . . . 71
Rapid Bioassessment Protocols . . . . . 72
Ohio EPA Invertebrate Community Index Method. . . . . 73
Standard Qualitative Collection Method. . . . . 73
Miscellaneous Qualitative Devices . . . . . 73
Suction Samplers. . . . . 74
Photography . . . . . 74
Scuba . . . . . 75
Brails. . . . . 76
Other Mussel Collecting Methods . . . . . 77
Literature Cited. . . . . 77

6. Sample Processing . . . . . 93
Sieving . . . . . 93
Preservation and Fixation . . . . . 95
Labelling and Record Keeping. . . . . 97
Sorting and Subsampling . . . . . 97
Preparation of Microscope Slide Mounts. . . . . 100
Drying Methods. . . . . 104
Organism Identification . . . . . 104
Biomass . . . . . 105
Literature Cited. . . . . 105

7. Data Evaluation . . . . . 109
Introduction. . . . . 109
Analyses of Qualitative Data. . . . . 109
Analyses of Semi-quantitative and Quantitative Data . . 111
Rapid Bioassessment Techniques. . . . . 117
Community Metrics and Pollution Indicators. . . . . 117
Statistical Methods . . . . . 123
Literature Cited. . . . . 159

8. Taxonomic Bibliography. . . . . 164

Appendices. . . . . 207

A. Pollution Tolerance of Selected Macroinvertebrates. . . . 207

B. Hilsenhoff's Family Level Pollution Tolerance Values for Aquatic Arthropods. . . . . 245

C. Examples of Macroinvertebrate Bench Sheets. . . . . 247

D. Example of Macroinvertebrate Summary Sheet. . . . . 250

E. List of Equipment and Supplies. . . . . 253

## FIGURES


| Number | | Page |
|---|---|---|
| 1. | Example of transect sampling scheme in rivers and streams | 23 |
| 2. | Example of transect sampling scheme lakes, reservoirs, and coastal waters | 23 |
| 3. | Illustration of how sighting lines are used to locate fixed sampling locations in lakes, reservoirs, or estuaries | 24 |
| 4. | Example of grid sampling scheme in rivers | 25 |
| 5. | Grab samplers (Ponar, Ekman, Wildco Box Corer) | 41 |
| 6. | Grab samplers (Petersen) | 43 |
| 7. | Grab samplers (Smith-McIntyre, Van Veen, Orange Peel, Shipek) | 46 |
| 8. | Stream-net samplers | 51 |
| 9. | Artificial substrate samplers | 62 |
| 10. | Core samplers | 70 |
| 11. | Great Lakes sieve bucket | 94 |
| 12. | Imhoff cone subsampler | 99 |
| 13. | Five-segmented palp of a water mite | 101 |
| 14. | Water mite showing dorsum separated from venter | 102 |
| 15. | Top (A) and side (B) views of the double cover-glass technique for mounting aquatic water mites | 102 |

TABLES


| Number | | Page |
|---|---|---|
| 1. | Categories for field evaluation of substrate characteristics | 27 |
| 2. | Substrate particle size classification of sieve analysis | 28 |
| 3. | Summary criteria for grab samplers | 35 |
| 4. | Summary criteria for stream-net samplers | 48 |
| 5. | Summary criteria for artificial substrate samplers | 59 |
| 6. | Summary criteria for coring devices | 67 |
| 7. | Typical responses to various types of stress by parameters of benthic community structure | 113 |
| 8. | Example of calculation of mean diversity | 114 |
| 9. | Pooled Stenonema data from three riffle stations | 131 |
| 10. | Stenonema data from three riffle stations | 132 |
| 11. | Occurrence of three species of midges | 133 |
| 12. | Macroinvertebrate biomass collected at different times of day from the Little Miami River at Milford, Ohio | 135 |
| 13A. | Generalized ANOVA | 136 |
| 13B. | Completed ANOVA Table Using Macroinvertebrate Biomass Data | 137 |
| 14A. | Macroinvertebrate biomass (grams wet. wt.) | 139 |
| 14B. | $Log_{10}$ transformed data | 139 |
| 15. | Treatment totals for the data of Table 14B | 140 |
| 16. | Analysis of variance table for field study data of Table 14 | 142 |

Number | Page

17. Macroinvertebrate Biomass Collected at Different Times of Day from the Little Miami River at Milford, Ohio . . . . . 145

18. Example of Shapiro-Wilk's Test: Centered Observations. . . . . 145

19. Example of Shapiro-Wilk's Test: Ordered Observations. . . . . 146

20. Coefficients for the Shapiro-Wilks Test . . . . . 146

21. Example of Shapiro-wilk's Test: Table of Coefficients and Differences. . . . . 147

22. Quantiles of the Shapiro-Wilks Test Statistic . . . . . 149

23. Functions for calculating species diversity and (for perfectly random sampling) its standard error logarithms are to base 10. Table values are accurate to within ± 1 in the eighth significant figure. . . . . 152

24. The diversity of species, $\bar{d}$, characteristic of MacArthur's model for various numbers of hypothetical species,s'*. . . . . 158

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## SECTION 1

## INTRODUCTION

1.1 Benthic invertebrates comprise a heterogenous assemblage of animal groups (taxa) that inhabit the sediment or live on or in other bottom substrates in the aquatic environment. They vary in size from forms small and difficult to see without magnification to other individuals large enough to see without difficulty.

1.2 The benthic invertebrates that are large enough to be seen by the unaided eye and which can be retained by a U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings) and live at least part of their life cycles within or upon available substrates in a body of water or water transport system are defined as **macroinvertebrates**. If a more representative sample of the benthos such as chironomids and other small forms (e.g., naidid and tubificid oligochaetes or aquatic worms) is desired, a U.S. Standard No. 60 sieve (60 meshes per inch, 0.250 mm openings) may be used.

1.2.1 Benthos (n.), Benthic (adj.)--the community of organisms living in or on the bottom or other substrate in an aquatic environment.

1.2.2 Benthic invertebrate--an invertebrate of the benthos.

1.2.3 Habitat--the place where an organism lives; for example mud, gravel, rocks, shoreline, vegetation, twigs, leaf packs, riffle/run, pool, etc.

1.2.4 Microhabitat--a smaller and more restricted area in a habitat; the immediate environment of the organism.

1.3 The standard opening for estuarine and marine benthic animals is also U.S. Standard No. 30 sieve (28 meshes per inch, 0.595 mm openings), and new benthic programs should use the No. 30 sieve for collecting these animals. To accommodate some historical data bases, a 1.0 mm screen, U.S. Standard No. 18 sieve may be used.

1.4 Any available substrate may provide suitable habitat for benthos, including bottom sediments, submerged logs, debris, pilings, pipes, conduits, vascular aquatic plants, root masses, filamentous algae, etc. The major taxonomic groups of freshwater macroinvertebrates include the insects, annelids, mollusks, flatworms, and crustaceans. The major invertebrate groups in estuarine and marine water are the mollusks, annelids, crustaceans, roundworms, cnidarians (coelenterates), sponges, bryozoans, and echinoderms.

1.5 The macroinvertebrates are important members of food webs, and their well-being is reflected in the well-being of the higher forms such as fish. Many invertebrates, such as the marine and freshwater shellfish (clams and mussels), are important commercial and recreational species. Some, such as mosquitoes, black flies, biting midges, leeches, Asiatic clams, and zebra mussels, are of considerable public health significance or are considered pests. Many forms are important for digesting organic material and recycling nutrients.

1.6 Benthic macroinvertebrates are frequently used as environmental indicators of biological integrity because they are found in most aquatic habitats. They are of a size that makes them easily collected. They can be used to describe the water quality conditions or health of the ecosystem components and to identify causes of impaired conditions.

1.6.1 A community of macroinvertebrates in an aquatic lentic or lotic ecosystem is very sensitive to stress; and, thus, its characteristics serve as a useful tool for detecting environmental perturbation resulting from introduced point and non-point sources of pollution. Because of the limited mobility of these benthic organisms and because many species have life cycles of a year or more, their characteristics are a function of conditions during the recent past, including reactions to infrequently discharged pollutants that would be difficult to detect by periodic chemical sampling.

1.6.2 Macroinvertebrates show responses to a wide array of potential pollutants (agricultural, domestic, industrial, mining, etc.), including those with synergistic or antagonistic effects that adversely affect the physiological, biochemical, and reproductive functions of the species. The analysis of changes in the makeup of different aquatic communities is one way to detect water quality problems. Knowledge of changes in the community structure (abundance and composition) and function (see Section 1.7) of benthic macroinvertebrates helps to indicate water quality status and trends in the aquatic environment. Also the regular sampling of macroinvertebrates can be used to document both spatial and temporal changes in the biological integrity of surface waters. Different types of environmental stress will often produce different macroinvertebrate communities.

1.6.3 In addition, because of the phenomenon of "biological magnification" and relatively long-term retention of toxic substances by benthic organisms, toxic materials such as metals, pesticides, radioactive materials, which are only periodically discharged into the environment or which are present at undetectable levels in the water or sediment, may be detected by chemical analyses of selected components of the macroinvertebrate community.

1.7 Individuals or groups of macroinvertebrates can be separated into trophic levels, such as herbivores, omnivores, or carnivores and, in stream ecosystems, functional feeding relationships (Cummins, 1973, 1974, 1975; Cummins and Klug, 1979; Cummins et al., 1984; Cummins and Wilzbach, 1985). In a well-balanced system, all three types will likely be present. They include deposit and detritus feeders, collectors, shredders, grazers or scrapers, parasites, scavengers, and predators.

1.8 In most biomonitoring studies, identification at, or near the species level will be required to determine water quality conditions (Resh and Unzicker, 1975). Tolerant species (Appendix A) will usually become dominant only in polluted waters.

1.9 In pollution-oriented studies of macroinvertebrate communities, there are basically three sampling approaches--qualitative, semi-quantitative, and quantitative--that may be utilized singly or in combination. These sampling approaches are used to link ecosystem endpoints to stresses (e.g., physical

habitat alterations, inert solids, eutrophication, organic enrichment, thermal disruptions, ambient toxic wastes, and cumulative impacts) measured by bioindicator methods and techniques. See Section 5, Sampling Methods and Section 7, Data Evaluation.

1.10 During studies of water quality accommodations should be made for stream size, geographic location, and seasonality (Lenat, 1983). Also, flow conditions are related to the relative impact due to point and nonpoint sources of pollution. High flow usually increases the impact of nonpoint sources, while it reduces the impact of point sources. In streams with low flow, the reverse is often true. In addition, the presence, distribution, and abundance of aquatic macroinvertebrates, especially aquatic insects, may be subject to wide seasonal variations (Hilsenhoff, 1988). Thus, when conducting comparative studies, the investigator must be careful to avoid the confounding effects of these seasonal changes. Seasonal variations are particularly important in freshwater habitats dominated by aquatic insects having several life stages, not all of which are aquatic.

1.11 The design of macroinvertebrate studies should be based upon study goals and data quality objectives (DQOs) (See Section 2, Quality Assurance and Quality Control). To supplement the material contained in this manual, a number of basic references should be reviewed or available to investigators of the macroinvertebrate communities, particularly to investigators engaged in aquatic water quality and pollution studies. These include Armitage (1978), Benke, Gillespie, and Van Arsdall (1984), Brinkhurst (1974), Cairns and Dickson (1973), Cummins (1966, 1973, 1974, 1975), Cummins and Klug (1979), Cummins et al. (1984), Cummins and Wilzbach (1985), Edmondson and Winberg (1971), Elliott (1977), Goodnight and Whitley (1960), Hart and Fuller (1974), Hellawell (1978, 1986), Hilsenhoff (1977), Howmiller and Scott (1977), Hynes (1960, 1970), Holme and McIntyre (1971), Hulings and Gray (1971), Lenat (1983), Lind (1974), Merritt and Cummins (1984), Mason (1981), Metcalfe (1989), Milbrink (1983), Meyer (1990), Neuswanger, Taylor, and Regnolds (1982), Pennak (1989), Posey (1990), Resh (1979), Resh and Rosenberg (1984), Resh and Unzicker (1975), Reynoldson et al. (1989), Ward and Stanford (1979), Warren (1971), Waters (1977), Welch (1948), Welch (1980), and Winner et al. (1975).

1.12 This manual was composed to assist biologists and managers in USEPA and other Federal, state, and private water monitoring organizations in the use of macroinvertebrates for evaluating the biological integrity of surface waters. The manual contains laboratory and field methods that will aid in the monitoring, detection, and bioassessment of surface waters and the effects of environmental stress on macroinvertebrate communities. It will also facilitate the expansion of our knowledge of the ecological requirements of macroinvertebrate species in fresh, estuarine, and marine habitats. The manual includes sections on quality assurance and quality control, safety and health, sampling site selection, sampling methods and techniques, sample processing, data evaluation, and a taxonomic bibliography, containing the current taxonomy used for identifying the macroinvertebrates of North America. Information on the pollution tolerance of selected species and examples of bench and data summary sheets are provided in the Appendices.

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## SECTION 2

## QUALITY ASSURANCE AND QUALITY CONTROL

### 2.1 Introduction

2.1.1 A strong quality assurance (QA) program and effective quality control (QC) procedures are needed for operating an adequate macroinvertebrate bioassessment or monitoring laboratory to ensure that all data produced are valid and of known quality. The term "quality assurance" refers to the quality control functions and involves the totally integrated program for ensuring the reliability of monitoring data; the term "quality control" refers to the routine application and procedures for obtaining prescribed standards of performance and for controlling the measurement process (USEPA, 1978). Quality assurance programs have two primary functions in a macroinvertebrate laboratory. First, the program should continually monitor the reliability of the data generated to determine the accuracy, precision, completeness, comparability, and representativeness of the data. The second function is the control of the quality of the data so as to meet the requirements for reliability that the program demands. Quality assurance and control must be a continuous process that includes all aspects of the program, including field collection and preservation, sample processing, and data analysis; otherwise the data generated may not be reliable and useful for decision making and the results will be of little use in establishing the biological integrity of the water body under study. In order to support the operation of a consistent plan, the persons responsible for QA should consult the EPA Quality Assurance manual (USEPA, 1984a). All EPA QA programs should be based on USEPA order 5360.1 (USEPA, 1984b) which describes the policy, objectives and responsibilities of all USEPA program and regional offices.

2.1.2 Components of the QA program (USEPA, 1979) should include the following:

2.1.2.1 Collection, preservation and analysis of all samples should follow approved methodology.

2.1.2.2 Sampling equipment, flow measuring devices, and other measuring instruments such as pH, DO, and conductivity meters should be calibrated according to manufacturer's instructions, and documented.

2.1.2.3 Assurance that representative samples are collected (See Section 4, Selection of Sampling Sites).

2.1.2.4 Determination of precision of sampling and analysis procedures.

2.1.2.5 Use of replication in all phases of the sampling and analysis program.

2.1.2.6 Participation in interlaboratory investigations and use of quality control samples.

2.1.2.7 Accurate and timely recording, maintenance, and storage of data in a log book, computer, or other data storage and retrieval system.

## 2.2 Data Quality Objectives (DQOs)

2.2.1 A full assessment of the data quality needed to meet the study objectives should be made prior to preparation and implementation of the QA plan. Data quality is a measure or description of the type and amount of error associated with a set of data. Determination of data quality is accomplished through the development of data quality objectives (DQOs), which are statements of the level of uncertainty a decision-maker is willing to accept or the quality of the data needed to support a specific environmental decision or action. Both qualitative and quantitative descriptors of data quality must be considered in order to determine whether data are appropriate for a particular application. Data quality objectives are target values for data quality and are not necessarily criteria for the acceptance or rejection of data.

2.2.2 Data quality objectives are developed in three stages. During the first stage, the decision-maker determines what information is needed, reasons for the need, how the information will be used, and specifies time and resource constraints. The second stage involves the technical staff and decision-maker interacting to establish a detailed and clarified specification of the problem, how the information will be used, any constraints imposed on the data collection, and what limitations of the information will be acceptable. The third stage involves the analysis of possible approaches to collection and analysis of the data and a determination of the quality of the data that can be expected to result from each approach. The best approach is selected based on the criteria agreed upon in the second stage. It may be necessary to modify the objectives of the study during the development of these DQOs. Details for developing DQOs are described in two U.S. Environmental Protection Agency documents (USEPA, 1984c and 1986) available from the Quality Assurance Management Staff, Office of Research and Development, Washington, DC 20460.

2.2.3 After the final DQOs are established, the detailed project QA plan should be finalized stating specific quantitative and qualitative data quality goals and QC procedures that will be used to control and characterize error (USEPA, 1980). The goals based on the DQOs will be the criteria for measuring the success of the QA program.

2.2.4 The Quality Assurance Management Staff, Office of Modeling, Monitoring Systems, and Quality Assurance, is responsible for providing guidance for the inclusion of DQOs in quality assurance program and project plans, and for providing guidance to the regions on the application of the DQOs development process. The EPA regional offices are responsible for ensuring that state QA program and project plans conform with grant requirements specified in 40 CFR Part 30, and for assisting the states in developing DQOs requirements that meet state needs.

2.2.5 Regional and state laboratories or monitoring personnel in need of assistance in preparing Quality Assurance Project Plans or development of DQOs for bioassessment projects can contact personnel of the Aquatic Biology Branch in the Quality Assurance Research Division, Environmental Monitoring Systems Laboratory-Cincinnati, for assistance (FTS 684-8114 or COML 513-533-8114, FAX FTS 684-8181 or COML 513-533-8181).

## 2.3 Facilities And Equipment

2.3.1 Laboratory and field facilities and utility services must be in place and operating consistent with their designed purposes so that quality environmental data may be generated and processed in an efficient and cost-effective manner. Suitability of the facilities for the execution of both the technical and QA aspects of the study should be assessed prior to initiation of the study. Adequate space, lighting, temperature, noise levels, and humidity should be provided. Satisfactory safety and health maintenance features must also be provided (see Section 3, Safety and Health).

2.3.2 Equipment and supplies necessary to adequately collect, preserve and process biological samples must be available and in good operating condition. See Appendix E for a list of recommended equipment and supplies.

2.3.2 To ensure data of consistently high quality, a plan of routine inspection and preventive maintenance should be developed for all facilities and equipment. All inspections, calibrations, and maintenance must be documented in individually bound notebooks. This documentation should include detailed descriptions of all calibrations performed, adjustments made, and parts replaced and each entry should be signed and dated.

2.3.3 Taxonomists and aquatic biologists who are capable of identifying organisms are expected to have at their disposal adequate taxonomic references to perform the level of identification required. See Section 8, Taxonomic Bibliography, for a list of selected taxonomic references. Aquatic biologists should check this list and obtain those references that will be needed for the identification of specimens to the lowest taxonomic level possible.

2.3.4 Representative specimens of all taxa identified should be verified by a specialist who is a recognized authority in that particular taxonomic group. These specimens should be properly labeled as reference or voucher specimens, including the name of the verifying authority, permanently preserved, and stored in the laboratory for future reference.

## 2.4 Calibration, Documentation, and Record Keeping

2.4.1 Quality assurance plans should contain mechanisms for demonstrating the reproducibility of each measuring process. Regular calibration of instruments, proper documentation, and permanent record keeping are essential aspects of such plans.

2.4.2 Each measuring device must be calibrated before each use according to

the manufacturer's instructions, and routine checks using National Institute of Standards and Technology, or other standards of known accuracy, should be made to demonstrate that variables are within predetermined acceptance limits. Permanent records giving dates and details of these calibrations and checks must be kept. Documentation is necessary to identify each specific measuring device, where and when it is used, what maintenance was performed, and the dates and steps used in instrument calibration. Each sample collected should also be documented by assigning a unique identification number and label (See Section 6, Sample Processing). Data should be documented to allow complete reconstruction, from initial field record through data storage system retrieval.

2.4.3 Whenever samples are collected to be used as evidence in a court of law, it is imperative that laboratories and field operations follow written chain-of-custody procedures for collecting, transferring, storing, analyzing, and disposing of the samples. The primary objective of chain-of-custody procedures is to create written record which can be used to trace the possession of the sample from the moment of collection through the introduction of the analytical data into evidence. Explicit procedures must be followed to maintain the documentation necessary to satisfy legal requirements. All survey participants should receive a copy of the study plan and be knowledgeable of its contents prior to implementing the field work. A presurvey briefing should be held to reappraise all participants of the survey objectives and chain-of-custody procedures. After all chain-of-custody samples are collected, a debriefing should be held in the field to check adherence to chain-of-custody procedures. Chain-of-custody procedures are detailed in three USEPA manuals (USEPA, 1974, 1982, and 1990).

2.4.4 Field and laboratory personnel should keep complete and permanent records of all conditions and activities that apply to each individually numbered sample sufficient to satisfy legal requirements for any potential enforcement or judicial proceedings. All field and laboratory data sheets should be dated and signed by the sampler and analyst, respectively. Notebooks, data sheets, and all other records that may be needed to document the integrity of the data should be kept permanently filed in a safe and fireproof place.

## 2.5 Qualifications and Training

2.5.1 All personnel need to have adequate education, training, and experience in the areas of their technical expertise and in QA to fulfill their designated responsibilities. Because no formal academic programs in research QA exist, most QA experience will have to be acquired through on-the-job training.

2.5.2 At least one professional biologist with training and experience in biological sampling methods and macroinvertebrate identification should be on the staff and should be personally involved in the field work as well as the laboratory analysis of the samples. Statistical expertise should be readily available and consulted during every phase of the project.

2.5.3 Management should periodically assess the training needs of all

personnel engaged in QA and recommend and support their participation in appropriate and relevant seminars, training courses, and professional meetings. Biologists and technicians should be expected to participate regularly in evaluation and/or certification programs where appropriate.

2.5.4 The laboratory should have on file an up-to-date resume for each person who is responsible for the analysis, evaluation and reporting of biological data.

## 2.6 Standard Operating Procedures (SOPs)

2.6.1 Each laboratory must define the precise methods to be used during each step of the sample collection, analysis, and data evaluation process. These written procedures become the standard operating procedures (SOPs) describing the operation of the laboratory. Standard operating procedures for a macroinvertebrate laboratory should describe in stepwise language, easily understood by the potential user, the sampling methodology, details of preservation and labeling the samples, use of taxonomic keys, use and calibration of measuring instruments, replication and QC requirements, sample custody and handling procedures, and data evaluation and handling. The SOPs should include a listing of the taxonomic keys and references that should be used for each level of identification required and for each taxonomic group. It should provide an outline of the steps to be taken to assure the quality of the data.

2.6.2 The SOPs should stress the need for the traceability of the samples. At a minimum it should specify that each sample be assigned a unique identification number and be properly labeled with the sample number, sampling location, and name of the collector. It should describe procedures to ensure that each sample collected, as accurately and precisely as possible, represents the community sampled.

2.6.3 The SOPs should be approved by the proper authority and should be easily accessible to personnel for referral.

2.6.4 The laboratory SOPs should be followed as closely as possible. Any deviations should be documented as to the reason for the deviation and any possible effect the deviation might have on the resulting data.

## 2.7 Literature Cited

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USEPA. 1978. Quality Assurance Newsletter. Environmental Monitoring and Support Laboratory - Cincinnati, OH 45268.

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## SECTION 3

## SAFETY AND HEALTH

### 3.1 Introduction

3.1.1 Collection and analysis of benthic samples involve significant risks to personal safety and health. While safety is often not considered an integral part of benthic sampling routine, the biologist must be aware of unsafe working conditions, hazards connected with the operation of sampling gear, and other risks. Management should assign health and safety responsibilities and establish a program for training in safety, accident reporting, and medical and first aid treatment. Written safety policies should be available to all persons involved in the sampling and analysis of macroinvertebrate samples and this should include a copy of the USEPA (1986) safety manual.

### 3.2 General Precautions

3.2.1 Basic good housekeeping practice should be followed both in the field and in the laboratory. These practices should be aimed at protecting the staff from physical injury, preventing or reducing exposure to hazardous or toxic substances, avoiding interferences with laboratory operations, and producing valid data.

3.2.2 Operation of benthic sampling devices involves hazards that must be addressed by the person using the equipment. Some grab samplers (e.g., Ekman, Smith-McIntyre) have spring loaded cocking devices that can cause serious injury if not handled and operated carefully. Other grabs (e.g., Ponar) have safety locking pins that must be put in place to prevent injury. Persons using these devices should become familiar with the hazards involved and establish appropriate safety practices prior to using them.

3.2.3 Field personnel should known how to swim. Waders should always be worn with a belt to prevent them from filling with water in case of a fall. A life jacket at dangerous wading stations is advisable if one is not a strong swimmer because of the possibility of sliding into deep holes.

3.2.4 Many hazards lie out of sight in the bottoms of lakes, rivers and streams. Broken glass or sharp pieces of metal embedded in the substrate can cause serious injury if care is not exercised when walking or working with the hands in such environments. Infectious agents and toxic substances that may be absorbed through the skin or inhaled may also be present in the water or sediment.

3.2.5 Personnel must consider and prepare for hazards associated with the operation of motor vehicles, boats, winches, tools, and other incidental equipment. Boat operators should be familiar with U.S. Coast Guard rules and regulations for safe boating contained in a pamphlet, "Federal Requirements for Recreational Boats," available from your local U.S. Coast Guard Director or Auxiliary or State Boating Official (U.S. Coast Guard, 1987).

3.2.6 Prior to a sampling trip, personnel should determine that all necessary equipment is in safe working condition and that the operators are properly

trained to use the equipment.

3.2.7 Safety equipment and first aid supplies should be available in the laboratory and in the field at all times. A snake bite kit should be carried on all field trips in areas that may be infested with poisonous snakes. All motor vehicles and boats with motors should have fire extinguishers.

### 3.3 Safety Equipment and Facilities

3.3.1 Necessary and appropriate safety apparel such as waders, lab coats, gloves, safety glasses, and hard hats should be available.

3.3.2 First aid kits, fire extinguishers and blankets, safety showers, and emergency spill kits should be readily available in the laboratory at all times.

3.3.3 A properly installed and operating hood should be provided in the laboratory for use when working with volatile chemicals that may produce dangerous fumes.

3.3.4 Communication equipment should be available to field personnel and those working in mobile labs in remote areas for use in case of an emergency.

3.3.5 Facilities and supplies should be available for cleaning of exposed body parts that may have been contaminated by pollutants in the water. Soap and an adequate supply of clean water or ethyl alcohol may be suitable for this purpose.

### 3.4 Field and Laboratory Operations

3.4.1 At least two persons should be involved in all field collecting trips and no one should be left alone while in the field.

3.4.2 All surface waters should be considered potential health hazards due to toxic substances or pathogens and exposure to them should be minimized as much as possible. Exposed body parts should be cleaned immediately after contact with these waters.

3.4.3 All electrical equipment should bear the approval of Underwriters Laboratories and be properly grounded to protect against electric shock.

3.4.4 Staff training in basic first aid and cardio-pulmonary resuscitation is strongly recommended.

3.4.5 Before transporting grab sampling devices, be sure all safety lock pins are in place or transport them in the closed position. Read and follow all safety instructions provided by the manufacturer.

3.4.6 Use a winch for retrieving samples collected with heavy sampling devices such as the Ponar grab and use care in lifting heavy items to prevent back injury.

3.4.7 Heavy gloves should be used when hands are used to agitate the substrate

during collection of square-foot type samples and when turning over rocks during hand picking.

3.4.8 Persons working in areas where poisonous snakes may be encountered should check with the local Drug and Poison Control Center for recommendations on what should be done in case of a bite from a poisonous snake. If local advice is not available and medical assistance is over an hour away, carry a snake bite kit and be familiar with its use. Any person allergic to bee stings or other insect bites should take proper precautions and have any needed medications handy.

3.4.9 Personnel dealing in field activities on a regular or infrequent basis should be in sound physical condition and have a physical exam annually in accordance with Regional or State Safety Officer's requirements.

3.4.10 Hypothermia--all field personnel should be familiar with the symptoms of hypothermia and know what to do in case symptoms should occur. Hypothermia can kill a person at temperataure much above freezing (up to 50°F) if he or she is exposed to wind and rain or otherwise becomes wet.

## 3.5 Disease Prevention

3.5.1 Because it is not known what pollutants may be present in surface waters and sediments, they should be considered potential health hazards and exposure to them kept to a minimum.

3.5.2 Personnel, who may be exposed to water known or suspected to contain human wastes, should be immunized against tetanus, hepatitis, typhoid fever, and polio.

## 3.6 Literature Cited

US Coast Guard. 1987. Federal requirements for recreational boats. U.S. Department of Transportation, United States Coast Guard, Washington, DC 20593.

USEPA. 1986. Occupational health and safety manual. Office of Planning and Management, U.S. Environmental Protection Agency, Washington, DC 20460.

## SECTION 4

## SELECTION OF SAMPLING STATIONS

### 4.1 Introduction

4.1.1 The design of monitoring programs is one of the major sources of error or uncertainty in water quality data (Thornton et al., 1982). Proper selection of sampling sites (overall sampling areas) should be directed toward minimizing uncertainty or, at least, provide a means by which variability may be reduced.

4.1.2 If samples are taken at random over the whole stream, river or lake bottom, the sample sites may differ physically and species counts can be highly variable. A reasonable sample size would be expected to detect only a population density difference of more than 200% of the mean between two sites (Schwenneker and Hellenthal, 1984). If, however, the potential sampling areas are restricted to those of similar physical nature, this variability will be reduced so that differences of 50% or better can often be obtained. Mason et al. (1973) found that three artificial substrate replicate samples could be expected to give estimates within 20% of the true mean at the 95% confidence level.

4.1.3 Chutter and Noble (1966) studied the effects of sample site selection using the Surber square foot sampler and concluded that the closer the sampling site is defined the more reliable will be the sampling data in terms of a single species per square foot. Therefore, selection of sampling sites with similar substrate types (e.g., particle size), current velocity, depth and other physical characteristics will aid greatly in reducing variability.

4.1.4 Most organisms, even in a selected and defined habitat type, are not evenly distributed over the bottom of a waterbody so replicate samples will be needed to evaluate this variability (Cairns and Dickson, 1971). The crucial question is how many samples should be taken. The answer will depend on the purpose of the study, data quality objectives, physical characteristics of the sampling location, the type of sampler to be employed, and the time available. Mackey et al. (1984) considered four replicates in each distinctive environmental zone along the river to be adequate when using pond nets. A minimum of two replicate samples at each station are required when using drift nets (Lewis et al., 1975). Two (Mason et al., 1973) or three replicate samples are required for artificial substrate type samplers, and three replicate samples are the absolute minimum when using Surber and Hess type samplers (Needham and Usinger, 1956) or grab samplers (Lewis et al., 1982). In most cases collecting five replicate samples at each station will increase the statistical precision and accuracy. Additional replicate samples may be necessary to characterize the benthic community in some aquatic environments. The number of replicate samples needed should be determined during the reconnaissance or pilot study. The samples from various habitats should be processed and analyzed separately. The data can be

aggregated later after individual samples are analyzed and tabulated, but potentially important comparisons among habitats are lost if samples are composited.

4.1.5 A sound sampling design requires substantial understanding of the organisms being sampled and the types and limitations of the sampling devices to be employed. Data reduction techniques also, should be included in the study plans. Knowledge of locations of possible sources of pollution as well as insight into the intensity of the expected effects of the environmental changes that may be occurring at the site are also of great value. Other factors that will need to be involved in proper selection of the sampling sites include objectives of the study, accessibility, flow and mixing characteristics of effluents, personnel and facilities available to conduct the study, and historical data from previous studies. The primary concern in designing a sampling scheme is to gain an accurate measurement with high precision with the least effort possible to optimize productivity of available person-hours (Downing, 1979).

## 4.2 Location of Sampling Stations (Sampling Locations Within Each Site)

4.2.1 After determining the specific data quality objectives of the study and defining clearly what information is needed, it is necessary to select specific reaches of the stream or areas of the lake to use as sampling sites. Reconnaissance of the waterway (pilot study) at this time, using the Rapid Bioassessment Protocol I (Plafkin et al., 1989) or similar techniques, is important. Note possible sources of pollution, access points, bottom types, flow characteristics, and other physical characteristics that will need to be considered in selecting the sampling sites. The results of the pilot study may be used to obtain estimates of variances needed to establish sample size. Other advantages of the pilot study are that it accomplishes a detailed reconnaissance and it provides the opportunity to obtain experience in the actual field situation where the final study will be made. Information obtained and difficulties encountered may often be used to avoid costly and needless expenditures during the full scale study. Although the number and location of sampling stations will vary with each individual study, the following basic rules modified from Cairns and Dickson (1971), if carefully followed, should result in a sound survey design.

4.2.1.1 Always have at least one reference station (control station) away from all possible discharge points to provide a basis for comparison between areas above and below the point of discharge. This station should be directly above the effluent discharge in streams or just outside the zone influenced by the discharge in lakes and estuaries. It is advisable to add a second reference station well above or outside the zone of influence. See Section 4.3, Selecting Control Stations.

4.2.1.2 Establish a station directly below the source of pollution in streams or at the point of discharge into lakes. If the discharge does not mix completely immediately on entering a stream, left-bank, midchannel, and right bank substations should be established.

4.2.1.3 Establish stations at various distances downstream from the discharge in streams or away from the discharge point in lakes. Effort should be made to space the collecting stations approximately exponentially farther apart going down stream from the pollution source to determine the extent of the recovery zone.

4.2.1.4 All sampling stations should be as ecologically similar as possible in order to compare the benthic fauna collected at these sites. Decreasing station similarity with regard to habitat parameters generally indicate decreasing station comparability. The ability to control or measure inherent natural variability will enhance the overall assessment of benthic community structure and function. Bottom substrate, depth, temperature, flow velocity, bank cover, and salinity, etc. should be similar at each site. Where stations cannot be located in areas of similar habitats it may be necessary to use artificial substrate samplers to collect the samples.

4.2.1.5 Sampling stations for macroinvertebrates should be close to the sites where sampling for chemical and physical analyses will be located.

4.2.1.6 Sampling stations should be located in areas where benthos is not influenced by atypical conditions, such as those created by bridges or dams unless effects of atypical conditions make up part of the study objectives. For instance, urbanized areas include these structures as typical, and, in some cases, may provide the best suitable habitat that is available.

4.2.1.7 Sampling stations should be located so that samples can be collected from all the stations in a study on approximately the same day. If samples are collected on different days, emergence of adults may occur at a later collection site resulting in erroneous conclusions.

4.2.1.8 The sampling stations should be in places that are easily accessible. Long hiking distances and steep banks should be avoided if at all possible. If a boat will be needed for sample collection, the station should be located near a boat dock or launch ramp. In some habitats, such as a large lake, estuary, or ocean, sampling stations will, of necessity, often be miles from the boat launch ramp. If artificial substrate samplers are being used, the possibility of vandalism should be taken into account when selecting stations for installing these sampling devices.

4.2.2 Sampling to assess the effects of non-point sources of pollution requires a number of stations along the stream in the impacted area. Samples should also be collected in the unimpacted upstream area and the downstream recovery zone of the impacted stream.

### 4.3 Selecting Control Stations

4.3.1 Selecting appropriate control stations is a critical step because the control condition is the best estimate of integrity available to the investigator. The control station must be at a representative site at

which conditions adequately reflect or approximate the conditions of the water body being investigated. Four basic approaches available as estimates of control conditions are: (1) consult historical records, (2) use pristine or least disturbed areas, (3) use ecoregion reference sites, and (4) use computer simulation techniques to create a hypothetical benthic community as a reference station.

4.3.1.1 Historical records may be incomplete or nonexistent but, if available, can often provide valuable information on the status of previous conditions at the site. Usefulness of computer simulation techniques will depend on the quality and quantity of data available on the site in question. The most viable option most of the time is the use of least disturbed areas as controls in combination with the other three approaches.

4.3.1.2 The investigator, therefore, must look for the least impacted areas as close to the impacted area as possible or to an ecoregion reference station as the control site. The ecoregion reference station represents the best attainable conditions for all streams (or other water bodies) with similar physical characteristics for a given ecoregion (Plafkin et al., 1989). Ecoregions are geographic patterns of similarity among ecosystems, grouped on the basis of environmental variables such as climate, soil type, physiography and vegetation. From the data base that has been generated at the ecoregion reference station it would be theoretically possible to determine the expected aquatic community structure that would exist in the study area if not impacted (or in its pristine condition). If the ecoregion reference station or a station in an adjacent area is used as the control site, a second control station should be sampled in the least impacted area of the water body under study for comparison. Care must be taken because most navigable waterways have been altered by channelization, dredging, bridge building, etc.

## 4.4 Study Design

4.4.1 Once the sampling stations are chosen, the investigator will need to determine exactly where the samples will be collected at each station in order to determine the biological integrity of the aquatic community. Two types of sampling plans are discussed: 1) random sampling is used when quantitative data is needed, and 2) non-random sampling may be used to generate qualitative data or semi-quantitative data.

### 4.4.2 Random Sampling

4.4.2.1 In biological studies using the quantitative sampling approach, the exact location of sample collection (sampling units) and number of samples to be collected at each station must be selected with some known probability that a certain measure of precision will be obtained. Usually, random selection is the only feasible means of satisfying this criterion. Only by knowing the probability of selecting a specific sample can one extrapolate from the sample to the population in an objective way. The probability allows one to place a weight upon an observation in making an extrapolation to the population. There is no other quantifiable

measure of how well the selected sample represents the population. Thus the study plan should include an appropriate effort to define the problem in such a way as to allow a person to estimate the parameter of interest using a sample of known probability called a random sample.

4.4.2.2 There is a fundamental distinction between a "haphazardly-selected" sample and a "randomly-selected" sample. The distinction is that a haphazardly-selected sample is one where there is no conscious bias, whereas a randomly-selected sample is one where there is consciously no bias. There is consciously no bias because the randomization is planned and, therefore, bias is planned out of the study. This is usually accomplished with the aid of a table of random numbers. A sample selected according to a plan that includes random selection of experimental units is the only sample validly called a random sample.

4.4.2.3 Quantitative sampling in biological field studies is most often aimed at explaining spatial distributions of population densities or of some parameter related to population densities and the measurement of rates of change which permit prediction of some future course of a biologically-related parameter. In these cases the sampling unit is a unit of space (volume, area). Even in cases where the sampling unit is not a unit of space, the problem may often be stated in such a manner that a unit of space may be used, so that random sampling may be more easily carried out.

4.4.2.4 It is not always a simple or straightforward matter to define sampling units, because of the dynamic nature of the hydrology of streams and living populations. Many aquatic organisms are mobile, and even rooted or sessile forms change with time, so that changes occurring during the study often make data interpretation difficult. Thus, the benefit to be derived from any attempt to consider such factors in the planning stage will be considerable.

4.4.2.5 Random sample selection is a subject apart from the selection of the study site. It is of use only after the study objectives have been defined, the type of measurements have been selected, and the number of samples has been determined. At this point, random sampling provides an objective means of obtaining information to achieve the objectives of the study.

4.4.2.6 One satisfactory method of random sample selection is to number the universe, or entire set of sampling units available, from which the sample will be selected. This could be accomplished by marking off equal distances on a line transect across the stream and numbering each mark consecutively or by dividing a section of a water body (the sampling station) into grids as in figure 4 and numbering each intercept. The total number of marks or intercepts is "N". Then from a table of random numbers select as many random numbers, n, as there are sampling units selected for the sample. Select a starting point in the table and read the numbers consecutively in any direction (across, diagonal, down, up). For example, if "N" is twenty, select only numbers less than or equal to 20, ignoring any number greater than "N" or any number that has already

been selected. These numbers will be the numbers of the sampling units to be selected (Cummins, 1962).

4.4.2.7 If a random starting point is chosen along the transect to introduce randomness needed to guarantee freedom from bias and allow statistical inference and the samples are collected at points chosen systematically along the transect, the data collected could be considered quantitative. To avoid arbitrariness, randomization should also be employed in transect placement.

4.4.2.8 Simple Random Sampling is used when there is no reason to subdivide the population from which the sample is drawn. The sample is drawn such that every unit of the population (numbered section or grid) has an equal chance of being selected. This may be accomplished by using the random selection scheme already described. Because the spacial distribution of benthic communities is so closely related to physical factors such as substrate type, current velocity, depth, and salinity, a design using simple random sampling is seldom meaningful. Therefore, it is usually best to stratify the habitat on the basis of known physical habitat differences and select sampling units by an appropriate randomization procedure in each habitat type; a procedure known as stratified random sampling.

4.4.2.9 Stratified Random Sampling is usually the preferred sampling design because of a resulting increase in precision. If any knowledge of the expected size or variation of the observations is available, it can often be used as a guide in subdividing the population (potential sampling points or units) into subpopulations (strata) (Gaufin et al., 1956). Information obtained during the pilot study will be useful in determining what strata to sample. The pilot study planning should be done carefully, perhaps stratifying based upon suspected variability in community structure. To maximize precision, strata should be constructed such that the observations are alike within strata and different among strata. In practice, the information used to form strata will usually be from previously obtained data or the pilot study. In aquatic field situations, stratification may be based upon bottom type, depth, isotherms, and numerous other variables suspected of being correlated with the characteristic of interest.

4.4.2.10 Stratification may also be done on other bases such as convenience or administrative imperative, but except where these correspond with criteria which minimize the variation within strata, no gain in precision may be expected.

4.4.2.11 Number of strata - In aquatic biological field studies, the use of knowledge of biological cause-and-effect may help define reasonable strata (e.g., thermoclines, sediment types, etc., may markedly affect the organisms so that the environmental features may be the obvious choice for the strata divisions). Where a gradient is suspected and where stratification is based on a factor correlated to an unknown degree with the characteristic of interest, the answer to the question of how many strata to form and where to locate their boundaries is not clear. Usually

as many strata are selected as may be needed to meet the data quality objectives of the study. In practice, gains in efficiency with increasing stratification usually become negligible after only a few divisions unless the characteristic used as the basis of stratification is very highly correlated with the characteristic of interest.

4.4.2.12 For many quantitative studies, it is often necessary in the interest of economy and efficiency and within the limitations of the available gear, to sample primarily at sites having substrates which normally support the most abundant and varied fauna, and devote a minimum effort to those substrates supporting little or no life. For instance, in many large, swiftly flowing rivers of the midwest and southeast, the areas of "scour" with a substrate of shifting sand or hardpan may be almost devoid of macroinvertebrates; sampling effort may be reduced there in favor of the more productive areas of "deposition" on the inside of bends or in the vicinity of obstructions. Just the opposite situation may occur in many of the swiftly-flowing upland streams, where most of the effort may be devoted to sampling the productive rubble and gravel riffle areas instead of the pools.

4.4.2.13 When the location of sampling stations and placement of the samplers at these stations are done in a non-random manner, as is often done in practice, the sample is best considered a semi-quantitative sample even though a quantitative sampling device is used in the study.

4.4.3 Systematic Sampling

4.4.3.1 If quantitative data are not needed, some type of systematic sampling is generally employed for synoptic surveys and reconnaissance studies. Line transects established at discrete intervals across a river or stream and sampled at quarter points, or more frequent intervals, are a form of systematic sampling (Fig. 1). Use of this type of sampling assures an adequate cross section while maintaining relative ease of sampling. In lakes, reservoirs, wetlands, and estuaries, transects may be established along the short or long axis or may radiate out from a source of pollution (Fig. 2). The method of placement of the transect should be given a great deal of thought so that sampling stations will be as representative as possible. The confounding effects of changes in physical characteristics of the environment along the transect must be fully recognized and accounted for. A topographical map with fixed bench marks, a surveyor's sighting instrument mounted on a tripod, and surveying stakes marked off in centimeters are useful for establishing a line transect. The sampling points should be marked so that the fixed stations can be visited during each sampling visit. These fixed stations can be marked on a rope extended between poles on each side of a stream or buoys can be attached to weights on the bottom.

4.4.3.2 In lakes, reservoirs and estuaries the stations may be marked by use of sighting stakes or dabs of paint on rocks established on the shore. Two sighting lines should be established for each station so that they intersect at the fixed site (Fig. 3).

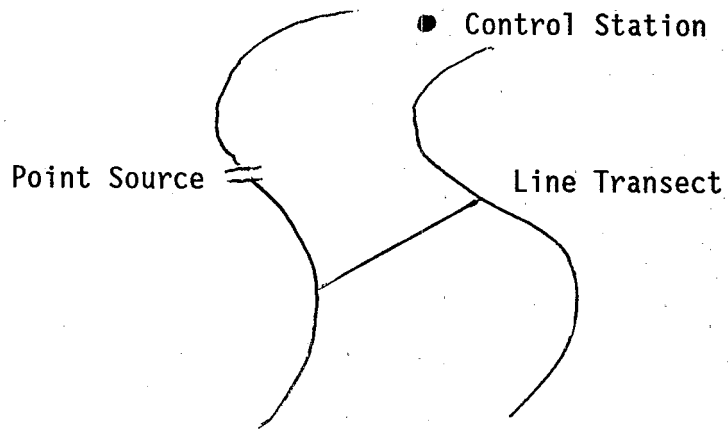


Figure 1. Example of transect sampling scheme in rivers and streams.

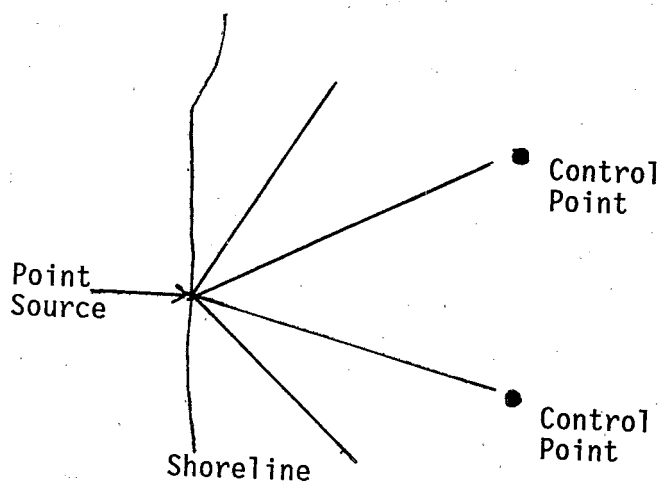


Figure 2. Example of transect sampling scheme in lakes, reservoirs, and coastal waters.

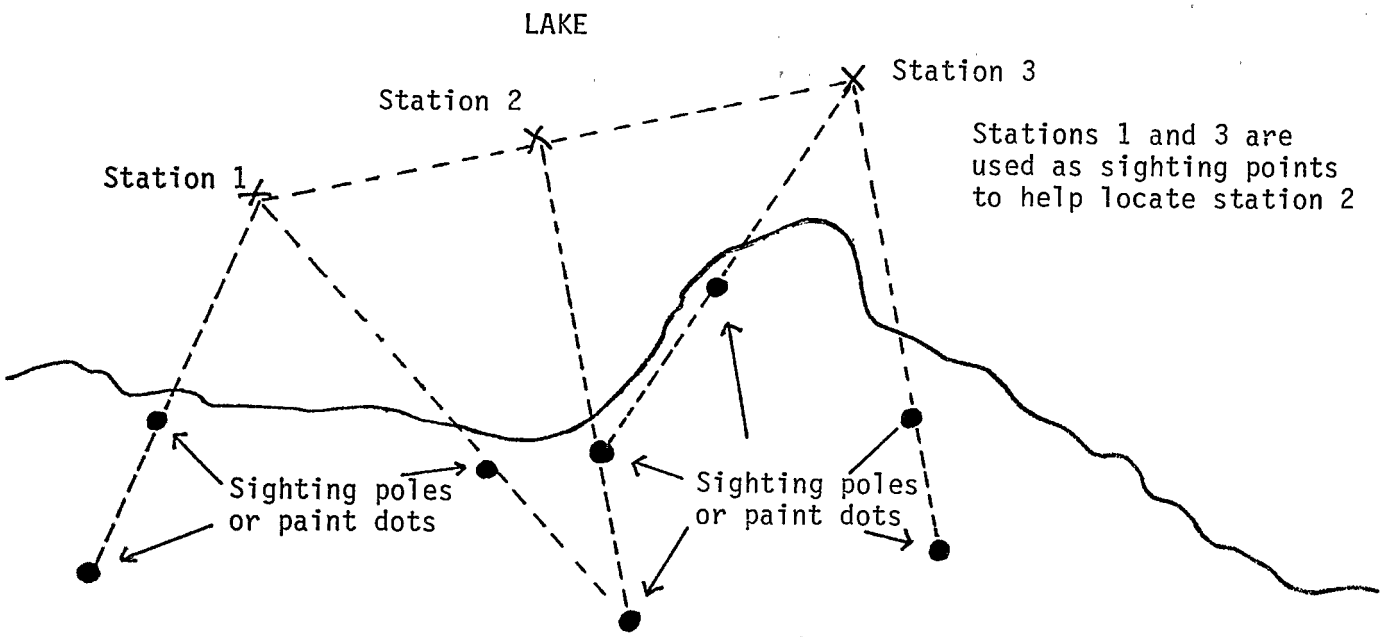


Figure 3. Illustration of how sighting lines are used to locate fixed sampling locations in lakes, reservoirs, or estuaries.

4.4.3.3 Two other methods of locating stations on large water bodies are the Loran-C and Navstar/GPS methods of sighting longitude and latitude (USEPA, 1987).

4.4.3.4 Loran is an acronym for long range navigation. It is a pulsed low-frequency electronic navigation system that operates at 90 to 110 KHz in the hyperbolic mode. Loran-C has a nominal absolute accuracy of 185-460 meters over short distances using ground waves, whereas repeatable accuracy varies from 15-90 meters. Loran-C is frequently used for coastal monitoring programs, however, it can be used up to 160 Km inland if overland transmission of signals is used. User capability is unlimited.

4.4.3.5 Navstar/GPS is an acronym for Navstar Global Positioning System (GPS). It is a second generation satellite navigation system currently under development by the U.S. Department of Defense. Its purpose is to provide precise, continuous, worldwide, all-weather, three-dimensional navigation for land, sea, and air applications. More information on these and other systems can be found in "Evaluation of Survey Positioning

Methods for Nearshore Marine and Estuarine Waters" (USEPA, 1987).

4.4.3.6 Instead of line transects, the investigator may employ the grid sampling scheme in rivers lakes, reservoirs, wetlands, and estuaries as another type of systematic sampling (Fig. 4). Grid sampling may be either random or non-random depending on the method of choosing the sampling points within the grid as discussed above for transect sampling (See section 4.4.2.6).

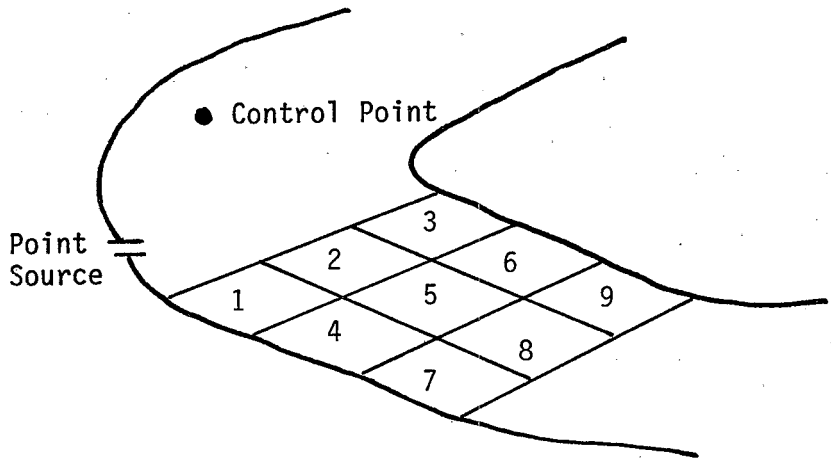


Figure 4. Example of grid sampling scheme in rivers.

4.4.3.7 In another form of systematic sampling, the investigator, using a variety of gear, consciously selects and intensively samples all recognizable habitat types. Such a non-random sampling plan may be used for collecting qualitative data. Non-random sampling is often employed during the reconnaissance phase of the study to gain a general idea of the type of benthic organisms that will be sampled during the main phase of the study. Use of kick nets in riffle areas and hand picking from rocks in pool areas are typical collection methods employed during this phase of the sampling program. These non-random sampling methods are also commonly used in rapid bioassessment studies (Plafkin et al., 1989).

4.4.3.8 In conducting synoptic surveys or other qualitative studies and taking into account the limitations of available sampling devices, sampling stations should be selected to include all substrate types. If these qualitative samples are to be used for determining the effects of pollutants where the pollutant does not have a direct effect on the substrate, the investigator must bear in mind that only the fauna from sites having similar substrates in terms of organic content, particle size, vegetative cover, and detritus will provide valid data for comparison.

## 4.5 Consideration of Abiotic Factors

4.5.1 Regardless of the method used to select the sampling unit, the biologist must consider and account for those natural environmental variations that may affect the distribution of organisms in the waterbody under investigation. Among the more important environmental variables in freshwater habitats are substrate type and stability, gradient, current velocity, flow rate, water depth (spates and drought in lotic waters), light and temperature regimes, and water quality characteristics such as dissolved oxygen, turbidity, acidity, hardness, alkalinity, sulfates, and nutrient concentration. In mountain ranges the elevation is an important consideration because it affects water temperature and other stream characteristics. In estuaries, additional variables that must be accounted for are the salinity gradient and tidal cycles.

4.5.2 Substrate Type is one of the most important factors for controlling the characteristics of the community of macroinvertebrates found at a given location in a body of water (Scott, 1958). Over a period of time, the natural substrates may be greatly altered by the discharge of particulate mineral or organic matter, and the location and expanse of various substrate types (silt, sand, gravel, etc.) may change because of normal variations in hydrologic factors such as current velocity and stream flow. The biologist, therefore, must be cognizant of changes in the nature and properties of the substrate which may provide clues on the quality and quantity of pollutants and other factors which affect the normal distribution of the benthic fauna.

4.5.2.1 Where the pollutant has a direct effect on the characteristics of the substrate, the effects of these changes may be inseparable from the effects of changes in water quality. Where substrate has deteriorated, faunal effects may be so obvious that extensive sampling may not be required and special attention should be given to the physical and/or chemical characterization of the deposits.

4.5.2.2 Because of the importance of substrate (in terms of both organic content and particle size) in macroinvertebrate studies, it is suggested that one or more unsieved substrate samples be collected from each station for use in conducting an analysis of substrate characteristics.

4.5.2.3 The mineral and organic matter content of the stream, lake, or estuary bottom at each sampling station should be classified and recorded on suitable forms, on a percentage basis, using the categories shown in Table 1, which should be applicable to most situations with only slight modification.

4.5.2.4 It is often desirable to further evaluate the inorganic components of the substrate by conducting a wet and dry particle size analysis in the laboratory. This analysis should be conducted on replicate samples from each sampling site with the use of standard sieves following the modified Wentworth classification shown in Table 2. Methods for separating the coarse fractions are given in Welch (1948). The silt-clay fraction may be considered "silt" if it is of a fine, loose

consistency upon drying, and "clay" if it is of a sticky consistency forming hard clumps on drying (Lewis et al., 1982). If it is desirable to further separate the silt-clay fraction, a Coulter counter as described by Walker et al. (1974) is recommended.

TABLE 1. CATEGORIES FOR FIELD EVALUATION OF SUBSTRATE CHARACTERISTICS*

| Type | Size or characteristic |
|---|---|
| Inorganic Components | |
| Bed rock or solid rock | |
| Boulders | >256 mm (10 in.) in diameter |
| Rubble/cobble | 64 to 256 mm in diameter |
| Gravel | 2 to 64 mm in diameter |
| Sand | 0.06 to 2.0 mm in diameter |
| Silt | <0.06 mm in diameter, of a loose consistency easily disturbed |
| Clay-Marl/hard pan | <0.004 mm in diameter, of a sticky consistency not easily disturbed, slick feeling when rubbed between fingers |
| Organic Components | |
| Detritus | Wood, sticks, and other undecayed coarse plant materials |
| Peat | Variously decomposed, green to brown, plant remains |
| Muck | Completely decomposed, black, fine organic matter |

*Modified from Roelofs, 1944.

4.5.2.5 Analysis of Organic Content - The organic content may be determined by drying and ashing a weighed amount of a representative sample of the sediment.

4.5.2.6 Dry weight is determined by weighing the sample in a tared porcelain crucible, drying in an oven at 105 degrees C to a constant weight (24 hours), and weighing.

4.5.2.7 Ash-free weight is determined after the dry weight is done. Place the same crucible with the dried sample in a muffle furnace at 500 degrees C for one hour. Cool, rewet the ash with distilled water, and bring to constant weight (about 24 hours) at 105 degrees C. The ash is wetted to reintroduce the water of hydration of the clay and other minerals that, though not driven off at 105 degrees C, is lost at 500 degrees C. This water loss often amounts to ten percent of the weight lost during ignition and, if not corrected for, will be interpreted as organic matter. Subtract the weight of the crucible from the dry weight to obtain ash-free weight.

4.5.3 Gradient is the percent of slope of the stream bed which affects velocity and the ability of the stream to maintain substrate quality. Gradient is particularly important in streams and rivers where it influences siltation and scouring.

TABLE 2. SUBSTRATE PARTICLE SIZE CLASSIFICATION FOR SIEVE ANALYSIS*

| Name | Particle Size (mm) | U.S. Standard Sieve Number |
|---|---|---|
| Boulder | >256 | |
| Rubble | 64 to 256 | |
| Coarse Gravel | 32 to 64 | |
| Medium Gravel | 8 to 32 | Available but not U.S. Standard |
| Fine Gravel | 2 to 8 | 10 |
| Coarse Sand | 0.5 to 2 | 35 |
| Medium Sand | 0.25 to 0.5 | 60 |
| Fine Sand | 0.125 to 0.25 | 120 |
| Very Fine Sand | 0.0625 to 0.125 | 230 |
| Silt | 0.0039 to 0.0625 | See Text |
| Clay | <0.0039 | See Text |

*Modified from Wentworth, 1922; see Cummins, 1962.

4.5.4 Current velocity affects the distribution of organisms in lotic environments and along the windswept shores of lentic environments, both directly because of differing species requirements and indirectly by sorting of bottom sediments. Therefore, it is of critical importance that velocity be considered when sampling stations are selected, and when data are analyzed. Only stations with similar velocity should be compared. In addition, windswept and protected shores of lakes may not be comparable. At the actual time of sampling, velocity should be determined at each sampling point. Relatively inexpensive current meters are commercially available (See equipment list in Appendix E). Current velocity may also be determined by use of a home-made velocity head tube described by Ciborowski (1989).

4.5.4.1 At depths greater than three feet, use the two-point method; take readings at two points, 0.2 and 0.8 of the depth below the surface. The average of these two observations is taken as the velocity.

4.5.4.2 At depths less than three feet, take one reading at 0.6 of the depth. Where artificial substrate samplers or drift nets are being utilized, take the reading directly upstream of the sampler and at the same depth as the sampler.

4.5.5 Flow rate may be a factor in the distribution of benthic organisms in that it indirectly effects other factors such as current velocity and water depth. Also, flow rate is a factor in the dilution of toxic substances in the water. During periods of low flow a toxic material will cause greater stress on the organisms present because of higher concentration of the substance. For this reason it is often desirable to

sample areas of suspected problems during low flow conditions in order to determine if an effluent is causing a stress on the aquatic community. Comparison of a sampling station during the sample period from year to year may not be valid if there is a large difference in flow rate between the two years.

4.5.6 Depth indirectly affects the distribution of aquatic macroinvertebrates as a result of its influence on the availability of light for plant growth, water temperature, the zonation of bottom deposits, water chemistry (particularly oxygen), and on phototactic responses of organisms. In selecting sampling stations for both qualitative and quantitative studies, depth must be measured and included as an independent variable in the study design.

4.5.7 Turbidity is defined as a cloudy condition in water due to the suspension of silt or finely divided organic matter. It is an important factor in that it directly effects light penetration and indirectly effects the productivity of algae and aquatic plants. The settling out of solids can also eliminate all life from a stream or river, or reduce its amount without greatly altering its composition simply by shading out all or some of the plant life, smothering out all algal growth, and altering the nature of the substratum.

4.5.8 Salinity is an important factor in marine and estuarine environments. The salinity of freshwater is generally a few parts per million compared to approximately 35 parts per thousand for sea water. Where sea water and fresh water meet in estuaries, there may be wide fluctuations of salinity due to variations in tides and river discharge, and a salt wedge may extend upstream under the fresh water layer for a significant distance. This area may be inhabited to some extent by both freshwater and saltwater forms, but the number of species is usually less than that under more stable conditions of salinity (Macan, 1963). Since movement and location of many species is governed by tides and salinity, these must be taken into account in determining sampling location as well as time of sampling.

4.5.8.1 Because of the extreme spatial and temporal fluctuations of salinity in the estuaries, simple, rapid instrumental measurements are more desirable than slower, more precise chemical methods (Mangelsdorf, 1967). Wide range, temperature-compensated conductivity salinometers are recommended for determining both horizontal and vertical salinity profiles at high-slack and low-slack tide levels in the area of estuary or reach of river being studied.

4.5.9 Tidal inundation (the amount of time that a particular stratum is inundated in marine intertidal zones) affects the kinds of organisms that can live within the substrate. Organisms that can resist desiccation and temperature change are able to colonize the intertidal zone. Organisms that cannot, will be restricted to the sublittoral zone or area below the tidal reach.

4.5.10 Chemical factors such as alkalinity, pH, hardness and sulfates are

also important factors to consider. They affect the numbers and composition of macroinvertebrates in the stream. Alkalinity is closely related to primary productivity. An increase in sulfates causes deterioration in water quality and adversely affects the macroinvertebrate community.

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## SECTION 5

## SAMPLING METHODS

### 5.1 Introduction

5.1.1 Aquatic macroinvertebrates are good indicators of environmental water quality in fresh, estuarine, and marine waters. The analysis of faunal assemblages is an excellent way to detect water quality problems. Different kinds of stress will often produce different communities of benthic macroinvertebrates. The sampling equipment and methods discussed can be used to study and analyze macroinvertebrate communities for ambient or special studies, and the resulting data and information can be used to document both spatial and temporal changes in water quality. The sampling devices and methods of this section relate to qualitative, semi-quantitative, and quantitative sampling.

5.1.1.1 Qualitative and semi-quantitative sampling of macroinvertebrates are relatively easy. The current methodology discussed here is well developed, and the equipment needed for sampling is not elaborate. Many effective methods of data analysis, including pollution indices and diversity indices, have been developed for use with macroinvertebrates (also, see Section 7, Data Evaluation).

5.1.1.2 Quantitative sampling is more difficult. Random sampling and the patchy distribution of macroinvertebrates within the substrate often means larger numbers of samples are needed in order to be able to make reasonable estimates of community structure and population densities. However, this is not a problem confined only to macroinvertebrates, but to other aquatic animals as well. Also, see Section 4, Selection of Sampling Sites and Section 7, Data Evaluation.

5.1.2 The sampling methods employed should depend on the data quality objectives (DQOs) (see Section 2, Quality Assurance and Quality Control) of the study determined by interaction of the decision making authority and biomonitoring expertise of qualified aquatic biologists.

5.1.3 A list of equipment, supplies, and companies that can provide sampling gear for collecting benthic macroinvertebrates can be found in Appendix E.

### 5.2 Qualitative Sampling

5.2.1 The objective of qualitative studies is to make within or between site comparisons to determine the presence or absence of benthic macroinvertebrates having varying degrees of tolerance to pollution and to obtain information on the richness of taxa, at or near the species level (taxa present and relative abundance). Samples are obtained with the use of a wide variety of collecting methods and gear, many of which are not amenable to quantification on a unit-area or volume basis. Any collecting device (e.g., dip or hand nets, kick nets, screens, dredges,

grab samplers, stream-net samplers, and artificial substrate samplers) can be used for qualitative collections of macroinvertebrates. The use of several methods of collection at each station can increase the total number of taxa collected. When conducting qualitative studies, an attempt is usually made to collect as many taxa as possible in the time available by exhaustive sampling in all available habitat types. No habitat should be overlooked at the site if the objective of the study is to obtain a representative collection of the macroinvertebrates.

5.2.2 Experience and skill are required in selecting suitable collecting techniques and recognizing and locating various types of habitats where qualitative samples can be collected.

5.2.3 When conducting comparative studies of the macrobenthos, a major drawback is the confounding effect of the differences in physical habitat among the different stations being studied. This problem is particularly inherent in qualitative studies when an attempt is made to systematically collect as many species as possible at the sampling stations or reaches of streams being compared. Unfortunately, differences in habitat unrelated to the effects of pollution may render such comparisons meaningless. To minimize this drawback, the investigator should carefully record, in the field, the habitats from which specimens are collected (a habitat assessment) and then base comparisons only on stations with like habitats in which the same amount of collecting effort has been expended. Appropriate sampling methods, such as the use of artificial substrates, should be utilized to eliminate the problem of comparing different physical habitats among stations being studied.

5.2.4 Advantages of qualitative sampling are the wide latitude in collecting methods, the types of habitats that can be sampled are relatively unrestricted, and the processing of the samples is usually less time consuming.

5.2.5 Limitations of qualitative sampling include collecting techniques that are subjective and depend on the skill and experience of the sample collector, sampling results of one investigator can be difficult to compare with those of another, and no information on standing crop or biomass can be generated from a qualitative study.

### 5.3 Semi-quantitative Sampling

5.3.1 Semi-quantitative sampling data can be generated based on methods that measure the collection of benthic macroinvertebrates by level of effort (e.g., time expended per habitat) or when quantitative sampling devices are used to collect samples in a non-random manner. Examples of some semi-quantitative methods include the 10 rock method (Lewis, personal communication), traveling kick method (Hornig and Pollard, 1978; Pollard, 1981), and Rapid Bioassessment Protocols II and III (Plafkin et al., 1989). See Section 7, Data Evaluation.

### 5.4 Quantitative Sampling

5.4.1 Quantitative methods essentially provide an estimation of the numbers or biomass (standing crop) of the various components of the macroinvertebrate community per unit area, volume, or sampling unit. The method also provides information on the species composition, richness of species, and distribution of individuals among the species. The high variability often associated with some macroinvertebrate populations makes them difficult to study quantitatively (Schwenneker and Hellenthal, 1984), but multi-metric assessment endpoints are used to avoid the difficulty of utilizing only population-based measurement endpoints. Section 7, Data Evaluation and Elliott (1971) should provide statistical principles for sampling and data analyses of benthic macroinvertebrates.

5.4.2 Quantitative estimates are obtained by using devices that sample a unit area or volume of the habitat. The major considerations are the size of the sampling units, the number of sampling units in each sample, and the location of sampling units in the sampling area. Grab samplers, stream-net samplers (e.g., Surber and related type samplers, Hess and related type samplers, and drift nets), and artificial substrate type samplers, are examples of devices that are used to collect samples quantitatively.

5.4.3 Sampling precision in the study of macroinvertebrate populations is affected by the substrate area encompassed by the sampling device and the patchiness in distribution of the organisms. The smaller the substrate surface area encompassed by a sampling device, the larger the number of sampling units required to obtain a desired level of precision (Elliott, 1971). Precision can be increased by collecting larger sampling units or by increasing the numbers of sampling units collected. A quantitative approach necessitates that a measure of the precision be obtained by replicate sampling. Replicate sampling in each habitat (habitat niche, microhabitat, or strata) selected for study is an absolute requirement.

5.4.3.1 For measurement of precision, three replicate random sampling units at each sampling station are an absolute minimum. Five replicates at each station would increase the statistical precision and accuracy. A series of single sampling units taken at discrete points along a transect do not represent replicate samples of benthic organisms unless it can be demonstrated that the physical characteristics of the habitat do not change along the transect.

5.4.4 The total number of samples depends on the degree of precision required, which will depend on the type of study and data quality objectives (DQOs). A reconnaissance or pilot study of the station may be necessary to help determine the sample size. Southwood (1966) gives a formula for determining the number of sampling units required for a specific level of precision.

5.4.5 The data from properly designed quantitative studies are amenable to the use of simple but powerful statistical tools that aid in maintaining the objectivity of the data evaluation process (see Section

7, Data Evaluation). The measures of precision and probability statements that can be attached to quantitative data reduce the possibilities of bias in the data evaluation process and make the results of different investigators more readily comparable. The advantages of quantitative methods are that they provide a measure of invertebrate diversity, biomass, and productivity, and their associated precision, thereby providing objective comparisons within, between, and among studies or intra- and interstudy comparisons.

5.4.6 No one sampling device is completely adequate to sample all types of habitat. When either qualitative, semi-quantitative, or quantitative devices are used, only selected portions of the environment are usually sampled. Also, because of the potential use of these data, experienced and skilled biologists are needed for sample collections.

## 5.5 Sampling Devices

### 5.5.1 Grab Samplers (Grabs)

5.5.2 Grabs are devices designed to penetrate the substrate by virtue of their own weight and leverage and have spring- or gravity-activated closing mechanisms. The jaws of grabs are forced shut by weights, lever arms, springs, or cables. All grabs are designed to take discrete "bites" or "scoops" of a defined area into the bottom sediment of a lake, stream, estuary, ocean, or similar habitats to sample the benthos. Scoops are grab samplers that scoop sediment with a rotating container. In shallow waters, some of these devices may be rigged on poles or rods and physically pushed into the substrate to a predetermined depth.

5.5.2.1 The number and kinds of macroinvertebrates collected by a particular grab may be affected by the habitat sampled, substrate type sampled, depth of penetration, angle of closure, completeness of closure of the jaws and loss of sample material during retrieval, creation of a "shock" wave and consequent "washout" of organisms at the surface of the substrate, and the effect of the high-flow velocities often encountered in rivers and wave action in large lakes and oceans on the stability of the sampler.

### 5.5.2 Selecting Grab Sampling Devices

5.5.2.1 Table 3 summarizes criteria for selecting grabs.

TABLE 3. SUMMARY CRITERIA FOR GRAB SAMPLERS

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1. Ponar Grab (Standard)

   A. Habitats and Substrates Sampled: Freshwater lakes, rivers, estuaries, and reservoirs with hard and soft sediments such as clay, hard pan, sand, gravel and muck; somewhat less efficient in softer sediments.

TABLE 3. SUMMARY CRITERIA FOR GRAB SAMPLERS (Continued)

B. Effectiveness of the Device: Not entirely adequate for deep burrowing organisms in soft sediments; very efficient for hard sediments; collects both qualitative and quantitative samples.

C. Advantages: Better penetration than other grabs; side plates and screens reduce washout, shock waves and substrate disturbance; best quantitative grab sampler for freshwater use.

D. Limitations: A very heavy grab that requires use of a boat with winch and cable; stones, pebbles and other debris can hold jaws open causing loss of sample.

2. Petite Ponar Grab

A. Habitats and Substrates Sampled: Freshwater lakes, rivers and reservoirs and estuaries with moderately hard sediments such as sand, silt and mud; will not penetrate clay; somewhat less efficient in soft sediments and coarse gravel.

B. Effectiveness of the Device: Not entirely adequate for deep burrowing organisms in soft sediments; not useful in clay.

C. Advantages: Good penetration for such a small grab; side plates and screens reduce washout, shock waves and substrate disturbance; can be operated by hand without boat or winch.

D. Limitations: Jaws can be blocked by stones, sticks and other debris causing loss of part of the sample; not efficient in swiftly flowing water of over one meter per second velocity.

Selected Literature: APHA, 1989; ASTM, 1990; Brinkhurst, 1967, 1974; Elliott et al., 1978, 1980, 1981b; Flannagan, 1970; Howmiller, 1971; Hudson, 1970; Lewis et al., 1982; Powers and Robertson, 1967; USEPA, 1973.

3. Ekman Grab (Standard, Tall, Large, and Extra-large)

A. Habitats and Substrates Sampled: Freshwater rivers, lakes and reservoirs where there is little current; soft sediments such as muck and silt.

B. Effectiveness of the Device: Efficient only in soft sediments but weights can be added for deeper penetration in fine sand; collects both qualitative and quantitative samples.

C. Advantages: Easy to operate by hand without winch, can be pushed into substrate in shallow water; hinged doors at top reduce washout, shock waves and disturbance of the substrate; comes in a range of sizes.

D. Limitations: Light weight so that jaw will not penetrate hard substrates; jaws often do not close completely due to blocking of jaws or failure of closing mechanism; inefficient in deep water or where there is even moderate current.

Wildco box corer resembles a heavy duty Ekman grab that has been designed to penetrate harder substrates with the addition of a frame and weights. The device can be used to collect infauna of lakes and estuaries. The box corer may also be used to sample finely divided muck, clays, mud, ooze, submerged marl, or fine peaty bottoms. The sampler weighs about 14 kg, but a maximum of 49 kg (12 removable weights) may be used. The sample area is 150 x 150 x 225 mm; a removable acrylic liner is included.

Selected Literature: APHA, 1989; ASTM, 1990; Beattie, 1979; Burton and Flannagan, 1973; Ekman, 1911, 1947; Flannagan, 1970; Howmiller, 1971; Hudson, 1970; Lanz, 1931; Lewis et al., 1982; Lind, 1974; Milbrink and Wiederholm, 1973; Paterson and Fernando, 1971; Rowe and Clifford, 1973; Rawson, 1947; Schwoerbel, 1970; Welch, 1948; USEPA. 1973.

4. Petersen Grab (Standard and Baby)

A. Habitats and Substrates Sampled: Freshwater lakes, reservoirs and rivers and estuaries with sand, gravel, clay and hard pan substrates.

B. Effectiveness of the Device: Less effective in most substrates than the Ponar, Baby Petersen effective in moderately soft sediments.

C. Advantages: Can give quantitative samples if used properly; range of sizes available.

D. Limitations: Standard grab is heavy and requires boat with winch; can cause washout if dropped rapidly to the bottom; shallow bite by jaws so that deeper burrowing organisms are not sampled; jaws are easily blocked by debris causing loss of sample; hard to use in adverse weather; of questionable value as a quantitative sampler.

Selected Literature: APHA, 1989; ASTM, 1990; Barnes, 1959; Birkett, 1958; Brinkhurst, 1974; Davis, 1925; Edmondson and Winberg, 1971; Elliott and Tullett, 1978; Holme and McIntyre, 1971; Hudson, 1970; Howmiller, 1971; Jensen, 1981; Lewis et al., 1982; Petersen, 1918; Petersen and Tensen, 1911.

5. Smith-McIntyre Grab

A. Habitats and Substrates Sampled: Marine and estuaries; adaptable

to large rivers, lakes and reservoirs with sand, gravel, clay and similar substrates.

B. Effectiveness of the Device: Limited penetration; has been widely used for sampling in marine and estuarine habitats.

C. Advantages: Provides reasonably quantitative samples; trigger plates help penetrate the substrate.

D. Limitations: Very heavy, needs boat and power winch; spring loaded jaws could be hazardous; inefficient for collecting deep burrowing organisms; jaws can be blocked by debris.

Selected Literature: APHA, 1989; ASTM, 1990; Carey and Heyamoto, 1972; Carey and Paul, 1968; Elliott and Tullett, 1978; Holme, 1964; Hopkins, 1964; Hunter and Simpson, 1976; McIntyre, 1971; Smith and McIntyre, 1954; Tyler and Shackley, 1978; Wigley, 1967; Word, 1976, 1977; Word et al., 1976.

6. Van Veen Grab

A. Habitats and Substrates Sampled: Marine and estuaries with sand, gravel, mud, clay and similar substrates; could be adapted to freshwater.

B. Effectiveness of the Device: Penetrates to a depth of 5 to 7 cm.

C. Advantages: Jaws close better than the Petersen Grab; samples most types of sediments; comes in a range of sizes.

D. Limitations: A very heavy grab that requires a large boat and power winch; jaws may become blocked by debris such as rocks and sticks; not useful for deep burrowing organisms.

Selected Literature: APHA, 1989; ASTM, 1990; Barnes, 1959; Beukema, 1974; Birkett, 1958; Elliott and Drake, 1981b; Elliott and Tullett, 1978; Holme, 1971; Lassig, 1965; Longhurst, 1959; McIntyre, 1956; Nichols and Ellison, 1966; Schwoerbel, 1970; Ursin, 1954; Wigley, 1967; Word, 1976a, 1976b; Word et al., 1976.

7. Orange-Peel Grab

A. Habitats and Substrates Sampled: Marine waters and deep lakes with sandy substrates containing cobble, rubble and coarse gravel.

B. Effectiveness of the Device: For qualitative use only; sampling area not constant.

TABLE 3. SUMMARY CRITERIA FOR GRAB SAMPLERS (Continued)

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C. Advantages: Comes in a range of sizes; works well in deep water; closes relatively well to prevent loss of sample; good for reconnaissance.

D. Limitations: Very heavy so that large boat with power winch and cable lines is required; does not sample constant area and depth.

Selected Literature: APHA, 1989; ASTM, 1990; Briba and Reys, 1966; Elliott and Tullett, 1978; Hartman, 1955; Hopkins, 1964; Merna, 1962; Packard, 1918; Reish, 1959; Thorson, 1957, Word, 1976, 1977.

8. Shipek Grab

A. Habitats and Substrates Sampled: Estuaries and large deep lakes with sand, gravel, mud and clay substrates.

B. Effectiveness of the Device: A relatively good quantitative sampler.

C. Advantages: Good for collecting a small sample in deep water.

D. Limitations: A heavy grab that requires the use of a boat with a power winch; must be lowed vertically so is not effective in moving water; inefficient for collecting deep burrowing organisms; samples small area.

Selected Literature: APHA, 1989; ASTM, 1990; Barnes, 1959; Elliott and Tullett, 1978; Flannagan, 1970; Holme, 1964, 1971; Holme and McIntyre, 1971.

5.5.3 Precautions

5.5.3.1 Always inspect grabs for mechanical defects prior to use.

5.5.3.2 Exercise caution at all times when handling grabs.

5.5.4 Significance and Use of Grabs

5.5.4.1 Qualitative and quantitative samples of macroinvertebrates inhabiting sediments or substrates are may be taken by grabs. Grab samplers, if used correctly, are devices that sample a unit area of the habitat. In view of the advantages and limitations regarding the penetration of the sediment by many grabs and their closing mechanisms, it is not possible to recommend any single instrument as suitable for general use. However, the Petersen grab is considered the least effective bottom grab sampler and, therefore, has limited application. The type and size of the grab sampler or device selected for use will depend on such factors as the size of boat, hoisting gear available, the type of substrate or sediment to be sampled, depth of water, current

velocity, and whether sampling is in sheltered areas or in open waters of large rivers, reservoirs, lakes, or oceans. The choice of grab will depend largely on what is available, what is suitable for the sampling area, and what can be used with the least difficulty.

## 5.6 Commonly Used Grabs

5.6.1 The ponar grab sampler (Fig. 5A,B) is most commonly used for sampling macroinvertebrates from sediments in lakes, rivers, reservoirs, estuaries, and oceans with coarse and hard substrates, such as coarse sand, gravel, and similar substrates, rather than soft sediments, such as mud, fine sand, or sludge. The sampler can be used in moderate currents and deep waters.

5.6.1.1 The Ponar grab sampler has paired jaws that must penetrate beneath the surface of the substrate without disturbing the water surface boundary layer, close when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The device has side plates and a screen on the top of the sample compartment to prevent loss of the sample during closure. With one set of weights, this heavy steel sampler can weigh 20 Kg. Word et al. (1976a) reports that the large amount of surface disturbance associated with Ponar grabs can be greatly reduced by simply installing hinges rather than fixed screen tops, which will reduce the pressure wave associated with the sampler's descent into the sediment. The standard Ponar takes a sample area of 523 $cm^2$. A small version, the petite Ponar grab, takes a sample area of 232 $cm^2$ and can be used in habitats where there may be an unusual abundance of macroinvertebrates, thus eliminating the need to subsample.

5.6.1.2 When not in use, a safety pin lock attached to the lever bar prevents closing of the sampler until the pin is removed.

5.6.1.3 The weight of the standard Ponar grab makes it necessary to use a winch and cable or portable crane for retrieving the sample, and ideally the samples should be taken from a stationary boat or platform. The smaller version, petite Ponar grab, is designed for hand-line operation, but it may be used with a winch and cable.

5.6.2 The Ekman grab sampler (Fig. 5C) is used to obtain samples of macroinvertebrates from soft sediments, such as very fine sand, mud, silt, and sludge, in lakes, reservoirs, estuaries, and similar habitats where there is little current. This grab is inefficient in deep waters, under adverse weather conditions, and in waters of moderate to strong currents or wave action. The Wildco box corer (Fig. 5D) is like a heavy duty Ekman with a frame and weights and is used to collect macroinvertebrates in lakes and estuaries. Because of its weight a winch is necessary for retrieving the sample from a stationary boat or platform.

5.6.2.1 The Ekman grab sampler is a box-shaped device with two scoop-like jaws that must penetrate the intended substrate without disturbing

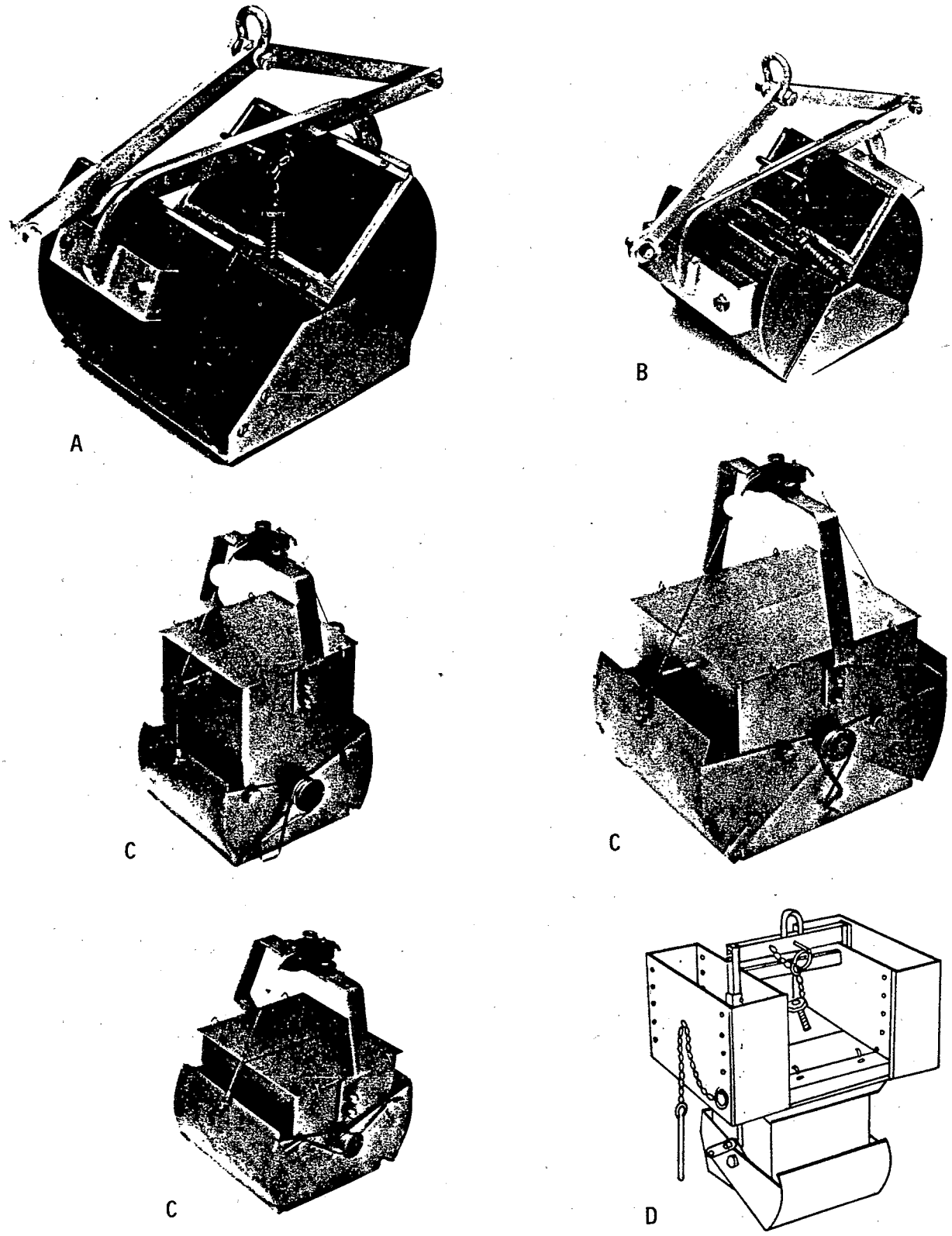



Figure 5. Grab Samplers. (A) Standard Ponar; (B) Petite Ponar; (C) Large, tall, and standard Ekman grabs; (D) Wildco box corer

the water surface boundary layer, close when positioned properly on the bottom, and retain a discrete sample of sediment while it is brought to the surface for processing. Hinged doors on the top of the grab prevents washout during sample lowering and retrieval. The grab is made of 12 to 20 gauge brass or stainless steel and weighs approximately 3.2 Kg. The box-like part holding the sample has spring-operated jaws on the bottom that must be manually set. The sampler is available in several sizes; however, in very soft substrates only a tall model should be used, either a 23 cm or a 30.5 cm model. Ekman is not used with a winch very often but can be operated from a boat with a winch and cable.

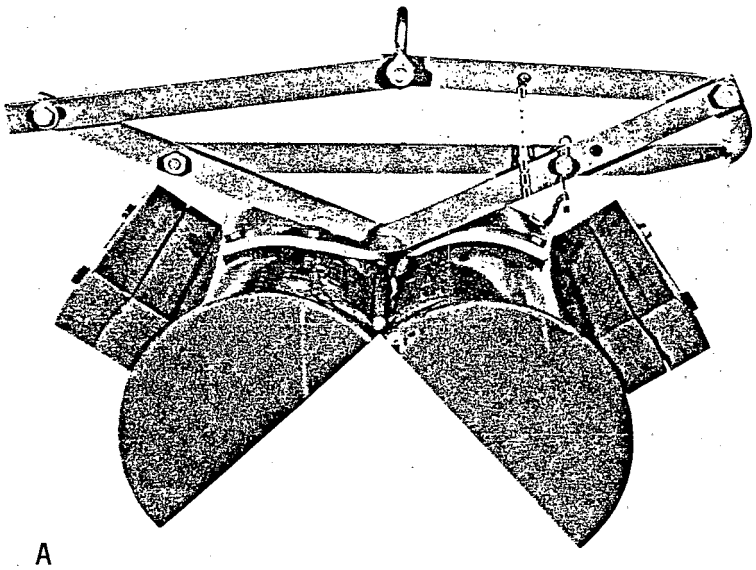
5.6.2.2 Exercise caution at all times once the grab is loaded or cocked because a safety lock is not part of the standard design.

5.6.3 The Petersen grab sampler (Fig. 6A,B) is designed to obtain samples of macroinvertebrates from sediments in lakes, reservoirs, and similar habitats and is adaptable to rivers, estuaries, and oceans. This grab sampler has limited application, and is not recommended for quantitative benthic work and must be used with due consideration of its defects when quantitative estimates are attempted. It is useful for sampling sand, gravel, marl, and clay in moderate currents and deep waters, the sampler cannot be used under adverse weather conditions. This sampler is available in a range of sizes that will sample an area from 0.06 to 0.099 $m^2$. A consensus of aquatic biologists consider the use of this device the least preferable grab sampler and would use it only in limited applications.

5.6.3.1 The Petersen grab sampler has paired jaws that must penetrate the intended substrate without disturbing the water surface boundary layer, close when positioned properly on the bottom, and retain the sample of sediment while it is brought to the surface for processing. This heavy steel device can weigh 13.7 Kg, but may weigh as much as 31.8 Kg when auxiliary weights are bolted to its side. The extra weights are to make the grab stable in swift current and to give additional cutting force in firm bottom sediments. It has been suggested that users of this device modify it by the addition of end plates and by cutting large strips out at the top of each side and adding hinged 30 mesh screen as in the Ponar grab. It is necessary to use a winch and cable to lower and raise the sampler.

5.6.3.2 Newer versions of the Petersen grab sampler may have a screened window at the top of each jaw to allow water to escape while the grab is descending and closing. While some modifications may close or function better, the sampling characteristics remain the same. Most of the modified versions are intended for use in estuarine and marine waters.

5.6.3.3 Ideally a stationary boat or platform should be used when taking samples. The modified Petersen devices are designed to be quite heavy and require heavy gear and a large vessel for efficient operation. A small version can be hauled aboard by hand and held with one hand for washing procedures.



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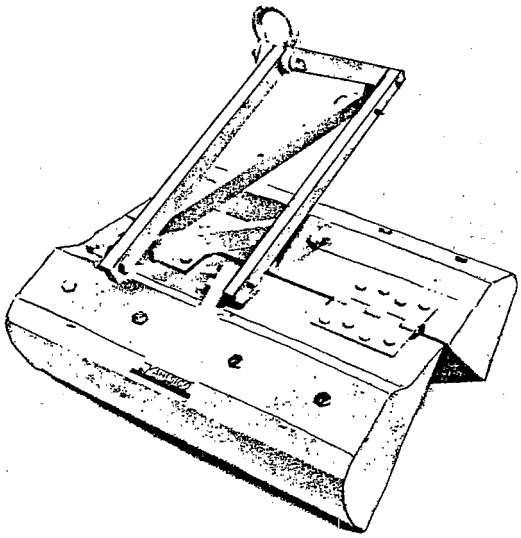


B

Figure 6. Grab Samplers: (A) Original Petersen; (B) Modified Petersen

5.6.4 The Smith-McIntyre grab sampler (Fig. 7A) is designed to obtain samples of macroinvertebrates from sediments in rough weather and deep water in lakes, rivers, estuaries, and oceans. This device samples a surface area of 0.1 $m^2$ and is useful for sampling macroinvertebrates from sand, gravel, mud, clay, and similar substrates.

5.6.4.1 The Smith-McIntyre grab sampler has paired jaws that are forced to penetrate into the intended substrate by two "loaded" strong coiled springs, must close when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The device is heavy and can weigh 45.4 Kg or more. The chief advantages of the sampler are its stability and easier control in deep and rough waters. The spring-loaded jaws of the Smith-McIntyre grab must be considered a hazard and caution should be exercised when using the device. Due to the weight and size, this device must be used from a vessel with boom and lifting capabilities.

5.6.4.2 The Smith-McIntyre grab sampler is fitted with gauze panels or free swinging panels on the top to reduce the shock wave during descent.

5.6.4.3 Larger Smith-McIntyre grabs can be constructed depending on the type of bottom to be sampled and additional weights can be fitted to the frame of the grab sampler for additional penetration into the sediment.

5.6.5 The Van Veen grab sampler (Fig. 7B) is used to obtain samples of macroinvertebrates from sediments in estuaries and other marine habitats, and is adaptable to freshwater areas. It can also be used for qualitative sampling. This device is useful for sampling sand, gravel, mud, clay and similar substrates and is available in two sizes, 0.1 $m^2$ and 0.2 $m^2$. Larger and double versions of this grab are available, and their use is dependent upon the type of bottom to be sampled, and the type of vessel available to deploy this sampler.

5.6.5.1 The Van Veen grab sampler has paired jaws that must penetrate the intended substrate without disturbing the water surface boundary layer of the substrate, close by pincher-like action of two long arms when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The long arms give added leverage for penetrating hard sediments. The advantage of using the twin Van Veen is that with a single lowering, two separate bottom sediment sampling units can be collected from the same station.

5.6.5.2 The Van Veen is basically an improved version of the Petersen grab in that long arms have been attached to the jaws to stabilize the grab on the bottom in the open sea just prior to or during closure of the device. Additional weights can be applied to the jaws to effect greater penetration in sediments.

5.6.6 The Orange-Peel grab sampler (Fig. 7C) is used primarily in marine waters and deep lakes where it has advantages over other grabs when sandy substrates are sampled, but it cannot be used under adverse

weather conditions. This grab should not be used in critical quantitative work that is to be compared with results of other areas and is recommended as a reconnaissance sampler only. The sampler is available in a range of sizes but the 1600 $cm^3$ is generally used, although larger sizes are available.

5.6.6.1 The Orange-Peel grab sampler has four curved jaws that close to encircle a hemisphere of sediment. It must penetrate the intended substrate without disturbing the water surface boundary layer, close when positioned properly on the bottom, and retain discrete samples of sediment while it is brought to the surface for processing. The top of the sampler is enclosed by a canvas bag, serving as a portion of the sample compartment. When taking samples, a stationary boat or platform should be used.

5.6.6.2 A recent modification of the Orange-Peel, described by Reish (1959) has a new trigger mechanism and more efficient closing jaws, and the volume of sample to surface-area sampled relationship has been worked out.

5.6.6.3 The surface area sampled by this device varies with penetration depth or volume sampled. The device penetrates to a maximum depth of 18 cm, but depth of penetration will vary.

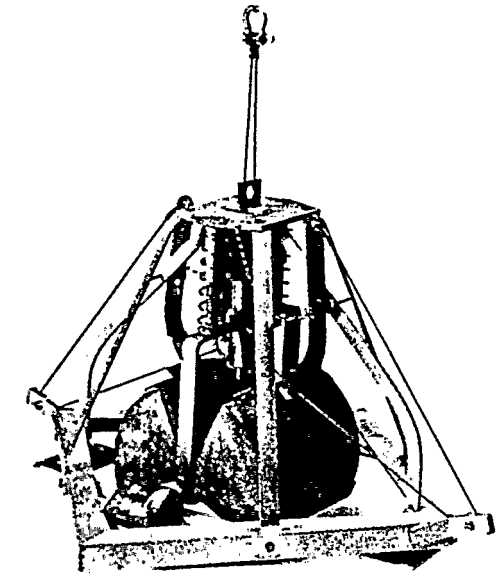
5.6.7 The Shipek (scoop) grab sampler (Fig. 7D) is designed to obtain samples of macroinvertebrates from sediments in marine waters and large inland bodies of water. This device is useful for sampling macroinvertebrates from sand, gravel, mud, clay, and similar substrates. It is designed to take a sediment sample with a surface area of 20 $cm^2$ to approximately 10 cm deep at the center.

5.6.7.1 The Shipek (scoop) grab sampler consists of a semi-cylindrical scoop that must be positioned properly on the bottom to take a scoop and retain discrete samples of sediment through 180°. Holmes and McIntyre (1971) report that this device is usually used by geologists to collect small samples rather than by biologists. However, it can be used in marine waters and large inland lakes, reservoirs, and rivers. Unlike many other types of samplers, closure of the device is made at the side, rather than at the bottom. This sampler cannot be used under adverse wind and wave conditions. The sampler requires a vessel with a winch and cable.

5.6.8 General Operating Procedures

5.6.8.1 Most grabs are heavy sampling devices that should be operated using a hand or powered winch and cable from a boat. In large bodies of water ships are used for this operation.

5.6.8.2 Grabs must be lowered slowly because free-fall may airplane the device, causing the device to land improperly or causing a pressure wave and blowout of the surface layer of sediment when the grab reaches the



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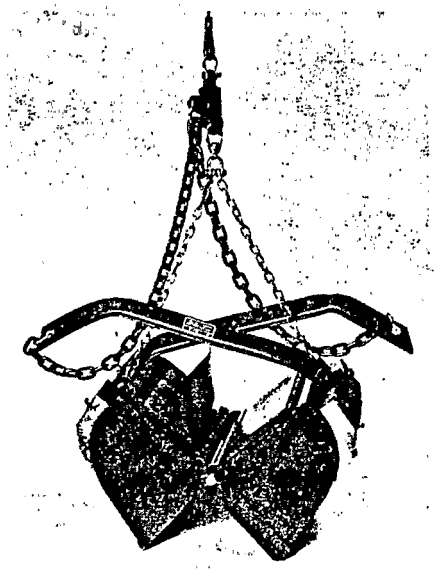

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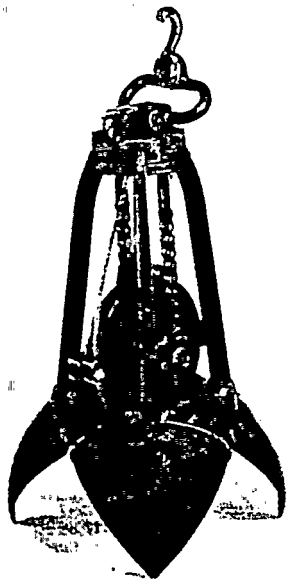

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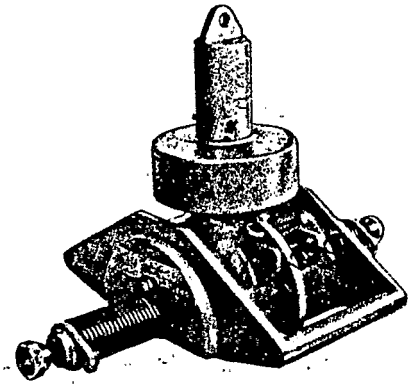

D

Figure 7. Grab Samplers: (A) Smith-McIntyre; (B) Van Veen; (C) Orange-Peel; (D) Shipek

bottom. In order for the device to operate effectively, it must bite vertically.

5.6.8.3 When most grabs reach the bottom, their weight will cause them to penetrate the substrate, and the slack-off on the cable allows the locking lever to release, therefore permitting the movement that allows the horizontal locking bar to drop out of the locking notch and allow the jaws to close as the device is raised. Other grabs are closed by spring action or some other mechanical device after penetrating the substrate.

5.6.8.4 In the Ekman grab the jaws are cocked by raising them upward into the cocked position using the attached cable and securing the cable to the catch pin located at the top of the sampler. Once on the bottom, indicated by a slack line, a messenger is sent down the line tripping the catch mechanism, causing the spring loaded jaws to close the bottom of the sampler and contain the sediment.

5.6.8.5 The Smith-McIntyre grab is "loaded" by compressing the large coil springs mounted on the instrument with the loading bar. As soon as the spring is loaded, the safety pin is inserted to prevent the accidental triggering of the bottom plates. Once the device is overboard, just prior to lowering to the bottom, the safety pins are removed. When the trigger plates contact the bottom, pressure on these plates releases the two coiled springs that drive the buckets (jaws) into the sediment. Closure of the sampler is made at the side, rather than at the bottom. After closure the sample is given optimum protection from washout during the return trip to the surface by the cylindrical configuration of the sampler. Once on deck, the sampler is placed on a stand; the sample buckets can be disengaged from the rest of the device by releasing two retaining latches at each end of the upper semicylinder, and the sample is dumped into a large basin or washtub and prepared for processing. After the sample has been removed, the springs may then be loaded and the safety pins installed.

5.6.8.6 The chains from the jaws of the Van Veen are attached to the counter balance mechanism, as are the slackened wires from the long arms. Tension is carefully applied to the trigger mechanisms as the sampler is winched off its platform, and once the tension is firmly changed from the jaws, the grab is relatively stable in the cocked position. Care should be exercised in lowering the Van Veen through the surface of the water as occasionally contact will produce slack in the chain that will trip the counter balance mechanism. The grab is lowered slowly to the bottom, and once it makes contact with the bottom, the grab is winched in initially closing the jaws containing the sediment. Retrieve the grab slowly to prevent washout.

5.6.8.7 The Shipek grab is composed of two concentric half cylinders, the inner semicylinder is rotated at high torque by two spirally wound external springs. Upon contact with the bottom, the two external springs are automatically released by the inertia of a self-contained weight upon a sear mechanism which trips the catch and the scoop rotates

upward. At the end of its 180° travel, the sample bucket is stopped and held at the closed position by residual spring torque. After closure the sample is given optimum protection from washout. The scoop is disengaged from the upper semicylinder by releasing the two retaining latches at each end of the upper semicylinder.

5.6.8.8 Once on board, the sample is placed into either a suitable container or a sieving device directly for processing (see Section 6). Thoroughly wash or hose the grab with water, so that all sediment materials are included in the sample before a replicate sample is taken.

## 5.7 Stream-Net Samplers

5.7.1 Stream-net samplers are lotic collecting devices, fitted with a net of various mesh sizes that collect organisms from flowing water passing through the sampler.

5.7.2 Selecting Stream-Net Sampling Devices

5.7.2.1 Table 4 summarizes criteria for selecting stream-net sampling devices.

TABLE 4. SUMMARY CRITERIA FOR STREAM-NET SAMPLERS

1. Surber Sampler

   A. Habitats and Substrates Sampled: Shallow, flowing streams, less than 32 cm in depth with good current; rubble substrate, mud, sand, gravel.

   B. Effectiveness of Device: Relatively quantitative when used by experienced biologist; performance depends on current and substrate.

   C. Advantages: Encloses area sampled; easily transported or constructed; samples a unit area.

   D. Limitations: Difficult to set in some substrate types, that is, large rubble; cannot be used efficiently in still, slow moving streams.

2. Portable Invertebrate Box Sampler, Hess Sampler, Hess Stream Bottom Sampler, and Stream-Bed Fauna Sampler

   A. Habitats and Substrates Sampled: Same as Surber.

   B. Effectiveness of Device: Same as Surber.

   C. Advantages: Same as above except completely enclosed with stable platform; can be used in weed beds.

TABLE 4. SUMMARY CRITERIA FOR STREAM-NET SAMPLERS (continued)

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D. Limitations: Same as Surber.

Selected Literature: APHA, 1989; ASTM, 1990; Canton and Chadwick, 1984; Elliott and Tullett, 1978; Ellis and Rutter, 1973; Hess, 1941; Kroger, 1972; Lane, 1974; Merritt et al., 1984; Needham and Usinger, 1956; Pollard and Kinney, 1979; Rutter and Ellis, 1977; Rutter and Poe, 1978; Rutter and Ettinger, 1977; Resh, 1979; Resh et al., 1984; Schwenneker and Hellenthal, 1984; Surber, 1937, 1970; Usinger, 1963; Waters and Knapp, 1961; Welch, 1948; Winner et al., 1980.

3. Drift Nets

A. Habitats and Substrates Sampled: Flowing rivers and streams; all substrate types.

B. Effectiveness: Relatively quantitative and effective in collecting all taxa which drift in the water column; performance depends on current velocity and sampling period.

C. Advantages: Low sampling error; less time, money, effort; collects macroinvertebrates from all substrates, usually collects more taxa.

D. Limitations: Unknown where organisms come from; terrestrial species may make up a large part of sample in summer and periods of wind and rain; does not collect non-drifting organisms.

Selected Literature: Allan, 1984; Allan and Russek, 1985; APHA, 1989, ASTM, 1990; Bailey, 1964; Berner, 1951; Brittain and Eikeland, 1988; Chaston, 1969; Clifford, 1972a,b; Coutant, 1964; Cushing, 1963, 1964; Dimond, 1967; Edington, 1965; Elliott, 1965, 1967; 1969, 1970; 1971; Elliott and Minshall, 1968; Ferrington, 1984; Hales and Gaufin, 1969; Hemsen, 1956; Hildebrand, 1974; Holt and Waters, 1967; Hynes, 1970; Keefer and Maughan, 1985; Larimore, 1972, 1974; Larkin and McKone, 1985; Lehmkuhl and Anderson, 1972; McLay, 1970; Merritt et al., 1984; Minshall and Winger, 1968, Modde and Schulmbach, 1973, Muller, 1965, 1974, Mullican et al., 1967; Mundie, 1959, 1964; Pearson and Franklin, 1968; Pearson and Kramer, 1969, 1972; Pearson et al., 1968; Pfitzer, 1954; Radford and Hartland-Rowe, 1971; Reisen and Prins, 1972; Resh, 1979; Resh et al., 1984; Spence and Hynes, 1971; Tanaka, 1960; Tranter and Smith 1968; USEPA, 1973; Waters, 1961, 1962, 1964, 1965; 1966; 1968, 1969a,b, 1972; Wilson and Bright, 1973; Winner et al., 1980; Wojtalik and Waters, 1970.

5.7.3 The Surber, portable invertebrate box, Hess, Hess stream bottom, and stream-bed fauna samplers (Fig. 8A-E) were designed as quantitative samplers when carefully used by an experienced biologist; however, they

are more often used to collect qualitative samples or semi-quantitative samples because of the large number of samples needed for an acceptable level of precision (Needham and Usinger, 1956). They outline a definite unit-area for collecting the macroinvertebrates within the area. They are designed to be placed by hand onto or in some cases into sand, gravel, or rubble substrate types (usually in riffle/run areas) in shallow streams, or shallow areas of rivers. The drift net sampler (Fig. 8F) is a qualitative and quantitative collecting device used to capture drifting organisms in flowing waters. It differs from the other net type samplers in that it collects from a unit volume of water rather than from a unit area of bottom.

5.7.4 Significance and Use of Stream-Net Samplers

5.7.4.1 The significance of using stream-net samplers is to collect macrobenthos inhabiting a wide range of habitat types from shallow flowing streams or shallow areas in rivers. The stream-net devices (Surber, portable invertebrate box, Hess, Hess stream bottom, and stream-bed fauna samplers) are unit area samplers used for collecting benthic organisms in certain types of substrates. They may be used to obtain estimates of the standing crop, for example, biomass, number of individuals and number of taxa of benthic macroinvertebrates per unit area of stream bottom. Efficiency of the sampler depends on the experience and ability of the user. Drift net samplers are designed to collect emigrating or dislodged benthic macroinvertebrates inhabiting all substrate types that either actively or passively enter the water column in flowing streams and rivers and is used to determine drift density and drift rate.

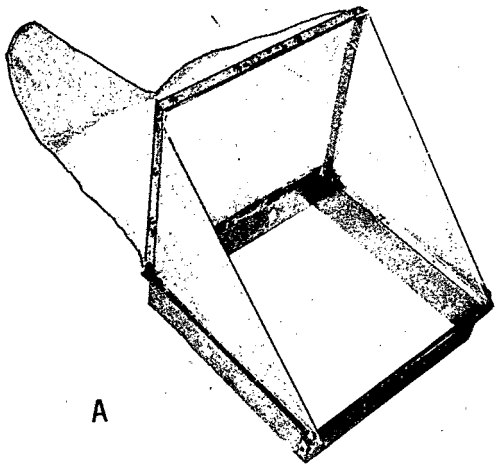
5.7.5 Description of Surber Type Samplers

5.7.5.1 The Surber sampler consists of two 30.5-cm frames, hinged together; one frame rests on the substrate, the other remains upright and holds the nylon net. The sampler is positioned with its net mouth open, facing upstream. When in use, the two frames are locked at right angles, one frame marking off the area of substrate to be sampled and the other frame supporting a net to strain out organisms washed into it from the sample area.

5.7.5.2 Modification of the Surber sampler to overcome some of the limitations of its use (for example, loss of organisms due to backwash) has resulted in the design and construction of a number of related sampling devices, such as the four-sided (enclosed) portable invertebrate box sampler, the cylindrical Hess sampler, the cylindrical Hess stream bottom sampler, and the cylindrical stream-bed fauna sampler. These devices sample 0.1 $m^2$.

5.7.5.3 Operation of the portable invertebrate box, Hess, Hess stream bottom, and stream-bed fauna samplers are similar to the Surber sampler.

5.7.5.4 The net used to collect macroinvertebrates can vary in mesh size, length, taper, and material, for example, canvas, taffeta, or



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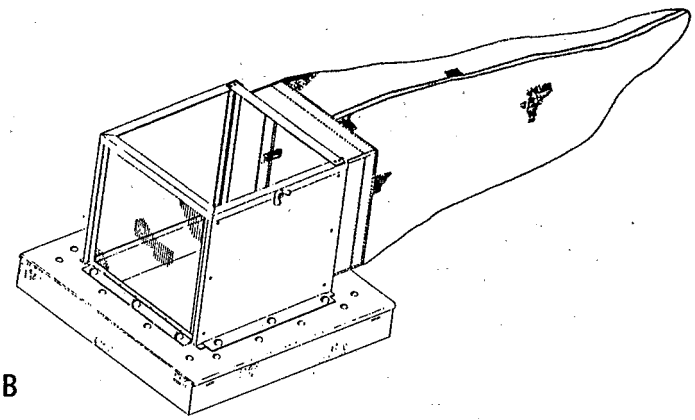

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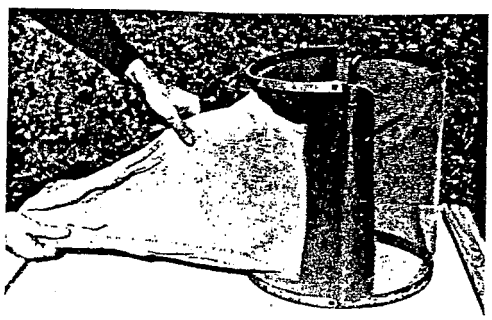

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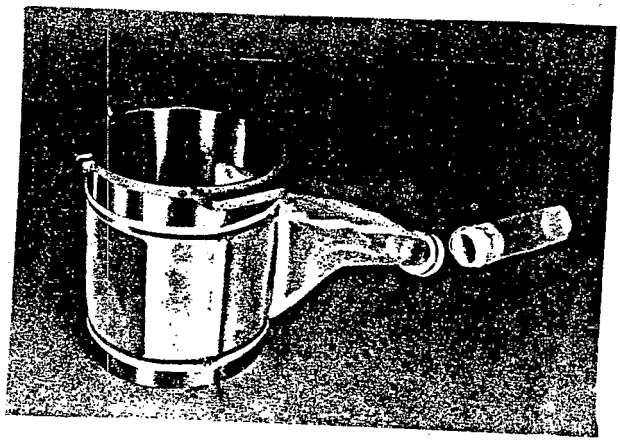

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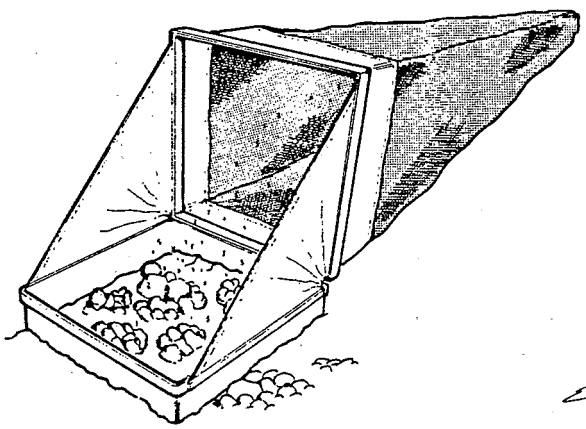

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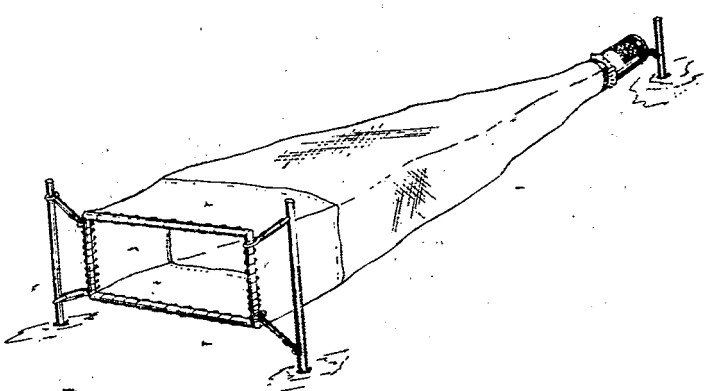

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Figure 8. Stream-Net Samplers: (A) Surber sampler; (B) Portable invertebrate box sampler; (C) Hess sampler; (D) Hess stream bottom sampler; (E) Stream-bed fauna sampler (F) Drift net

nylon monofilament. It is usually made of nylon, and a variety of mesh sizes is available. The mesh size used will depend on the objectives of the study. A mesh size of 0.35 mm, for example, will retain most instars of aquatic insects.

5.7.5.6 While a smaller mesh size might increase the number of smaller invertebrates and young instars collected, it will clog more easily and exert more resistance to the current than a larger mesh, possibly resulting in a loss of organisms due to backwashing from the sample net.

5.7.5.7 The polyester foam base of the portable invertebrate box sampler conforms to a variety of substrates to prevent the loss of organisms from beneath the sampler. The Hess, Hess stream bottom, and stream-bed fauna samplers can be "turned" into most sediment types to a depth of several centimeters. The Surber sampler rests on the surface of most sediments.

5.7.5.8 When sampling is completed, the net of the portable invertebrate box sampler slides out for cleaning or exchange with a different net. Hess-type samplers may have a mason jar ring and an adapter with a fixed or removable cloth net bucket. Some of the stream-net samplers have fixed nets.

5.7.5.9 These samplers cannot be used as efficiently in still or deep water of more than 30.48 cm (1-ft) depth. If the water depth is greater than 30.48 cm (1-ft), benthic organisms may wash over the top of the net rather than into it.

5.7.5.10 While there can be large sampling errors associated with their use by an inexperienced operator, these samplers can provide data which are precise and comparable if they are used consistently by one experienced person in similar habitats.

5.7.5.11 If the water velocity is very great, resistance provided by the small mesh of the net or debris washed into it, or both, may result in a backwashing effect that washes benthic organisms out of the sample area of the Surber sampler or over the top of the other samplers.

5.7.6 General Operating Procedures

5.7.6.1 Position these samplers securely on the substrate, parallel to the flow of the water, with the net pointing downstream.

5.7.6.2 The samplers are brought down quickly to reduce the escape of rapidly moving organisms.

5.7.6.3 There should be no gaps under the edges of the frame that would allow for washing of water under the net and loss of benthic organisms. Eliminate gaps that may occur along the edge of the Surber sampler frame by careful shifting of rocks and gravel along the outside edge of the sampler. This is also true of the cylindrical-type samplers if they are on rubble substrate that makes turning into the bottom difficult. The

portable invertebrate box sampler polyester foam pad can conform to a relief of 7.6 cm (3 in.).

5.7.6.4 Take care not to disturb the substrate upstream from the sampler, to avoid excessive drift into the sampler from outside the sample area.

5.7.6.5 Once the sampler is positioned on the stream bottom, it should be maintained in position during sampling so that the area delineated remains constant.

5.7.6.6 Hold the Surber sampler with one hand or brace with the knees from behind. The Hess, Hess stream bottom, and stream-bed fauna samplers, and the portable invertebrate box samplers can be held with one hand or braced with the knees from the sides. The portable invertebrate box sampler also can be sat upon for convenience while sampling; this provides the collector with a stable sampling platform that allows maximum manipulation of the substrate with little sampler movement.

5.7.6.7 Heavy gloves should be required when handling dangerous debris; for example, glass or other sharp objects present in the sediment.

5.7.6.8 Turn over and examine carefully all rocks and large stones and rub carefully in front of the net with the hands or a soft brush to dislodge the organisms and pupal cases, etc. clinging to them before discarding. Scrape attached algae, insect cases, etc., from the stones into the sample net.

5.7.6.9 Wash larger components of the substrate within the enclosure with stream water; water flowing through the sampler should carry dislodged organisms into the net.

5.7.6.10 Stir the remaining gravel and sand vigorously with the hands to a depth of 10 cm (4.0 in.) where applicable, depending upon the substrate, to dislodge bottom-dwelling organisms.

5.7.6.11 It may be necessary to hand pick some of the heavier mussels and snails that are not carried into the net by the current.

5.7.6.12 Remove the sample by inverting the net (or washing out sample bucket, if applicable) into the sample container (wide-mouthed jar) with 10% buffered formalin fixative or 70-80% ethanol.

5.7.6.13 Examine the net carefully for small organisms clinging to the mesh, and remove them (preferably with forceps to avoid damage) for inclusion in the sample.

5.7.6.14 Rinse the sampler net after each use.

**5.8 Drift Nets**

5.8.1 Significance and Use of Drift Nets

5.8.1.1 Macroinvertebrate drift is a normal feature of flowing waters (Brittain and Eikeland, 1988). Drift of organisms may be used to assess environmental stress or pollution in some situations. Stress, fluctuations in water level, changes in light intensity, and changes in temperature are the basic factors that influence the extent of macroinvertebrate drift.

5.8.1.2 One source of drifting macroinvertebrates is the immature insects in the final stages of metamorphosis that actively seek to reach the water surface where emergence to the adult stage occurs. Regular periodic downstream drift rate of immature insects and other macroinvertebrate fauna in slow-moving streams or rivers is markedly reduced in comparison to lotic habitats with rapidly flowing water.

5.8.2.3 Drift insects are about evenly distributed at all levels in a stream, but in large rivers drift is more abundant near the bottom in the shore-line zone.

5.8.2.4 It is generally found that there are pulses of drift organisms that move from top to bottom of the water column, at least during periods of low flow.

5.8.2.5 Drift collections can be used to determine drift density, rate, and periodicity of drift organisms, and interesting aspects of the organisms' life histories, for example, period of transformation.

5.8.2.6 Drift nets are useful for collecting macroinvertebrates that actively or passively enter the water column or that are dislodged from the substrate; naturally or by stress. They are particularly well-suited for synoptic surveys because they are light weight and easily transported.

5.8.2.7 The first step in interpreting drift data is to determine the respective contributions of constant, behavioral, and catastrophic drift to the samples being analyzed.

5.8.2.8 Only constant and behavioral drift are usually utilized in a synoptic survey, but catastrophic drift is extremely important in testing for recent discharges of toxic materials.

5.8.2.9 Bear in mind that the drift density may not be a function of the total bottom population density or of production; however, species composition of the drift is useful as an index of species composition of the benthos.

5.8.2.10 Density and composition of invertebrate drift are influenced by many factors that also must be considered when interpreting the data, including stage of life cycle, weather, time of day, light intensity, population density, temperature, turbidity, water level fluctuation, season, current velocity, growth rate, photoperiod, and proximity to

tributary streams.

5.8.2.11 In an enriched stream there is usually a marked increase in total numbers and biomass of drifting organisms as the stream becomes more polluted. Intolerant forms decrease and pollution tolerant forms increase proportional to changing water quality.

5.8.2.12 Thousands of organisms, including larvae of stoneflies, mayflies, caddisflies, and midges and other Diptera, may be collected in a sampling period of only a few hours.

5.8.2.13 The drift net efficiently collects organisms originating from all types of substrates upstream and a wide spectrum of microhabitats in lotic (flowing) waters.

5.8.2.14 The device is restricted to flowing rivers or streams with a current velocity of more than 0.05 m/s.

5.8.3 Advantages of Using Drift Nets

5.8.3.1 A benthic sample shows only which taxa were existing in the particular area (usually some fraction of a square meter, etc.) that was sampled. The great variation among benthic samples, even in a limited area, illustrates the necessity of several samples and the influence of selecting the collecting stations. One drift sample might be adequate for collecting the majority of invertebrate taxa in a stream reach, whereas a large number of benthic samples would be needed to cover the variety of bottom habitats even in an uniform reach of the stream.

5.8.3.2 Quantitative benthic sampling is seldom extended to include stream banks, organic substrates (logs, etc.), and areas of dense vegetation. The drift net collects organisms from all these areas.

5.8.3.3 Drift net collections often require much less sorting work than a series of grab samples. Drift samples do not require the laborious, time-consuming job of washing out silts, clays, and other materials and of sorting and picking through much of the debris for the organisms in the samples.

5.8.3.4 Nets are light-weight and easy to set up in a stream and usually yield a light-weight sample free from most debris. Benthic sampling in flowing water often procures samples heavy with inorganic materials.

5.8.3.6 A drift net is inexpensive to construct, whereas bottom samplers are often costly and more than one kind may be required to adequately sample the multiple habitat types present in a stream or river.

5.8.4 Limitations of Use of Drift Nets

5.8.4.1 Certain aquatic organisms enter the drift only sporadically and

might be missed even though common in the benthos.

5.8.4.2 The relative abundance of macroinvertebrates in a drift sample often differs significantly from their "relative" abundance on the stream bottom.

5.8.4.3 A slight current is necessary if a drift collection is to be taken (greater than 0.05 m/s).

5.8.4.4 Most species drift more abundantly at night, so that the best collections are usually taken in the dark. Time of sampling depends on the purpose of the study. Day samples are usually adequate for showing effects of pollution on the stream reach.

5.8.4.5 There is a waiting period while the drifting organisms accumulate in the net, but not as long as with using artificial substrates.

5.8.4.6 Tree leaves in the autumn, floating and anchor ice in the winter, and heavy debris (logs) during floods may interfere with drift net collecting and make processing difficult.

5.8.4.7 The abundance and composition of drift changes, daily, hourly, or seasonally and might prevent direct comparison of collections taken at different times. At times certain life stages of an organism might not be fairly represented in the drift. The same holds true for other types of sampling.

5.8.4.8 Drift collections give little precise habitat information for individual organisms, since the exact source of the individual is not known.

5.8.4.9 Collections of drift, with the organisms originating an indefinite distance above the collecting site, may not show local or temporary deleterious effects imposed on an aquatic community, whereas bottom samples might reveal the destruction or reduction of benthos in a small area. Studies have shown that most drift organisms originate from only several meters upstream from the nets (Elliott, 1967).

5.8.5 Description of Drift Nets

5.8.5.1 The typical drift net consists of a bag of nylon or nylon monofilament. The drift net generally preferred is the simple rectangular net which is light-weight, easy to install, and gives an adequate sample of the drifting macroinvertebrates. The U.S. Standard No. 30 (0.595-mm mesh openings) net is often used for collecting macroinvertebrates.

5.8.5.2 Drift nets vary in size, but the type recommended for use in water pollution surveys or other ecological assessments has an upstream opening of 15 by 30 cm, and the collection bag is 1.3 m long. A variety of mesh sizes is available, and mesh size should be selected based on

the objectives of the study; the finer the mesh, the more organisms (instars) will be collected.

5.8.5.3 The frame typically consists of a 0.045-$m^2$ (15 by 30-cm) brass rod structure anchored into the stream bed by a pair of steel rods.

5.8.5.4 Drift nets are anchored in the stream by driving 1/2-in. steel rods into the stream bottom or mounting the rods in concrete slabs that are weighted down with stones. Use cable clamps to secure the nets to the rods.

5.8.5.5 The drift net frame can be fitted anteriorly with a mouth reducing rectangular plexiglass enclosure (Rutter and Ettinger 1977) to increase filtration efficiency and volume of water passing through the net.

5.8.5.6 Alternatives to the typical drift net include the waterwheel drift sampler (Pearson and Kramer, 1969) which might be useful in large rivers or streams with slow flow which can be reached by automobile.

5.8.5.7 An automatic drift sampler (Muller, 1965) can be constructed that eliminates the need of an attendant at the sampling site during collection of as many as eight consecutive samples.

5.8.5.8 A modified emergence-trap drift sampler (Mundie, 1964; Cushing, 1964) is useful in streams with extremely high drift, where water is very turbid, or where a long sampling period is desired without clogging.

5.8.5.9 The average volume of water passing through the net is determined by measuring the water velocity at the mouth of the drift net with a current meter at the beginning of the sampling period and at the end of the sampling period using the average, and recording the total time the drift net is set in the water column. Results are expressed as numbers per $cm^3$ of water passing through the net.

5.8.5.10 The efficiency of the net is determined by the simultaneous measurement of the water velocity passing by the set drift net.

5.8.6 General Operating Procedures

5.8.6.1 Because the performance and sampling efficiency of a drift net sampler varies with local stream conditions, seasonal changes, and water level, make a preliminary test before the start of regular drift sampling in order to determine the best sampling stations, best sampling interval, number of nets needed, mesh size, and best sampling depth.

5.8.6.2 For synoptic surveys, one net set above each of the major areas of population concentrations is usually adequate; but for definitive studies a minimum of two drift nets should be set at each station so that drift from above a pollution source, drift from the polluted reach,

and drift from the zone of clean water downstream from the recovery zone can be compared.

5.8.6.3 Take into consideration the fact that the drift net will collect drifting organisms that may have entered the drift from an indefinite distance upstream or a tributary stream. Nets located 80 to 100 m below the effluent will generally sample the polluted reach efficiently. A drift net below a riffle collects more animals than one below a pool.

5.8.6.4 For definitive studies, install four nets at each station - two about 25 cm from the bottom and two about 10 cm below the surface in water not exceeding 3 m in depth.

5.8.6.5 If the objective of the study is to relate pupal exuviae to pollution, or to collect terrestrial organisms that may float on the surface, then extend one net slightly above the surface.

5.8.6.6 Ideally, collect 24-h drift samples; but this is usually not practicable unless one resorts to the use of a water-wheel, automatic drift sampler, or a modified drift sampler with a restricted opening to solve the clogging problem or by changing the nets at regular intervals.

5.8.6.7 Although the sampling interval will vary with time of day, current velocity, density of drift organisms, and floating debris, collect 1-3 hours daytime drift samples when either a 24-h or overnight sampling period is not prudent.

5.8.6.8 Drift nets have also been used from small boats in large rivers (Rutter and Ettinger, 1977).

5.8.6.9 Because the size of the catch varies as the flow of water through the net varies, it is necessary to measure the current velocity at the entrance of each net at the beginning and end of each sampling period so that the catch can be converted into number of organisms per volume of water flowing through the net.

5.8.6.10 At the end of the specified sampling period, remove the net from the water by loosening the cable clamps and raising the net over the top of the steel rods, taking care not to disturb the bottom upstream of the net.

5.8.6.11 Concentrate the material in the net in one corner by swishing up and down in the water and then wash into a bucket half-filled with water. Then sieve and handle the sample in the regular manner.

5.8.6.12 Subdividing the sample substantially reduces analysis time with large samples (Waters, 1969a and USEPA, 1973).

5.8.6.13 Reporting data as numbers of individuals per net is meaningless because no two drift net samples are collected under exactly the same conditions of current velocity, stream discharge, and sampling

interval. Conversion equations and other statistical aspects of drift sampling are given by Elliott (1970). An equation for converting the data to number per 100 $m^3$ of water flow is:

$$X = 100a/bdc$$

where:
X = number of organisms per 100 $m^3$,
a = number of organisms in the net (density)
b = number of minutes of the sampling interval,
c = current velocity, m/min, and
d = area of the net opening in $m^2$.

## 5.9 Artificial Substrate Samplers

5.9.1 Artificial substrate samplers are devices made of natural or artificial materials of various composition and configuration that are placed in water for a predetermined period of exposure and depth for the colonization of indigenous macroinvertebrate communities. They are used in obtaining qualitative and quantitative samples of macroinvertebrates in rivers, streams, lakes, and reservoirs.

5.9.2 Artificial substrate sampling can effectively augment bottom substrate sampling because many of the physical variables encountered in bottom sampling are minimized (e.g., variable depth and light penetration, temperature differences, and substrate types).

5.9.3 Samples usually contain negligible amounts of extraneous material, permitting quick laboratory processing.

5.9.4 Selecting Artificial Substrate Samplers

5.9.4.1 Table 5 summarizes criteria for selecting artificial substrate samplers.

TABLE 5. SUMMARY CRITERIA FOR ARTIFICIAL SUBSTRATE SAMPLERS

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1. Multiplate (Modified Hester-Dendy) Sampler

   A. Habitats and Substrates Sampled: All types of habitats in rivers, streams, lakes and reservoirs; not efficient in wetlands; uses hardboard or porcelain substrate.

   B. Effectiveness of the Device: Colonization depends on type of substrate; selective for certain types of organisms; three replicates considered adequate.

   C. Advantages: Excellent for water quality monitoring; uniform substrate type; high level of precision; samples contain negligible amount of debris; provides habitats of known area for a known time at a known depth.

TABLE 5. SUMMARY CRITERIA FOR ARTIFICIAL SUBSTRATE SAMPLERS (Continued)

D. Limitations: Requires trip for installation and trip for collection; subject to vandalism; biased for aquatic insects; need to use caution in reuse of plates that may have been contaminated with toxicants, oil, etc.; may require additional weight for stability; up to eight weeks wait for results.

Selected Literature: APHA, 1989; Beck et al., 1973; Beckett and Miller, 1982; Cairns, 1982; Flannagan and Rosenberg, 1982; Fullner, 1971; Greeson et al., 1977; Hall, 1982; Harrold, 1978; Hester and Dendy, 1962; Hellawell, 1978; Jacobi, 1971; Mason et al., 1973; McConville, 1975; McDaniel, 1974; Merritt and Cummins, 1984; Ohio EPA, 1987; Rosenberg and Resh, 1982; USEPA, 1973; Wefring and Teed, 1980.

2. Basket Sampler

A. Habitats and Substrates Sampled: All types of habitats in rivers, streams, lakes and reservoirs; may be used in areas where other methods are not feasible; not efficient for sampling in wetlands.

B. Effectiveness of the Device: Colonization depends on type of artificial substrate used in the basket (rocks, 3M Conservation Webbing, etc.); selective of certain types of fauna; three replicates considered adequate.

C. Advantages: Excellent for water quality monitoring; uniform substrate type at each station for better comparison and high level of precision; gives quantitatively comparable data; samples contain negligible amounts of debris; does not require additional weight for stability; samples a known area at a known depth for a known exposure time.

D. Limitations: Require trip for installation and another for collection; biased for insects; samplers and floats often difficult to anchor; may be navigation hazard; susceptible to vandalism; records only biotic community present during exposure period; no measure of past conditions; size and texture of limestone substrates may vary from study to study; up to eight weeks wait for results.

Selected Literature: Anderson and Mason, 1968; APHA, 1989; Benfield et al., 1974; Bergensen and Galat, 1975; Bull, 1968; Cairns, 1982; Flannagan and Rosenberg, 1982; Hall, 1982; Hanson, 1965; Hellawell, 1978; Leopold, 1970; Lium, 1974; Mason et al., 1967, 1973; Merritt and Cummins, 1984; Newlon and Rabe, 1977; Rabeni and Gibbs, 1978; Rabeni et al., 1985; Rosenberg and Resh, 1982; USEPA, 1973; Voshell and Simmons, 1977; Zillich, 1967.

5.9.5 Significance and Use of Artificial Substrate Samplers

5.9.5.1 Multiple-plate and basket samplers (Figure 9A-F) are usually colonized by a wide variety of invertebrates which have some means of mobility (active or passive) that are borne in the current. The organisms that colonize the artificial substrates are primarily aquatic insects, aquatic oligochaetes, crustaceans, cnidarians, turbellarians, bryozoans, and mollusks. The colonization of these organisms should be relatively equal in similar habitats and reflect the capacity of the water to support aquatic life. Although these samplers may exclude certain mollusks or worms, they collect a sufficient diversity of benthic fauna to be useful in assessing water quality.

5.9.5.2 Recovery techniques are critical for insuring collection of all organisms retained on the sampler.

5.9.5.3 Uniform substrate type reduces the effects of substrate differences.

5.9.5.4 Optimum time for substrate colonization is 6 weeks for most water in the United States.

5.9.5.5 Quantitatively comparable data can be obtained in environments from which it is virtually impossible to obtain samples with conventional devices.

5.9.6 Description of Multiple-Plate Samplers

5.9.6.1 Multiple-plate samplers consist of standardized, reproducible artificial substrate surfaces for colonization by aquatic organisms. Their uniform shape and texture compared to natural substrates greatly simplifies the problem of sampling. The sampler is constructed from readily available materials.

5.9.6.2 The modified multiple-plate sampler (Fig. 9A,B) is constructed of 0.125 in (0.3 cm) tempered hardboard or ceramic material with 3 in (7.6 cm) round or square plates and 1 in (2.5 cm) round spacers that have 5/8 in holes drilled in the center (Fullner, 1971). The plates are separated by spacers on a 0.25 in (0.63 cm) diameter eyebolt, held in place by a nut at the top and bottom. A total of 14 large plates and 24 spacers are used in each sampler. The top nine plates are each separated by a single spacer, plates 9 and 10 are separated by two spacers, plates 11 and 12 are separated by three spacers, and plates 13 and 14 are separated by four spacers. The hardboard sampler is about 5.5 in (14 cm) long, 3 in (7.6 cm) diameter, exposes approximately 1,160 $cm^2$ (.116 $m^2$) of surface area for the attachment of organisms, and weighs about 1 lb (0.45 kg). The ceramic sampler is 6.5 in. long and weighs 2.2 lbs (1 kg). The ceramic plates can be chemically cleaned, oven dried and reused indefinitely as they are stable and unaffected by long-term immersion in water. The sampler will not warp with time; therefore, the spacings between plates do not change, assuring replicate and efficient sampling. Each sampler is supplied with a 6 m (20′) long nylon suspension rope. The total weight is 1 Kg (2.2 lbs.). Sturdy

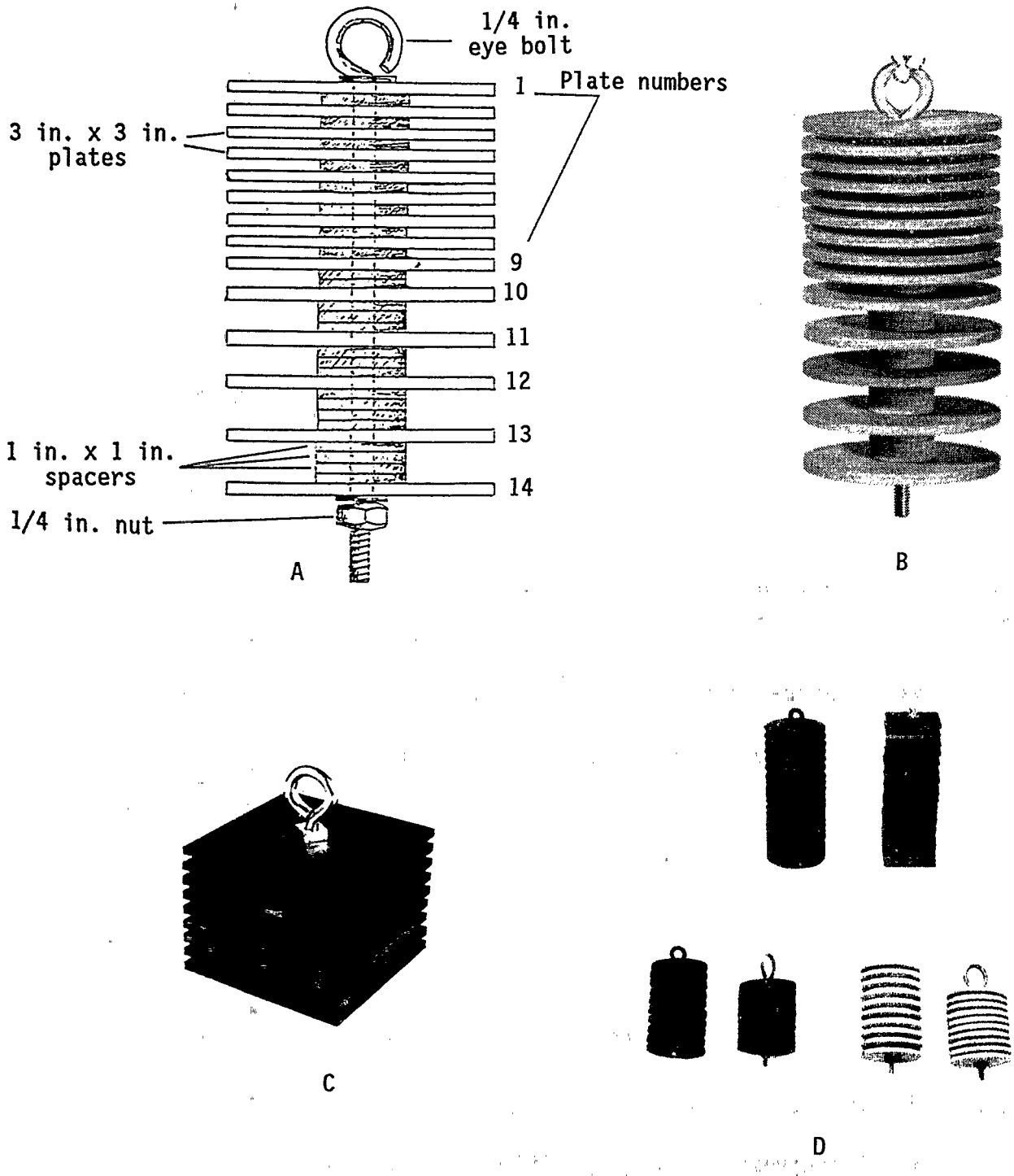
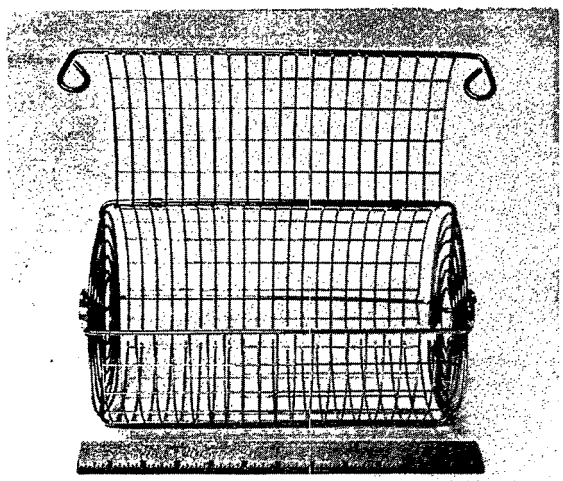



Figure 9. Artificial Substrate Samplers: (A) Schematic drawing of multiplate Sampler; (B) Typical round multiplate type; (C) Original Hester-Dendy multiplate, square design; (D) Jumbo and standard hardboard and porcelain multiplate designs



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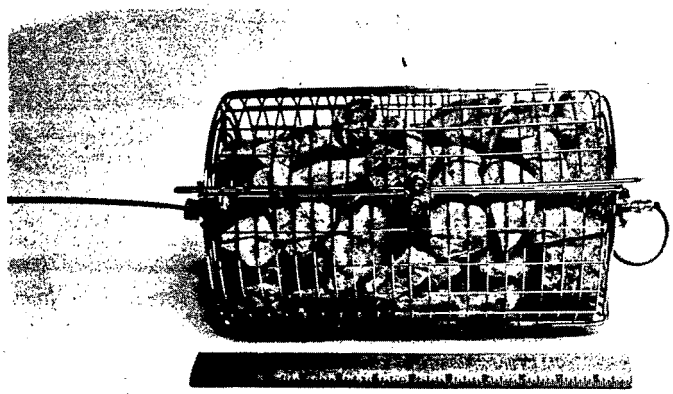

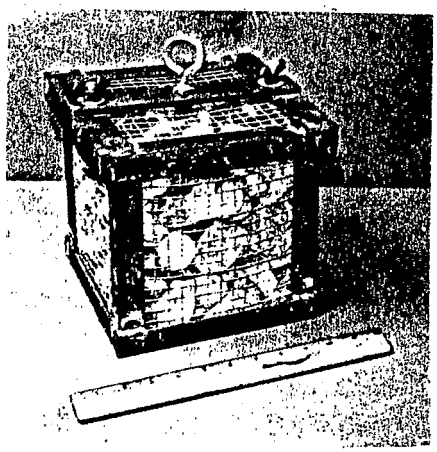

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Figure 9. Artificial Substrate Samplers: (E) Barbecue basket; (F) Basket samplers, cylindrical and square types

wire stakes for holding the sampler above the riverbed are recommended accessories.

5.9.6.3 When the samplers are suspended from the eyebolt, whether in strong currents or not, a 5 lb weight, such as a brick, is attached by .6 m wire to a 1/4 in turnbuckle. The turnbuckle is screwed tightly onto the shank of the multiplate eyebolt. The weight serves to stabilize the sampler and to lessen undue disturbance to the organisms. Upon retrieval, the weight is gently cut free before the sampler is bagged. Care should be taken not to reuse samplers exposed to oils and chemicals that may inhibit colonization during the next sampling period. Due to its cylindrical configuration, the sampler fits a wide mouth container for shipping and storage purposes. The sampler is inexpensive, compact, and light weight which are valuable attributes in water quality surveys.

5.9.7 Description of a Basket Sampler

5.9.7.1 The typical type of basket sampler (Fig. 9E) used is the one described by Mason et al. (1967). It is a cylindrical "barbecue" basket 11 in (28 cm) long and 7 in (17.8 cm) in diameter and is filled with approximately 17 lbs (7.7 kg) of natural rocks that vary from 1 to 3 in (2.5 to 7.6 cm) in diameter. A hinged door on the side allows access to the contents. An estimated 3.2 square ft (0.3 sq. m) of surface area is provided for colonization by macroinvertebrates. A 1/8 inch wire cable is passed through the long axis of the basket; one end is fastened with a cable clamp, and the other end is attached to a 5 gallon metal container filled with polyurethane foam used as a float. A 3/8 inch steel rod that is threaded at each end is passed through the long axis of the float and fastened at each end by nuts. Three inch long 1-1/8 by 1/8 inch strap iron secured on the rods by nuts serves as swivels at each end. The wire cable used to suspend the basket is attached to the swivels by holes drilled for that purpose. The float can be attached to a stationary structure or the basket can be anchored to the bottom in shallow water. The rugged construction of this particular basket sampler is heavy enough to resist movement by most water currents. In using the basket as a method of collecting macroinvertebrates, special consideration should be given to the types of substrates placed within the basket. Substrates tested have varied from limestone, tin cans, concrete cones, #200 3M Conservation Webbing (3M Corporation, St. Paul, MN), and porcelain spheres. Since each type of substrate will result in a different species diversity, the type of substrate used should be determined by the study objectives, weighing the advantages and disadvantages of each substrate type. For most investigations, a basket filled with 30 5-8 cm diameter rocks or rock-like material is recommended.

5.9.8 Precautions

5.9.8.1 Physical factors such as stream velocity and installation depth may variably affect degree of colonization.

5.9.8.2 The sampling method is selective for drifting organisms and for those which preferentially attach to hard surfaces.

5.9.8.3 Recovery techniques are critical for insuring collection of all organisms retained on the sampler.

5.9.8.4 Samplers are vulnerable to vandalism and often lost.

5.9.8.5 Caution should be exercised in reuse of samplers that may be subjected to contamination by toxicants, oils, etc.

5.9.8.6 The sampler provides no measure of the biota and the condition of the natural substrate at a station or of the effect of pollution on that substrate.

5.9.8.7 Sampler and floats must be anchored or fixed in place. This is sometimes difficult, and they may present a navigation hazard.

5.9.8.8 The sampler only records the community that develops during the sampling period, thus reducing the value of the collected fauna as indicators of prior conditions.

5.9.9 General Operating Procedures

5.9.9.1 Artificial substrate samplers are usually positioned in the euphotic zone of good light penetration (one to three feet, or .3-.9 m) for maximum abundance and diversity of macroinvertebrates (Mason, et al. 1973). Optimum time for substrate colonization is six weeks for most types of water in the United States. For uniformity of depth, suspend sampler from floats on 1/8 in. or 3.2 mm steel cable. If water fluctuation is not expected during sampling period, the samplers may be suspended from stationary objects. If vandalism is a problem, use subsurface floats or place sampler on supports placed on the bottom. Regardless of installation technique, use uniform procedures (e.g., same exposure period, sunlight, current velocity and habitat type). At shallow water stations (less than 1.2 m deep), install samplers so that the exposure occurs midway in the water column at low flow. If the samplers are installed in July when the water depth is about four feet and the August average low flow is two feet, the correct installation depth in July is one foot above the bottom. The sampler will receive sunlight at optimum depth (one foot) and will not be exposed to air anytime during the sampling period. Care should be exercised not to allow the samplers to touch bottom which may permit siltation, thereby, increasing the sampling error. In shallow streams with sheet rock bottoms, artificial substrate samplers are secured to 3/8 in. (.95 cm) steel rods that are driven into the substrate or secured to rods that are mounted on low, flat rectangular blocks (Hilsenhoff, 1969). These must, however, be securely anchored to the rock bottom to avoid loss during floods.

5.9.9.2 Artificial substrate samplers can be attached to floats, cement structures, a weight, or a rod driven into the stream-bed or lake-bed.

At least two or three samplers should be installed at each collecting site. Leave the samplers in place for at least 6 weeks to allow for organism colonization. The exposure time should be consistent among sites during the study. If study time limitations reduce this period, the data must be evaluated with caution, and in no case should data be compared from samplers exposed for different time periods.

5.9.9.3 The samplers may be installed in pools or riffles/runs suspended below the water surface. Make the collections as representative of the reach as possible by insuring that the samplers are not to close to the bank. In streams up to a few meters in width, install the devices about midstream. In larger streams install the devices at about one-quarter of the total width from the nearest bank.

5.9.9.4 To minimize losses of animals when retrieving multiplate and basket samplers, approach from downstream, lift the sampler quickly and place the entire sampler in a polyethylene jug or bag containing 10% formalin or 70-80% ethanol. Once the sampler is touched it must be removed from the water at once or many of the animals will leave the sampler. If the sampler must be disturbed during the recovery process so that it cannot be lifted straight up out of the water, a net should be used to enclose the sampler before it is disturbed.

5.9.9.5 The organisms can be removed in the field by disassembling the sampler in a tub or bucket partially filled with water and scrubbing the rocks or plates with a soft-bristle brush to remove clinging organisms. Pour the contents of the bucket through a No. 30 or 60 sieve and wash the contents of the sieve into a jar and preserve with 10% formalin or 70-80% ethanol. If the organisms are not removed in the field, place the sampler and the detached portion of sample into a wide-mouth container or sturdy plastic bag containing preservative for transporting to the laboratory. Label the sample with the location, habitat, date, and time of collection. The exposed multiplate sampler can be taken to the laboratory where the plates are removed from the bolt and cleaned with a soft-bristled brush. The basket samplers are usually disassembled in the field; however, they can be taken to the laboratory and disassembled if placed in preservative in a water-tight container.

5.9.9.6 Cleaned samplers can be reused unless there is reason to believe that contamination by toxicants (e.g., chemicals or oils) has occurred. These substances may be toxic to the macroinvertebrates or may inhibit colonization. Do not reuse hardboard, porcelain plates, or any other substrate that have been exposed to preservatives. Clean the multiple-plates before reassembly and use.

## 5.10 Coring Devices

5.10.1 Included in this category are single and multiple-head coring devices, tubular inverting devices, and open-ended stovepipe devices.

5.10.2 Selecting Coring Devices

5.10.2.1 Table 6 summarizes criteria for selecting coring devices

TABLE 6. SUMMARY CRITERIA OF CORING DEVICES

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1. KB Core Sampler

   A. Habitats and Substrates Sampled: Freshwater rivers,lakes, estuaries; soft sediments only, 40% silty clay.

   B. Effectiveness of the Device: Permits analysis of stratification in quantitative and qualitative samples; uses 5.08 cm (2 inch) pipe core tube; used in shallow to medium shallow water up to 30.5 m (100 feet) or deeper.

   C. Advantages: Samples a variety of substrates up to harder types; sampling tube can be modified for various diameters up to 100 $cm^2$ substrate surface; least disturbance to water/bottom interface; standard and heavy models available; wide variety of core tubes. liner tubes, core catchers, and nosepieces.

   D. Limitations: Gravity operated; samples limited surface area; standard KB core sampler head, without core tube weights approximately 8 kg (18 pounds), but additional weight can be added to sampler; requires boat and powered winch.

2. Ballchek Single and Multiple Tube Core Sampler

   A. Habitats and Substrates Sampled: Same as KB Core Sampler.

   B. Effectiveness of the Device: Samples deep burrowing organisms in soft sediment, particularly effective for sampling oligochaetes; uses 5.08 cm (2 inch) or 7.62 cm (3 inch) pipe core tube; used in shallow or deep waters, 3 m to 183 m (10-600 feet); multiple core sampler weight approximately 38 kg (84 pounds); check valves work automatically, prevent loss of sample.

   C. Advantages: Good penetration in soft sediments; small volume of sample allows for greater number of replicates to be analyzed in a short period of time; single or multiple (four) core tube sampler available; three inch pipe for larger cores and/or deep water lakes and oceans available; wide variety of core tubes, liner tubes, core catchers, and nosepieces.

   D. Limitations: Heavy device, approximately 38 kg, requires boat and winch; gravity operated; does not retain sand unless bronze core retainers are used which require additional weight to insure penetration.

3. Phelger Core Sampler

A. Habitats and Substrates Sampled: Same as above core samplers.

B. Effectiveness of the Device: Similar to KB core sampler.

C. Advantages: Similar to KB core sampler.

D. Limitations: Gravity operated or can be messenger operated with a suspension-release device; styles and weights vary among manufacturers, some use interchangeable weights, between 7-35 kg, others use fixed weights up to 41 kg; length core taken varies with substrate texture.

4. Box Core Sampler

A. Habitats and Substrates Sampled: Same as above core samplers, also oceans.

B. Effectiveness of the Device: Same as above core samplers; samples a surface area of 100 $cm^2$ and a sediment depth of 20 cm.

C. Advantages: Same as above core samplers.

D. Limitations: Same as above core samplers; also deployed from ships or other platforms; diver collected cores are preferred.

5. Hand-Operated Core Samplers

A. Habitats and Substrates Sampled: Same as above core samplers.

B. Effectiveness of the Device: Sampled by hand or by diver.

C. Advantages: Can be used in shallow water. In deep water can be used with a diver, usually a trained biologist, who can collect and recognize substrate and bottom changes to stratify sampling; can be used with extension handles of 5, 10, or 15 feet; used with pipe fitting for driving from a pontoon boat, dock, or bridge.

D. Limitations: Limited area sampled.

Selected Literature: APHA, 1989; Brinkhurst, 1967, 1974; Burton, 1974; Coler and Haynes, 1966; Edmondson and Winberg, 1971; Flannagan, 1970; Gale, 1977; Hamilton et al., 1972; Holme, 1964; Holme and McIntyre, 1971; Miller and Bingham, 1987; Poole, 1974; Schwoerbel, 1970.

5.10.2.2 Coring devices can be used at various depths in any substrate that is sufficiently compacted so that an undisturbed sample is retained; however, they are best suited for sampling the relatively homogenous soft sediments, such as clay, silt, or sand of the deeper portions of lakes, reservoirs, and oceans. Because of the small area sampled, data from coring devices are likely to provide very imprecise estimates of the standing crop of macrobenthos.

5.10.2.3 KB type, Ballchek, and Phleger corers (Fig. 10A,B,C) are examples of devices used in shallow and deep water; they depend on gravity to drive them into the sediment. The cores are designed so that they retain the sample as it is withdrawn from the sediment and returned to the surface. Hand corers (Fig. 10D) designed for manual operation are used in shallow water. Sections of the core can be extruded and preserved separately or the entire core can be retained in the tube and processed in the field or laboratory. Intact cores can also be preserved by freezing and processed later.

5.10.2.4 Additional replication with corers is feasible because of the small amount of material per sample that must be handled in the laboratory. Multiple-head corers have been used in an attempt to reduce the field sampling effort that must be expended to collect large series of core samples (Flannagan, 1970).

5.10.2.5 The Dendy inverting sampler (Welch, 1948) is a highly efficient coring-type device used for sampling at depths to 2 or 3 meters in nonvegetated substrates ranging from soft muds through coarse sand. Because of the small surface area sampled, data obtained by this sampler suffer from the same lack of precision (Kajak, 1963) as the coring devices described above. Since the per-sample processing time is reduced, as with the corers, large series of replicates can be collected. The Dendy sampler is highly recommended for use in habitats for which it is suitable.

5.10.2.6 Stovepipe-type devices include the Wilding sampler (Wilding, 1940; APHA, 1989) and any tubular material such as 60-to-75 cm sections of standard 17-cm-diameter stovepipe (Kajak, 1963) or 75-cm sections of 30-cm-diameter aluminum irrigation pipe fitted with handles. In use, the irrigation pipe or commercial stovepipe is manually forced into the substrate, after which the contained vegetation and coarse substrate materials are removed by hand. The remaining materials are repeatedly stirred into suspension, removed with a long-handled dipper, and poured through a wooden-framed floating sieve. Because of the laborious and repetitive process of stirring, dipping, and sieving large volumes of material, the collection of a sample often requires 20 to 30 minutes.

5.10.2.7 The use of stovepipe samplers is limited to standing or slowly moving waters having a maximum depth of less than 60 cm. Since problems relating to depth of sediment penetration, changes in cross-sectional area with depth of penetration, and escapement of organisms are circumvented by stovepipe samplers, they are recommended for quantitative sampling in all shallow-water benthic habitats. They probably

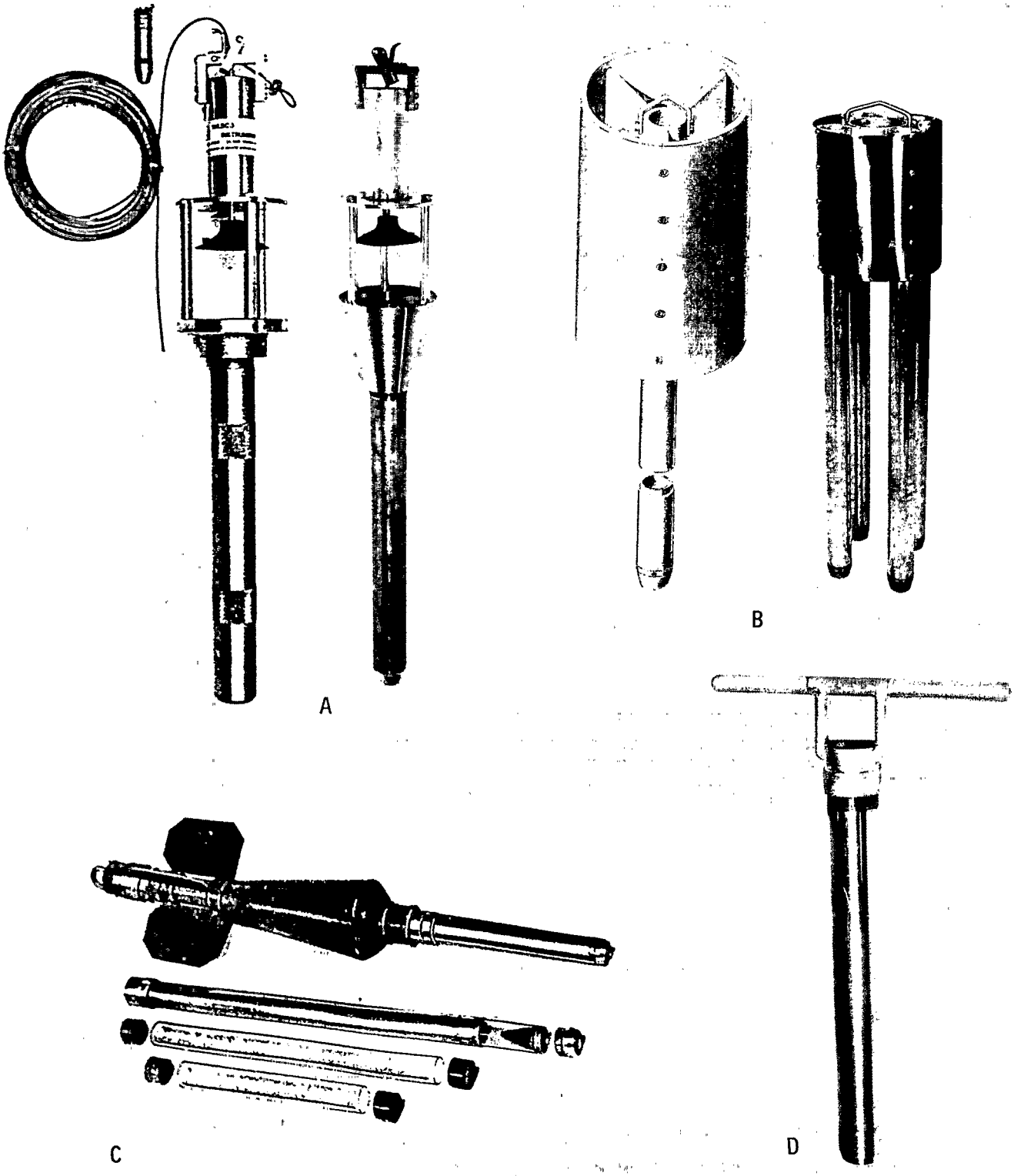


Figure 10. Core Samplers: (A) KB corer, standard and heavy duty; (B) Ballchek corer, single and multiple types; (C) Phleger corer; (D) Hand-operated corer

represent the only quantitative device suitable for sampling shallow-water habitats containing stands of rooted vascular plants and they will collect organisms inhabiting the vegetative substrates as well as those living in sediments.

5.10.2.8 In marine waters benthic macrofauna are generally collected using various box cores deployed from ships or other platforms, or diver collected cores. A box coring device consisting of a rectangular corer having a cutting arm which can seal the sample prior to retraction from the bottom should be used. In order to sample a sufficient number of individuals and species, and to integrate the patchy distribution of fauna, each sample should have a surface area of no less than 100 $cm^2$ and a sediment depth of at least 20 cm. In sediments having deep, burrowing fauna, a box corer capable of sampling deeper sediment may be needed. In sandier sediments, it may be necessary to substitute a grab sampler for the box corer in order to achieve adequate sediment penetration. Sufficient replicates (usually 3 to 10) should be taken to produce an asymptotic cumulative species curve. Visual inspection of each sample is necessary to insure an undisturbed and adequate amount of sample is collected.

## 5.11 Frames

5.11.1 For estimating the populations of attached marine organisms on a rocky shore, 0.1 $m^2$ or 1 $m^2$ square-shaped metal frames can be used for delineating percent coverage of the colonial forms. At least ten frames should be counted for characterizing the distribution statistically. Samples of the algae and macroinvertebrates should be removed from a measured area for species identification and weighed for biomass determination. It is important to note the attitude of the sampling frame relative to the horizontal and vertical axis in order to relate the data with the zonation patterns. A vertical plane is apt to have a dramatically different species array compared to a horizontal plane even with both being at the same level with the intertidal zone.

5.11.2 Attaching a 35 mm SLR camera to a sampling frame so that the focal distance is fixed is an excellent method for documenting the population present at each sampling site. Species enumeration and percent cover can be estimated from the developed photographs. This method is especially useful for documenting temporal changes at a particular sampling site.

5.11.3 For sampling the infauna of beaches, a 0.1 $m^2$ square metal frame with a 15 cm lip is useful. The frame can be deliberately thrown near a fixed position (see Section 4.4.3, Systematic Sampling). Stovepipe or large coffee can work very well in most sandy, sandy-mud beaches but have limited use in cobble beaches. All of the substrate is removed and screened in fine-meshed screens. The animals retained are washed or picked from the screens and preserved for later identification and enumeration.

5.11.4 Edged frames (.1 $m^2$) or corers can be utilized for

systematically sampling the substrates around fixed positions on the flats. At least five replicate samples should be collected at each site for statistically delineating the distribution patterns of the infauna populations. The substrate is then washed through fine meshed screens. The invertebrates can be washed or picked from the screens and preserved. Flats represent areas of quiet, low velocity waters with the settling of suspended materials. Flats near pollution sources are good sites to observe the impact of all settled materials, non-toxic and toxic. Some flats are so poorly drained as to require snowshoes or similar devices for walking out to the sampling area. In such areas, it may be easier to sample at high tide from a boat using a conventional benthic grab.

**5.12 Rapid Bioassessment Protocols (RBPs) for Macroinvertebrates (see Plafkin et al., 1989 and Section 7, Data Evaluation.)**

5.12.1 The methods describe three different protocols (I,II, and III) for use in wadable streams and rivers to determine water quality. The RBPs are considered qualitative and semi-quantitative sampling techniques for assessing the health of benthic macroinvertebrate communities. The protocols consist of three basic components--water quality and physical characteristics, habitat assessment, and biosurvey. The biological assessment involves integrated data analyses of both functional and structural components of the macroinvertebrate communities through the use of metrics. The protocols describe guidelines for a rapid means of detecting water quality and aquatic life impairments and assessing their relative severity. The RBPs are not intended to replace traditional biomonitoring methods but provide an option which may be cost effective. These RBPs work very well as a surveillance tool to prioritize sites for more intensive evaluations (quantitative biological surveys) but are not always comparable to the results obtained with more traditional methods such as artificial substrate samplers or drift nets. The same metrics (RBPs) may be used with these more traditional methods of collection and give qualitative or quantitative results.

5.12.1.1 Protocol I provides for basic qualitative information for a subjective judgment of macroinvertebrate abundance and presence. The method consists of habitat assessment and the collection of macroinvertebrates from all possible habitats. The specimens are identified to orders and counted in the field. The data are used to make a subjective assessment of stream water quality or impairment.

5.12.1.2 Protocol II provides a reasonably reproducible assessment of biological impact and consists of habitat assessment and collecting macroinvertebrates from all available habitats. The specimens are identified to families, and the list of families in a 100-organisms subsample is used in the evaluation. The study is based on established guidelines in scoring parameters, and the stream site would be classified as to water quality or degree of impact and possible cause.

5.12.1.3 The objectives of Protocol III are to assess the biological

impact and to establish the basis for trend monitoring of pollution effects over a period of time. The method consists also of specific guidelines for evaluating the habitat assessment parameters and collecting macroinvertebrates from all available habitats. The protocol is similar to Protocol II except that the specimens are identified to the lowest possible taxonomic level (genus, species). The data are categorized into parameters based on taxa richness, biotic index, percent composition, and functional group designations. The classification of stream sites is dependent on established guidelines.

**5.13 Ohio EPA Invertebrate Community Index method (ICI) (see Ohio EPA, 1987, 1989)**

5.13.1 The ICI semi-quantitative method uses 10 metrics to determine if wadable streams or rivers are polluted using benthic macroinvertebrates. Nine of the 10 metrics are based on multiple plate artificial substrate samples, and one is based on dip net sampling (Ohio EPA, 1987 and 1989). Also, see Section 7, Data Evaluation.

**5.14 Standard Qualitative Collection Method (see Lenat, 1988; Eagleson, et al., 1990, and NC DEM, 1990 and Section 7, Data Evaluation)**

5.14.1 The method emphasizes multiple-habitat sampling, field-picking of samples, and the use of both coarse- and fine-mesh samplers. This standard qualitative method consists of collecting macroinvertebrates in shallow streams, usually less than 1.5 m deep using two kick net samples, three dip net samples (sweeps), one leaf-pack sample, three aufwuchs samples, one sand sample, and visual search collections. The data resulting from this method, especially taxa richness, can be used to assign water quality ratings. The method is applicable for most between-site and/or between-date comparisons. Also, a secondary abbreviated qualitative method (EPT survey) can be used to quickly determine between-site differences in water quality. The number of collections is decreased from 10 samples in the standard quality collections to only four samples: one kick, one sweep, one leaf-pack and visual searches in the abbreviated method.

**5.15 Miscellaneous Qualitative Devices**

5.15.1 The investigator has an unlimited choice of gear for collecting qualitative samples. Any of the quantitative devices discussed previously, plus hand-held screens, dip nets, sweep nets, kick nets, rakes, tongs, post-hole diggers, bare hands, and forceps can be used for collecting benthic macroinvertebrates from freshwater, estuarine, and marine environments. For deep-water collecting, some of the conventional grabs described earlier and dredges are normally required. In water less than 2 meters deep, a variety of gear may be used for sampling the sediments including long-handled dip nets and post-hole diggers. Collections from vascular plants and filamentous algae may be made with a dip net, common garden rake, potato fork, or oyster tongs. Collections from floating debris and rocks may be made by hand, using forceps to catch the smaller organisms. In shallow streams, short

sections of common window screen may be fastened between two poles and held in place at right angles to the water flow to collect organisms dislodged from upstream materials that have been agitated.

5.15.2 Dip, hand, sweep, kick nets and screens are rapid devices for collecting macroinvertebrates in wadable streams and rivers or at low tide in the inter-tidal zone of tidal sites. Two approaches are generally used, one in which the investigator sweeps the dip or hand net through aquatic habitats (Slack, et al., 1976; Armitage, et al., 1981) and one in which the kick net or hand held screen is held stationary against the streambed, facing upstream, and the investigator physically disturbs the stream bottom just upstream from the net or screen. The investigator vigorously kicks with the feet four or five times into the streambed to disturb the habitat in an upstream direction (Hynes, 1961; Morgan and Egglishaw, 1965; Frost, et al., 1971; Armitage, et al., 1974; Armitage, 1978; Hornig and Pollard, 1978; Pollard, 1981; and Plafkin, et al., 1989). The kicks disturb the substrate, dislodging the macroinvertebrates and some detritus, and cause the benthos to be swept by the current into the net. The debris and organisms in the kick net are then washed down into a sieve bucket and larger leaves and debris are removed.

5.15.3 Dredges are devices that are usually pulled by hand or power boat across or through the bottom sediment of a lake or stream to sample the benthos and prevent loss of active macroinvertebrates. The forward motion of the dredge carries macroinvertebrates into the net.

5.15.3.1 Elliott and Drake (1981a,b) compared four light-weight dredges for sampling in rivers. They indicated that the dredges are not suitable for quantitative sampling. Also, considerable variation existed in their effectiveness as qualitative samplers for estimating the total number of taxa per sample.

5.15.3.2 Dredges should be emptied after collection into a shallow tray, bucket, or sieving device if the sample is sorted on-site. The sample can be placed directly in labeled wide-mouth containers with preservative and transported back to the lab for processing.

## 5.16 Suction Samplers

5.16.1 Suction samplers have been used widely in sampling macroinvertebrates in fresh, estuarine, and marine waters (Brett, 1964; Larsen, 1974; Gale and Thompson, 1975). They can be placed directly on the sampling station and can be operated by hand in shallow water or by a scuba diver in deep water (see 5.18).

## 5.17 Photography

5.17.1 The use of photography is mainly limited to environments that have suitably clear water and are inhabited by sessile animals and rooted plants. Many estuarine habitats, such as those containing corals, sponges, and attached algal forms, fall in this category and can

be photographed before, during and after the introduction of stress. The technique has been used with success in south Florida to evaluate changes brought about by the introduction of heated effluents.

5.17.2 The technique for horizontal underwater photos using scuba gear involves placing a photographically identifiable 1.0 $m^2$ area frame or marker in the habitat to be photographed and an additional nearby marker on which the camera is placed each time a photograph is taken. By this means, identical areas can be photographed repeatedly over a period of time to evaluate on-site changes in sessile forms at both affected and control stations. Vertical, overhead photos may be taken under suitable conditions.

5.17.3 Photographs are also useful in documenting a habitat or alterations in a station over time (e.g., increase in canopy cover, changes in channelization of a stream, and effects of flooding, etc.).

5.18 **Scuba**

5.18.1 This equipment can be used in freshwater sampling of mollusks in large riverine systems or with diver collected cores.

5.18.2 The reader is referred to Simmons (1977), Sommers (1972), U.S. Department of the Navy, U.S. Navy diving manual (latest edition), and Gale and Thompson (1974) for much additional information on this subject. All USEPA diving operations should be conducted in accordance with standards set forth in the U.S. EPA Occupational Health and Safety Manual-1440, 1986, entitled Chapter 10, EPA Diving Safety Policy. Therefore, if the need for diving capability exists, approval must be obtained through an USEPA regional laboratory diving officer. Scuba gear can be used to improve aquatic sampling; in particular sampling of mussels, other benthos, and fish. Isom, et al., (1979) reported utilizing scuba in rediscovery of snails, which were thought to be extinct. Various investigators had sampled the same areas previously on numerous occasions.

5.18.3 Gale (1977) notes the numerous applications of scuba to sampling benthos including placement and retrieval of artificial substrate; use of suction samplers (Larsen, 1974; Gale and Thompson, 1975); sampling with a quadrate frame; and, perhaps most importantly, identifying and delineating substrate types for purpose of determining sampling effort (stratified sampling) and choice of samplers.

5.18.4 If pelecypods (freshwater mussels) are to be sampled with brails in areas which historically contained them and/or it is desired to sample quantitatively, scuba can be used effectively in taking quadrates. In large rivers, which have mussel beds with homogenous substrate, it is desirable to take at least 10 square meter quadrates (10,000 square cm each). In small rivers where the mussels' niche may be between rocks and it is generally difficult to place a square meter frame, then a 0.5 square meter frame (2500 square cm) should be utilized with no less than 3 square meters, or twelve 0.5 square meter samples

taken. Samples should be taken randomly in all cases, which in the latter instance, will result in collection of good representative diversity (see Section 2, Quality Assurance and Quality Control).

5.18.5 Scuba diving is safe if conducted by rigid safety standards, some of which are mandatory for scientific/educational diving (See Federal Register, July 22, 1977; 42, 41: pp. 37650-37673). Conformance with these and subsequent standards is costly but essential for safe conduct of scuba sampling. See references listed above for more in depth discussion of safety, the buddy system, etc. The need for observance of safety rules cannot be overemphasized.

5.19 Brails

5.19.1 This device is primarily limited to sampling of bivalve mussels in large (non-wadable) rivers.

5.19.2 The use of brails for commercial harvest of mussels has been the common practice since before 1900; however, this practice and scuba have been used by investigators to study mussel populations on a limited basis.

5.19.3 The reader is referred to Coker (1919), Van der Schalie (1941), Scruggs (1960), Lopinot (1967), Isom (1969), Bates (1970), Starrett (1971), and Buchanan (1980) for more information on collecting mussels, brails, and brailing. Coker (1919) describes how to make a brail.

5.19.5 Once the site to be sampled has been identified, reference should be made to historical literature for determination of species that may be encountered.

5.19.6 Quantitative sampling is accomplished with a crowfoot brail to determine the rate of catch per drag from a given area. All equipment can be made or rented from and fished by a commercial fisherman. Each brail sample consists of dragging a measured distance of 100 m, then sorting and counting the catch. The area sampled is calculated in square yards by multiplying the length of brail by 100 m. Catch success is expressed in terms of the average catch of mussels per square per drag. Brail sampling is randomized within fishing area and by time periods during two complete harvest seasons (March through August).

5.19.7 Brailing is also an effective qualitative sampling device, especially in large, deep rivers. Where possible, the services of a commercial mussel fisherman should be utilized. The experienced mussel fisherman is adept at using brails and only extensive experience would make an investigator's results equivalent to the general mussel fisherman. Maximum legal brail length is 16 feet (approximately 5 m) in some states; diameter of wire used for hooks is also controlled. These points can be worked out with the state permitting agency.

5.19.8 A minimum of six 100 m long hauls (drags) should be accomplished

where a single brail is used. Most commercial fisherman use two brails simultaneously; thus, only three hauls would be required. Record the time for each haul; however, take about 20 minutes to make each haul since a very slow speed is best for catching mussels. If the hauls are made too fast, the catch will be small. If a significant mussel population is found, then qualitative or quantitative scuba (see 3.18, Scuba) samples should be taken. A minimum of 10 $m^2$ samples should be taken by scuba at each station. All specimens should be identified to species, growth cessation rings counted, and measured for determination of population age structure.

5.19.9 Mussel fishing with brails is highly dependent on experience of the user; however, they are very efficient in the hands of experienced users as attested to by almost 100 years of continuous use.

5.19.10 Availability of brailing equipment may be a deterrent to its use; however, if the method is adopted more widely by the scientific community, suppliers may develop to meet the need.

## 5.20 Other Mussel Collecting Methods

5.20.1 Mussels found in small or medium sized streams and rivers that can be waded are often found most numerous on bars where the pools break off into shoals. Sometimes, there are constrictions in streams at these points where weed beds can be found. Sample into the lower end of pools, around the weed beds, and in riffles/runs and fast-flowing water. A long-handled rake modified with a rectangular collection basket of one-quarter inch wire mesh, dredge dip net, or using the hands are the best method for sampling mussels from these habitats (Starrett, 1971). It is advisable to wear gloves and place a net below the area being sampled to catch small mussels that might otherwise not be collected.

5.20.2 Other collection techniques and procedures can be found in the 1941 Annual Report of the American Malacological Union. Information on collecting snails can be found in the same publication.

5.20.3 If rare or endangered species are collected, they should be returned to their habitat since it is illegal to take such species.

## 5.21 Literature Cited

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## SECTION 6

## SAMPLE PROCESSING

### 6.1 Sieving

6.1.1 Samples collected with grabs, coring devices, and artificial substrates contain varying amounts of finely divided materials such as decomposed organic material, silts, clays, and fine sand. To reduce sample volume and expedite sample processing in the laboratory, these fines should be removed in the field by passing the sample through a U.S. Standard No. 30 sieve. Sieves may be commercial models or homemade sieves framed with wood or metal. Floating sieves with wooden frames reduce the danger of accidental loss of both sieve and sample when working over the side of a boat in deep waters. A sieve should contain no cracks or crevices in which small organisms can become lodged.

6.1.2 Sampling efficiency is increased by using sieves with smaller mesh openings (Mason et al., 1975; Barber and Kevern, 1974; and Zelt and Clifford, 1972). However, use of the smaller mesh size does not have an appreciable effect on the eutrophic classification based on common biotic indices. Precision based on coefficient of variation (CV) increased with smaller mesh size (Mason et al., 1975). Usually the increased length of time required to use the smaller mesh sieve sizes is not compensated for by the increased accuracy of results (Hummon, 1981). Also, organisms passing through the U.S. Standard No. 30 sieve are not macroinvertebrates by definition. (See Section 1, Introduction).

6.1.3 If at all possible, sieving should be done in the field immediately after the sample is collected and the captured organisms are still alive, but time can often be saved by returning to the laboratory with the samples unsieved and doing the sieving with a mechanical device such as the elutriation apparatus described by Worswick and Barbour (1974). If the sample is likely to include tubificid worms, leeches, or Turbellaria, a few representative specimens of each should be picked out before sieving and fixed in 10% buffered formalin or transported live to the laboratory for fixing or immediate identification. Once preserved, many organisms become quite fragile and if subjected to sieving will be broken up, lost, or rendered unidentifiable. Great care should be taken in sieving preserved samples containing mayflies, stoneflies and worms to reduce breaking the specimens or otherwise damaging body parts necessary for identification.

6.1.4 Sieving may be accomplished by one of several techniques depending upon the preference of the biologist. In one method, the sample is placed directly into a sieve and the sieve is then partially submerged in water and agitated until all fine materials have passed through. The sieve is agitated, preferably in a large tub of water but sieving may be done over the side of the boat if care is taken not to spill the sample. A variation of this technique is to place the original sample in a tub or bucket, add screened water, stir, and pour the resulting slurry through a U.S. Standard No. 30 sieve. Only a moderate amount of agitation is required to completely

clean the sample. Since this method requires considerably less effort, most biologists may prefer it. A sieve bucket (Fig.11) described by Hiltunen (1983) for use in the Great Lakes works well under most conditions and allows the sample to be sieved while the boat is under way to the next sampling site. The cycle sieve described by Mason (1976) works well in calm weather from a small boat but is cumbersome and impractical for use from large boats, bridges or other such structures. In all of the above methods, remove, carefully clean, and discard all the larger pieces of debris and rocks from the sample before stirring or agitating.

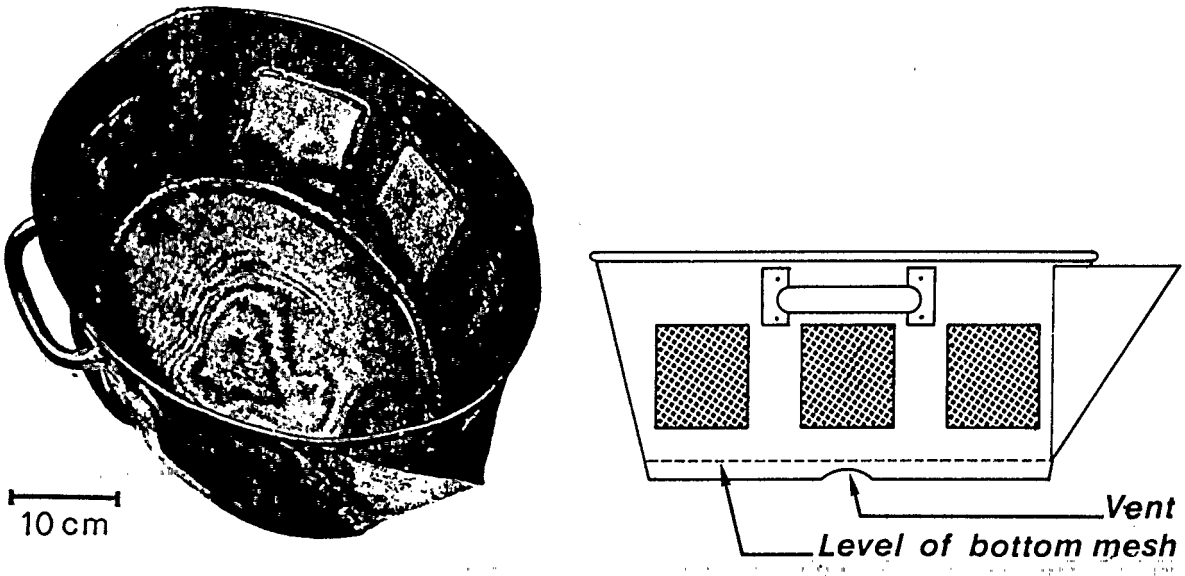


Figure 11. Great Lakes sieve bucket (From Hiltunen, 1983).

6.1.5 Artificial substrate samplers are placed intact into a bucket or tub of screened water and dismantled. Each individual piece of substrate is rinsed, gently but thoroughly cleaned under water with a soft brush such as a soft bristled toothbrush, examined visually, and laid aside. The water in the bucket or tub is then poured through a U.S. Standard No. 30 sieve to remove the fines. After most of the fines are washed from the sample, the organisms are left scattered over the surface of the screen. These organisms can be picked from the screen with forceps and placed in the sample container. A faster method is to concentrate them at one edge of the sieve by gently swirling the sieve in a little water, then tilting the sieve over a wide-mouth jar and gently backflush the organisms into the jar with water from a wash bottle directed through the screen.

6.1.6 Another way to separate the organisms from the detritus is the flotation method in which a concentrated aqueous solution of sugar, salt, or other chemical is poured over the sample in the tub or bucket causing the animals to float up out of the detritus due to the difference in the specific

gravity of the animals and solution. The organisms can then be poured or scooped into the sample container with a sieve spoon. Some organisms, such as clams and snails, must still be hand picked from the debris because they are too heavy to float. Two or three lbs. of sugar per gallon of water makes a good flotation solution (Anderson, 1959).

6.1.7 When drift net or Surber-type samplers are being used, it is usually possible to empty the bag directly into a white bottom enamel pan or small bucket and hand pick the organisms into a sample container filled three-fourths full of preservative.

6.1.8 Although the U.S. Standard No. 30 (600 μm) sieve is also commonly used in marine studies, some investigators (Grassle et al. 1985) have chosen to use a 300 μm sieve in order to more efficiently sample smaller and juvenile macrofauna. This practice requires more time and taxonomic expertise. The 600 μm sieve is usually adequate since the vast majority of macrofaunal biomass and production is associated with larger forms.

6.1.8.1 For marine work the use of more than one sieve in series, one on top of the other, allows benthic communities to be fractionated by size allowing comparisons of community size distributions between stations and over time. Commonly used sieve sizes are 300 μm, 500 μm, 600 μm, 1 mm, and 2 mm.

6.1.8.2 Sieving marine samples should be done by rinsing organisms with a gentle spray of water to minimize mechanical damage to the organisms. Direct heavy jets of water should not be used and an elutriate procedure that ensures that the major source of water is from the bottom of the sieves is recommended. Water used in sieving should be obtained from the sample site whenever possible. Fresh water should never be used to sieve unpreserved marine fauna because of osmotic effects that cause cell bursting.

## 6.2 Preservation and Fixation

6.2.1 All samples collected in the field should be preserved in 70-80% ethyl alcohol (ethanol), but ideally, and for ease in identification, representative specimens of leeches, aquatic oligochaetes, and other soft bodied organisms, if time permits, should first be fixed in 10% formalin to fix the tissue. After fixation (about 10 minutes), depending on size and number of organisms, or after returning to the laboratory, they may be preserved in 70-80% ethanol. This process should aid in their identification (see Section 6.5.4. and 6.5.5). Because wash water is contained in the sieved material, the stock preservative solution added to the sample should be over-strength (90%) so that the final solution will be sufficient to preserve the organisms. Grab samples collected from lakes, the muddy bottoms of large rivers, estuaries and oceans are often fixed and preserved in ten percent buffered formalin because they contain many worms which are difficult to identify after being preserved in ethanol. Formalin should be buffered to a neutral or slightly alkaline level with borax.

6.2.2 Since leeches dropped alive into preservatives such as 70-80% ethanol or 10% formalin solution contract strongly, some diagnostic features used

for species identification may be difficult to determine by the inexperience. Ideally, specimens should first be narcotized by direct placement into carbonated water, fixed in 10% formalin, and preserved in 70-80% ethanol. If this procedure is inconvenient in the field, the specimens should be preserved directly in 70-80% ethanol. Most specimens still can be identified to species but might take a little longer than usual. Additional collecting, narcotizing, and processing techniques can be found in Klemm (1982, 1985).

6.2.3 Turbellarians that require identification to species should be transported to the laboratory alive in a small amount of water (Pennak, 1978, 1989).

6.2.4 Although not always necessary, species identifications are easier and morphometric analyses are facilitated if marine organisms are relaxed after sieving and prior to fixation and preservation. Organisms to be relaxed are transferred from sieves to a fine mesh (approximately 100 $\mu$m) bag and placed in a solution of magnesium chloride (approximately 75 g/l) for about 10 minutes. The organisms may then be fixed and preserved.

6.2.4.1 A 10% (by weight) formalin solution is most commonly used to fix and preserve marine samples. The solution is buffered to keep the dissolution of molluskan shells to a minimum.

6.2.4.2 Because formaldehyde is a carcinogen, and because some individuals develop severe sensitivities to formaldehyde over time, some researchers prefer to transfer samples from formalin to ethanol for preservation. This is acceptable if samples are only to be used to do taxonomic studies. However, biomass measurements should not be done on samples preserved in ethanol. Although weight loss due to preservation in formalin is significant (10-20%) (Mills et al., 1982; Schram et al., 1981; Williams and Robins 1982), weight loss due to preservation in ethanol is greater.

6.2.5 Sample containers used for holding preserved samples should be large enough so that they are not over one-half full of the washed sample before the preservative is added. Quart or liter sized jars are adequate for most samples collected with artificial substrate, drift net, or square-foot type samplers, but two or more jars may be needed for a grab sample depending on the amount of detrital material mixed with the sample. Hand picked specimens are usually preserved by placing them directly into small screw-cap vials filled with 70-80% ethanol.

6.2.6 If the samples are not sorted within two or three weeks after collecting, the preservative should be poured off and replaced with fresh preservative for permanent storage (Cairns and Dickson, 1971).

6.2.7 After sorting and/or identification most macroinvertebrates should be stored in a solution of 70-80% ethanol and 5% glycerine in vials sealed with tightly fitting rubber stoppers. If screw-cap vials are used, they should be submerged in 70-80% ethanol in a larger container and should be checked yearly to replace alcohol lost because of evaporation or Teflon tape can be used to secure the screw-caps to prevent evaporation.

## 6.3 Labelling and Record Keeping

6.3.1 All sample containers must be labeled in the field immediately upon collection. Sample labels made of water-resistant paper should be placed inside each sample container. Write all information on the label with a soft-lead pencil or waterproof ink. Where the volume of sample is so great that several containers are needed, additional external labels with sample number and notations such as 1 of 2, 2 of 2, etc. are helpful for identifying the sample containers when the samples are logged in at the laboratory. All labels must include a sample identification number which corresponds to the number entered in the field notebook for that sample, the sampling date, water body and location from which the sample was collected, and the name of the collector. In addition to the information on the label, the field notebook should include the sampling method, weather, substrate characteristics, depth, and any other physical or environmental conditions noted.

6.3.2 Marine sample data sheets should include date of collection, time of day, station number, geographic coordinates, replicate number, core penetration depth, and the identification number and final storage location of each sample. These data sheets should also include space for comments on the visual appearance of each sample (e.g., obvious tubes or burrows, presence or absence of a surface flocculent layer, sediment color, apparent depth of the redox-potential discontinuity, etc.); ancillary data such as water temperature, salinity, secchi disk visibility, vertical profiles of dissolved oxygen; and other data potentially useful in the interpretations of benthic community data.

6.3.3 As soon as possible after returning to the laboratory, each sample should be assigned an ID number in sequence. This number identifies the sample in a bound ledger where all the information from the field label and field notebook are recorded for permanent record. The sample ID number must also be placed prominently on the sample container before storing so that it can be identified when needed. This sample ID number should be placed on all specimen vials, microscope slides, and other items connected with the sample.

## 6.4 Sorting and Subsampling

### 6.4.1 Sorting

6.4.1.1 Sort through the samples by hand in the laboratory using a low power (2X) scanning lens or a stereomicroscope. Place one or two tablespoonfuls of the sample in a white enamel pan (size 25 X 40 X 5 cm) filled about one-third full of water. Usually small insects and worms will float free of most of the debris when ethanol-preserved samples are transferred to the pan. These floating organisms should be removed before they soak up water and sink. They can be skimmed off with a sieve spoon or poured off. Addition of about one tablespoon full of sugar and stirring the sample will cause most of the other organisms to float free. Flotation in formalin-preserved samples is accomplished by adding sugar slowly to raise the specific gravity to 1.12 (Pask and Costa, 1971). Numerous other techniques have been proposed to aid recovery of the organisms from the sample debris, including solutions

of magnesium sulphate, D-mannitol, calcium chloride or sodium chloride; electricity; bubbling air through samples in a tube, etc. The efficacy of these techniques is affected both by the characteristics of the substrate material and the types of organisms present (Flannagan, 1973). Regardless of the sorting method used, heavy organisms such as clams and snails will not float and will have to be picked out with forceps.

6.4.1.2 Various staining methods have been devised to help speed the sorting process (Williams and Williams, 1974). Staining samples in the field with either rose bengal or phloxine B at a concentration of 100 g/L of ethanol or formalin significantly reduces sorting time for benthic samples (Mason and Yevich, 1967). Should the stain interfere with identifications where color patterns or internal organs must be examined, the stain can be removed by placing the organisms in 95% ethanol over night.

6.4.1.3 As soon as the sample is sorted, make note in the log book, including the date and the initials of the person who sorted the sample. It is often advisable to ask a co-worker to check the sample debris before discarding to be certain no organisms were overlooked. The organisms may be sorted and transferred to watch glasses or petri dishes for immediate identification and counting, or stored in vials for future identification.

6.4.2 Subsampling

6.4.2.1 Analysis time for samples containing large numbers of organisms can be substantially reduced if the samples are subdivided before sorting. There are several methods for subdividing the samples and each method has its advantages and disadvantages.

6.4.2.2 Welch (1948) described a method that has been used successfully for many years. The sample is thoroughly mixed and distributed evenly over the bottom of a shallow white-bottom pan. A divider, delineating one-quarter sections, is placed in the tray and one quarter or two opposite quarters are sorted.

6.4.2.3 An air driven subsampler (Figure 12) was described by Wrona et al. (1982) and modified by the State of Maine Department of Environmental Protection (Susan Davies, Personal communication). The sample is placed in a Imhoff-type settling cone that is filled with water to a total volume of one liter. The sample is gently agitated for two to five minutes by use of an air stone sealed into the bottom and connected to an air supply. One-quarter of the sample is removed with a wide-mouth 50 mL dipper or test tube in five aliquots and combined in a white-bottom pan for hand sorting. If less than 100 organisms are present in the one-quarter subsample, additional one-quarter subsamples are removed until the subsample contains at least 100 organisms. Large or heavy organisms that cannot be suspended by agitating the water are sorted and counted separately.

6.4.2.4 The Rapid Bioassessment Protocols II and III (Plafkin et al., 1989) use a modification of a subsampling method described by Hilsenhoff (1987).

All large detrital material (leaves, twigs, etc.) are rinsed, visually

inspected for organisms, and discarded. The sample is then poured into a white-bottom pan that has been marked with a grid pattern of 5-cm squares. Grids are randomly selected and all the organisms in the selected grids are picked in succession until approximately 100 organisms have been removed from the sample. All the organisms in the grid that contains the 100th organism are picked once that grid is started. Before using this method, live organisms should be narcotized with club soda or nicotine before sorting so they will not move from square to square.

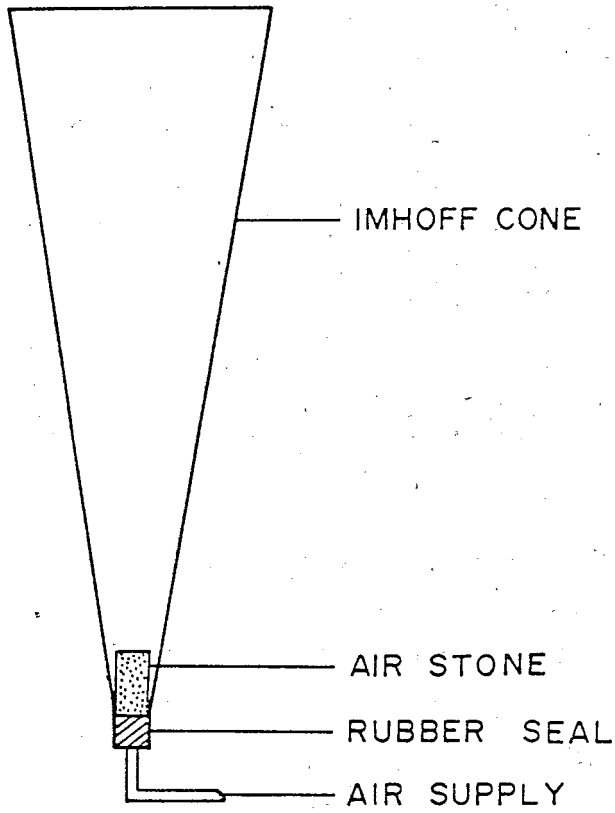


Figure 12. Imhoff cone subsampler (From Wrona et al., 1982).

6.4.2.5 Regardless of the method used for subsampling, the sorted sample should be labelled to reflect the portion sorted (e.g., 2X if half sorted, 4X if one-quarter sorted, 100 C if 100 count method was used, etc.) with the sample ID number. The unsorted portions of the sample should be combined, preserved, labeled and stored for future reference. It should be discarded only if there is no possible future need.

6.4.2.6 Experience has shown that, if less than one-quarter of the original sample is sorted, considerable error may result in estimating the total numbers of worms and other organisms that tend to clump. If the sample contains large numbers of a single taxonomic group (such as oligochaete worms

or midges) but few other organisms, it may be advisable to subsample the abundant taxa and pick all of the other organisms.

## 6.5 Preparation of Microscope Slide Mounts

6.5.1 To identify certain taxa of macroinvertebrates, it is often necessary to make slide mounts of all or parts of the organisms for examination under a compound microscope. Generally, if the organism is over 10 mm in length, it is best to carefully remove the important diagnostic structures (such as mouthparts or genitalia) with fine pointed forceps and mount them on microscope slides. Some large chironomids and tubificid worms that are too long to be mounted whole are cut in half and mounted under two separate cover glasses on the same slide.

6.5.2 Because most of the slides made for diagnostic purposes will be discarded after the organisms have been identified, we recommend mounting directly from the preservative using a water miscible mounting medium consisting of a mixture of two-thirds CMCP-9 and one-third CMCP-9AF (Beckett and Lewis, 1982). This mixture stains the organism a light red and contains a clearing agent providing optimum contrast for easy viewing of taxonomically important structures after about 12 hours clearing time. Because CMCP-9/9AF is a low viscosity medium, the specimen can be easily manipulated after the cover glass is in place by using pressure from forceps on the cover glass, rolling the specimen while viewing with a dissecting microscope until the best viewing position is obtained. The slides may be made permanent by ringing the cover glass with additional CMCP-9/9AF followed 24 hours later with polyurethane spar varnish or fingernail polish. Round 12 mm or 15mm cover glasses are recommended because they are less likely to trap air bubbles, are easier to manipulate, and less likely to break with pressure than the square ones. This method has proven very successful for making semi-permanent slides of whole chironomids and oligochaetes and parts of mayflies, caddisflies, and other macroinvertebrates.

6.5.3 Other slide-making techniques have been recommended for specific groups of organisms (Mason, 1973; Beck, 1975; Britton and Greeson, 1988). Although these methods are more time consuming and require more effort than the above method, they are thought to produce superior results by some taxonomists and are considered more permanent.

6.5.3.1 Many chironomid taxonomists use KOH to clear the midges before mounting them in Euparal (Mason, 1973) or CMCP-10 (Beck, 1975). The US Geological Survey (Britton and Greeson, 1988) has adopted a slightly modified version of this method for mounting midges and blackflies as follows:

1. Place the specimens in distilled water for 10 minutes to remove the preservative.
2. Transfer to crucibles containing 10% KOH and heat for 10 to 15 minutes to digest opaque tissue, taking care not to digest exoskeleton also.
3. Soak in distilled water for at least 3 minutes to remove KOH.
4. Soak in 95% ethyl alcohol for three to five minutes.
5. Mount in a drop of Euparal or CMCP-10.
6. Place specimen ventral side up and cover with a 12 mm cover glass.

7. Working under a stereoscopic microscope, apply pressure from a pencil eraser to roll ventral side up and flatten the head capsule.
8. Allow the slide to dry for about a week before storing on edge.

6.5.3.2 Water mites mounted using either of the above methods are nearly impossible to identify beyond family level. If identification to genus or species is needed, the mites should be dissected first to speed the clearing process and make it possible to examine sclerotized plates and other structures on both dorsal and ventral surfaces of the abdomen. First, using a dissecting microscope, forceps and a needle, separate one palp or the entire gnathostoma with palps from the body and mount the palps in the position shown in Figure 13. Next, separate the dorsum of the abdomen from the venter leaving a small section of the posterior body wall intact as shown in Figure 14, and mount with the venter and dorsum upward. Rather than dissect the very small specimens, pierce the body wall in the posterior-lateral areas to facilitate the clearing process and mount with the ventral surface upward (Britton and Greeson, 1988).

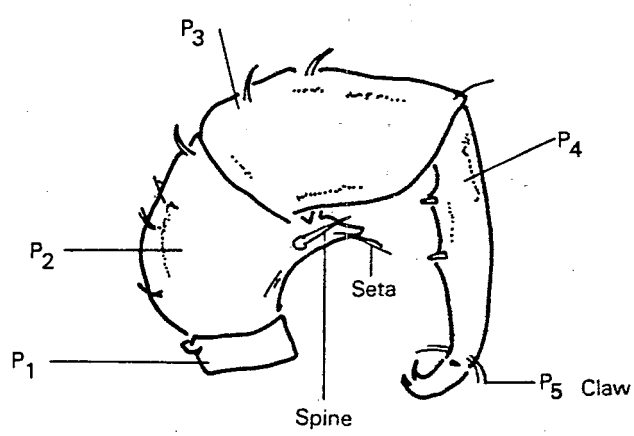


Figure 13. Five-segmented palp of a water mite (From Britton and Greeson, 1988).

6.5.3.3 When permanent slides are needed for the water mites, the double cover-glass glycerine method described by Mitchell and Cook (1952), modified by Britton and Greeson (1988), and illustrated in Figure 15 should be used.

6.5.4 Aquatic oligochaete worms--To identify oligochaete worms the specimens must be go through a clearing process and be side mounted. The identification of species requires a compound light microscope and some specimens require oil immersion (1000X). Some worm specialists make temporary mounts by placing oligochaete specimens on sides in Amman's lactophenol (100 g phenol, 100 ml lactic acid, 200 ml glycerine, 100 ml water), a medium which clears tissues and eliminates the risk of specimen desiccation if a more permanent

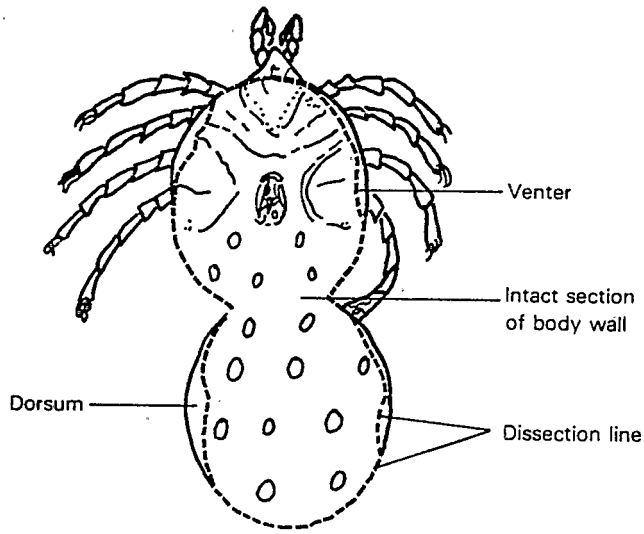


Figure 14. A water mite showing the dorsum separated from the venter, leaving a small section of the posterior body wall intact (From Britton and Greeson, 1988).

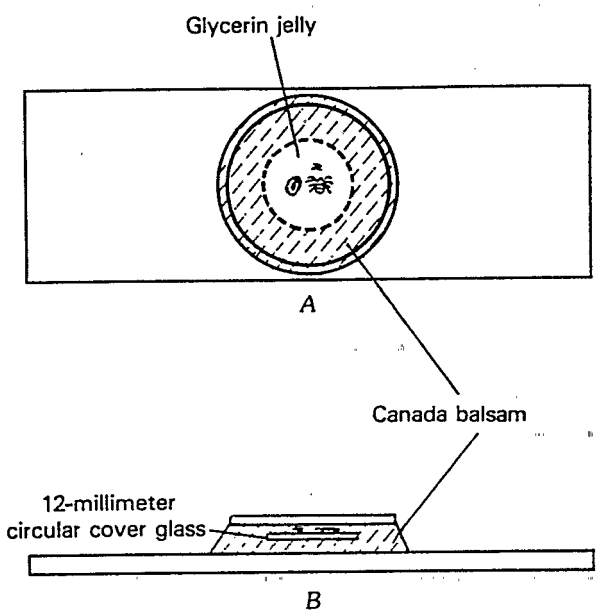


Figure 15. Top (A) and side (B) views of the double cover-glass technique for mounting aquatic water mites (From Britton and Greeson, 1988).

mount cannot be prepared immediately following extraction from the sample (Brinkhurst, 1986; Hiltunen and Klemm, 1980, Stimpson, et al., 1982; or Klemm, 1985). The clearing process usually takes a few hours to a few days depending on the size of the specimens. Gentle application of heat will speed the clearing process. If the specimens are preserved in 70-80% ethyl alcohol, they should be placed in 30% ethyl alcohol and then in water for a short time to leach out the alcohol before clearing. The alcohol retards the clearing process of Amman's lactophenol (Hiltunen and Klemm, 1980, Stimpson et al., 1982; Klemm, 1985). Do not leave specimens in the water too long (not more than two hours) because the worms will begin to deteriorate. Naidids and tubificids can be held indefinitely in Amman's lactophenol or 10% buffered formalin for later processing and mounting.

6.5.4.1 Non-resinous media are recommended for rapid processing of large numbers of specimens. For extremely important reference specimens, a permanent resinous mounting medium is best.

6.5.4.2 The non-resinous semi-permanent mounting media (CMCP-9 or 9AF, CMCP-10, or aquamount), which also contain clearing agents, are the simplest to use, allow for rapid processing of specimens, and are usually adequate for species identification. If Fuschin dye is added to the colorless mounting media (CMCP-9 or CMCP-10), only enough of the dye should be used (avoid overstaining) to slightly or partially stain the specimens. The specimens can be mounted directly on the slide using these media. However, the clearing process of these media takes approximately 24 hours. If the slides are to be semi-permanent, the edge of the cover slip should be sealed with finger nail lacquer to prevent the mounting medium from shrinking and forming bubbles under the cover slip. An 18 mm diameter, No. 0 or 1 round cover glass is appropriate because it will adequately accommodate the size range of the worms and the shape allows for maneuvering the specimen to rest in the most desired position by gentle rotation of the cover glass.

6.5.4.3 Place naidids or tubificids on their sides so that both dorsal and ventral fascicles of chaetae can be examined (Hiltunen and Klemm, 1980; Stimpson et al., 1982; Klemm, 1985). A variation from this is followed with specimens of Dero which must be viewed from the dorsal aspect, revealing the arrangement of the branchial apparatus (Hiltunen and Klemm, 1980, Klemm, 1985). The methods sections found in Hiltunen and Klemm (1980)and Klemm (1985) should be consulted for more specific information on identification of specimens.

6.5.4.4 Optimal resolution and longevity of mounted materials are achieved only in resinous media (e.g., Canada Balsam, Harleco's Xylene Coverbond, etc.). These mounting media require dehydration of the specimens through the alcohol series and clearing before mounting in Canada balsam or other resinous medium, but they produce the best permanent mounts (Knudsen, 1966; Klemm, 1985).

6.5.5 Leeches--species identification of most specimens do not require mounting on slides. A stereozoom microscope of 500X is needed for species identification. However, specialized slide-making techniques must be used for species identification of some leeches (See Klemm, 1982, 1985, 1990).

6.5.6 Regardless of the mounting method used or the permanence of the slides, proper labelling is a must. The label should include the date the slide was made, the sample ID number, and the initials of the person who made the slide. Labels on permanent slides should also include the location of the collecting site and name of the collector.

## 6.6 Drying Methods

6.6.1 Occasionally, alcohol-preserved specimens may require dry mounting on points or minutens for identification. The critical point drying method is recommended because the pigments colors are preserved, specimens do not collapse, and they are not brittle. Specimens to be dried are taken from 80% ethanol and passed through the alcohol series of washes in a small mesh screen basket with a lid, ending with two washes in 100% ethanol. After removal from the alcohol wash, the specimens, with the basket, are placed in the chamber of the critical point drier and processed according to dryer instructions (Gordh and Hall, 1979).

## 6.7 Organism Identification

6.7.1 The taxonomic level to which animals are identified depends on the needs, experience, and available resources. However, species level identification is very important in determining water quality and environmental pollution (Resh and Unzicker, 1975). The rapid bioassessment protocol II calls for organism identification only to the family level for use with Hilsenhoff's (1988) Family Biotic Index, whereas protocol III calls for identification to genus or species if possible (Plafkin et al., 1989). Many state programs carry most organism identifications to the genus level, while others (e.g., State of Maine) carry identification of certain taxa, such as stoneflies and mayflies, to species. Although the selective sensitivity of a family-level identification effort is often sufficient for differentiating non-impaired, moderately impaired, and severely impaired conditions, subtle differences in biological impairment will not be discerned except by species-level identification (Plafkin et al., 1989). In general, identifications should be carried to the lowest taxonomic level readily possible, and the taxonomic level to which identifications are carried in each major group should be constant throughout a given study.

6.7.1.1 Since the accuracy of identification depends on the availability of up-to-date taxonomic literature. A library of the basic taxonomic literature is essential for benthic laboratories. Basic references that should be available in a macroinvertebrate identification laboratory are listed in Section 8, Taxonomic Bibliography.

6.7.2 For comparative purposes and quality control checks, a reference collection of identified specimens should be established in each laboratory.

6.7.3 Most identifications to order and family can be made using a hand lens or a stereoscopic microscope with up to 50X magnification. Identification to genus and species often requires a compound microscope with phase contrast capable of 1000X magnification. Preparation of specimens for microscopic viewing is discussed in Section 6.5.

6.7.4 Insect larvae often comprise the majority of macroinvertebrates collected with artificial substrate samplers, drift nets, and other net type devices. In certain cases, identifications are facilitated if exuviae, pupae, and adults are available.

6.7.5 The life history stages of an insect can be positively associated only if specimens are reared individually. Small insect larvae can be reared individually in 6 to 12 dram vials half filled with stream water and aerated by use of a fine-drawn glass tubing. Mass rearing can be carried out by placing rocks and sticks containing the larvae in an aerated aquarium. Current can be provided in the aquarium by use of a magnetic stirrer (Mason and Lewis, 1970).

6.7.6 As organisms are identified, the individuals in each taxonomic category are counted and the numbers recorded on bench sheets (see Appendix C). Samples are compared by use of a summary sheet (see Appendix D) which provides room for comparing eight samples from the same sampling site.

## 6.8 Biomass

6.8.1 Macroinvertebrate biomass (weight of organisms per unit area) is a useful quantitative estimation of standing crop and is useful in assessing the biological integrity of surface waters. One study shows that biological assessments of water quality status using biomass estimates of wet, dry, and ash-free dry weights provide essentially similar results concerning impact of a sewage treatment plant discharge as did counts of individual organisms using a variety of commonly utilized biotic indices of water quality (Mason et al., 1983, 1985). To determine wet weights, soak the organisms in distilled or deionized water for 30 minutes, centrifuge for one minute at 140 g in wire mesh cones, and weigh to the nearest 0.1 mg. To obtain dry weight, dry the organisms to a constant weight at 105 degrees C for 4 hours or vacuum dry at 105 degrees C for 15 to 30 minutes at one-half atmosphere. Cool to room temperature for 15 minutes and weigh to nearest 0.1 mg. Freeze drying (-55 degrees C, 10 to 30 microns pressure) can be used. It has advantages over oven drying because the organisms remain intact for identification and reference, preservatives are not needed, and cooling the material in desiccators after drying is not required. The main disadvantage of freeze drying is the time (usually 24 hours) required for drying to a constant weight. To obtain ash-free dry weight, ash the dried organisms at 500 degrees C for one hour. Cool the ash to ambient temperature in a desiccator and weigh to the nearest 0.1 mg. Express the biomass as ash-free dry weight.

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## SECTION 7

## DATA EVALUATION

### 7.1 Introduction

7.1.1 One of the major concerns of USEPA, other federal, state and private agencies is to describe water quality and habitat quality in terms which are easily understood by the non-biologist. The purpose of this section is not to recommend one particular data evaluation method, but to point out a number of more common methods. Some of these methods may not be applicable to every stream or water body in the United States.

7.1.2 Water quality and habitat quality are reflected in the species composition and diversity, population density and biomass, and physiological condition of indigenous communities of aquatic organisms. A number of data interpretation methods have been developed based on these community characteristics to indicate the water quality and the degree of habitat degradation, and also to simplify communication problems regarding management decisions.

### 7.2 Analyses of Qualitative Data

7.2.1 As previously defined, qualitative data result from samples collected in such a manner that no estimates of numerical abundance or biomass can be calculated. The principle output is a list of taxa collected in the various habitats of the environment studied. The numerous schemes advanced for the analysis of qualitative data may be grouped under two categories; the indicator organism scheme and reference station methods.

7.2.2 Indicator Organism Scheme

7.2.2.1 For this technique, individual taxa are classified on the basis of their tolerance or intolerance to various levels of domestic wastes (Beck, 1954; Lewis, 1974; Chutter, 1972; Hilsenhoff, 1977; Howmiller and Scott, 1977; Milbrink, 1983; Reynoldson et al. 1989). Taxa are classified as tolerant or intolerant according to their presence or absence in different environments as determined by field studies. Beck (1955), reduced data, based on the presence or absence of indicator organisms, to a simple numerical form for ease in presentation. Clean water taxa are given twice the weight as tolerant organisms in the formula:

$$2\ (n\ \text{Class I}) + (n\ \text{Class II}) = \text{Biotic Index}$$

where "n" is the number of taxa in that class. Values less than 10 are considered to indicate a polluted stream.

7.2.3 Reference Station Methods

7.2.3.1 Reference station methods (Ohio EPA, 1989) compare the

characteristics of the fauna in clean water habitats with those of fauna in habitats subject to stress. Patrick (1950) compared stations on the basis of richness of species, and Wurtz (1955) used indicator organisms in comparing stations.

7.2.4 If adequate background data are available to an experienced investigator, both of these techniques can prove quite useful; particularly for demonstrating the effects of gross to moderate organic contamination on the macroinvertebrate community. To detect more subtle changes in the macroinvertebrate community, quantitative data on numbers or biomass of organisms are needed. Data on the presence of tolerant and intolerant taxa and richness of species may be effectively summarized for evaluation and presentation by means of line graphs, bar graphs, pie diagrams, histograms, or pictorial diagrams (Ingram and Bartsch, 1960).

7.2.5 Classification of representative macroinvertebrates according to their tolerance of organic wastes is presented in Appendix A. Hilsenhoff's (1977) original tolerance classification with a numerical range of 0 to 5 is followed in Appendix A. Later, Hilsenhoff (1987) modified his biotic index for Wisconsin taxa to include more intermediate values with a numerical ranged of 0-10. However, similar results can be obtained using index values of either 0-5 or 0-10, and adequate information is not available for many species that would allow use of the more definitive 0-10 tolerance range (Hilsenhoff, 1990, personal communication). In most cases, the taxonomic nomenclature used is that of the original authors listed at the end of Appendix A. The pollutional classifications were arbitrarily placed in three categories--tolerant, facultative, and intolerant--defined as follows:

- Tolerant: Organisms frequently associated with gross organic contamination, that are generally capable of thriving under anaerobic conditions. Tolerance values 4 and 5.

- Facultative: Organisms having a wide range of tolerance that frequently are associated with moderate levels of organic contamination. Tolerance values 2 and 3.

- Intolerant: Organisms that are usually not found associated with organic contaminants and are generally intolerant of even moderate reductions in dissolved oxygen. Tolerance values 0 and 1.

When evaluating qualitative data in terms of material such as that contained in Appendix A, the investigator should keep in mind the pitfalls mentioned earlier, as well as the following:

7.2.5.1 Since tolerant species may be found in both clean and degraded habitats, a simple record of their presence or absence is not of significance. However, the presence of intolerant organisms provides evidence of only one condition--clean water. But the fact that sensitive (intolerant) species may be totally absent, because of the discharge of toxic substances or thermal pollution, would indicate that absence of intolerant species may not be a reflection of the presence of organic wastes. The presence of tolerant organisms is a significant indicator of organic

pollution only when they are dominant in the sample.

7.2.5.2 The presence or absence of particular taxa may depend more on characteristics of the environment, such as velocity and substrate, than on the level of degradation by organic wastes. This affects both the original placement of the taxa in the classificatory scheme and its presence in study samples.

7.2.5.3 Because indicator species evaluations are based on the presence or absence of organisms, a single specimen has as much weight as a large population. Therefore, studies may be biased by the drift of organisms into the study area. The technique is totally subjective and dependent upon the skill and experience of the individual who makes the field collections. Therefore, results of one investigator are difficult to compare with those of another, particularly where data are summarized in an index such as that proposed by Beck (1955).

7.2.6 Biotic Index

7.2.6.1 Many of the problems discussed above can be overcome by use of the biotic index proposed by Chutter (1972) and modified by Hilsenhoff (1977) for use with the index values given in Appendix A. Any organisms not listed in Appendix A should be given an index of three (3) unless available information would suggest a different value. This same formula is used with the family level biotic index of Hilsenhoff (1988a) and the Rapid Bioassessment metric 2 of Protocol III (Plafkin et al., 1989) where pollution tolerance values of 0-10 are used. Appendix B gives the family level index values (Hilsenhoff, 1988a) for use with the family level biotic index. Results are comparable between stations in the same and nearby streams if similar habitats were sampled using similar methods and sampling effort (Hilsenhoff, 1988a,b). The formula to use is:

$$HBI = \Sigma \frac{n_i \, a_i}{N}$$

Where "$n_i$" is the number of individuals in the "$i^{th}$" taxa, "$a_i$" is the index value of that taxa, and "N" is the total number of individuals in the sample. Biotic index values below 1.75 indicate excellent water quality, 1.76-2.50 indicate good water quality, 2.51-3.75 indicate fair water quality, 3.76-4.00 indicate poor water quality, and over 4.00 would indicate serious water quality problems.

7.2.6.2 The following are water quality values for Hilsenhoff's (1988a) family level biotic index: 0.00-3.75 (excellent), 3.76-4.25 (very good), 4.25-5.00 (good), 5.01-5.75 (fair), 5.76-6.50 (fairly poor), 6.51-7.25 (poor), and 7.26-10.00 (very poor).

**7.3 Analyses of Semi-quantitative and Quantitative Data**

7.3.1 The high variability usually associated with benthic macroinvertebate

populations makes them difficult to study quantitatively because of the large number of samples needed to obtain normal levels of precision. For most benthic studies, it is generally impractical, due to large number of samples needed, to detect population changes of less than 100% of the mean. Many benthic populations exhibit such high variability (see Section 4.5.) that any reasonable number of replicate samples would be too small to detect a population density difference of more than 200% of the mean between two sites (Schwenneker and Hellenthal, 1984). It is important to keep this limitation in mind as one considers the methods to use in evaluating the data.

7.3.2 Data from quantitative samples may be used to obtain total standing crop of individuals, or biomass, or both and numbers or biomass, or both, of individual taxa per unit area or unit volume or sample unit. Data from quantitative samples may also be evaluated in the same manner as discussed for qualitative samples but results will be qualitative. In order to reduce the amount of time spent in field sampling, there has been a recent trend to collect data based on level of effort or other not strictly quantitative methods and treat the data as semi-quantitative. These data are then analyzed using the quantitative methods described in this section.

7.3.3 For purposes of comparison and to provide data useful for determining production, a uniform convention must be established for the units of data reported. For this purpose, USEPA biologists should adhere to the following units:

- Data from devices sampling a unit area of bottom are reported in grams dry weight or ash-free dry weight per square meter ($gm/m^2$), or numbers of individuals per square meter, or both.
- Data from multiplate samplers are reported in terms of the total surface area of the plates, as grams dry weight or ash-free dry weight or numbers of individuals per square meter, or both.
- Data from rock-filled basket samplers are reported as grams dry weight, ash-free dry weight, or numbers of individuals per sampler, or both.

7.3.4 Three informative parameters of benthic community structure which may be obtained from quantitative grab or artificial substrate sample data are standing crop (biomass or numbers), species richness, and species composition. Standing crop and species richness in a community are highly sensitive to natural environmental conditions and to anthropogenic perturbations resulting from the introduction of contaminants. These parameters, particularly standing crop, may vary considerably in unpolluted habitats, where they may range from the typically high standing crop of littoral zones of glacial lakes to the sparse fauna of torrential soft-water streams. Thus, it is important that comparisons be made only between truly comparable habitats. Typical responses of standing crop or species richness to various types of stress are shown in Table 7 below:

7.3.5 Organic enrichment and sludge deposits are frequently associated. The responses shown are by no means simple or fixed and may vary depending on a

number of factors including a combination of stresses acting together or in opposition, indirect effects (such as the destruction of highly productive vegetative substrate by temperature alterations, sludge deposits, turbidity, or chemical weed control) and the physical characteristics of the stressed environment; particularly in relation to substrate and current velocity.

Table 7. TYPICAL RESPONSES TO VARIOUS TYPES OF STRESS BY PARAMETERS OF BENTHIC COMMUNITY STRUCTURE

| Stress | Standing crop (Numbers or Biomass) | Number of Taxa |
|---|---|---|
| Toxic substance | Reduces | Reduces |
| Severe temperature changes | Variable | Reduces |
| Silt | Reduces | Reduces |
| Low pH | Reduces | Reduces |
| Inorganic nutrients | Increases | Variable |
| Organic enrichment (Low DO) | Increases | Reduces |
| Sludge deposits (Non toxic) | Increases | Reduces |

7.3.6 Data on standing crop and species richness may be presented in simple tabular form or pictorially with bar and line graphs, pie diagrams, and histograms. Whatever the method of presentation, the number of replicates and the sampling variability must be shown in the tables or graphs. Sampling variability may be shown as a range of values or as a calculated standard deviation, as discussed in Section 7.6.

7.3.7 Data on standing crop and species richness are amenable to simple but powerful statistical techniques of evaluation. Under grossly stressed situations, such analyses may be unnecessary; however, in some cases, the effects of environmental perturbations may be so subtle in comparison with sampling variation that statistical comparisons are a helpful and necessary tool for the evaluation process. For this purpose, biologists engaged in studies of macroinvertebrates should familiarize themselves with the simple statistical tools discussed in Section 7.6.

7.3.8 The usefulness of species composition as a parameter of environmental quality is based on the generally observed phenomenon that relatively undisturbed environments support communities having large numbers of species with no individual species present in overwhelming abundance. If the species found in a random sample from such a community are ranked on the basis of their numerical abundance, there will be relatively few species with large numbers of individuals and large numbers of species represented by only a few individuals. Many forms of stress alter species composition by making the environment unsuitable for some species or by giving other species a competitive advantage.

7.3.9 It is important for the investigator to keep in mind that there are naturally occurring severely stressed environments supporting communities

dominated by one or more species adapted to rigorous conditions. Examples include the profundal fauna of deep lakes and the black fly dominated communities of the high gradient, bedrock section of a torrential stream. Furthermore, because colonization is by chance, both species richness and species composition may be highly variable in a successional community; for this reason, data summarized from artificial substrate samples must be evaluated with caution. These confounding factors can be reduced by comparing data from similar environments and by exposing artificial substrate samplers long enough for a relatively stable community to develop.

7.3.10 Data on species composition may be summarized and evaluated using percent species composition tables, frequency distribution tables and/or graphs; however, for any appreciable number of samples, such methods of presentation are so voluminous that they are virtually impossible to compare and interpret. Fortunately, single numerical values which provide a measure of species composition can be extracted from indices of diversity as proposed by Margalef (1957) and subsequently utilized by numerous workers (McIntosh, 1967; Cairns and Dickson, 1971; Wilhm and Dorris, 1968). Mean diversity ($\bar{d}$) may be calculated using the machine formula presented by Lloyd, Zar, and Karr (1968) and better known as the Shannon-Weaver mean diversity (Shannon and Weaver, 1963).

$$\bar{d} = \frac{C}{N} (N \log_{10} N - \Sigma n_i \log_{10} n_i)$$

where C=3.321928 (converts base 10 log to base 2); N = total number of individuals; and $n_i$ = total number of individuals in the $i^{th}$ species. When their table (see Table 23) is used, the calculations are simple and straightforward, as shown in Table 8.

Table 8 EXAMPLE OF CALCULATION OF MEAN DIVERSITY

| | Taxa Number | Number of Individuals in each Taxon ($n_i$) | $n_i \log n_i$ (From Table 23) |
|---|---|---|---|
| | 1 | 41 | 66.1241 |
| | 2 | 5 | 3.4949 |
| | 3 | 18 | 22.5949 |
| | 4 | 3 | .4314 |
| | 5 | 1 | .000 |
| | 6 | 22 | 29.5333 |
| | 7 | 1 | .0000 |
| | 8 | 2 | .6021 |
| | 9 | 12 | 12.9502 |
| | 10 | 4 | 2.4082 |
| Totals | 10 | 109 | 139.1391 |

$N \log N$ (109) = 222.0795 (From Table 23)

$\Sigma n_i \log_{10} n_i$ = 139.1391 (From Column 3 above)

$$\bar{d} = \frac{3.321928}{109} (222.0795 - 139.1391)$$

$\bar{d} = 0.030476 \times 82.9404$

$\bar{d} = 2.5$

7.3.10.1 Mean diversity as calculated above is affected both by richness of species and by the distribution of individuals among the species (species composition) and may range from zero to 3.321928 log N. Since the calculated value of mean diversity is a result of the interaction of two parameters which may vary independently, it is often insensitive to subtle changes in community structure. Therefore, unless the environment has been grossly modified, mean diversity (d) often has limited value in detecting alterations in community structure and serves mainly as an intermediate step in the calculation of a single numerical value for species composition.

7.3.11 To evaluate the component of diversity due to the distribution of individuals among the species (species composition), the calculated d must be compared with a hypothetical maximum d based on an arbitrarily selected distribution. The measure of redundancy proposed by Margalef (1957) is based on the ratio between d and a hypothetical maximum computed as though all species were equally abundant. In nature, equality of species is quite unlikely, so Lloyd and Ghelardi (1964), proposed the term "equitability" and compared d with a maximum based on the distribution obtained from MacArthur's (1957) broken stick model. The MacArthur model results in a distribution quite frequently observed in nature; one with a few relatively abundant species and increasing numbers of species represented by only a few individuals. Sample data are not expected to conform to the MacArthur model, since it is only being used as a yardstick against which the distribution of abundances is being compared. Lloyd and Ghelardi (1964) devised a table for determining equitability by comparing the number of species (s) in the sample with the number of species expected (s′) from a community that conforms to the MacArthur model. In the table (reproduced as Table 24) the proposed measure of equitability is:

$$e = \frac{s'}{s}$$

where s = the number of taxa in the sample and s′ = the tabulated value.

7.3.11.1 For the example given above:

$$e = \frac{s'}{s} = \frac{8}{10} = 0.8$$

where "s′" is found from Table 24 using $\bar{d}$ of 2.5. Equitability "e", as calculated, may range from 0 to 1 except in the unusual situation where the distribution in the sample is more equitable than the distribution resulting from the MacArthur model. Such an eventuality will result in values of "e" greater than 1, and this occasionally occurs in samples containing only a few specimens with several taxa represented. The value of "e" is not entirely

sample size independent and should not be used for samples containing fewer than five taxa.

7.3.11.2 Equitability ("e") is very sensitive to slight changes in community structure. Since the sample is a representation of the community sampled, a usable index must be sensitive to sample differences and within station variability must be handled by proper study design and adequate replication. Equitability above 0.5 is indicative of waters not affected by oxygen demand wastes. Even slight levels of degradation have been found to reduce equitability below 0.5, generally below 0.3.

7.3.12 Quantitative data can also be produced using the biotic index described in 7.2.6 as long as quantitative methods were used in sample collection and analysis, and proper assumptions are made concerning the subjective nature of the pollution tolerance values.

7.3.13 A rather simple technique for evaluating quantitative data is the sequential comparison index (SCI) which estimates relative differences in biological diversity (Cairns and Dickson, 1971). The method requires no taxonomic expertise on the part of the investigator and is based on differences in the shape, color, and size of the organisms. It should be stressed that the method is useful only as a technique to evaluate the diversity of the bottom community rapidly producing numerical data which can be interpreted statistically. However, it should not be used to replace other more exact techniques providing information on the identity and pollution tolerance of the organisms and requiring persons trained in aquatic ecology.

7.3.14 Wilhm's Species Diversity Index (Wilhm and Dorris, 1968) is based upon information theory and is an attempt to give a numerical value to the environmental changes caused by waste dischargers. This index takes into account not only the number of species encountered, but also the relative abundances of the different species and is very similar to that described in section 7.3.10. Results from this system indicate that values of "d" less than one are indicative of heavy pollution, values from one to three indicate moderate pollution and values above three are found in clean water areas.

7.3.15 Harkins and Austin (1973) have also developed a method that appears to be universal in scope and has worked well in diverse situations. This method is based on average diversity per individual and redundancy which are reduced to a single index value per sample utilizing a nonparametric discrimination technique which then gives a unique distance value from a predefined "biological desert" condition (control values). This condition exists as the case of no organisms present or only one species containing "n" organisms.

7.3.15.1 Computer programs have been written to perform the needed calculations as well as the analysis of variance which can be used with this method. Harkin and Austin's method then is essentially an objective method for reducing several biological indexes to a single meaningful value that will reflect subtle changes in the structure of aquatic communities. The resulting sets of standardized distance values can be compared subjectively

or can be subjected to statistical evaluation and probability level of differences assessed. With this method any changes of quality will be detected and can be plotted for long-term trend analysis.

## 7.4 Rapid Bioassessment Techniques

7.4.1 Rapid Bioassessment Techniques (Plafkin et al., 1989) are generally considered both qualitative and semi-quantitative. The protocols were established as a rapid means of detecting aquatic life impairments and assessing their relative severity and are not intended to replace traditional biomonitoring methods. The three protocols each consist of three basic components: water quality/physical characteristics, habitat assessment, and a biosurvey. The biological assessment in each protocol involves an integrated analysis of both functional and structural components of the aquatic communities through use of metrics for benthic macroinvertebrates and fish.

7.4.1.1 Rapid Bioassessment Protocol I consists of an estimation of the level of diversity of the aquatic biota; an estimation of the relative abundance of major macrobenthic taxa, using a qualitative sampling process to include as many habitats as possible; observations of the presence of fish, plants and physical structures; observations on habitat alterations; and observation on possible sources of impact.

7.4.1.2 Rapid Bioassessment Protocol II consists of an in the field estimation of the abundance level of the major aquatic biota, a list of families found in a 100-organisms subsample based on field identification, the number of individuals in each family, and separation of these into scraper and filtering collector functional feeding groups, collection of a course particulate organic material (CPOM) sample, and observations as in Protocol I.

7.4.1.3 Rapid bioassessment Protocol III is similar to Protocol II except that the subsampling and identifications are done in the laboratory and the organisms are identified to genus or species.

7.4.1.4 Rapid Bioassessment Protocols IV and V are based on fish surveys conducted by fishery personnel usually with assistance from the aquatic biologist involved with Protocols I to III.

## 7.5 Community Metrics and Pollution Indicators

7.5.1 Biological impairment of the benthic community may be assessed by use of metrics including community, population and functional parameters. Metrics measure different components of the community structure and have different ranges of sensitivity to stress. It is advisable, therefore, to use several metrics because an integrated approach provides more assurance of a valid assessment. A few of the more useful metrics are briefly described.

7.5.2 Species (or Taxa) Richness reflects the health of the community through a measurement of the variety of taxa (total number of families and/or

genera and/or species) present. Richness generally increases with increasing water quality, habitat diversity, and/or habitat suitability. Sampling of highly similar habitats will reduce the variability in this metric attributable to factors such as current speed and substrate type. Some pristine headwater streams may be naturally unproductive, supporting only a very limited number of taxa. In these situations, organic enrichment may result in an increase in number of taxa.

7.5.3 The modified Hilsenhoff Biotic Index (HBI) (Plafkin et al. 1989) was developed to summarize overall pollution tolerance of the benthic arthropod community with a single value. This index was developed as a means of detecting organic pollution in communities inhabiting rock or gravel riffles/runs. Although Hilsenhoff's (1977) biotic index using tolerance values of 0-5 was originally developed for use in Wisconsin, it is successfully used by several states and should prove reliable for extensive use, perhaps requiring regional modification in some instances. Based on an in depth study of 53 Wisconsin streams Hilsenhoff (1988a) expanded the scale for tolerance values to 0-10. The 0-10 scale was adopted for use with the Rapid Bioassessment Protocol III and was modified to include non-arthropod species.

7.5.3.1 Although it may be applicable for other types of pollutants, use of the HBI in detecting non-organic pollution effects has not been thoroughly evaluated. The state of Wisconsin is conducting a study to evaluate the ability of Hilsenhoff's index to detect non-organic effects. Winget and Mangum (1979) have developed a tolerance classification system applicable to the assessment of nonpoint source impact.

7.5.3.2 Invertebrate Community Index (ICI)--Ohio EPA (1989) measures the condition of the macroinvertebrate community by use of the Invertebrate Community Index (ICI). This index is a modification of the Index of Biotic Integrity (IBI) used for fish (Karr, 1981) consisting of ten community metrics. Scoring of each metric varies with drainage area and ecoregion (Ohio EPA, 1987), and all but one metric is generated from artificial substrate (multiplate) samplers. Metric 10 is based solely on qualitative sample data.

7.5.4 Ratio of Scraper and Filtering Collector Functional Feeding Groups reflect the riffle/run community food base and provides insight into the nature of potential disturbance factors. The proportion of the two feeding groups is important because predominance of a particular feeding type may indicate an unbalanced community responding to an overabundance of a particular food source. The predominant feeding strategy reflects the type of impact detected.

7.5.4.1 A description of the functional feeding group concept can be found in Cummins (1973). Genus-level functional feeding group designations for most aquatic insects can be found in Merritt and Cummins (1984). Within a functional feeding group individual taxa may be either specialists which are restricted to the utilization of a specific food resource or be facultative and thus be able to exploit a broader range of food resources. The trophic generalists (see Merritt and Cummins, 1984) are expected to be better able

to tolerate disturbance to aquatic habitats and thus become numerically dominant because of their more flexible ability to utilize available resources.

7.5.4.2 The relative abundance of scrapers and filtering collectors in the riffle/run habitat provides an indication of the periphyton community composition and availability of suspended fine particulate organic material (FPOM) associated with organic enrichment. Scrapers increase with increased abundance of diatoms and decrease as filamentous algae and aquatic mosses (which cannot be efficiently harvested by scrapers) increase. However, filamentous algae and aquatic mosses provide good attachment sites for filtering collectors, and the organic enrichment often responsible for over abundance of filamentous algae provide FPOM utilized by the filterers.

7.5.4.3 Filtering collectors are also sensitive to toxicants bound to fine particles and may decrease in abundance when exposed to sources of such bound toxicants. The scraper-to-filtering-collector ratio may not be a good indication of organic enrichment if adsorbing toxicants are present. This situation is often associated with point source discharges where certain toxicants adsorb readily to dissolved organic matter forming FPOM during flocculation. Toxicants thus become available to filterers via FPOM.

7.5.5 Ratio of Shredder Functional Feeding Group and Total Number of Individuals collected in a coarse particulate organic material (CPOM) sample is also based on the functional feeding group concept. The abundance of the shredder functional group relative to the abundance of all other functional groups allows evaluation of potential impairment as indicated by the CPOM-based shredder community. Shredders are sensitive to riparian zone impacts and are particularly good indicators of toxic effects when the toxicants involved are readily adsorbed to the CPOM and either affect the microbial communities colonizing the CPOM or the shredders directly (Plafkin et al. 1989).

7.5.5.1 The degree a toxicant effects shredders versus filterers depends on the nature of the toxicant and the organic particle adsorption efficiency. Generally, as the size of the particle decreases, the adsorption efficiency increases as a function of the increased surface to volume ratio (Hargrove 1972). Toxicants of a terrestrial source (pesticides and herbicides) accumulate on CPOM prior to leaf fall thus having a substantial effect on shredders. The focus of this approach is on a comparison to the reference community, which should have an abundance and diversity of shredders representative of the particular area under study. This allows for an examination of shredder or collector "relative" abundance as indicators of toxicity.

7.5.6 Ratio of Ephemeroptera-Plecoptera-Trichoptera (EPT) and Chironomidae abundance uses relative abundance of these indicator groups as a measure of community balance. Good biotic condition is reflected in communities having a fairly even distribution among all four major groups and with substantial representation in the sensitive groups Ephemeroptera, Plecoptera, and Trichoptera. Skewed populations having a disproportionate number of the generally tolerant Chironomidae relative to the more sensitive insect groups

may indicate environmental stress (Ferrington 1987). Certain species of some genera such as Cricotopus are highly tolerant (Lenat, 1983; Mount et al., 1984), opportunistic,and may become numerically dominant in habitats exposed to metal discharges where EPT taxa are not abundant, thereby providing a good indicator of toxicant stress (Winner et al., 1980; Clements et al., 1988).

7.5.6.1 Chironomids tend to become increasingly dominant in terms of percent taxonomic composition and relative abundance along a gradient of increasing enrichment or heavy metals concentration (Ferrington 1987).

7.5.7 The EPT Index (the total number of distinct taxa within the orders Ephemeroptera, Plecoptera, and Trichoptera) compared to total taxa present generally increases with increasing water quality. This value summarizes taxa richness within the insect orders that are generally considered to be pollution sensitive. Headwater streams which are naturally unproductive may experience an increase in taxa (including EPT taxa) in response to organic enrichment.

7.5.8 An alternative to the ratio of EPT and Chironomidae abundance metric is the Indicator Assemblage Index (IAI) developed by Shackleford (1988). The IAI integrates the relative abundances of the EPT taxonomic groups and the relative abundances of chironomids and annelids upstream and downstream of a pollution source to evaluate impairment. The IAI may be a valuable metric in areas where the annelid community may fluctuate substantially in response to pollutant stress.

7.5.9 Percent Contribution of Dominant Taxon to the total number of organisms is an indication of community balance at the lowest possible taxonomic level. (The lowest positive taxonomic level is assumed to be genus or species in most instances). A community dominated by relatively few species would indicate environmental stress. Shackleford (1988) has modified this metric to reflect "dominants in common" (DIC) utilizing the dominant five taxa at the stations of comparison. The DIC will provide a measure of replacement or substitution between the reference community and the downstream station.

7.5.10 Community Similarity Indices are used in situations where reference communities exist. The reference community can be derived through sampling an upstream station or prediction for a region using a reference data base. Data sources or ecological data files may be available to establish a reference community for comparison. Several of the many similarity indices available are discussed below:

7.5.10.1 Community Loss Index measures the loss of benthic species between a reference station and the station of comparison. The community loss index was developed by Courtemanch and Davies (1987) and is an index of dissimilarity with values increasing as the degree of dissimilarity from the reference station increases. Values range from zero (0) to "infinity." Based on preliminary data analysis, this index provides greater discrimination than the following two community similarity indices. The formula for determining community loss index is:

$$I = \frac{a - c}{b}$$

where I = Coefficient of Community Loss, "a" is the number of taxa at the unimpacted site, "b" is the number of taxa at the study site, and "c" is the taxa common to "a" and "b". The result is a ratio of the number of taxa assumed lost due to the pollution source (a-c) to the number of taxa remaining including any new taxa.

7.5.10.2 Jaccard Coefficient of Community measures the degree of similarity in taxonomic composition between two stations in terms of taxa presence or absence and discriminates between highly similar collections (Jaccard, 1912). Coefficient values, ranging from 0 to 1.0, increase as the degree of similarity with the reference station increases. See Boesch (1977), and USEPA (1983) for more detail. The formula for the Jaccard Coefficient is:

$$\text{Jaccard Coefficient} = \frac{a}{a + b + c}$$

where

a = number of species common to both samples
b = number of species present in Sample B but not A
c = number of species present in Sample A but not B

Sample A = reference station
Sample B = station of comparison

7.5.10.3 The Index of Similarity (S) Between Two Samples has been used to determine whether shifts in community assemblages have occurred along a stream gradient or above and below a pollutional impact. The Index of Similarity can also be used as a quality assurance tool when evaluating variance in community assemblages between two control or reference sites. The inverse of the Index of Similarity is known as the Index of Dissimilarity. Both are reported as percentages and the formula is ( Odum, 1971):

$$S = \frac{2C}{A + B}$$

Where A = Number of Species in Sample 1
B = Number of Species in Sample 2
C = Number of Species Common to both Species
1 - S = Index of Dissimilarity

7.5.10.4 The Pinkham and Pearson Community Similarity Index measures the degree of similarity in taxonomic composition in terms of taxa abundances and can be calculated with either percentages or numbers. A weighting factor can be added that assigns more significance to dominant species. See Pinkham and Pearson (1976) and USEPA (1983) for more detail. The formula is:

$$S.I._{ab} = \Sigma \frac{\text{Min}\ (X_{ia}, X_{ib})}{\text{Max}\ (X_{ia}, X_{ib})} \left[ \frac{X_{ia}}{X_a} \cdot \frac{X_{ib}}{X_b} / 2 \right]$$

Weighting factor

where $X_{ia}$, $X_{ib}$ = number of individuals in the $i^{th}$ species in sample A or B.

7.5.10.5 A Percent Similarity Method described by Gauch and Whittaker (1972) matches the benthic community structure of the site under study with an unimpacted site (control). It is a calculation of the degree to which the distribution of individuals within specific taxa in one site is similar to the distribution in another matched site. The value may range from zero (0) for sites with no taxa in common, to one (1) for identical communities.

$$P.S. = \frac{2\ \Sigma\ \text{min.}\ (P_{ij}, P_{ik})}{(P_{ij} + P_{ik})}$$

where P.S. = Percent similarity, $P_{ij}$ = Percentage of taxa "i" in community "j", and $P_{ik}$ = Percentage of organisms of taxa "i" in community "k".

7.5.10.6 Other Community Similarity Indices include Spearman's Rank Correlation (Snedecor and Cochran, 1980); Moriseta's Index (Moriseta, 1959); Biotic Condition Index (Winget and Mangum, 1979); and Bray-Curtis Index (Bray and Curtis, 1957; Whittaker, 1952). Calculation of a chi-square "goodness of fit" (Cochran, 1952) may also be appropriate.

7.5.11 Presence and/or Absence of Specific Indicator Organisms is usually based upon a classification of organisms as either pollution sensitive (intolerant), facultative (variable), or tolerant (see paragraph 7.2.5). For example, usually stoneflies, mayflies, and caddisflies are considered sensitive or facultative and, therefore, are usually the first to suffer in a polluted environment. Sludgeworms and bloodworms, on the other hand, can tolerate very heavy pollutional loads.

7.5.11.1 The method differs from the biotic index of Hilsenhoff (1977, 1987) in that only selected indicator species are used to make decisions, whereas his biotic index used all the organisms in the samples.

7.5.11.2 A classic example of a system using the presence/absence criteria, is the Saprobien system (Kolkwitz and Marsson, 1908) which recognizes three basic zones of pollution ranging from a zone of heavy pollution (polysaprobic) characterized by a lack of dissolved oxygen, an abundance of bacteria, and the presence of a few tolerant species, to a zone of recovery (oligosaprobic) characterized by relatively pure water with a somewhat stable species diversity and dissolved oxygen concentration. This system was developed for use in Europe. Its usefulness is limited to organic pollutants in slow moving streams and is not always applicable to rivers and streams of

the United States. A modification of the method was used in studies of the Illinois River (Richardson, 1928) and of a stream in southern Ohio (Gaufin and Tarzwell, 1956). A further modification of this method in combination with the biotic index was recently used by Rabeni et al. (1985) in the study of a Maine river. The results appear to be encouraging for wide use in this country. This approach is highly subjective and would naturally vary from one stream to another. It is also restricted to organic-type wastes.

7.5.12 Mean Number of Individuals per Sample is a simple means of comparing biological data. All of the individuals in all the replicate samples from one station are counted and divided by the number of replicates to yield the number of individuals per sample.

## 7.6 Statistical Methods

### 7.6.1 Graphical Examination of Data

Often the most elementary techniques are of the greatest use in data interpretation. Visual examination of data can point the way for more discriminatory analyses, or on the other hand, interpretations may become so obvious that further analysis is superfluous. In either case, graphical examination of data is often the most effortless way to obtain an initial examination of data and affords the chance to organize the data. Therefore, it is often done as a first step. Some commonly used techniques are presented below.

#### 7.6.1.1 Raw Data

It is of utmost importance that raw data be recorded in a careful, logical, interpretable manner together with appropriate, but not superfluous, annotations. Note that although some annotations may be considered superfluous to the immediate intent of the data, they may not be so for other purposes. Any note that might aid in determining whether the data are comparable to other similar data, etc., should be recorded if possible.

#### 7.6.1.2 Frequency Histograms

To construct a frequency histogram (see Freund, 1986) from the data, examine the raw data to determine the range, then establish intervals. Choose the intervals with care so they will be optimally integrative and differentiable. If the intervals are too wide, too many observations will be integrated into one interval and the picture will be hidden; if too narrow, too few will fall into one interval and a confusing overdifferentiation or overspreading of the data will result. It is often enlightening if the same data are plotted with the use of several interval sizes. Construct the intervals so that no doubt exists as to which interval an observation belongs, i.e., the end of one interval must not

be the same number as the beginning of the next.

Although a frequency table contains all the information that a comparable histogram contains, the graphical value of a histogram is usually worth the small effort required for its construction. Histograms are more immediately interpretable. The height of each bar is the frequency of the interval; the width is the interval width.

#### 7.6.1.3 Frequency Polygon

Another way to present essentially the same information as that in a frequency histogram is the use of a frequency polygon. Plot points at the height of the frequency and at the midpoint of the interval, and connect the points with straight lines.

#### 7.6.1.4 Cumulative Frequency

Cumulative frequency plots are often useful in data interpretation. The height of a bar (frequency) is the sum of all frequencies up to and including the one being plotted. Thus, the first bar will be the same as the frequency histogram, the second bar equals the sum of the first and second bars of the frequency histogram, etc., and the last bar is the sum of all frequencies.

Closely related to the cumulative frequency histogram is the cumulative frequency distribution graph, a graph of relative frequencies. To obtain the cumulative graph, merely change the scale of the frequency axis on the cumulative frequency histogram. The scale change is made by dividing all values on the scale by the highest value on the scale.

The value of the cumulative frequency distribution graph is to allow relative frequency to be read, i.e., the fraction of observations less than or equal to some chosen value. Exercise caution in extrapolating from a cumulative frequency distribution to other situations. Always bear in mind that in spite of a planned lack of bias, each sample, or restricted set of samples, is subject to influences not accounted for and is therefore unique. This caution is all the more pertinent for cumulative frequency plots because they tend to smooth out some of the variation noticed in the frequency histogram. In addition, the phrase "fraction of observations less than or equal to some chosen value" can easily be read "fraction of time the observation is less than or equal to some chosen value." It is tempting to generalize from this reading and extend these results beyond their range of applicability.

#### 7.6.1.5 Two-dimensional Graphs

Often data are taken where the observations are recorded as a pair (biomass and nutrient concentration). Here a quick plot of the set of pairs will usually be of value. The peaks and troughs, their frequency, together with intimate knowledge of the conditions of the study, might suggest something of biological interest, further statistical analysis, or further field or laboratory work.

7.6.1.6 In summary, carefully prepared tables and graphs may be important and informative steps in data analysis. The added effort is usually small, whereas gains in interpretive insight may be large. Therefore, graphic examination of data is a recommended procedure in the course of most investigations.

## 7.6.2 Sample Mean and Variance

### 7.6.2.1 Notation

Knowledge of certain computations and computational notations is essential to the use of statistical techniques. Some of the more basic of these will be briefly reviewed here.

To illustrate the computations, let us assume we have a set of data, i.e., a list of numeric values written down. Each of these values can be labeled by a set of numerals beginning with 1. Thus, the *first* of these values can be called $X_1$, the *second* $X_2$, etc., and the *last* one we call $X_n$,. The data values are labeled with consecutive numbers (recall from the definitions that these numeric values are observations), and there are n values in the set of data. A typical observation is $X_i$, where i may take any value between 1 and n, inclusive, and the subscript indicates which X is being referenced.

The sum of the numbers in a data set, such as our sample, is indicated in statistical computations by capital sigma, Σ. Associated with Σ are an operand (here, $X_i$), a subscript (here, i = 1), and a superscript (here, n).

$$\sum_{i=1}^{n} X_i$$

The subscript i= 1 indicates that the value of the operand X is to be the number labeled $X_i$ in our data set and that this is to be the first observation of the sum. The superscript n indicates that the last number of the summation is to be the value of $X_n$ the last X in our data set.

### 7.6.2.2 Calculation of the Sample Mean and Variance

Computations for the mean, variance, standard deviation, variance of the mean, and standard deviation of the mean (standard error) are presented below. Note that these are computations for a sample of n observations, i.e., they are statistics.

Note: The $X_i$'s are squared, then the summation is performed in the first term

$$\text{Mean } (\overline{X}): \quad \overline{X} = \frac{\sum_{i=1}^{n} X_i}{n}$$

$$\text{Variance } (s^2): \quad s^2 = \frac{\sum_{i=1}^{n} X_i^2 - \frac{(\sum_{i=1}^{n} X_i)^2}{n}}{n-1}$$

of the numerator; in the second term, the sum of the $X_i$'s is first formed, then the sum is squared, as indicated by the parenthesis.

$$\text{Standard deviation } (s): \quad s = \sqrt{s^2}$$

$$\text{Variance of the mean } (s_{\overline{x}}^2): \quad s_{\overline{x}}^2 = \frac{s^2}{n}$$

$$\text{Standard deviation of the mean } (s_{\overline{x}}): \quad s_{\overline{x}} = \sqrt{s_{\overline{x}}^2} = \frac{s}{\sqrt{n}}$$

### 7.6.3 Rounding

The questions of rounding and the number of digits to carry through the calculations always arise in making statistical computations. Measurement data are approximations, since they are rounded when the measurements were taken; count data and binomial data are not subject to this type of approximation.

Observe the following rules when working with measurement or continuous data.

* When rounding numbers to some number of decimal places, first look at the digit to the right of the last place to be retained. If this number is greater than 5, the last place to be retained is rounded up by 1; if it is less than 5, do not change the last place – merely drop the extra places. To round to 2 decimal places:

| Unrounded | Rounded |
|---|---|
| 1.239 | 1.24 |
| 28.5849 | 28.58 |

* If the digit to the right of the last place to be retained is 5, then look at the second digit to the right of the last place to be kept, provided that the unrounded number is recorded with that digit as a significant digit. If the second digit to the right is greater than 0, then round the number up by 1 in the last place to be kept; if the second digit is 0, then look at the third digit, etc. To round to 1 place:

| Unrounded | Rounded |
|---|---|
| 13.251 | 13.3 |
| 13.25001 | 13.3 |

* If the number is recorded to only one place to the right of the last place to be kept, a special rule (odd-even rule) is followed to ensure that upward rounding occurs as frequently as downward rounding. The rule is: if the digit to the right of the last place to be kept is 5, and is the last digit of significance, round up when the last digit to be retained is *odd* and drop the 5 when the last digit to be retained is even. To round to 1 place:

| Unrounded | Rounded |
|---|---|
| 13.25 | 13.2 |
| 13.3500 | 13.4 |

Caution: all rounding must be made in 1 step to avoid introducing bias. For example the number 5.451 rounded to a whole number is clearly 5, but if the rounding were done in two steps it would first be rounded to 5.5 then 6.

Retention of significant figures in statistical computations can be summarized in three rules:

* Never use more significance for a raw data value than is warranted.

* During intermediate computations keep all significant figures for each data value, and carry the computations out in full.

* Round the final result to the accuracy set by the least accurate data value.

### 7.6.4 Tests of Hypotheses

#### 7.6.4.1 Introduction

Often in biological field studies some aspect of the study is directed to answering a hypothetical question about a population (Allan, 1984). If the hypothesis is quantifiable, such as: "At the time of sampling, the standing crop

of macroinvertebrates per basket at station 1 was the same as at station 2", then the hypothesis can be tested statistically. The question of drawing a sample in such a way that there is freedom from bias, so that such a test may be made, was discussed in Section 4, Selection of Sampling Stations.

There are many different types of hypothesis tests. Two basic categories of hypothesis tests are parametric tests, those based on the data following a specific distribution, and nonparametric tests, those based on relative rankings of the data. Three standard parametric tests of hypotheses will be presented here: the t-test, the $\chi^2$ test, and the F-test. For information concerning nonparametric tests see Conover, 1980.

### 7.6.4.2 T-test

The t-test is used to compare a sample statistic (such as the mean) with some value for the purpose of making a judgment about the population as indicated by the sample. The comparison value may be the mean of another sample (in which case we are using the two samples to judge whether the two populations are the same). The form of the t-statistic is

$$t = \frac{\phi - \theta}{s_\phi}$$

where $\phi$ = some sample statistic; $S_\phi$ = the standard deviation of the sample statistic; and $\theta$ = the value to which the sample statistic is compared (the value of the null hypothesis).

The use of the t-test requires the use of t-tables. The t-table is a two-way table usually arranged with the column headings being the probability, $\alpha$, of rejecting the null hypothesis when it is true, and the row headings being the degrees of freedom. Entry of the table at the correct probability level requires a discussion of two types of hypotheses testable using the t-statistic.

The null hypothesis is a hypothesis of no difference between a population parameter and another value. Suppose the hypothesis to be tested is that the mean, $\mu$, of some population equals 10. Then we would write the null hypothesis (symbolized $H_o$) as

$$H_o: \mu = 10$$

Here 10 is the value of $\theta$ in the general form for the t-statistic. An alternative to the null hypothesis is now required. The investigator, viewing the experimental situation, determines the way in which this is stated. If the investigator merely wants to answer whether the sample indicates that $\mu$ = 10 or

not, then the alternate hypothesis, $H_a$, is

$$H_a : \mu \neq 10$$

If it is known, for example, that $\mu$ cannot be less than 10, the $H_a$ is

$$H_a : \mu > 10$$

and by similar reasoning the other possible $H_a$ is

$$H_a : \mu < 10$$

Hence, there are two types of alternate hypotheses: one where the alternative is simply that the null hypothesis is false $H_a$: $\mu \neq 10$; the other, that the null hypothesis is false and, in addition, that the population parameter lies to one side or the other of the hypothesized value [$H_a$ : $\mu$ (> or <) 10]. In the case of $H_a$: $\mu \neq 10$, the test is called a two-tailed test; in the case of either of the second types of alternate hypotheses,, the t-test is called a one-tailed test.

To use a t-table, it must be determined whether the column headings (probability of a larger value, or percentage points, or other means of expressing $\alpha$) are set for one-tailed or two-tailed tests. Some tables are presented with both headings, and the terms "sign ignored" and "sign considered" are used. "Sign ignored" implies a two-tailed test, and "sign considered" implies a one-tailed test. Where tables are given for one-tailed tests, the column for any probability (or percentage) is the column appropriate to twice the probability for a two-tailed test. Hence, if a column heading 0.025 and the table is for one tailed tests, use this same column for 0.05 in a two tailed test (double any one-tailed test heading to get the proper two-tailed test heading; or conversely, halve the two-tailed test heading to obtain proper headings for one-tailed tests).

Testing $H_o$ : $\mu$ = M (the population mean equals some value M):

$$t = \frac{\overline{X} - M}{s_{\overline{X}}}$$

where $\overline{X}$ is given by the sample mean; M = the hypothesized population mean; and $s_{\overline{X}}$ is given by the standard deviation (standard error) of the mean. The t-table is entered at the chosen probability level (often 0.05) and n-1 degrees of freedom, where n is the number of observations in the sample.

When the computed t-statistic exceeds the tabular value there is said to

be a $1-\alpha$ probability that $H_o$ is false.

Testing $H_o$: $\mu_1 = \mu_2$ (the mean of the population from which sample 1 was taken equals the mean of the population from which sample 2 was taken):

$$t = \frac{\overline{X}_1 - \overline{X}_2}{s_{\overline{X}_1} - s_{\overline{X}_2}}$$

where $\overline{X}_1$ and $\overline{X}_2$ are the means from sample 1 and sample 2 respectively and $s_{\overline{X}_1} - s_{\overline{X}_2}$ is the standard error for the difference $\overline{X}_1 - \overline{X}_2$ calculated as follows:

$$s_{\overline{X}_1 - \overline{X}_2} = \sqrt{\frac{(n_1-1)\,s_1^2 + (n_2-1)\,s_2^2}{(n_1+n_2-2)} \cdot \left(\frac{n_1+n_2}{n_1 \cdot n_2}\right)}$$

where $s_1^2$ and $s_2^2$ are variances of samples one and two respectively, and $n_1$ and $n_2$ are the number of observations for each sample.

For all conditions to be met where the t-test is applicable, the sample should have been selected from a population distributed as a normal distribution. Even if the population is not distributed normally, however, as sample size increases, the t-test approaches to applicability. If it is suspected that the population deviates too drastically from the normal, exercise care in the use of the t-test. Another assumption of the t-test is that the variances of the two populations are equal. Both the normality assumption and the equal variance assumption should be formally tested prior to using the t-test.

### 7.6.4.3 Chi-Square Test ($\chi^2$)

The chi-square test is useful for statistically testing a hypothesis. Like t, $\chi^2$ values may be found in mathematical and statistical tables tabulated in a
two-way arrangement. Usually, the column headings are probabilities of obtaining a larger $\chi^2$ value when $H_o$ is true, and the row headings are degrees of freedom.

If the calculated $\chi^2$ exceeds the tabular value, then the null hypothesis is rejected. The chi square test is often used with the assumption of approximate normality in the population.

Chi-Square appears in two forms that differ not only in appearance, but that provide formats for different applications.

One form is useful in tests regarding hypotheses about $\sigma^2$:

$$\chi^2 = \frac{(n-1)s^2}{\sigma_o^2} \quad where\ H_o: \sigma^2 = \sigma_o^2$$

The other form:

$$\chi^2 = \sum_{i=1}^{k} \frac{(O_i - E_i)^2}{E_i}$$

where O = an observed value, and E = an expected (hypothesized) value, is especially useful in sampling from binomial and multinomial distributions, i.e., where the data may be classified into two or more categories (k).

Consider first a binomial situation. Suppose the Stenonema mayflies (2 species) from three stream riffle stations are pooled and the hypothesis of an equal ratio of the two species is tested based on the hypothetical data in Table 9.

Table 9. POOLED STENONEMA DATA FROM THREE RIFFLE STATIONS

| Stenonema sp. 1 | Stenonema sp. 2 | Total |
|---|---|---|
| 892* (919**) | 946* (919**) | 1838 |

* Observed value.
** Expected or hypothesized value.

To compute the hypothesized values (919 above) it is necessary to have formulated a null hypothesis. In this case it was $H_o$:No. Sp. 1 = No. Sp. 2 = (0.5) (Total).

Expected values are always computed based upon the null hypothesis. The computation for $\chi^2$ is

$$\chi^2 = \frac{(892-919)^2 + (946-919)^2}{919} = 1.59\ n.s.^*$$

*n.s. = not significant at $\alpha = 0.05$

There is one degree of freedom for this test. Since the computed $\chi^2$ is not

greater than the tabulated $\chi^2$ (3.84) for $\alpha$ =0.05, the null hypothesis is not rejected. This test, of course, applies equally well to data that has not been pooled, i.e., where the values are from two unpooled categories.

The information contained in each of the collections is partially obliterated by pooling. If the identity of the collections is maintained, two types of tests may be made; a test of the null hypothesis for each collection separately; and a test of interaction, i.e., whether the ratio depends upon the riffle from which the sample was obtained (Table 10).

With the use of the same null hypothesis, the following results are obtained. All tests were made at the $\alpha$ =0.01 level of significance. (Note: A significance level of 0.01 is used, instead of 0.05, to allow for the fact that multiple tests are being made within one experiment)

The individual $\chi^2$'s were computed, using the second form of chi square above, in separate tests of the hypothesis for each riffle. Note that the first two are not significant whereas the third is significant. This points to probable ecological differences among riffles, a possibility that would not have been discerned by pooling the data.

Table 10. STENONEMA DATA FROM THREE RIFFLE STATIONS

| Riffle | Sp. 1 | Sp. 2 | Total | $\chi^2$ | |
|---|---|---|---|---|---|
| 1 | 346* (354)+ | 362 (354) | 708 | 0.36 | n.s. |
| 2 | 302 (288) | 274 (288) | 576 | 1.30 | n.s. |
| 3 | 244 (277) | 310 (277) | 554 | 7.88 | |
| Total | 892 (919) | 946 (919) | 1838 | 1.59 | n.s. |

* Observed values.
\+ Expected, or hypothesized values.

The test for interaction (dependence) is made by summing the individual $\chi^2$'s and subtracting the $\chi^2$ obtained using totals, i.e.,

$$\chi^2 \text{ (interactions)} = \Sigma\, \chi^2 \text{ (individuals)} - \chi^2 \text{ (total)}$$

$$= 0.36 + 1.30 + 7.88 - 1.59$$

$$= 7.95$$

The degrees of freedom for the interaction $\chi^2$ are the number of individual $\chi^2$'s minus one; in this case, two. This interaction $\chi^2$ is significant, which indicates that the dominant species is indeed dependent upon the riffle.

Another $\chi^2$ test may be illustrated by the following example. Suppose that comparable techniques were used to collect from four streams. With the use of three species common to all streams, it is desired to test the hypothesis that the three species occur in the same ratio regardless of stream, i.e., that their ratio is independent of stream (Table 11).

TABLE 11. OCCURRENCE OF THREE SPECIES OF MIDGES

| Stream | Number of organisms | | | Frequency |
|---|---|---|---|---|
| | Species 1 | Species 2 | Species 3 | |
| 1 | 24* (21.7)+ | 12 (12.5) | 30 (31.7) | 66 |
| 2 | 15 (18.5) | 14 (10.6) | 27 (26.9) | 56 |
| 3 | 28 (27.4) | 15 (15.7) | 40 (39.9) | 83 |
| 4 | 20 (19.4) | 9 (11.2) | 30 (28.4) | 59 |
| Total | 87 | 50 | 127 | 264 |
| Expected ratio | 87/264 | 50/264 | 127/264 | |

* Observed values.
\+ Expected or hypothesized.

To discuss the table above, $O_{ij}$ = the observation for the $i^{th}$ stream and the $j^{th}$ species. Hence, $O_{23}$ is the observation for stream two and species three. A similar indexing scheme applies to the expected values, $E_{ij}$. For the totals, a subscript replaced by a dot $E_{i.}$ symbolizes that summation has occurred for the observations indicated by that subscript. Hence, $O_{.2}$ is the total for species two (50); $O_{3.}$ is the total for stream three (83); and $O_{..}$ is the grand total (264).

Computations of expected values make use of the null hypothesis that the ratios are the same regardless of stream. The best estimate of this ratio for any species is $O_{.j}/O_{..}$, the ratio of the sum for species j to the total of all species. This ratio multiplied by the total for stream i gives the expected

number of organisms of species j in stream i:

$$E_{ij} = \frac{O_{\cdot j}}{O_{\cdot\cdot}} \cdot (O_{i\cdot})$$

For example,

$$E_{12} = \frac{O_{\cdot 2}}{O_{\cdot\cdot}} \cdot (O_{1\cdot}) = \frac{50}{264} \cdot (66) = 12.5$$

$\chi^2$ is computed as

$$\chi^2 = \sum_i \sum_j \frac{(O_{ij} - E_{ij})^2}{E_{ij}} = 2.69 \quad (n.s.)$$

For this type of hypothesis, there are (rows − 1) (columns − 1) degrees of freedom, in this case

$$(4-1)\ (3-1) = 6$$

In the example, since the computed $\chi^2$ is not greater than the tabulated $\chi^2(12.59)$ for $\alpha$=0.05 the null hypothesis cannot be rejected. Thus, there is no evidence that the ratios among the organisms are different for different streams.

Tests of two types of hypotheses by $\chi^2$ have been illustrated. The first type of hypothesis was one where there was a theoretical ratio, i.e., the ratio of sp.1 to sp.2 is 1:1. The second type of hypothesis was one where equal ratios were hypothesized, but the values of the ratios themselves were computed from the data. To draw the proper inference, it is important to make a distinction between these two types of hypotheses.

### 7.6.4.4 Analysis of Variance

Another form of hypothesis testing is the analysis of variance (ANOVA). The ANOVA is a powerful and general technique applicable to data from virtually any experimental or field study. There are restrictions, however, in the use of the technique. Experimental errors are assumed to be normally (or approximately normally) distributed about a mean of zero and have a common variance; they are also assumed to be independent (i.e., there should be no correlations among responses that are unaccounted for by the identifiable factors of the study or by the model). The effects tested must be assumed to be linearly additive. In practice these assumptions are rarely completely fulfilled, but the analysis of variance can be used unless significant departures from normality, or

correlations among adjacent observations, or other types of measurement bias are suspected. It would be prudent, however, to check with a statistician regarding any uncertainties about the applicability of the test before issuing final reports or publications. Two simple but potentially useful examples of the analysis of variance are presented to illustrate the use of this technique.

7.6.4.4.1 Randomized Design

The analysis of variance for completely randomized designs provides a technique often useful in field studies. This test is commonly used for data derived from highly-controlled laboratory or field experiments where treatments are applied randomly to all experimental units, and the interest lies in whether or not the treatments significantly affected the response of the experimental units. This case may be of use in water quality studies, but in these studies the treatments are the conditions found, or are classifications based upon ecological criteria. Here the desire is to detect any differences in some type of measurement that might exist in conjunction with the field situation or the classifications or criteria.

For example, suppose it is desired to test whether the biomass of organisms in drift nets in a stream varies due to sampling time. Data from such a study are presented in Table 12.

In testing with the analysis of variance, as with other methods, a null hypothesis should be formulated. In this case the null hypothesis could be:

$H_o$: There are no differences in the biomass of organisms that may be attributed to time of sampling.

TABLE 12. MACROINVERTEBRATE BIOMASS COLLECTED AT DIFFERENT TIMES OF DAY FROM THE LITTLE MIAMI RIVER AT MILFORD, OHIO

| Sampling Time (Time) | Replicate number | Biomass (mg dry wt.) |
|---|---|---|
| 9:00AM - 1:00PM | 1 | 1678 |
| | 2 | 1211 |
| | 3 | 1644 |
| | 4 | 1137 |
| 1:00AM - 4:00PM | 1 | 1604 |
| | 2 | 1639 |
| | 3 | 2077 |
| | 4 | 2581 |
| 4:00PM - 7:00PM | 1 | 4276 |
| | 2 | 2400 |
| | 3 | 3183 |
| | 4 | 3451 |

In utilizing the analysis of variance, the test for whether there are differences across time is made by comparing two types of variances, most often called "mean squares" in this context. Two mean squares are computed: one based upon the means for times; and one that is free of the effect of the means. In our example, a mean square for times is computed with the use of the averages (or totals) from the sampling time. The magnitude of this mean square is affected both by differences among the means and by differences among nets of the same time. The mean square within time is computed that has no contribution due to time differences. If the null hypothesis is true, then differences among sampling time do not exist and, therefore, they make no contribution to the mean square for times. Thus, both mean squares (between times and within times) are estimates of the same variance, and with repeated sampling, they would be expected to average to the same value. If the null hypothesis ($H_o$) is true, the ratio of these values is expected to equal one. If $H_o$ is not true, i.e., if there are real differences due to the effect of times, then the mean square between times is affected by these differences and is expected to be the larger. The ratio in the second case is expected to be greater than one. The ratio of these two variances forms an F-test.

The analysis of variance is presented in Table 13A.

TABLE 13A. Generalized ANOVA Table

| Source | df | SS |
|---|---|---|
| Total | $N-1$ * | $\sum_i \sum_j X_{ij}^2 - C$ |
| Between Times | $t-1$ | $[(\sum_i X_{i\cdot}^2)/r_i] - C$ |
| Within Times | $\sum_i (r_i - 1)$ | Total SS − Stream SS |

*The symbols are defined as N=total number of observations (nets); t=number of sampling times; $r_i$=number of nets for sample time i; $X_{ij}$=an observation (biomass of net j at sampling time i); $X_i$=sum of the observations for sampling time i; and

C=correction for mean = $\frac{(\sum_i \sum_j X_{ij})^2}{N}$

TABLE 13B. Completed ANOVA Table Using Macroinvertebrate Biomass Data

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Total | 11 | 10,381,723 | | |
| Between Times | 2 | 7,717,020 | 3,858,510 | 13.03** |
| Within Times | 9 | 2,664,703 | 296,078 | |

** Significant at the 0.05 probability level.

The computations are:

$$C = \frac{(5670+7901+13310)^2}{12} = 60,215,680$$

$$\sum_i \sum_j X_{ij}^2 = (1678)^2 + (1211)^2 + \ldots + (3451)^2 = 70,597,403$$

$$Total\ SS = 70,597,403 - 60,215,680 = 10,381,723$$

$$\sum_i \frac{X_{i.}^2}{r_i} = \frac{(5670)^2}{4} - \frac{(7901)^2}{4} + \frac{(13310)^2}{4} = 67,932,700$$

$$Between\ Times\ SS = 67,932,700 - 60,215,680 = 7,717,020$$

$$Within\ Times\ SS = Total\ SS - Between\ Times\ SS$$
$$= 10,381,723 - 7,717,020 = 2,664,703$$

The mean squares (MS column) are computed by dividing the sums of squares (SS column) by its corresponding degrees of freedom (df column). The F-test is performed by computing the ratio, (Between Times MS)/(Within Times MS), in this case:

$$\frac{3,858,510}{296,078} = 13.03$$

When the calculated F value (13.03) is compared with the F values in the table (tabular F values) where df = 2 for the numerator and df = 9 for the denominator, we find that the calculated F exceeds the value of the tabular F for probability greater than 0.95. Thus the conclusion is that there are significant differences in biomass due to time of sampling.

Note that this analysis presumes good biological procedure and obviously cannot discriminate differences in sampling time from differences arising, for example, from the net having been placed in riffles with different current velocity. In general, the form of any analysis of variance derives from a model describing an observation in the experiment. In the example, the model, although not stated explicitly, assumed only one factor affecting a biomass measurement -- sampling time. If the model had included other factors, a more complicated analysis of variance would have resulted.

### 7.6.4.4.2 Factorial Design

Another application of a simple analysis of variance may be made where the factors are arranged factorially. Suppose a field study was conducted where the effect of a suspected toxic effluent upon the macroinvertebrate fauna of a river above and below a sewage treatment plant (STP) was in question (Tables 14A and 14B). Five samples were taken about one-quarter mile upstream and five one-quarter mile downstream in the spring, and the sampling scheme was repeated again in the summer. Standard statistical terminology refers to each of the combinations $P_1T_1$, $P_2T_1$, $P_1T_2$, and $P_2T_2$ as treatments or treatment combinations.

In planning for this field study, a null and alternate hypothesis should have been formed. In fact, whether stated explicitly or not, the null hypothesis was:

$H_o$: The toxic effluent has no effect upon the macroinvertebrate biomass collected.

This hypothesis is not stated in statistical terms and, therefore, only implicitly tells us what test to make. Let us look further at the analysis before attempting to state a null hypothesis in statistical terms.

In this study two factors are identifiable: times and positions. A study could have been done on each of the two factors separately, i.e., an attempt could have been made to distinguish whether there was a difference associated with times, assuming all other factors insignificant, and likewise with the positions. The example, used here, however, includes both factors simultaneously. Data are given for times and for positions but with the complication that we cannot assume that one is insignificant when studying the other. For the purpose of this study, whether there is a significant difference with times or on the other hand with positions, are questions that are of little

interest. Of interest to this study is whether the above-below the STP difference varies with times. This type of contrast is termed a positions-times interaction. Thus, our null hypothesis is, in statistical terminology:

$H_o$: There is no significant interaction effect

An analysis of variance may be used to test this hypothesis. In order to meet the normality and homogeneity of variance assumptions of the analysis, the raw data were $\log_{10}$ transformed (Table 14B). All calculations are on the transformed data.

TABLE 14A. MACROINVERTEBRATE BIOMASS (GRAMS WET WT.)

| Time Collected | Collected above STP | Collected below STP |
|---|---|---|
| Spring | 437 | 193 |
| | 343 | 86 |
| | 337 | 119 |
| | 635 | 505 |
| | 373 | 171 |
| Summer | 888 | 28 |
| | 1778 | 18 |
| | 4332 | 117 |
| | 1078 | 26 |
| | 859 | 78 |

TABLE 14B. $LOG_{10}$ TRANSFORMED DATA

| Time Collected | Collected above STP | Collected below STP |
|---|---|---|
| Spring | 2.64 | 2.28 |
| | 2.54 | 1.93 |
| | 2.53 | 2.08 |
| | 2.80 | 2.70 |
| | 2.57 | 2.23 |
| Summer | 2.95 | 1.45 |
| | 3.25 | 1.26 |
| | 3.64 | 2.07 |
| | 3.03 | 1.41 |
| | 2.93 | 1.89 |

TABLE 15. TREATMENT TOTALS FOR THE DATA OF TABLE 14B

| Total | Positions | | Times totals |
|---|---|---|---|
| | Above | Below | |
| Spring | 13.08 | 11.22 | 24.3 |
| Summer | 15.8 | 8.08 | 23.88 |
| | Positions | | Grand |
| totals | 28.88 | 19.3 | 48.18 |

Symbolically, an observation must have three indices specified to be completely identified: position, time, and sample number. Thus there are three subscripts: $X_{ijk}$ is an observation at position i, time j, and from sample k. A value of 1 for i is above the STP; 2, below the STP; 1 for j is spring; 2, summer. A particular example is $X_{123}$, the third sample above the STP for the summer, or 3.64. A total (Table 15) is specified by using the dot notation. For the value of $X_{ij\cdot}$, then the individually sampled values for position i, time j are totaled. It is a total for a treatment combination. For example, the value of $X_{11\cdot}$, is 13.08, and the value of $X_{1\cdot\cdot}$, where sampling and times are both totaled to give the total for above the STP is 28.88. Treatment totals are presented in Table 15.

For a slight advantage in generality, let the following additional symbols apply: t = number of times of sampling (in this case t = 2); p = number of positions sample (in this case p = 2); s = number of samples per treatment combination; and n = the total number of observations.

The computations are:

Correction for the mean (CT):

$$CT = \frac{(\sum_i \sum_j \sum_k X_{ijk})^2}{n} = \frac{(48.18)^2}{20} = 116.06$$

$$TSS = \sum_i \sum_j \sum_k X_{ijk}^2 - CT = (2.64)^2 + (2.54)^2 + \cdots + (1.89)^2 - 116.06 = 7.54$$

Note that the divisor (5) may be factored out here, if desired, but where a different number of samples is taken for each treatment combination it should be left as above.

Position Sum of Squares (SSP):

$$SSP = \frac{(\sum_i X_{i..})^2}{st} - CT = \frac{(28.88)^2}{10} + \frac{(19.3)^2}{10} - 116.06 = 4.59$$

Times Sum of Squares (SST):

$$SST = \frac{(\sum_j X_{.j.})^2}{sp} - CT = \frac{(24.3)^2}{10} + \frac{(23.88)^2}{10} - 116.06 = 0.01$$

Interaction of Positions and Times of Sums Squares (SSPT):

$$SSPT = \frac{(\sum_i \sum_j X_{ij.})^2}{s} - SPS - SST - CT$$

$$\frac{(13.08)^2}{5} + \frac{(11.22)^2}{5} + \frac{(15.80)^2}{5} + \frac{(8.08)^2}{5} - 4.59 - 0.01 - 116.06 = 1.72$$

Error Sums of Squares (SSE):

$$SSE = TSS - SSP - SST - SSPT = 7.54 - 4.59 - 0.01 - 1.72 = 1.22$$

The completed ANOVA, including F tests, is given in Table 16. Although not important to this example, the main effects, positions and times, are tested for significance. The F table is entered with df = 1 for effect tested, and df = 16 for error. The positions effect is significant and the times effect is not significant, both tested at $\alpha$=0.05. The interaction effect is significant, and we, therefore, conclude that there is a significant effect of the effluent changes across time on biomass.

TABLE 16. ANALYSIS OF VARIANCE TABLE FOR FIELD STUDY DATA OF TABLE 14

| Source | df | SS | MS | F |
|---|---|---|---|---|
| Positions | 1 | 4.59 | 4.59 | 57.38 ** |
| Times | 1 | 0.01 | 0.01 | 0.125 |
| Positions X Times | 1 | 1.72 | 1.72 | 21.51 ** |
| Error | 16 | 1.22 | 0.08 | |
| Total | 19 | 7.54 | | |

** Significant at the 0.05 probability level.

### 7.6.5 Confidence Interval for Means

When means are computed in field studies, the desire often is to report them as intervals rather than as fixed numbers. This is entirely reasonable because computed means are virtually always derived from samples and are subject to the same uncertainty that is associated with the sample.

The correct computation of confidence intervals requires that the distribution of the observations be known. But very often approximations are close enough to correctness to be of use, and often are, or may be made to be, conservative. For computation of confidence intervals for the mean, the normal distribution is usually assumed to apply for several reasons: the central limit theorem assures us that with large samples the mean is likely to be approximately normally distributed; the required computations are well known and are easily applied; and when the normal distribution is known not to apply, suitable transformation of the data often is available to allow a valid application.

The confidence interval for a mean is an interval within which the true mean is said to have some stated probability of being found. If the probability of the mean not being in the interval is $\alpha$ ($\alpha$ could equal 0.1, 0.05, 0.01, or any probability value), then the statement may be written:

$$P\,(CL_1 < \mu < CL_2) = 1 - \alpha$$

This is read, "The probability that the lower confidence limit ($CL_1$) is less than the true mean ($\mu$) and that the upper confidence limit ($CL_2$) is greater than the true mean, equals $1-\alpha$." However, we never know whether or not the true

mean is actually included in the interval. So the confidence interval statement is really a statement about our procedure rather than about $\mu$. It says that if we follow the procedure for repeated experiments, a proportion of those experiments equal to $\alpha$ will, by chance alone, fail to include the true mean between our limits. For example, if $\alpha$=0.05, we can expect 5 of 100 confidence intervals to fail to include the true mean.

To compute the limits, the sample mean, $\bar{X}$,; the standard error, $s_x$; and the degrees of freedom, n-1; must be known. A $t_{\alpha/2,n-1}$ value from tables of Student's t is obtained corresponding to n-1 degrees of freedom and probability $\alpha$. The computation is:

$$CL_1 = \bar{X} - (t_{\alpha/2}) \cdot (s_{\bar{X}})$$
$$CL_2 = \bar{X} + (t_{\alpha/2}) \cdot (s_{\bar{X}})$$

### 7.6.6 Validating Normality and Homogeneity of Variance Assumptions[1]

#### 7.6.6.1 Introduction

The t-test and the analysis of variance are parametric procedures based on the assumptions that the observations within treatments are independent and normally distributed, and that the variance of the observations is homogeneous across all groups of observations. These assumptions should be checked prior to using these tests, to determine if they have been met. Tests for validating the assumptions are provided in the following discussion. If the tests fail (if the data do not meet the assumptions), a non-parametric procedure such as Friedman's Test or Wilcoxon's Rank Sum Test may be more appropriate. However, the decision on whether to use parametric or non-parametric tests may be a judgment call, and a statistician should be consulted in selecting the analysis.

#### 7.6.6.2 Test for Normal Distribution of Data

A formal test for normality is the Shapiro-Wilk's Test. The test statistic is obtained by dividing the square of an appropriate linear combination of the sample order statistics by the usual symmetric estimate of variance. The calculated W must be greater than zero and less than or equal to one. This test is recommended for a sample size of 50 or less. If the sample size is greater than 50, the Kolomogorov "D" statistic is recommended. An example of the Shapiro-Wilk's test is provided below.

The example uses macroinvertebrate biomass data. The same data are used

[1]Adapted and modified from USEPA, 1989

in the discussion of the homogeneity of variance determination and the one-way analysis of variance example. The data and the mean and standard deviation of the observations at each time are listed in Table 17.

The first step of the test for normality is to center the observations by subtracting the mean of all the observations within a concentration from each observation in that concentration. The centered observations are listed in Table 18.

Calculate the denominator, D, of the test statistic:

$$D = \sum_i X_i^2 - \frac{(\sum_i X_i)^2}{n}$$

$$D = 2{,}664{,}705 - \frac{(-3)^2}{12} = 2{,}664{,}704$$

Where: $X_i$ = The $i^{th}$ centered observations.

n = The total number of observations.

Order the centered observations from smallest to largest.

$$X^{(1)} - X^{(2)} \quad . \quad . \quad . \quad - \quad X^{(n)}$$

Where $X^{(i)}$ denotes the ith ordered observation. The ordered observations are listed in Table 19.

From Table 21, for the number of observations, n, obtain the coefficients $a_1$, $a_2$, ...., $a_k$, where k is approximately n/2. For the data in this example, n = 12, k = 6. The $a_i$ values are listed in Table 20.

Compute the test statistic, W, as follows:

$$W = \frac{1}{D}\left[\sum_{i=1}^{k} a_i (X^{(n-i+1)} - X^{(i)})\right]$$

$$W = \frac{1}{2{,}664{,}704}(1610)^2 = 0.973$$

The differences, $X^{(n-i+1)} - X^{(i)}$, are listed in Table 20.

The decision rule for this test is to compare the critical value from Table 22 to the computed W. If the computed value is less than the critical value, conclude that the data are not normally distributed. For this example, the critical value at a significance level of 0.01 and 12 observations (n) is 0.805. The calculated value, 0.973, is not less than the critical value. Thus, the conclusion of the test is that the data are normally distributed.

TABLE 17. MACROINVERTEBRATE BIOMASS COLLECTED AT DIFFERENT TIMES OF DAY FROM THE LITTLE MIAMI RIVER AT MILFORD, OHIO

| Sampling Time | Replicate number | Biomass (mg dry wt.) | $S^2$ | $\bar{X}$ |
|---|---|---|---|---|
| 9:00AM – 1:00PM | 1 | 1678 | 80,161 | 1418 |
| | 2 | 1211 | | |
| | 3 | 1644 | | |
| | 4 | 1137 | | |
| 1:00AM – 4:00PM | 1 | 1604 | 209,392 | 1975 |
| | 2 | 1639 | | |
| | 3 | 2077 | | |
| | 4 | 2581 | | |
| 4:00PM – 7:00PM | 1 | 4276 | 598,680 | 3328 |
| | 2 | 2400 | | |
| | 3 | 3183 | | |
| | 4 | 3451 | | |

TABLE 18. EXAMPLE OF SHAPIRO-WILK'S TEST: CENTERED OBSERVATIONS

| Sampling Time | Replicate | | | |
|---|---|---|---|---|
| | 1 | 2 | 3 | 4 |
| 9:00AM – 1:00PM | 260 | –207 | 226 | –281 |
| 1:00PM – 4:00PM | –371 | –336 | 102 | 606 |
| 4:00PM – 7:00PM | 948 | –928 | –145 | 123 |

TABLE 19. EXAMPLE OF SHAPIRO-WILK'S TEST: ORDERED OBSERVATIONS

| $i$ | $X_{(i)}$ | $i$ | $X_{(i)}$ |
|---|---|---|---|
| 1 | -928 | 7 | 102 |
| 2 | -371 | 8 | 123 |
| 3 | -336 | 9 | 226 |
| 4 | -280 | 10 | 260 |
| 5 | -207 | 11 | 606 |
| 6 | -145 | 12 | 948 |

TABLE 20. EXAMPLE OF SHAPIRO-WILK'S TEST: TABLE OF COEFFICIENTS AND DIFFERENCES

| $i$ | $a_i$ | $X^{(n-i-1)} - X^{(i)}$ | |
|---|---|---|---|
| 1 | .5475 | 1876 | $X^{(12)}-X^{(1)}$ |
| 2 | .3325 | 977 | $X^{(11)}-X^{(2)}$ |
| 3 | .2347 | 596 | $X^{(10)}-X^{(3)}$ |
| 4 | .1586 | 507 | $X^{(9)}-X^{(4)}$ |
| 5 | .0922 | 330 | $X^{(8)}-X^{(5)}$ |
| 6 | .0303 | 247 | $X^{(7)}-X^{(6)}$ |

TABLE 21. COEFFICIENTS FOR THE SHAPIRO-WILKS TEST[1]

| i \ n | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.7071 | 0.7071 | 0.6872 | 0.6646 | 0.6431 | 0.6233 | 0.6052 | 0.5888 | 0.5739 |
| 2 | — | 0.0000 | 0.1667 | 0.2413 | 0.2806 | 0.3031 | 0.3164 | 0.3244 | 0.3291 |
| 3 | — | — | — | 0.0000 | 0.0875 | 0.1401 | 0.1743 | 0.1976 | 0.2141 |
| 4 | — | — | — | — | — | 0.0000 | 0.0561 | 0.0947 | 0.1224 |
| 5 | — | — | — | — | — | — | — | 0.0000 | 0.0399 |

| i \ n | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.5601 | 0.5475 | 0.5359 | 0.5251 | 0.5150 | 0.5056 | 0.4968 | 0.4886 | 0.4808 | 0.4734 |
| 2 | 0.3315 | 0.3325 | 0.3325 | 0.3318 | 0.3306 | 0.3290 | 0.3273 | 0.3253 | 0.3232 | 0.3211 |
| 3 | 0.2260 | 0.2347 | 0.2412 | 0.2460 | 0.2495 | 0.2521 | 0.2540 | 0.2553 | 0.2561 | 0.2565 |
| 4 | 0.1429 | 0.1586 | 0.1707 | 0.1802 | 0.1878 | 0.1939 | 0.1988 | 0.2027 | 0.2059 | 0.2085 |
| 5 | 0.0695 | 0.0922 | 0.1099 | 0.1240 | 0.1353 | 0.1447 | 0.1524 | 0.1587 | 0.1641 | 0.1686 |
| 6 | 0.0000 | 0.0303 | 0.0539 | 0.0727 | 0.0880 | 0.1005 | 0.1109 | 0.1197 | 0.1271 | 0.1334 |
| 7 | — | — | 0.0000 | 0.0240 | 0.0433 | 0.0593 | 0.0725 | 0.0837 | 0.0932 | 0.1013 |
| 8 | — | — | — | — | 0.0000 | 0.0196 | 0.0359 | 0.0496 | 0.0612 | 0.0711 |
| 9 | — | — | — | — | — | — | 0.0000 | 0.0163 | 0.0303 | 0.0422 |
| 10 | — | — | — | — | — | — | — | — | 0.0000 | 0.0140 |

| i \ n | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.4643 | 0.4590 | 0.4542 | 0.4493 | 0.4450 | 0.4407 | 0.4366 | 0.4328 | 0.4291 | 0.4254 |
| 2 | 0.3185 | 0.3156 | 0.3126 | 0.3098 | 0.3069 | 0.3043 | 0.3018 | 0.2992 | 0.2968 | 0.2944 |
| 3 | 0.2578 | 0.2571 | 0.2563 | 0.2554 | 0.2543 | 0.2533 | 0.2522 | 0.2510 | 0.2499 | 0.2487 |
| 4 | 0.2119 | 0.2131 | 0.2139 | 0.2145 | 0.2148 | 0.2151 | 0.2152 | 0.2151 | 0.2150 | 0.2148 |
| 5 | 0.1736 | 0.1764 | 0.1787 | 0.1807 | 0.1822 | 0.1836 | 0.1848 | 0.1857 | 0.1864 | 0.1870 |
| 6 | 0.1399 | 0.1443 | 0.1480 | 0.1512 | 0.1539 | 0.1563 | 0.1584 | 0.1601 | 0.1616 | 0.1630 |
| 7 | 0.1092 | 0.1150 | 0.1201 | 0.1245 | 0.1283 | 0.1316 | 0.1346 | 0.1372 | 0.1395 | 0.1415 |
| 8 | 0.0804 | 0.0878 | 0.0941 | 0.0997 | 0.1046 | 0.1089 | 0.1128 | 0.1162 | 0.1192 | 0.1219 |
| 9 | 0.0530 | 0.0618 | 0.0696 | 0.0764 | 0.0823 | 0.0876 | 0.0923 | 0.0965 | 0.1002 | 0.1036 |
| 10 | 0.0263 | 0.0368 | 0.0459 | 0.0539 | 0.0610 | 0.0672 | 0.0728 | 0.0778 | 0.0822 | 0.0862 |
| 11 | 0.0000 | 0.0122 | 0.0228 | 0.0321 | 0.0403 | 0.0476 | 0.0540 | 0.0598 | 0.0650 | 0.0697 |
| 12 | — | — | 0.0000 | 0.0107 | 0.0200 | 0.0284 | 0.0358 | 0.0424 | 0.0483 | 0.0537 |
| 13 | — | — | — | — | 0.0000 | 0.0094 | 0.0178 | 0.0253 | 0.0320 | 0.0381 |
| 14 | — | — | — | — | — | — | 0.0000 | 0.0084 | 0.0159 | 0.0227 |
| 15 | — | — | — | — | — | — | — | — | 0.0000 | 0.0076 |

[1]Taken from Conover, 1980.

TABLE 21. COEFFICIENT FOR THE SHAPIRO-WILKS TEST (Continued)

| i \ n | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.4220 | 0.4188 | 0.4156 | 0.4127 | 0.4096 | 0.4068 | 0.4040 | 0.4015 | 0.3989 | 0.3964 |
| 2 | 0.2921 | 0.2898 | 0.2876 | 0.2854 | 0.2834 | 0.2813 | 0.2794 | 0.2774 | 0.2755 | 0.2737 |
| 3 | 0.2475 | 0.2462 | 0.2451 | 0.2439 | 0.2427 | 0.2415 | 0.2403 | 0.2391 | 0.2380 | 0.2368 |
| 4 | 0.2145 | 0.2141 | 0.2137 | 0.2132 | 0.2127 | 0.2121 | 0.2116 | 0.2110 | 0.2104 | 0.2098 |
| 5 | 0.1874 | 0.1878 | 0.1880 | 0.1882 | 0.1883 | 0.1883 | 0.1883 | 0.1881 | 0.1880 | 0.1878 |
| 6 | 0.1641 | 0.1651 | 0.1660 | 0.1667 | 0.1673 | 0.1678 | 0.1683 | 0.1686 | 0.1689 | 0.1691 |
| 7 | 0.1433 | 0.1449 | 0.1463 | 0.1475 | 0.1487 | 0.1496 | 0.1505 | 0.1513 | 0.1520 | 0.1526 |
| 8 | 0.1243 | 0.1265 | 0.1284 | 0.1301 | 0.1317 | 0.1331 | 0.1344 | 0.1356 | 0.1366 | 0.1376 |
| 9 | 0.1066 | 0.1093 | 0.1118 | 0.1140 | 0.1160 | 0.1179 | 0.1196 | 0.1211 | 0.1225 | 0.1237 |
| 10 | 0.0899 | 0.0931 | 0.0961 | 0.0988 | 0.1013 | 0.1036 | 0.1056 | 0.1075 | 0.1092 | 0.1108 |
| 11 | 0.0739 | 0.0777 | 0.0812 | 0.0844 | 0.0873 | 0.0900 | 0.0924 | 0.0947 | 0.0967 | 0.0986 |
| 12 | 0.0585 | 0.0629 | 0.0669 | 0.0706 | 0.0739 | 0.0770 | 0.0798 | 0.0824 | 0.0848 | 0.0870 |
| 13 | 0.0435 | 0.0485 | 0.0530 | 0.0572 | 0.0610 | 0.0645 | 0.0677 | 0.0706 | 0.0733 | 0.0759 |
| 14 | 0.0289 | 0.0344 | 0.0395 | 0.0441 | 0.0484 | 0.0523 | 0.0559 | 0.0592 | 0.0622 | 0.0651 |
| 15 | 0.0144 | 0.0206 | 0.0262 | 0.0314 | 0.0361 | 0.0404 | 0.0444 | 0.0481 | 0.0515 | 0.0546 |
| 16 | 0.0000 | 0.0068 | 0.0131 | 0.0187 | 0.0239 | 0.0287 | 0.0331 | 0.0372 | 0.0409 | 0.0444 |
| 17 | — | — | 0.0000 | 0.0062 | 0.0119 | 0.0172 | 0.0220 | 0.0264 | 0.0305 | 0.0343 |
| 18 | — | — | — | — | 0.0000 | 0.0057 | 0.0110 | 0.0158 | 0.0203 | 0.0244 |
| 19 | — | — | — | — | — | — | 0.0000 | 0.0053 | 0.0101 | 0.0146 |
| 20 | — | — | — | — | — | — | — | — | 0.0000 | 0.0049 |

| i \ n | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.3940 | 0.3917 | 0.3894 | 0.3872 | 0.3850 | 0.3830 | 0.3808 | 0.3789 | 0.3770 | 0.3751 |
| 2 | 0.2719 | 0.2701 | 0.2684 | 0.2667 | 0.2651 | 0.2635 | 0.2620 | 0.2604 | 0.2589 | 0.2574 |
| 3 | 0.2357 | 0.2345 | 0.2334 | 0.2323 | 0.2313 | 0.2302 | 0.2291 | 0.2281 | 0.2271 | 0.2260 |
| 4 | 0.2091 | 0.2085 | 0.2078 | 0.2072 | 0.2065 | 0.2058 | 0.2052 | 0.2045 | 0.2038 | 0.2032 |
| 5 | 0.1876 | 0.1874 | 0.1871 | 0.1868 | 0.1865 | 0.1862 | 0.1859 | 0.1855 | 0.1851 | 0.1847 |
| 6 | 0.1693 | 0.1694 | 0.1695 | 0.1695 | 0.1695 | 0.1695 | 0.1695 | 0.1693 | 0.1692 | 0.1691 |
| 7 | 0.1531 | 0.1535 | 0.1539 | 0.1542 | 0.1545 | 0.1548 | 0.1550 | 0.1551 | 0.1553 | 0.1554 |
| 8 | 0.1384 | 0.1392 | 0.1398 | 0.1405 | 0.1410 | 0.1415 | 0.1420 | 0.1423 | 0.1427 | 0.1430 |
| 9 | 0.1249 | 0.1259 | 0.1269 | 0.1278 | 0.1286 | 0.1293 | 0.1300 | 0.1306 | 0.1312 | 0.1317 |
| 10 | 0.1123 | 0.1136 | 0.1149 | 0.1160 | 0.1170 | 0.1180 | 0.1189 | 0.1197 | 0.1205 | 0.1212 |
| 11 | 0.1004 | 0.1020 | 0.1035 | 0.1049 | 0.1062 | 0.1073 | 0.1085 | 0.1095 | 0.1105 | 0.1113 |
| 12 | 0.0891 | 0.0909 | 0.0927 | 0.0943 | 0.0959 | 0.0972 | 0.0986 | 0.0998 | 0.1010 | 0.1020 |
| 13 | 0.0782 | 0.0804 | 0.0824 | 0.0842 | 0.0860 | 0.0876 | 0.0892 | 0.0906 | 0.0919 | 0.0932 |
| 14 | 0.0677 | 0.0701 | 0.0724 | 0.0745 | 0.0765 | 0.0783 | 0.0801 | 0.0817 | 0.0832 | 0.0846 |
| 15 | 0.0575 | 0.0602 | 0.0628 | 0.0651 | 0.0673 | 0.0694 | 0.0713 | 0.0731 | 0.0748 | 0.0764 |
| 16 | 0.0476 | 0.0506 | 0.0534 | 0.0560 | 0.0584 | 0.0607 | 0.0628 | 0.0648 | 0.0667 | 0.0685 |
| 17 | 0.0379 | 0.0411 | 0.0442 | 0.0471 | 0.0497 | 0.0522 | 0.0546 | 0.0568 | 0.0588 | 0.0608 |
| 18 | 0.0283 | 0.0318 | 0.0352 | 0.0383 | 0.0412 | 0.0439 | 0.0465 | 0.0489 | 0.0511 | 0.0532 |
| 19 | 0.0188 | 0.0227 | 0.0263 | 0.0296 | 0.0328 | 0.0357 | 0.0385 | 0.0411 | 0.0436 | 0.0459 |
| 20 | 0.0094 | 0.0136 | 0.0175 | 0.0211 | 0.0245 | 0.0277 | 0.0307 | 0.0335 | 0.0361 | 0.0386 |
| 21 | 0.0000 | 0.0045 | 0.0087 | 0.0126 | 0.0163 | 0.0197 | 0.0229 | 0.0259 | 0.0288 | 0.0314 |
| 22 | | — | 0.0000 | 0.0042 | 0.0081 | 0.0118 | 0.0153 | 0.0185 | 0.0215 | 0.0244 |
| 23 | — | — | — | — | 0.0000 | 0.0039 | 0.0076 | 0.0111 | 0.0143 | 0.0174 |
| 24 | — | — | — | — | — | — | 0.0000 | 0.0037 | 0.0071 | 0.0104 |
| 25 | — | — | — | — | — | — | — | — | 0.0000 | 0.0035 |

TABLE 22. QUANTILES OF THE SHAPIRO-WILKS TEST STATISTIC[1]

| *n* | *0.01* | *0.02* | *0.05* | *0.10* | *0.50* | *0.90* | *0.95* | *0.98* | *0.99* |
|---|---|---|---|---|---|---|---|---|---|
| **3** | 0.753 | 0.756 | 0.767 | 0.789 | 0.959 | 0.998 | 0.999 | 1.000 | 1.000 |
| **4** | 0.687 | 0.707 | 0.748 | 0.792 | 0.935 | 0.987 | 0.992 | 0.996 | 0.997 |
| **5** | 0.686 | 0.715 | 0.762 | 0.806 | 0.927 | 0.979 | 0.986 | 0.991 | 0.993 |
| **6** | 0.713 | 0.743 | 0.788 | 0.826 | 0.927 | 0.974 | 0.981 | 0.986 | 0.989 |
| **7** | 0.730 | 0.760 | 0.803 | 0.838 | 0.928 | 0.972 | 0.979 | 0.985 | 0.988 |
| **8** | 0.749 | 0.778 | 0.818 | 0.851 | 0.932 | 0.972 | 0.978 | 0.984 | 0.987 |
| **9** | 0.764 | 0.791 | 0.829 | 0.859 | 0.935 | 0.972 | 0.978 | 0.984 | 0.986 |
| **10** | 0.781 | 0.806 | 0.842 | 0.869 | 0.938 | 0.972 | 0.978 | 0.983 | 0.986 |
| **11** | 0.792 | 0.817 | 0.850 | 0.876 | 0.940 | 0.973 | 0.979 | 0.984 | 0.986 |
| **12** | 0.805 | 0.828 | 0.859 | 0.883 | 0.943 | 0.973 | 0.979 | 0.984 | 0.986 |
| **13** | 0.814 | 0.837 | 0.866 | 0.889 | 0.945 | 0.974 | 0.979 | 0.984 | 0.986 |
| **14** | 0.825 | 0.846 | 0.874 | 0.895 | 0.947 | 0.975 | 0.980 | 0.984 | 0.986 |
| **15** | 0.835 | 0.855 | 0.881 | 0.901 | 0.950 | 0.975 | 0.980 | 0.984 | 0.987 |
| **16** | 0.844 | 0.863 | 0.887 | 0.906 | 0.952 | 0.976 | 0.981 | 0.985 | 0.987 |
| **17** | 0.851 | 0.869 | 0.892 | 0.910 | 0.954 | 0.977 | 0.981 | 0.985 | 0.987 |
| **18** | 0.858 | 0.874 | 0.897 | 0.914 | 0.956 | 0.978 | 0.982 | 0.986 | 0.988 |
| **19** | 0.863 | 0.879 | 0.901 | 0.917 | 0.957 | 0.978 | 0.982 | 0.986 | 0.988 |
| **20** | 0.868 | 0.884 | 0.905 | 0.920 | 0.959 | 0.979 | 0.983 | 0.986 | 0.988 |
| **21** | 0.873 | 0.888 | 0.908 | 0.923 | 0.960 | 0.980 | 0.983 | 0.987 | 0.989 |
| **22** | 0.878 | 0.892 | 0.911 | 0.926 | 0.961 | 0.980 | 0.984 | 0.987 | 0.989 |
| **23** | 0.881 | 0.895 | 0.914 | 0.928 | 0.962 | 0.981 | 0.984 | 0.987 | 0.989 |
| **24** | 0.884 | 0.898 | 0.916 | 0.930 | 0.963 | 0.981 | 0.984 | 0.987 | 0.989 |
| **25** | 0.888 | 0.901 | 0.918 | 0.931 | 0.964 | 0.981 | 0.985 | 0.988 | 0.989 |
| **26** | 0.891 | 0.904 | 0.920 | 0.933 | 0.965 | 0.982 | 0.985 | 0.988 | 0.989 |
| **27** | 0.894 | 0.906 | 0.923 | 0.935 | 0.965 | 0.982 | 0.985 | 0.988 | 0.990 |
| **28** | 0.896 | 0.908 | 0.924 | 0.936 | 0.966 | 0.982 | 0.985 | 0.988 | 0.990 |
| **29** | 0.898 | 0.910 | 0.926 | 0.937 | 0.966 | 0.982 | 0.985 | 0.988 | 0.990 |
| **30** | 0.900 | 0.912 | 0.927 | 0.939 | 0.967 | 0.983 | 0.985 | 0.988 | 0.990 |
| **31** | 0.902 | 0.914 | 0.929 | 0.940 | 0.967 | 0.983 | 0.986 | 0.988 | 0.990 |
| **32** | 0.904 | 0.915 | 0.930 | 0.941 | 0.968 | 0.983 | 0.986 | 0.988 | 0.990 |
| **33** | 0.906 | 0.917 | 0.931 | 0.942 | 0.968 | 0.983 | 0.986 | 0.989 | 0.990 |
| **34** | 0.908 | 0.919 | 0.933 | 0.943 | 0.969 | 0.983 | 0.986 | 0.989 | 0.990 |
| **35** | 0.910 | 0.920 | 0.934 | 0.944 | 0.969 | 0.984 | 0.986 | 0.989 | 0.990 |
| **36** | 0.912 | 0.922 | 0.935 | 0.945 | 0.970 | 0.984 | 0.986 | 0.989 | 0.990 |
| **37** | 0.914 | 0.924 | 0.936 | 0.946 | 0.970 | 0.984 | 0.987 | 0.989 | 0.990 |
| **38** | 0.916 | 0.925 | 0.938 | 0.947 | 0.971 | 0.984 | 0.987 | 0.989 | 0.990 |
| **39** | 0.917 | 0.927 | 0.939 | 0.948 | 0.971 | 0.984 | 0.987 | 0.989 | 0.991 |
| **40** | 0.919 | 0.928 | 0.940 | 0.949 | 0.972 | 0.985 | 0.987 | 0.989 | 0.991 |
| **41** | 0.920 | 0.929 | 0.941 | 0.950 | 0.972 | 0.985 | 0.987 | 0.989 | 0.991 |
| **42** | 0.922 | 0.930 | 0.942 | 0.951 | 0.972 | 0.985 | 0.987 | 0.989 | 0.991 |
| **43** | 0.923 | 0.932 | 0.943 | 0.951 | 0.973 | 0.985 | 0.987 | 0.990 | 0.991 |
| **44** | 0.924 | 0.933 | 0.944 | 0.952 | 0.973 | 0.985 | 0.987 | 0.990 | 0.991 |
| **45** | 0.926 | 0.934 | 0.945 | 0.953 | 0.973 | 0.985 | 0.988 | 0.990 | 0.991 |
| **46** | 0.927 | 0.935 | 0.945 | 0.953 | 0.974 | 0.985 | 0.988 | 0.990 | 0.991 |
| **47** | 0.928 | 0.936 | 0.946 | 0.954 | 0.974 | 0.985 | 0.988 | 0.990 | 0.991 |
| **48** | 0.929 | 0.937 | 0.947 | 0.954 | 0.974 | 0.985 | 0.988 | 0.990 | 0.991 |
| **49** | 0.929 | 0.937 | 0.947 | 0.955 | 0.974 | 0.985 | 0.988 | 0.990 | 0.991 |
| **50** | 0.930 | 0.938 | 0.947 | 0.955 | 0.974 | 0.985 | 0.988 | 0.990 | 0.991 |

[1]Taken from Conover, 1980.

### 7.6.6.3 Test for Homogeneity of Variance

For the analysis of variance, the variances of the data obtained for each group of observations are assumed to be equal. Bartlett's Test is a formal test of this assumption. In using this test, it is assumed that the data are normally distributed.

The data used in this example are biomass data from the one-way analysis of variance example and the Shapiro-Wilk's Test example. These data are listed in Table 17, together with the calculated sample variance for each group of observations.

The test statistic for Bartlett's Test (Snedecor and Cochran, 1980) is as follows:

$$B = \frac{[(\sum_{i=1}^{p} V_i)\ln\bar{S}^2 - \sum_{i=1}^{p}(V_i \ln S_i^2)]}{C}$$

Where: $V_i$ = Degrees of freedom for each time
$p$ = Number of levels of times

$\bar{S}^2$ = The average of the individual variances.
ln = $\text{Log}_e$

$$C = 1 + [\frac{1}{3(p-1)}][\sum_{i=1}^{p}\frac{1}{V_i} - \frac{1}{\sum_{i=1}^{p} V_i}]$$

Since B is approximately distributed as chi-square with p - 1 degrees of freedom when the variances are equal, the appropriate critical value is obtained from a table of the chi-square distribution for p - 1 degrees of freedom and a significance level of $\alpha$. If B is less than the critical value then the variances are assumed to be equal.

For the data in this example, $V_i = 4 - 1 = 3$, $p = 3$, $\bar{S}^2 = 296{,}078$, and $C = 1.148$. The calculated value is:

$$B = \frac{[(\sum_{i=1}^{3} 3)\ln\bar{S}^2 - 3\sum_{i=1}^{3}(\ln S_i^2)]}{1.148}$$

$$B = \frac{9\,(12.598) - 3\,(36.846)}{1.148} = 2.477$$

Since B is approximately distributed as chi-square with 2 degrees of freedom when the variances are equal, the appropriate critical value for the test is 9.210 (see a $\chi^2$ table) for a significance level of 0.01. Since B = 2.477 is less than the critical value of 9.210, conclude that the variances are not different.

### 7.6.6.4 Transformations of the Data

When the assumptions of normality and/or homogeneity of variance are not met, transformations of the data may remedy the problem, so that the data can be analyzed by parametric procedures, rather than a non-parametric technique such as Friedman's Test or Wilcoxon's Rank Sum Test. Examples of transformations include log, square root, arc sine square root, and reciprocals. After the data have been transformed, Shapiro-Wilk's and Bartlett's test should be performed on the transformed observations to determine whether the assumptions of normality and/or homogeneity of variance are met.

Table 23 is reproduced here with permission from Lloyd, Zar, and Karr (1968) for use in calculating mean diversity ($\bar{d}$) (see 7.3.10, page 114). To use the table, find the number of individuals (n) in column 1 and read the log of that number in column 3 (n log n).

TABLE 23. FUNCTIONS FOR CALCULATING SPECIES DIVERSITY AND (FOR PERFECTLY RANDOM SAMPLING) ITS STANDARD ERROR LOGARITHMS ARE TO BASE 10. TABLE VALUES ARE ACCURATE TO WITHIN ±1 IN THE EIGHTH SIGNIFICANT FIGURE.

| n | log n! | n log n | n log² n | n | log n! | n log n | n log² n |
|---|---|---|---|---|---|---|---|
| 1 | .0000 | .0000 | .0000 | 14 | 10.9404 | 16.0458 | 18.3905 |
| 2 | .3010 | .6021 | .1812 | 15 | 12.1165 | 17.6414 | 20.7479 |
| 3 | .7782 | 1.4314 | .6829 | 16 | 13.3206 | 19.2659 | 23.1985 |
| 4 | 1.3802 | 2.4082 | 1.4499 | 17 | 14.5511 | 20.9176 | 25.7381 |
| 5 | 2.0792 | 3.4949 | 2.4428 | 18 | 15.8063 | 22.5949 | 28.3628 |
| 6 | 2.8573 | 4.6689 | 3.6331 | 19 | 17.0851 | 24.2963 | 31.0690 |
| 7 | 3.7024 | 5.9157 | 4.9993 | 20 | 18.3861 | 26.0206 | 33.8536 |
| 8 | 4.6055 | 7.2247 | 6.5246 | 21 | 19.7083 | 27.7666 | 36.7135 |
| 9 | 5.5598 | 8.5882 | 8.1952 | 22 | 21.0508 | 29.5333 | 39.6462 |
| 10 | 6.5598 | 10.0000 | 10.0000 | 23 | 22.4125 | 31.3197 | 42.6490 |
| 11 | 7.6012 | 11.4553 | 11.9295 | 24 | 23.7927 | 33.1251 | 45.7196 |
| 12 | 8.6803 | 12.9502 | 13.9756 | 25 | 25.1906 | 34.9485 | 48.8559 |
| 13 | 9.7943 | 14.4813 | 16.1313 | 26 | 26.6056 | 36.7893 | 52.0559 |

| n | log n! | n log n | n log² n | n | log n! | n log n | n log² n |
|---|---|---|---|---|---|---|---|
| 27 | 28.0370 | 38.6468 | 55.3177 | 84 | 126.5204 | 161.6395 | 311.0395 |
| 28 | 29.4841 | 40.5204 | 58.6395 | 85 | 128.4498 | 164.0006 | 316.4259 |
| 29 | 30.9465 | 42.4095 | 62.0196 | 86 | 130.3843 | 166.3669 | 321.8364 |
| 30 | 32.4237 | 44.3136 | 65.4566 | 87 | 132.3238 | 168.7382 | 327.2709 |
| 31 | 33.9150 | 46.2322 | 68.9490 | 88 | 134.2683 | 171.1145 | 332.7291 |
| 32 | 35.4202 | 48.1648 | 72.4952 | 89 | 136.2177 | 173.4957 | 338.2108 |
| 33 | 36.9387 | 50.1110 | 76.0942 | 90 | 138.1719 | 175.8818 | 343.7157 |
| 34 | 38.4702 | 52.0703 | 79.7445 | 91 | 140.1310 | 178.2728 | 349.2437 |
| 35 | 40.0142 | 54.0424 | 83.4451 | 92 | 142.0948 | 180.6685 | 354.7946 |
| 36 | 41.5705 | 56.0269 | 87.1948 | 93 | 144.0632 | 183.0689 | 360.3680 |
| 37 | 43.1387 | 58.0235 | 90.9925 | 94 | 146.0364 | 185.4740 | 365.9640 |
| 38 | 44.7185 | 60.0318 | 94.8372 | 95 | 148.0141 | 187.8837 | 371.5821 |
| 39 | 46.3096 | 62.0515 | 98.7280 | 96 | 149.9964 | 190.2980 | 377.2223 |
| 40 | 47.9116 | 64.0824 | 102.6638 | 97 | 151.9831 | 192.7169 | 382.8844 |
| 41 | 49.5244 | 66.1241 | 106.6439 | 98 | 153.9744 | 195.1402 | 388.5682 |
| 42 | 51.1477 | 68.1765 | 110.6674 | 99 | 155.9700 | 197.5679 | 394.2734 |
| 43 | 52.7811 | 70.2391 | 114.7334 | 100 | 157.9700 | 200.0000 | 400.0000 |
| 44 | 54.4246 | 72.3119 | 118.8412 | 101 | 159.9743 | 202.4365 | 405.7477 |
| 45 | 56.0778 | 74.3946 | 122.9900 | 102 | 161.9829 | 204.8772 | 411.5164 |
| 46 | 57.7406 | 76.4869 | 127.1791 | 103 | 163.9958 | 207.3222 | 417.3059 |
| 47 | 59.4127 | 78.5886 | 131.4078 | 104 | 166.0128 | 209.7715 | 423.1160 |
| 48 | 61.0939 | 80.6996 | 135.6755 | 105 | 168.0340 | 212.2249 | 428.9466 |
| 49 | 62.7841 | 82.8196 | 139.9814 | 106 | 170.0593 | 214.6824 | 434.7976 |
| 50 | 64.4831 | 84.9485 | 144.3250 | 107 | 172.0887 | 217.1441 | 440.6686 |
| 51 | 66.1906 | 87.0861 | 148.7056 | 108 | 174.1221 | 219.6098 | 446.5597 |
| 52 | 67.9066 | 89.2322 | 153.1227 | 109 | 176.1595 | 222.0795 | 452.4706 |
| 53 | 69.6309 | 91.3866 | 157.5757 | 110 | 178.2009 | 224.5532 | 458.4013 |
| 54 | 71.3633 | 93.5493 | 162.0642 | 111 | 180.2462 | 227.0309 | 464.3514 |
| 55 | 73.1037 | 95.7199 | 166.5874 | 112 | 182.2955 | 229.5124 | 470.3210 |
| 56 | 74.8519 | 97.8985 | 171.1450 | 113 | 184.3485 | 231.9979 | 476.3098 |
| 57 | 76.6077 | 100.0849 | 175.7365 | 114 | 186.4054 | 234.4872 | 482.3178 |
| 58 | 78.3712 | 102.2788 | 180.3613 | 115 | 188.4661 | 236.9803 | 488.3447 |
| 59 | 80.1420 | 104.4803 | 185.0191 | 116 | 190.5306 | 239.4771 | 494.3905 |
| 60 | 81.9202 | 106.6891 | 189.7093 | 117 | 192.5988 | 241.9777 | 500.4550 |
| 61 | 83.7055 | 108.9051 | 194.4316 | 118 | 194.6707 | 244.4821 | 506.5380 |
| 62 | 85.4979 | 111.1283 | 199.1854 | 119 | 196.7462 | 246.9901 | 512.6395 |
| 63 | 87.2972 | 113.3585 | 203.9705 | 120 | 198.8254 | 249.5017 | 518.7594 |
| 64 | 89.1034 | 115.5955 | 208.7863 | 121 | 200.9082 | 252.0170 | 524.8974 |
| 65 | 90.9163 | 117.8394 | 213.6326 | 122 | 202.9945 | 254.5359 | 531.0535 |
| 66 | 92.7359 | 120.0899 | 218.5088 | 123 | 205.0844 | 257.0583 | 537.2275 |
| 67 | 94.5619 | 122.3470 | 223.4148 | 124 | 207.1779 | 259.5843 | 543.4194 |
| 68 | 96.3945 | 124.6106 | 228.3500 | 125 | 209.2748 | 262.1138 | 549.6290 |
| 69 | 98.2333 | 126.8806 | 233.3143 | 126 | 211.3751 | 264.6467 | 555.8561 |
| 70 | 100.0784 | 129.1569 | 238.3071 | 127 | 213.4790 | 267.1831 | 562.1007 |
| 71 | 101.9297 | 131.4393 | 243.3282 | 128 | 215.5862 | 269.7229 | 568.3627 |
| 72 | 103.7870 | 133.7279 | 248.3772 | 129 | 217.6967 | 272.2661 | 574.6420 |
| 73 | 105.6503 | 136.0226 | 253.4540 | 130 | 219.8107 | 274.8126 | 580.9383 |
| 74 | 107.5196 | 138.3231 | 258.5580 | 131 | 221.9280 | 277.3625 | 587.2517 |
| 75 | 109.3946 | 140.6296 | 263.6891 | 132 | 224.0485 | 279.9158 | 593.5821 |
| 76 | 111.2754 | 142.9418 | 268.8469 | 133 | 226.1724 | 282.4723 | 599.9292 |
| 77 | 113.1619 | 145.2598 | 274.0312 | 134 | 228.2995 | 285.0320 | 606.2930 |
| 78 | 115.0540 | 147.5834 | 279.2417 | 135 | 230.4298 | 287.5951 | 612.6735 |
| 79 | 116.9516 | 149.9125 | 284.4781 | 136 | 232.5634 | 290.1613 | 619.0704 |
| 80 | 118.8547 | 152.2472 | 289.7401 | 137 | 234.7001 | 292.7307 | 625.4837 |
| 81 | 120.7632 | 154.5873 | 295.0275 | 138 | 236.8400 | 295.3033 | 631.9134 |
| 82 | 122.6770 | 156.9327 | 300.3400 | 139 | 238.9830 | 297.8791 | 638.3592 |
| 83 | 124.5961 | 159.2835 | 305.6774 | 140 | 241.1291 | 300.4579 | 644.8212 |

## TABLE 23. (Continued)

| n | log n! | n log n | n log² n |
|---|---|---|---|
| 141 | 243.2783 | 303.0399 | 651.2991 |
| 142 | 245.4306 | 305.6249 | 657.7930 |
| 143 | 247.5860 | 308.2131 | 664.3027 |
| 144 | 249.7443 | 310.8042 | 670.8281 |
| 145 | 251.9057 | 313.3984 | 677.3692 |
| 146 | 254.0700 | 315.9955 | 683.9258 |
| 147 | 256.2374 | 318.5956 | 690.4979 |
| 148 | 258.4076 | 321.1987 | 697.0853 |
| 149 | 260.5808 | 323.8048 | 703.6880 |
| 150 | 262.7569 | 326.4137 | 710.3060 |
| 151 | 264.9359 | 329.0255 | 716.9390 |
| 152 | 267.1177 | 331.6402 | 723.5871 |
| 153 | 269.3024 | 334.2578 | 730.2501 |
| 154 | 271.4899 | 336.8782 | 736.9280 |
| 155 | 273.6803 | 339.5014 | 743.6207 |
| 156 | 275.8734 | 342.1274 | 750.3281 |
| 157 | 278.0693 | 344.7562 | 757.0501 |
| 158 | 280.2679 | 347.3878 | 763.7867 |
| 159 | 282.4693 | 350.0221 | 770.5377 |
| 160 | 284.6735 | 352.6592 | 777.3032 |
| 161 | 286.8803 | 355.2990 | 784.0830 |
| 162 | 289.0898 | 357.9414 | 790.8770 |
| 163 | 291.3020 | 360.5866 | 797.6852 |
| 164 | 293.5168 | 363.2344 | 804.5075 |
| 165 | 295.7343 | 365.8849 | 811.3438 |
| 166 | 297.9544 | 368.5379 | 818.1941 |
| 167 | 300.1771 | 371.1936 | 825.0582 |
| 168 | 302.4024 | 373.8520 | 831.9362 |
| 169 | 304.6303 | 376.5129 | 838.8280 |
| 170 | 306.8608 | 379.1763 | 845.7334 |
| 171 | 309.0938 | 381.8423 | 852.6524 |
| 172 | 311.3293 | 384.5109 | 859.5850 |
| 173 | 313.5674 | 387.1820 | 866.5311 |
| 174 | 315.8079 | 389.8556 | 873.4906 |
| 175 | 318.0509 | 392.5317 | 880.4634 |
| 176 | 320.2965 | 395.2102 | 887.4496 |
| 177 | 322.5444 | 397.8913 | 894.4489 |
| 178 | 324.7948 | 400.5748 | 901.4615 |
| 179 | 327.0477 | 403.2607 | 908.4871 |
| 180 | 329.3030 | 405.9491 | 915.5257 |
| 181 | 331.5606 | 408.6398 | 922.5774 |
| 182 | 333.8207 | 411.3330 | 929.6419 |
| 183 | 336.0832 | 414.0285 | 936.7193 |
| 184 | 338.3480 | 416.7265 | 943.8096 |
| 185 | 340.6152 | 419.4268 | 950.9125 |
| 186 | 342.8847 | 422.1294 | 958.0282 |
| 187 | 345.1565 | 424.8344 | 965.1564 |
| 188 | 347.4307 | 427.5417 | 972.2973 |
| 189 | 349.7071 | 430.2513 | 979.4506 |
| 190 | 351.9859 | 432.9632 | 986.6164 |
| 191 | 354.2669 | 435.6774 | 993.7946 |
| 192 | 356.5502 | 438.3938 | 1000.9852 |
| 193 | 358.8358 | 441.1126 | 1008.1880 |
| 194 | 361.1236 | 443.8335 | 1015.4031 |
| 195 | 363.4136 | 446.5567 | 1022.6304 |
| 196 | 365.7059 | 449.2822 | 1029.8698 |
| 197 | 368.0003 | 452.0098 | 1037.1213 |
| 198 | 370.2970 | 454.7397 | 1044.3849 |
| 199 | 372.5959 | 457.4718 | 1051.6604 |
| 200 | 374.8969 | 460.2060 | 1058.9478 |
| 201 | 377.2001 | 462.9424 | 1066.2471 |
| 202 | 379.5054 | 465.6810 | 1073.5583 |
| 203 | 381.8129 | 468.4217 | 1080.8812 |
| 204 | 384.1226 | 471.1646 | 1088.2159 |
| 205 | 386.4343 | 473.9095 | 1095.5622 |
| 206 | 388.7482 | 476.6566 | 1102.9202 |
| 207 | 391.0642 | 479.4059 | 1110.2897 |
| 208 | 393.3822 | 482.1572 | 1117.6709 |
| 209 | 395.7024 | 484.9106 | 1125.0635 |
| 210 | 398.0246 | 487.6661 | 1132.4675 |
| 211 | 400.3489 | 490.4236 | 1139.8829 |
| 212 | 402.6752 | 493.1832 | 1147.3098 |
| 213 | 405.0036 | 495.9449 | 1154.7479 |
| 214 | 407.3340 | 498.7085 | 1162.1973 |
| 215 | 409.6664 | 501.4743 | 1169.6579 |
| 216 | 412.0009 | 504.2420 | 1177.1296 |
| 217 | 414.3373 | 507.0118 | 1184.6126 |
| 218 | 416.6758 | 509.7835 | 1192.1066 |
| 219 | 419.0162 | 512.5573 | 1199.6116 |
| 220 | 421.3587 | 515.3330 | 1207.1277 |
| 221 | 423.7031 | 518.1107 | 1214.6547 |
| 222 | 426.0494 | 520.8904 | 1222.1926 |
| 223 | 428.3977 | 523.6720 | 1229.7415 |
| 224 | 430.7480 | 526.4556 | 1237.3011 |
| 225 | 433.1002 | 529.2411 | 1244.8716 |
| 226 | 435.4543 | 532.0285 | 1252.4528 |
| 227 | 437.8103 | 534.8179 | 1260.0447 |
| 228 | 440.1682 | 537.6091 | 1267.6473 |
| 229 | 442.5281 | 540.4023 | 1275.2606 |
| 230 | 444.8898 | 543.1974 | 1282.8844 |
| 231 | 447.2534 | 545.9944 | 1290.5188 |
| 232 | 449.6189 | 548.7932 | 1298.1637 |
| 233 | 451.9862 | 551.5939 | 1305.8192 |
| 234 | 454.3555 | 554.3965 | 1313.4850 |
| 235 | 456.7265 | 557.2009 | 1321.1613 |
| 236 | 459.0994 | 560.0072 | 1328.8479 |
| 237 | 461.4742 | 562.8154 | 1336.5448 |
| 238 | 463.8508 | 565.6253 | 1344.2521 |
| 239 | 466.2292 | 568.4371 | 1351.9696 |
| 240 | 468.6094 | 571.2507 | 1359.6973 |
| 241 | 470.9914 | 574.0661 | 1367.4352 |
| 242 | 473.3752 | 576.8833 | 1375.1833 |
| 243 | 475.7608 | 579.7023 | 1382.9415 |
| 244 | 478.1482 | 582.5231 | 1390.7098 |
| 245 | 480.5374 | 585.3457 | 1398.4881 |
| 246 | 482.9283 | 588.1700 | 1406.2764 |
| 247 | 485.3210 | 590.9961 | 1414.0747 |
| 248 | 487.7154 | 593.8240 | 1421.8829 |
| 249 | 490.1116 | 596.6536 | 1429.7010 |
| 250 | 492.5096 | 599.4850 | 1437.5291 |
| 251 | 494.9093 | 602.3181 | 1445.3669 |
| 252 | 497.3107 | 605.1529 | 1453.2146 |
| 253 | 499.7138 | 607.9895 | 1461.0720 |
| 254 | 502.1186 | 610.8278 | 1468.9392 |
| 255 | 504.5252 | 613.6677 | 1476.8161 |
| 256 | 506.9334 | 616.5094 | 1484.7026 |
| 257 | 509.3433 | 619.3528 | 1492.5988 |
| 258 | 511.7549 | 622.1979 | 1500.5047 |
| 259 | 514.1682 | 625.0446 | 1508.4201 |
| 260 | 516.5832 | 627.8931 | 1516.3450 |
| 261 | 518.9999 | 630.7432 | 1524.2795 |
| 262 | 521.4182 | 633.5949 | 1532.2234 |
| 263 | 523.8381 | 636.4484 | 1540.1769 |
| 264 | 526.2597 | 639.3034 | 1548.1397 |
| 265 | 528.6830 | 642.1602 | 1556.1119 |
| 266 | 531.1078 | 645.0185 | 1564.0936 |
| 267 | 533.5344 | 647.8785 | 1572.0845 |
| 268 | 535.9625 | 650.7401 | 1580.0847 |
| 269 | 538.3922 | 653.6034 | 1588.0943 |
| 270 | 540.8236 | 656.4682 | 1596.1130 |
| 271 | 543.2566 | 659.3347 | 1604.1410 |
| 272 | 545.6912 | 662.2027 | 1612.1782 |
| 273 | 548.1273 | 665.0724 | 1620.2245 |
| 274 | 550.5651 | 667.9437 | 1628.2800 |
| 275 | 553.0044 | 670.8165 | 1636.3446 |
| 276 | 555.4453 | 673.6909 | 1644.4182 |
| 277 | 557.8878 | 676.5669 | 1652.5009 |
| 278 | 560.3318 | 679.4445 | 1660.5927 |
| 279 | 562.7774 | 682.3236 | 1668.6934 |
| 280 | 565.2246 | 685.2042 | 1676.8031 |
| 281 | 567.6733 | 688.0865 | 1684.9217 |
| 282 | 570.1235 | 690.9702 | 1693.0492 |
| 283 | 572.5753 | 693.8556 | 1701.1856 |
| 284 | 575.0287 | 696.7424 | 1709.3309 |
| 285 | 577.4835 | 699.6308 | 1717.4850 |
| 286 | 579.9399 | 702.5207 | 1725.6479 |
| 287 | 582.3977 | 705.4121 | 1733.8196 |
| 288 | 584.8571 | 708.3050 | 1742.0001 |
| 289 | 587.3180 | 711.1995 | 1750.1893 |
| 290 | 589.7804 | 714.0954 | 1758.3871 |
| 291 | 592.2443 | 716.9929 | 1766.5937 |
| 292 | 594.7097 | 719.8918 | 1774.8089 |
| 293 | 597.1766 | 722.7922 | 1783.0327 |
| 294 | 599.6449 | 725.6941 | 1791.2651 |
| 295 | 602.1147 | 728.5975 | 1799.5061 |
| 296 | 604.5860 | 731.5023 | 1807.7557 |
| 297 | 607.0588 | 734.4087 | 1816.0138 |
| 298 | 609.5330 | 737.3164 | 1824.2803 |
| 299 | 612.0087 | 740.2257 | 1832.5554 |
| 300 | 614.4858 | 743.1364 | 1840.8389 |
| 301 | 616.9644 | 746.0485 | 1849.1308 |
| 302 | 619.4444 | 748.9621 | 1857.4312 |
| 303 | 621.9258 | 751.8771 | 1865.7399 |
| 304 | 624.4087 | 754.7936 | 1874.0570 |
| 305 | 626.8930 | 757.7115 | 1882.3824 |
| 306 | 629.3787 | 760.6308 | 1890.7162 |
| 307 | 631.8659 | 763.5515 | 1899.0582 |
| 308 | 634.3544 | 766.4736 | 1907.4085 |
| 309 | 636.8444 | 769.3972 | 1915.7670 |
| 310 | 639.3357 | 772.3221 | 1924.1337 |
| 311 | 641.8285 | 775.2485 | 1932.5087 |
| 312 | 644.3226 | 778.1762 | 1940.8918 |
| 313 | 646.8182 | 781.1054 | 1949.2831 |
| 314 | 649.3151 | 784.0359 | 1957.6825 |
| 315 | 651.8134 | 786.9678 | 1966.0900 |
| 316 | 654.3131 | 789.9011 | 1974.5056 |
| 317 | 656.8142 | 792.8358 | 1982.9293 |
| 318 | 659.3166 | 795.7718 | 1991.3610 |
| 319 | 661.8204 | 798.7092 | 1999.8007 |
| 320 | 664.3255 | 801.6480 | 2008.2484 |
| 321 | 666.8320 | 804.5881 | 2016.7041 |
| 322 | 669.3399 | 807.5296 | 2025.1678 |
| 323 | 671.8491 | 810.4724 | 2033.6394 |
| 324 | 674.3596 | 813.4166 | 2042.1189 |
| 325 | 676.8715 | 816.3621 | 2050.6064 |
| 326 | 679.3847 | 819.3089 | 2059.1016 |
| 327 | 681.8993 | 822.2571 | 2067.6048 |
| 328 | 684.4152 | 825.2066 | 2076.1157 |
| 329 | 686.9324 | 828.1575 | 2084.6345 |
| 330 | 689.4509 | 831.1096 | 2093.1611 |
| 331 | 691.9707 | 834.0631 | 2101.6954 |
| 332 | 694.4918 | 837.0178 | 2110.2375 |
| 333 | 697.0143 | 839.9739 | 2118.7874 |
| 334 | 699.5380 | 842.9313 | 2127.3449 |
| 335 | 702.0631 | 845.8900 | 2135.9102 |
| 336 | 704.5894 | 848.8500 | 2144.4831 |
| 337 | 707.1170 | 851.8113 | 2153.0636 |
| 338 | 709.6460 | 854.7738 | 2161.6518 |
| 339 | 712.1762 | 857.7377 | 2170.2477 |
| 340 | 714.7076 | 860.7028 | 2178.8510 |
| 341 | 717.2404 | 863.6692 | 2187.4620 |
| 342 | 719.7744 | 866.6369 | 2196.0806 |
| 343 | 722.3097 | 869.6059 | 2204.7067 |
| 344 | 724.8463 | 872.5761 | 2213.3403 |
| 345 | 727.3841 | 875.5476 | 2221.9814 |
| 346 | 729.9232 | 878.5203 | 2230.6299 |
| 347 | 732.4635 | 881.4943 | 2239.2860 |
| 348 | 735.0051 | 884.4696 | 2247.9495 |
| 349 | 737.5479 | 887.4461 | 2256.6204 |
| 350 | 740.0920 | 890.4238 | 2265.2988 |
| 351 | 742.6373 | 893.4028 | 2273.9845 |
| 352 | 745.1838 | 896.3830 | 2282.6776 |
| 353 | 747.7316 | 899.3645 | 2291.3780 |
| 354 | 750.2806 | 902.3472 | 2300.0858 |
| 355 | 752.8308 | 905.3311 | 2308.8009 |
| 356 | 755.3823 | 908.3162 | 2317.5233 |
| 357 | 757.9349 | 911.3026 | 2326.2531 |
| 358 | 760.4888 | 914.2901 | 2334.9900 |
| 359 | 763.0439 | 917.2789 | 2343.7342 |
| 360 | 765.6002 | 920.2689 | 2352.4857 |
| 361 | 768.1577 | 923.2601 | 2361.2444 |
| 362 | 770.7164 | 926.2525 | 2370.0102 |
| 363 | 773.2764 | 929.2461 | 2378.7832 |
| 364 | 775.8375 | 932.2409 | 2387.5634 |
| 365 | 778.3997 | 935.2369 | 2396.3508 |
| 366 | 780.9632 | 938.2341 | 2405.1453 |
| 367 | 783.5279 | 941.2324 | 2413.9469 |
| 368 | 786.0937 | 944.2320 | 2422.7556 |

## TABLE 23. (Continued)

| n | log n! | n log n | n log² n |
|---|---|---|---|
| 369 | 788.6608 | 947.2327 | 2431.5714 |
| 370 | 791.2290 | 950.2346 | 2440.3942 |
| 371 | 793.7983 | 953.2377 | 2449.2241 |
| 372 | 796.3689 | 956.2420 | 2458.0610 |
| 373 | 798.9406 | 959.2474 | 2466.9050 |
| 374 | 801.5135 | 962.2540 | 2475.7559 |
| 375 | 804.0875 | 965.2617 | 2484.6139 |
| 376 | 806.6627 | 968.2706 | 2493.4787 |
| 377 | 809.2390 | 971.2807 | 2502.3506 |
| 378 | 811.8165 | 974.2919 | 2511.2294 |
| 379 | 814.3952 | 977.3043 | 2520.1151 |
| 380 | 816.9749 | 980.3178 | 2529.0077 |
| 381 | 819.5559 | 983.3324 | 2537.9072 |
| 382 | 822.1379 | 986.3482 | 2546.8135 |
| 383 | 824.7211 | 989.3651 | 2555.7268 |
| 384 | 827.3055 | 992.3832 | 2564.6469 |
| 385 | 829.8909 | 995.4024 | 2573.5738 |
| 386 | 832.4775 | 998.4227 | 2582.5075 |
| 387 | 835.0652 | 1001.4441 | 2591.4480 |
| 388 | 837.6540 | 1004.4667 | 2600.3953 |
| 389 | 840.2440 | 1007.4904 | 2609.3493 |
| 390 | 842.8351 | 1010.5152 | 2618.3102 |
| 391 | 845.4272 | 1013.5411 | 2627.2777 |
| 392 | 848.0205 | 1016.5681 | 2636.2520 |
| 393 | 850.6149 | 1019.5963 | 2645.2330 |
| 394 | 853.2104 | 1022.6255 | 2654.2206 |
| 395 | 855.8070 | 1025.6558 | 2663.2150 |
| 396 | 858.4047 | 1028.6873 | 2672.2160 |
| 397 | 861.0035 | 1031.7198 | 2681.2237 |
| 398 | 863.6034 | 1034.7534 | 2690.2380 |
| 399 | 866.2044 | 1037.7882 | 2699.2589 |
| 400 | 868.8064 | 1040.8240 | 2708.2865 |
| 401 | 871.4096 | 1043.8609 | 2717.3206 |
| 402 | 874.0138 | 1046.8989 | 2726.3613 |
| 403 | 876.6191 | 1049.9379 | 2735.4086 |
| 404 | 879.2255 | 1052.9781 | 2744.4624 |
| 405 | 881.8329 | 1056.0193 | 2753.5228 |
| 406 | 884.4415 | 1059.0616 | 2762.5897 |
| 407 | 887.0510 | 1062.1049 | 2771.6631 |
| 408 | 889.6617 | 1065.1493 | 2780.7430 |
| 409 | 892.2734 | 1068.1948 | 2789.8293 |
| 410 | 894.8862 | 1071.2414 | 2798.9222 |
| 411 | 897.5001 | 1074.2890 | 2808.0215 |
| 412 | 900.1150 | 1077.3376 | 2817.1272 |
| 413 | 902.7309 | 1080.3874 | 2826.2394 |
| 414 | 905.3479 | 1083.4381 | 2835.3580 |
| 415 | 907.9660 | 1086.4900 | 2844.4830 |
| 416 | 910.5850 | 1089.5428 | 2853.6143 |
| 417 | 913.2052 | 1092.5967 | 2862.7521 |
| 418 | 915.8264 | 1095.6517 | 2871.8962 |
| 419 | 918.4486 | 1098.7077 | 2881.0467 |
| 420 | 921.0718 | 1101.7647 | 2890.2035 |
| 421 | 923.6961 | 1104.8228 | 2899.3666 |
| 422 | 926.3214 | 1107.8819 | 2908.5360 |
| 423 | 928.9478 | 1110.9420 | 2917.7117 |
| 424 | 931.5751 | 1114.0031 | 2926.8938 |
| 425 | 934.2035 | 1117.0653 | 2936.0820 |
| 426 | 936.8329 | 1120.1285 | 2945.2766 |
| 427 | 939.4633 | 1123.1927 | 2954.4774 |
| 428 | 942.0948 | 1126.2579 | 2963.6844 |
| 429 | 944.7272 | 1129.3242 | 2972.8976 |
| 430 | 947.3607 | 1132.3914 | 2982.1171 |
| 431 | 949.9952 | 1135.4597 | 2991.3428 |
| 432 | 952.6307 | 1138.5290 | 3000.5746 |
| 433 | 955.2672 | 1141.5993 | 3009.8126 |
| 434 | 957.9047 | 1144.6705 | 3019.0568 |
| 435 | 960.5431 | 1147.7428 | 3028.3071 |
| 436 | 963.1826 | 1150.8161 | 3037.5636 |
| 437 | 965.8231 | 1153.8904 | 3046.8261 |
| 438 | 968.4646 | 1156.9657 | 3056.0948 |
| 439 | 971.1071 | 1160.0419 | 3065.3696 |
| 440 | 973.7505 | 1163.1192 | 3074.6505 |
| 441 | 976.3949 | 1166.1974 | 3083.9374 |
| 442 | 979.0404 | 1169.2766 | 3093.2305 |
| 443 | 981.6868 | 1172.3568 | 3102.5295 |
| 444 | 984.3342 | 1175.4380 | 3111.8346 |
| 445 | 986.9825 | 1178.5202 | 3121.1458 |
| 446 | 989.6318 | 1181.6033 | 3130.4629 |
| 447 | 992.2822 | 1184.6875 | 3139.7861 |
| 448 | 994.9334 | 1187.7725 | 3149.1152 |
| 449 | 997.5857 | 1190.8586 | 3158.4504 |
| 450 | 1000.2389 | 1193.9456 | 3167.7915 |
| 451 | 1002.8931 | 1197.0336 | 3177.1385 |
| 452 | 1005.5482 | 1200.1226 | 3186.4915 |
| 453 | 1008.2043 | 1203.2125 | 3195.8505 |
| 454 | 1010.8614 | 1206.3033 | 3205.2154 |
| 455 | 1013.5194 | 1209.3952 | 3214.5862 |
| 456 | 1016.1783 | 1212.4880 | 3223.9629 |
| 457 | 1018.8382 | 1215.5817 | 3233.3455 |
| 458 | 1021.4991 | 1218.6764 | 3242.7339 |
| 459 | 1024.1609 | 1221.7720 | 3252.1283 |
| 460 | 1026.8237 | 1224.8686 | 3261.5284 |
| 461 | 1029.4874 | 1227.9661 | 3270.9345 |
| 462 | 1032.1520 | 1231.0646 | 3280.3464 |
| 463 | 1034.8176 | 1234.1640 | 3289.7641 |
| 464 | 1037.4841 | 1237.2643 | 3299.1876 |
| 465 | 1040.1516 | 1240.3656 | 3308.6169 |
| 466 | 1042.8200 | 1243.4678 | 3318.0521 |
| 467 | 1045.4893 | 1246.5710 | 3327.4930 |
| 468 | 1048.1595 | 1249.6750 | 3336.9396 |
| 469 | 1050.8307 | 1252.7801 | 3346.3921 |
| 470 | 1053.5028 | 1255.8860 | 3355.8503 |
| 471 | 1056.1758 | 1258.9928 | 3365.3142 |
| 472 | 1058.8498 | 1262.1006 | 3374.7838 |
| 473 | 1061.5246 | 1265.2093 | 3384.2592 |
| 474 | 1064.2004 | 1268.3189 | 3393.7403 |
| 475 | 1066.8771 | 1271.4295 | 3403.2271 |
| 476 | 1069.5547 | 1274.5409 | 3412.7196 |
| 477 | 1072.2332 | 1277.6533 | 3422.2177 |
| 478 | 1074.9126 | 1280.7665 | 3431.7216 |
| 479 | 1077.5930 | 1283.8807 | 3441.2310 |
| 480 | 1080.2742 | 1286.9958 | 3450.7462 |
| 481 | 1082.9564 | 1290.1118 | 3460.2669 |
| 482 | 1085.6394 | 1293.2287 | 3469.7933 |
| 483 | 1088.3234 | 1296.3465 | 3479.3253 |
| 484 | 1091.0082 | 1299.4651 | 3488.8630 |
| 485 | 1093.6940 | 1302.5847 | 3498.4062 |
| 486 | 1096.3806 | 1305.7052 | 3507.9550 |
| 487 | 1099.0681 | 1308.8266 | 3517.5094 |
| 488 | 1101.7565 | 1311.9489 | 3527.0693 |
| 489 | 1104.4458 | 1315.0720 | 3536.6349 |
| 490 | 1107.1360 | 1318.1961 | 3546.2059 |
| 491 | 1109.8271 | 1321.3210 | 3555.7825 |
| 492 | 1112.5191 | 1324.4468 | 3565.3646 |
| 493 | 1115.2119 | 1327.5735 | 3574.9523 |
| 494 | 1117.9057 | 1330.7011 | 3584.5454 |
| 495 | 1120.6003 | 1333.8296 | 3594.1441 |
| 496 | 1123.2957 | 1336.9589 | 3603.7482 |
| 497 | 1125.9921 | 1340.0891 | 3613.3578 |
| 498 | 1128.6893 | 1343.2202 | 3622.9730 |
| 499 | 1131.3874 | 1346.3522 | 3632.5935 |
| 500 | 1134.0864 | 1349.4850 | 3642.2195 |
| 501 | 1136.7862 | 1352.6187 | 3651.8510 |
| 502 | 1139.4869 | 1355.7533 | 3661.4879 |
| 503 | 1142.1885 | 1358.8887 | 3671.1302 |
| 504 | 1144.8909 | 1362.0250 | 3680.7779 |
| 505 | 1147.5942 | 1365.1621 | 3690.4310 |
| 506 | 1150.2984 | 1368.3002 | 3700.0896 |
| 507 | 1153.0034 | 1371.4390 | 3709.7535 |
| 508 | 1155.7093 | 1374.5788 | 3719.4228 |
| 509 | 1158.4160 | 1377.7193 | 3729.0974 |
| 510 | 1161.1235 | 1380.8608 | 3738.7775 |
| 511 | 1163.8320 | 1384.0031 | 3748.4629 |
| 512 | 1166.5412 | 1387.1462 | 3758.1536 |
| 513 | 1169.2514 | 1390.2902 | 3767.8496 |
| 514 | 1171.9623 | 1393.4350 | 3777.5510 |
| 515 | 1174.6741 | 1396.5807 | 3787.2577 |
| 516 | 1177.3868 | 1399.7272 | 3796.9697 |
| 517 | 1180.1003 | 1402.8746 | 3806.6870 |
| 518 | 1182.8146 | 1406.0228 | 3816.4095 |
| 519 | 1185.5298 | 1409.1718 | 3826.1374 |
| 520 | 1188.2458 | 1412.3217 | 3835.8705 |
| 521 | 1190.9626 | 1415.4724 | 3845.6089 |
| 522 | 1193.6803 | 1418.6240 | 3855.3526 |
| 523 | 1196.3988 | 1421.7764 | 3865.1015 |
| 524 | 1199.1181 | 1424.9296 | 3874.8556 |
| 525 | 1201.8383 | 1428.0836 | 3884.6150 |
| 526 | 1204.5592 | 1431.2385 | 3894.3795 |
| 527 | 1207.2811 | 1434.3942 | 3904.1493 |
| 528 | 1210.0037 | 1437.5507 | 3913.9243 |
| 529 | 1212.7271 | 1440.7080 | 3923.7045 |
| 530 | 1215.4514 | 1443.8662 | 3933.4899 |
| 531 | 1218.1765 | 1447.0252 | 3943.2804 |
| 532 | 1220.9024 | 1450.1850 | 3953.0761 |
| 533 | 1223.6292 | 1453.3456 | 3962.8770 |
| 534 | 1226.3567 | 1456.5070 | 3972.6830 |
| 535 | 1229.0851 | 1459.6693 | 3982.4942 |
| 536 | 1231.8142 | 1462.8323 | 3992.3105 |
| 537 | 1234.5442 | 1465.9962 | 4002.1319 |
| 538 | 1237.2750 | 1469.1609 | 4011.9584 |
| 539 | 1240.0066 | 1472.3263 | 4021.7901 |
| 540 | 1242.7390 | 1475.4926 | 4031.6268 |
| 541 | 1245.4722 | 1478.6597 | 4041.4687 |
| 542 | 1248.2061 | 1481.8276 | 4051.3156 |
| 543 | 1250.9409 | 1484.9963 | 4061.1676 |
| 544 | 1253.6765 | 1488.1658 | 4071.0247 |
| 545 | 1256.4129 | 1491.3361 | 4080.8868 |
| 546 | 1259.1501 | 1494.5072 | 4090.7541 |
| 547 | 1261.8881 | 1497.6791 | 4100.6263 |
| 548 | 1264.6269 | 1500.8517 | 4110.5035 |
| 549 | 1267.3665 | 1504.0252 | 4120.3859 |
| 550 | 1270.1068 | 1507.1995 | 4130.2732 |
| 551 | 1272.8480 | 1510.3745 | 4140.1655 |
| 552 | 1275.5899 | 1513.5504 | 4150.0629 |
| 553 | 1278.3327 | 1516.7270 | 4159.9652 |
| 554 | 1281.0762 | 1519.9044 | 4169.8726 |
| 555 | 1283.8205 | 1523.0826 | 4179.7849 |
| 556 | 1286.5655 | 1526.2616 | 4189.7021 |
| 557 | 1289.3114 | 1529.4413 | 4199.6245 |
| 558 | 1292.0580 | 1532.6219 | 4209.5516 |
| 559 | 1294.8054 | 1535.8032 | 4219.4838 |
| 560 | 1297.5536 | 1538.9853 | 4229.4210 |
| 561 | 1300.3026 | 1542.1682 | 4239.3630 |
| 562 | 1303.0523 | 1545.3518 | 4249.3099 |
| 563 | 1305.8028 | 1548.5362 | 4259.2618 |
| 564 | 1308.5541 | 1551.7214 | 4269.2187 |
| 565 | 1311.3062 | 1554.9074 | 4279.1804 |
| 566 | 1314.0590 | 1558.0941 | 4289.1470 |
| 567 | 1316.8126 | 1561.2816 | 4299.1185 |
| 568 | 1319.5669 | 1564.4698 | 4309.0949 |
| 569 | 1322.3220 | 1567.6589 | 4319.0762 |
| 570 | 1325.0779 | 1570.8487 | 4329.0623 |
| 571 | 1327.8345 | 1574.0392 | 4339.0533 |
| 572 | 1330.5919 | 1577.2305 | 4349.0492 |
| 573 | 1333.3501 | 1580.4226 | 4359.0499 |
| 574 | 1336.1090 | 1583.6154 | 4369.0554 |
| 575 | 1338.8687 | 1586.8090 | 4379.0658 |
| 576 | 1341.6291 | 1590.0033 | 4389.0810 |
| 577 | 1344.3903 | 1593.1984 | 4399.1010 |
| 578 | 1347.1522 | 1596.3943 | 4409.1258 |
| 579 | 1349.9149 | 1599.5909 | 4419.1555 |
| 580 | 1352.6783 | 1602.7882 | 4429.1898 |
| 581 | 1355.4425 | 1605.9863 | 4439.2291 |
| 582 | 1358.2074 | 1609.1852 | 4449.2731 |
| 583 | 1360.9731 | 1612.3848 | 4459.3218 |
| 584 | 1363.7395 | 1615.5851 | 4469.3754 |
| 585 | 1366.5066 | 1618.7862 | 4479.4337 |
| 586 | 1369.2745 | 1621.9880 | 4489.4967 |
| 587 | 1372.0432 | 1625.1906 | 4499.5645 |
| 588 | 1374.8125 | 1628.3939 | 4509.6370 |
| 589 | 1377.5827 | 1631.5979 | 4519.7143 |
| 590 | 1380.3535 | 1634.8027 | 4529.7963 |
| 591 | 1383.1251 | 1638.0082 | 4539.8830 |
| 592 | 1385.8974 | 1641.2144 | 4549.9744 |
| 593 | 1388.6705 | 1644.4214 | 4560.0706 |
| 594 | 1391.4443 | 1647.6291 | 4570.1714 |
| 595 | 1394.2188 | 1650.8376 | 4580.2769 |
| 596 | 1396.9940 | 1654.0468 | 4590.3871 |

## TABLE 23. (Continued)

| n | log n! | n log n | n log² n |
|---|---|---|---|
| 597 | 1399.7700 | 1657.2567 | 4600.5020 |
| 598 | 1402.5467 | 1660.4673 | 4610.6215 |
| 599 | 1405.3241 | 1663.6787 | 4620.7457 |
| 600 | 1408.1023 | 1666.8907 | 4630.8746 |
| 601 | 1410.8811 | 1670.1035 | 4641.0081 |
| 602 | 1413.6608 | 1673.3171 | 4651.1463 |
| 603 | 1416.4411 | 1676.5313 | 4661.2891 |
| 604 | 1419.2221 | 1679.7463 | 4671.4365 |
| 605 | 1422.0039 | 1682.9620 | 4681.5886 |
| 606 | 1424.7863 | 1686.1784 | 4691.7452 |
| 607 | 1427.5695 | 1689.3955 | 4701.9065 |
| 608 | 1430.3534 | 1692.6134 | 4712.0724 |
| 609 | 1433.1380 | 1695.8319 | 4722.2429 |
| 610 | 1435.9234 | 1699.0512 | 4732.4180 |
| 611 | 1438.7094 | 1702.2712 | 4742.5977 |
| 612 | 1441.4962 | 1705.4919 | 4752.7819 |
| 613 | 1444.2836 | 1708.7133 | 4762.9707 |
| 614 | 1447.0718 | 1711.9354 | 4773.1641 |
| 615 | 1449.8607 | 1715.1582 | 4783.3620 |
| 616 | 1452.6503 | 1718.3817 | 4793.5645 |
| 617 | 1455.4405 | 1721.6059 | 4803.7715 |
| 618 | 1458.2315 | 1724.8309 | 4813.9831 |
| 619 | 1461.0232 | 1728.0565 | 4824.1992 |
| 620 | 1463.8156 | 1731.2828 | 4834.4198 |
| 621 | 1466.6087 | 1734.5099 | 4844.6450 |
| 622 | 1469.4025 | 1737.7376 | 4854.8746 |
| 623 | 1472.1970 | 1740.9660 | 4865.1088 |
| 624 | 1474.9922 | 1744.1952 | 4875.3475 |
| 625 | 1477.7880 | 1747.4250 | 4885.5906 |
| 626 | 1480.5846 | 1750.6555 | 4895.8383 |
| 627 | 1483.3819 | 1753.8867 | 4906.0905 |
| 628 | 1486.1798 | 1757.1187 | 4916.3470 |
| 629 | 1488.9785 | 1760.3512 | 4926.6082 |
| 630 | 1491.7778 | 1763.5845 | 4936.8737 |
| 631 | 1494.5779 | 1766.8185 | 4947.1437 |
| 632 | 1497.3786 | 1770.0532 | 4957.4182 |
| 633 | 1500.1800 | 1773.2885 | 4967.6971 |
| 634 | 1502.9821 | 1776.5246 | 4977.9804 |
| 635 | 1505.7849 | 1779.7613 | 4988.2682 |
| 636 | 1508.5883 | 1782.9987 | 4998.5604 |
| 637 | 1511.3924 | 1786.2368 | 5008.8571 |
| 638 | 1514.1973 | 1789.4756 | 5019.1581 |
| 639 | 1517.0028 | 1792.7150 | 5029.4636 |
| 640 | 1519.8089 | 1795.9552 | 5039.7734 |
| 641 | 1522.6158 | 1799.1960 | 5050.0877 |
| 642 | 1525.4233 | 1802.4375 | 5060.4064 |
| 643 | 1528.2316 | 1805.6796 | 5070.7294 |
| 644 | 1531.0404 | 1808.9225 | 5081.0568 |
| 645 | 1533.8500 | 1812.1660 | 5091.3886 |
| 646 | 1536.6602 | 1815.4102 | 5101.7248 |
| 647 | 1539.4711 | 1818.6551 | 5112.0653 |
| 648 | 1542.2827 | 1821.9006 | 5122.4102 |
| 649 | 1545.0950 | 1825.1468 | 5132.7594 |
| 650 | 1547.9079 | 1828.3937 | 5143.1130 |
| 651 | 1550.7215 | 1831.6412 | 5153.4709 |
| 652 | 1553.5357 | 1834.8894 | 5163.8331 |
| 653 | 1556.3506 | 1838.1383 | 5174.1997 |
| 654 | 1559.1662 | 1841.3878 | 5184.5706 |
| 655 | 1561.9824 | 1844.6380 | 5194.9459 |
| 656 | 1564.7993 | 1847.8889 | 5205.3254 |
| 657 | 1567.6169 | 1851.1404 | 5215.7092 |
| 658 | 1570.4351 | 1854.3926 | 5226.0973 |
| 659 | 1573.2540 | 1857.6455 | 5236.4897 |
| 660 | 1576.0735 | 1860.8990 | 5246.8865 |
| 661 | 1578.8938 | 1864.1532 | 5257.2875 |
| 662 | 1581.7146 | 1867.4080 | 5267.6927 |
| 663 | 1584.5361 | 1870.6635 | 5278.1022 |
| 664 | 1587.3583 | 1873.9196 | 5288.5161 |
| 665 | 1590.1811 | 1877.1764 | 5298.9341 |
| 666 | 1593.0046 | 1880.4338 | 5309.3564 |
| 667 | 1595.8287 | 1883.6919 | 5319.7830 |
| 668 | 1598.6535 | 1886.9507 | 5330.2138 |
| 669 | 1601.4789 | 1890.2101 | 5340.6489 |
| 670 | 1604.3050 | 1893.4701 | 5351.0881 |
| 671 | 1607.1317 | 1896.7308 | 5361.5317 |
| 672 | 1609.9591 | 1899.9921 | 5371.9794 |
| 673 | 1612.7871 | 1903.2541 | 5382.4313 |
| 674 | 1615.6158 | 1906.5168 | 5392.8875 |
| 675 | 1618.4451 | 1909.7800 | 5403.3478 |
| 676 | 1621.2750 | 1913.0440 | 5413.8124 |
| 677 | 1624.1056 | 1916.3085 | 5424.2812 |
| 678 | 1626.9368 | 1919.5737 | 5434.7542 |
| 679 | 1629.7687 | 1922.8396 | 5445.2313 |
| 680 | 1632.6012 | 1926.1060 | 5455.7125 |
| 681 | 1635.4344 | 1929.3732 | 5466.1981 |
| 682 | 1638.2681 | 1932.6409 | 5476.6877 |
| 683 | 1641.1026 | 1935.9093 | 5487.1815 |
| 684 | 1643.9376 | 1939.1784 | 5497.6794 |
| 685 | 1646.7733 | 1942.4480 | 5508.1816 |
| 686 | 1649.6096 | 1945.7183 | 5518.6878 |
| 687 | 1652.4466 | 1948.9893 | 5529.1982 |
| 688 | 1655.2842 | 1952.2608 | 5539.7128 |
| 689 | 1658.1224 | 1955.5330 | 5550.2314 |
| 690 | 1660.9612 | 1958.8059 | 5560.7542 |
| 691 | 1663.8007 | 1962.0793 | 5571.2811 |
| 692 | 1666.6408 | 1965.3534 | 5581.8122 |
| 693 | 1669.4816 | 1968.6281 | 5592.3474 |
| 694 | 1672.3229 | 1971.9035 | 5602.8866 |
| 695 | 1675.1649 | 1975.1794 | 5613.4299 |
| 696 | 1678.0075 | 1978.4560 | 5623.9774 |
| 697 | 1680.8507 | 1981.7332 | 5634.5289 |
| 698 | 1683.6946 | 1985.0111 | 5645.0845 |
| 699 | 1686.5391 | 1988.2895 | 5655.6442 |
| 700 | 1689.3842 | 1991.5686 | 5666.2079 |
| 701 | 1692.2299 | 1994.8483 | 5676.7758 |
| 702 | 1695.0762 | 1998.1286 | 5687.3477 |
| 703 | 1697.9232 | 2001.4096 | 5697.9236 |
| 704 | 1700.7708 | 2004.6911 | 5708.5037 |
| 705 | 1703.6189 | 2007.9733 | 5719.0878 |
| 706 | 1706.4678 | 2011.2561 | 5729.6758 |
| 707 | 1709.3172 | 2014.5395 | 5740.2680 |
| 708 | 1712.1672 | 2017.8235 | 5750.8642 |
| 709 | 1715.0179 | 2021.1082 | 5761.4644 |
| 710 | 1717.8691 | 2024.3934 | 5772.0686 |
| 711 | 1720.7210 | 2027.6793 | 5782.6769 |
| 712 | 1723.5735 | 2030.9657 | 5793.2892 |
| 713 | 1726.4265 | 2034.2528 | 5803.9055 |
| 714 | 1729.2802 | 2037.5405 | 5814.5257 |
| 715 | 1732.1346 | 2040.8288 | 5825.1500 |
| 716 | 1734.9895 | 2044.1177 | 5835.7783 |
| 717 | 1737.8450 | 2047.4072 | 5846.4105 |
| 718 | 1740.7011 | 2050.6973 | 5857.0468 |
| 719 | 1743.5578 | 2053.9881 | 5867.6870 |
| 720 | 1746.4152 | 2057.2794 | 5878.3312 |
| 721 | 1749.2731 | 2060.5713 | 5888.9794 |
| 722 | 1752.1316 | 2063.8638 | 5899.6316 |
| 723 | 1754.9908 | 2067.1570 | 5910.2877 |
| 724 | 1757.8505 | 2070.4507 | 5920.9478 |
| 725 | 1760.7109 | 2073.7450 | 5931.6118 |
| 726 | 1763.5718 | 2077.0400 | 5942.2797 |
| 727 | 1766.4333 | 2080.3355 | 5952.9517 |
| 728 | 1769.2955 | 2083.6316 | 5963.6275 |
| 729 | 1772.1582 | 2086.9283 | 5974.3073 |
| 730 | 1775.0215 | 2090.2257 | 5984.9910 |
| 731 | 1777.8854 | 2093.5236 | 5995.6786 |
| 732 | 1780.7499 | 2096.8221 | 6006.3701 |
| 733 | 1783.6150 | 2100.1212 | 6017.0656 |
| 734 | 1786.4807 | 2103.4209 | 6027.7650 |
| 735 | 1789.3470 | 2106.7212 | 6038.4683 |
| 736 | 1792.2139 | 2110.0221 | 6049.1754 |
| 737 | 1795.0814 | 2113.3235 | 6059.8865 |
| 738 | 1797.9494 | 2116.6256 | 6070.6015 |
| 739 | 1800.8181 | 2119.9282 | 6081.3203 |
| 740 | 1803.6873 | 2123.2314 | 6092.0430 |
| 741 | 1806.5571 | 2126.5353 | 6102.7697 |
| 742 | 1809.4275 | 2129.8397 | 6113.5002 |
| 743 | 1812.2985 | 2133.1447 | 6124.2345 |
| 744 | 1815.1701 | 2136.4503 | 6134.9727 |
| 745 | 1818.0422 | 2139.7564 | 6145.7148 |
| 746 | 1820.9150 | 2143.0631 | 6156.4608 |
| 747 | 1823.7883 | 2146.3705 | 6167.2105 |
| 748 | 1826.6622 | 2149.6784 | 6177.9642 |
| 749 | 1829.5367 | 2152.9869 | 6188.7216 |
| 750 | 1832.4117 | 2156.2959 | 6199.4829 |
| 751 | 1835.2874 | 2159.6056 | 6210.2480 |
| 752 | 1838.1636 | 2162.9158 | 6221.0170 |
| 753 | 1841.0404 | 2166.2266 | 6231.7898 |
| 754 | 1843.9178 | 2169.5380 | 6242.5664 |
| 755 | 1846.7957 | 2172.8499 | 6253.3469 |
| 756 | 1849.6742 | 2176.1625 | 6264.1311 |
| 757 | 1852.5533 | 2179.4756 | 6274.9191 |
| 758 | 1855.4330 | 2182.7892 | 6285.7110 |
| 759 | 1858.3132 | 2186.1035 | 6296.5066 |
| 760 | 1861.1941 | 2189.4183 | 6307.3060 |
| 761 | 1864.0754 | 2192.7337 | 6318.1093 |
| 762 | 1866.9574 | 2196.0497 | 6328.9163 |
| 763 | 1869.8399 | 2199.3662 | 6339.7271 |
| 764 | 1872.7230 | 2202.6833 | 6350.5416 |
| 765 | 1875.6067 | 2206.0010 | 6361.3600 |
| 766 | 1878.4909 | 2209.3192 | 6372.1821 |
| 767 | 1881.3757 | 2212.6380 | 6383.0079 |
| 768 | 1884.2611 | 2215.9574 | 6393.8376 |
| 769 | 1887.1470 | 2219.2773 | 6404.6710 |
| 770 | 1890.0335 | 2222.5978 | 6415.5081 |
| 771 | 1892.9205 | 2225.9189 | 6426.3489 |
| 772 | 1895.8082 | 2229.2405 | 6437.1935 |
| 773 | 1898.6963 | 2232.5627 | 6448.0419 |
| 774 | 1901.5851 | 2235.8855 | 6458.8940 |
| 775 | 1904.4744 | 2239.2088 | 6469.7498 |
| 776 | 1907.3642 | 2242.5327 | 6480.6094 |
| 777 | 1910.2547 | 2245.8571 | 6491.4727 |
| 778 | 1913.1456 | 2249.1821 | 6502.3396 |
| 779 | 1916.0372 | 2252.5077 | 6513.2103 |
| 780 | 1918.9293 | 2255.8338 | 6524.0847 |
| 781 | 1921.8219 | 2259.1604 | 6534.9628 |
| 782 | 1924.7151 | 2262.4877 | 6545.8446 |
| 783 | 1927.6089 | 2265.8154 | 6556.7301 |
| 784 | 1930.5032 | 2269.1438 | 6567.6193 |
| 785 | 1933.3981 | 2272.4727 | 6578.5122 |
| 786 | 1936.2935 | 2275.8021 | 6589.4088 |
| 787 | 1939.1895 | 2279.1321 | 6600.3090 |
| 788 | 1942.0860 | 2282.4626 | 6611.2129 |
| 789 | 1944.9831 | 2285.7937 | 6622.1205 |
| 790 | 1947.8807 | 2289.1254 | 6633.0317 |
| 791 | 1950.7789 | 2292.4576 | 6643.9467 |
| 792 | 1953.6776 | 2295.7903 | 6654.8652 |
| 793 | 1956.5769 | 2299.1236 | 6665.7875 |
| 794 | 1959.4767 | 2302.4575 | 6676.7134 |
| 795 | 1962.3771 | 2305.7918 | 6687.6429 |
| 796 | 1965.2780 | 2309.1268 | 6698.5761 |
| 797 | 1968.1794 | 2312.4623 | 6709.5129 |
| 798 | 1971.0814 | 2315.7983 | 6720.4534 |
| 799 | 1973.9840 | 2319.1349 | 6731.3975 |
| 800 | 1976.8871 | 2322.4720 | 6742.3452 |
| 801 | 1979.7907 | 2325.8096 | 6753.2965 |
| 802 | 1982.6949 | 2329.1478 | 6764.2515 |
| 803 | 1985.5996 | 2332.4866 | 6775.2100 |
| 804 | 1988.5049 | 2335.8258 | 6786.1722 |
| 805 | 1991.4106 | 2339.1657 | 6797.1380 |
| 806 | 1994.3170 | 2342.5060 | 6808.1074 |
| 807 | 1997.2239 | 2345.8469 | 6819.0804 |
| 808 | 2000.1313 | 2349.1884 | 6830.0569 |
| 809 | 2003.0392 | 2352.5303 | 6841.0371 |
| 810 | 2005.9477 | 2355.8729 | 6852.0209 |
| 811 | 2008.8567 | 2359.2159 | 6863.0082 |
| 812 | 2011.7663 | 2362.5595 | 6873.9992 |
| 813 | 2014.6764 | 2365.9036 | 6884.9937 |
| 814 | 2017.5870 | 2369.2483 | 6895.9918 |
| 815 | 2020.4982 | 2372.5934 | 6906.9935 |
| 816 | 2023.4099 | 2375.9391 | 6917.9987 |
| 817 | 2026.3221 | 2379.2854 | 6929.0074 |
| 818 | 2029.2348 | 2382.6322 | 6940.0198 |
| 819 | 2032.1481 | 2385.9795 | 6951.0357 |
| 820 | 2035.0619 | 2389.3273 | 6962.0551 |
| 821 | 2037.9763 | 2392.6757 | 6973.0781 |
| 822 | 2040.8911 | 2396.0246 | 6984.1047 |
| 823 | 2043.8065 | 2399.3741 | 6995.1347 |
| 824 | 2046.7225 | 2402.7240 | 7006.1683 |

## TABLE 23. (Continued)

| n | log n! | n log n | n log² n |
|---|---|---|---|
| 825 | 2049.6389 | 2406.0745 | 7017.2054 |
| 826 | 2052.5559 | 2409.4255 | 7028.2462 |
| 827 | 2055.4734 | 2412.7770 | 7039.2903 |
| 828 | 2058.3914 | 2416.1291 | 7050.3380 |
| 829 | 2061.3100 | 2419.4817 | 7061.3893 |
| 830 | 2064.2291 | 2422.8348 | 7072.4440 |
| 831 | 2067.1487 | 2426.1884 | 7083.5023 |
| 832 | 2070.0688 | 2429.5426 | 7094.5640 |
| 833 | 2072.9894 | 2432.8973 | 7105.6293 |
| 834 | 2075.9106 | 2436.2525 | 7116.6980 |
| 835 | 2078.8323 | 2439.6082 | 7127.7703 |
| 836 | 2081.7545 | 2442.9644 | 7138.8460 |
| 837 | 2084.6772 | 2446.3212 | 7149.9252 |
| 838 | 2087.6005 | 2449.6785 | 7161.0079 |
| 839 | 2090.5242 | 2453.0363 | 7172.0942 |
| 840 | 2093.4485 | 2456.3946 | 7183.1838 |
| 841 | 2096.3733 | 2459.7534 | 7194.2770 |
| 842 | 2099.2986 | 2463.1128 | 7205.3736 |
| 843 | 2102.2244 | 2466.4726 | 7216.4736 |
| 844 | 2105.1508 | 2469.8330 | 7227.5772 |
| 845 | 2108.0776 | 2473.1939 | 7238.6842 |
| 846 | 2111.0050 | 2476.5553 | 7249.7946 |
| 847 | 2113.9329 | 2479.9172 | 7260.9086 |
| 848 | 2116.8613 | 2483.2797 | 7272.0259 |
| 849 | 2119.7902 | 2486.6426 | 7283.1467 |
| 850 | 2122.7196 | 2490.0061 | 7294.2709 |
| 851 | 2125.6495 | 2493.3700 | 7305.3986 |
| 852 | 2128.5800 | 2496.7345 | 7316.5297 |
| 853 | 2131.5109 | 2500.0995 | 7327.6642 |
| 854 | 2134.4424 | 2503.4650 | 7338.8022 |
| 855 | 2137.3744 | 2506.8310 | 7349.9436 |
| 856 | 2140.3068 | 2510.1975 | 7361.0884 |
| 857 | 2143.2398 | 2513.5645 | 7372.2366 |
| 858 | 2146.1733 | 2516.9321 | 7383.3882 |
| 859 | 2149.1073 | 2520.3001 | 7394.5433 |
| 860 | 2152.0418 | 2523.6686 | 7405.7018 |
| 861 | 2154.9768 | 2527.0377 | 7416.8636 |
| 862 | 2157.9123 | 2530.4073 | 7428.0289 |
| 863 | 2160.8483 | 2533.7773 | 7439.1975 |
| 864 | 2163.7848 | 2537.1479 | 7450.3696 |
| 865 | 2166.7218 | 2540.5189 | 7461.5450 |
| 866 | 2169.6594 | 2543.8905 | 7472.7238 |
| 867 | 2172.5974 | 2547.2625 | 7483.9060 |
| 868 | 2175.5359 | 2550.6351 | 7495.0916 |
| 869 | 2178.4749 | 2554.0082 | 7506.2805 |
| 870 | 2181.4144 | 2557.3817 | 7517.4728 |
| 871 | 2184.3544 | 2560.7558 | 7528.6686 |
| 872 | 2187.2950 | 2564.1304 | 7539.8676 |
| 873 | 2190.2360 | 2567.5054 | 7551.0700 |
| 874 | 2193.1775 | 2570.8810 | 7562.2758 |
| 875 | 2196.1195 | 2574.2570 | 7573.4849 |
| 876 | 2199.0620 | 2577.6336 | 7584.6974 |
| 877 | 2202.0050 | 2581.0106 | 7595.9133 |
| 878 | 2204.9485 | 2584.3882 | 7607.1324 |
| 879 | 2207.8925 | 2587.7662 | 7618.3549 |
| 880 | 2210.8370 | 2591.1447 | 7629.5808 |
| 881 | 2213.7820 | 2594.5238 | 7640.8100 |
| 882 | 2216.7274 | 2597.9033 | 7652.0425 |
| 883 | 2219.6734 | 2601.2833 | 7663.2784 |
| 884 | 2222.6198 | 2604.6638 | 7674.5175 |
| 885 | 2225.5668 | 2608.0448 | 7685.7600 |
| 886 | 2228.5142 | 2611.4263 | 7697.0059 |
| 887 | 2231.4621 | 2614.8082 | 7708.2549 |
| 888 | 2234.4105 | 2618.1907 | 7719.5074 |
| 889 | 2237.3594 | 2621.5736 | 7730.7632 |
| 890 | 2240.3088 | 2624.9571 | 7742.0222 |
| 891 | 2243.2587 | 2628.3410 | 7753.2846 |
| 892 | 2246.2091 | 2631.7254 | 7764.5502 |
| 893 | 2249.1599 | 2635.1104 | 7775.8192 |
| 894 | 2252.1113 | 2638.4957 | 7787.0914 |
| 895 | 2255.0631 | 2641.8816 | 7798.3670 |
| 896 | 2258.0154 | 2645.2680 | 7809.6458 |
| 897 | 2260.9682 | 2648.6548 | 7820.9279 |
| 898 | 2263.9214 | 2652.0421 | 7832.2133 |
| 899 | 2266.8752 | 2655.4300 | 7843.5020 |
| 900 | 2269.8295 | 2658.8182 | 7854.7939 |
| 901 | 2272.7842 | 2662.2070 | 7866.0891 |
| 902 | 2275.7394 | 2665.5963 | 7877.3876 |
| 903 | 2278.6951 | 2668.9860 | 7888.6893 |
| 904 | 2281.6512 | 2672.3763 | 7899.9943 |
| 905 | 2284.6079 | 2675.7669 | 7911.3026 |
| 906 | 2287.5650 | 2679.1581 | 7922.6141 |
| 907 | 2290.5226 | 2682.5498 | 7933.9288 |
| 908 | 2293.4807 | 2685.9419 | 7945.2468 |
| 909 | 2296.4393 | 2689.3346 | 7956.5681 |
| 910 | 2299.3983 | 2692.7277 | 7967.8926 |
| 911 | 2302.3578 | 2696.1212 | 7979.2203 |
| 912 | 2305.3178 | 2699.5153 | 7990.5513 |
| 913 | 2308.2783 | 2702.9098 | 8001.8855 |
| 914 | 2311.2393 | 2706.3048 | 8013.2230 |
| 915 | 2314.2007 | 2709.7003 | 8024.5636 |
| 916 | 2317.1626 | 2713.0963 | 8035.9075 |
| 917 | 2320.1249 | 2716.4927 | 8047.2546 |
| 918 | 2323.0878 | 2719.8896 | 8058.6049 |
| 919 | 2326.0511 | 2723.2869 | 8069.9584 |
| 920 | 2329.0149 | 2726.6848 | 8081.3152 |
| 921 | 2331.9792 | 2730.0831 | 8092.6752 |
| 922 | 2334.9439 | 2733.4819 | 8104.0383 |
| 923 | 2337.9091 | 2736.8812 | 8115.4047 |
| 924 | 2340.8748 | 2740.2809 | 8126.7742 |
| 925 | 2343.8409 | 2743.6811 | 8138.1470 |
| 926 | 2346.8075 | 2747.0818 | 8149.5229 |
| 927 | 2349.7746 | 2750.4829 | 8160.9021 |
| 928 | 2352.7421 | 2753.8845 | 8172.2844 |
| 929 | 2355.7101 | 2757.2866 | 8183.6699 |
| 930 | 2358.6786 | 2760.6891 | 8195.0586 |
| 931 | 2361.6476 | 2764.0921 | 8206.4504 |
| 932 | 2364.6170 | 2767.4956 | 8217.8455 |
| 933 | 2367.5869 | 2770.8996 | 8229.2438 |
| 934 | 2370.5572 | 2774.3040 | 8240.6451 |
| 935 | 2373.5280 | 2777.7088 | 8252.0497 |
| 936 | 2376.4993 | 2781.1142 | 8263.4574 |
| 937 | 2379.4710 | 2784.5200 | 8274.8683 |
| 938 | 2382.4433 | 2787.9262 | 8286.2823 |
| 939 | 2385.4159 | 2791.3330 | 8297.6995 |
| 940 | 2388.3890 | 2794.7402 | 8309.1199 |
| 941 | 2391.3626 | 2798.1478 | 8320.5433 |
| 942 | 2394.3367 | 2801.5559 | 8331.9700 |
| 943 | 2397.3112 | 2804.9645 | 8343.3998 |
| 944 | 2400.2862 | 2808.3735 | 8354.8326 |
| 945 | 2403.2616 | 2811.7831 | 8366.2687 |
| 946 | 2406.2375 | 2815.1930 | 8377.7079 |
| 947 | 2409.2138 | 2818.6034 | 8389.1503 |
| 948 | 2412.1906 | 2822.0143 | 8400.5957 |
| 949 | 2415.1679 | 2825.4256 | 8412.0442 |
| 950 | 2418.1456 | 2828.8374 | 8423.4960 |
| 951 | 2421.1238 | 2832.2497 | 8434.9507 |
| 952 | 2424.1024 | 2835.6624 | 8446.4087 |
| 953 | 2427.0815 | 2839.0755 | 8457.8698 |
| 954 | 2430.0611 | 2842.4891 | 8469.3339 |
| 955 | 2433.0411 | 2845.9032 | 8480.8011 |
| 956 | 2436.0216 | 2849.3177 | 8492.2715 |
| 957 | 2439.0025 | 2852.7327 | 8503.7450 |
| 958 | 2441.9838 | 2856.1481 | 8515.2216 |
| 959 | 2444.9657 | 2859.5640 | 8526.7012 |
| 960 | 2447.9479 | 2862.9804 | 8538.1840 |
| 961 | 2450.9307 | 2866.3972 | 8549.6698 |
| 962 | 2453.9138 | 2869.8144 | 8561.1588 |
| 963 | 2456.8975 | 2873.2321 | 8572.6508 |
| 964 | 2459.8815 | 2876.6502 | 8584.1459 |
| 965 | 2462.8661 | 2880.0688 | 8595.6442 |
| 966 | 2465.8510 | 2883.4879 | 8607.1454 |
| 967 | 2468.8365 | 2886.9074 | 8618.6498 |
| 968 | 2471.8223 | 2890.3273 | 8630.1571 |
| 969 | 2474.8087 | 2893.7477 | 8641.6676 |
| 970 | 2477.7954 | 2897.1686 | 8653.1812 |
| 971 | 2480.7827 | 2900.5898 | 8664.6978 |
| 972 | 2483.7703 | 2904.0116 | 8676.2174 |
| 973 | 2486.7584 | 2907.4338 | 8687.7402 |
| 974 | 2489.7470 | 2910.8564 | 8699.2660 |
| 975 | 2492.7360 | 2914.2795 | 8710.7948 |
| 976 | 2495.7254 | 2917.7030 | 8722.3267 |
| 977 | 2498.7153 | 2921.1270 | 8733.8617 |
| 978 | 2501.7057 | 2924.5514 | 8745.3997 |
| 979 | 2504.6965 | 2927.9762 | 8756.9407 |
| 980 | 2507.6877 | 2931.4016 | 8768.4847 |
| 981 | 2510.6794 | 2934.8273 | 8780.0319 |
| 982 | 2513.6715 | 2938.2535 | 8791.5819 |
| 983 | 2516.6640 | 2941.6801 | 8803.1351 |
| 984 | 2519.6570 | 2945.1071 | 8814.6913 |
| 985 | 2522.6505 | 2948.5347 | 8826.2505 |
| 986 | 2525.6443 | 2951.9626 | 8837.8127 |
| 987 | 2528.6386 | 2955.3910 | 8849.3781 |
| 988 | 2531.6334 | 2958.8199 | 8860.9463 |
| 989 | 2534.6286 | 2962.2491 | 8872.5176 |
| 990 | 2537.6242 | 2965.6788 | 8884.0918 |
| 991 | 2540.6203 | 2969.1090 | 8895.6692 |
| 992 | 2543.6168 | 2972.5396 | 8907.2495 |
| 993 | 2546.6138 | 2975.9706 | 8918.8328 |
| 994 | 2549.6111 | 2979.4020 | 8930.4191 |
| 995 | 2552.6090 | 2982.8340 | 8942.0084 |
| 996 | 2555.6072 | 2986.2663 | 8953.6007 |
| 997 | 2558.6059 | 2989.6991 | 8965.1960 |
| 998 | 2561.6051 | 2993.1323 | 8976.7944 |
| 999 | 2564.6046 | 2996.5659 | 8988.3956 |
| 1000 | 2567.6046 | 3000.0000 | 9000.0000 |
| 1001 | 2570.6051 | 3003.4345 | 9011.6072 |
| 1002 | 2573.6059 | 3006.8694 | 9023.2174 |
| 1003 | 2576.6072 | 3010.3048 | 9034.8307 |
| 1004 | 2579.6090 | 3013.7406 | 9046.4469 |
| 1005 | 2582.6111 | 3017.1769 | 9058.0660 |
| 1006 | 2585.6137 | 3020.6136 | 9069.6881 |
| 1007 | 2588.6168 | 3024.0507 | 9081.3132 |
| 1008 | 2591.6202 | 3027.4882 | 9092.9413 |
| 1009 | 2594.6241 | 3030.9262 | 9104.5724 |
| 1010 | 2597.6284 | 3034.3646 | 9116.2063 |
| 1011 | 2600.6332 | 3037.8034 | 9127.8433 |
| 1012 | 2603.6384 | 3041.2427 | 9139.4832 |
| 1013 | 2606.6440 | 3044.6823 | 9151.1260 |
| 1014 | 2609.6500 | 3048.1225 | 9162.7719 |
| 1015 | 2612.6565 | 3051.5630 | 9174.4205 |
| 1016 | 2615.6634 | 3055.0040 | 9186.0723 |
| 1017 | 2618.6707 | 3058.4454 | 9197.7269 |
| 1018 | 2621.6784 | 3061.8872 | 9209.3845 |
| 1019 | 2624.6866 | 3065.3295 | 9221.0450 |
| 1020 | 2627.6952 | 3068.7722 | 9232.7084 |
| 1021 | 2630.7043 | 3072.2153 | 9244.3749 |
| 1022 | 2633.7137 | 3075.6588 | 9256.0441 |
| 1023 | 2636.7236 | 3079.1028 | 9267.7163 |
| 1024 | 2639.7339 | 3082.5471 | 9279.3915 |
| 1025 | 2642.7446 | 3085.9919 | 9291.0696 |
| 1026 | 2645.7557 | 3089.4372 | 9302.7506 |
| 1027 | 2648.7673 | 3092.8828 | 9314.4346 |
| 1028 | 2651.7793 | 3096.3289 | 9326.1213 |
| 1029 | 2654.7917 | 3099.7754 | 9337.8110 |
| 1030 | 2657.8046 | 3103.2223 | 9349.5038 |
| 1031 | 2660.8178 | 3106.6697 | 9361.1993 |
| 1032 | 2663.8315 | 3110.1174 | 9372.8977 |
| 1033 | 2666.8456 | 3113.5656 | 9384.5991 |
| 1034 | 2669.8601 | 3117.0142 | 9396.3033 |
| 1035 | 2672.8751 | 3120.4633 | 9408.0105 |
| 1036 | 2675.8904 | 3123.9127 | 9419.7206 |
| 1037 | 2678.9062 | 3127.3625 | 9431.4336 |
| 1038 | 2681.9224 | 3130.8128 | 9443.1494 |
| 1039 | 2684.9390 | 3134.2635 | 9454.8682 |
| 1040 | 2687.9560 | 3137.7147 | 9466.5897 |
| 1041 | 2690.9735 | 3141.1662 | 9478.3142 |
| 1042 | 2693.9914 | 3144.6181 | 9490.0416 |
| 1043 | 2697.0096 | 3148.0705 | 9501.7719 |
| 1044 | 2700.0284 | 3151.5233 | 9513.5050 |
| 1045 | 2703.0475 | 3154.9765 | 9525.2410 |
| 1046 | 2706.0670 | 3158.4301 | 9536.9799 |
| 1047 | 2709.0869 | 3161.8842 | 9548.7216 |
| 1048 | 2712.1073 | 3165.3386 | 9560.4662 |
| 1049 | 2715.1281 | 3168.7935 | 9572.2136 |
| 1050 | 2718.1493 | 3172.2487 | 9583.9640 |

TABLE 24. THE DIVERSITY OF SPECIES, $\bar{d}$, CHARACTERISTIC OF MacARTHUR'S MODEL FOR VARIOUS NUMBERS OF HYPOTHETICAL SPECIES, s'*

| s' | $\bar{d}$ | s' | $\bar{d}$ | s' | $\bar{d}$ | s' | $\bar{d}$ |
|---|---|---|---|---|---|---|---|
| 1 | 0.0000 | 51 | 5.0941 | 102 | 6.0792 | 205 | 7.0783 |
| 2 | 0.8113 | 52 | 5.1215 | 104 | 6.1069 | 210 | 7.1128 |
| 3 | 1.2997 | 53 | 5.1485 | 106 | 6.1341 | 215 | 7.1466 |
| 4 | 1.6556 | 54 | 5.1749 | 108 | 6.1608 | 220 | 7.1796 |
| 5 | 1.9374 | 55 | 5.2009 | 110 | 6.1870 | 225 | 7.2118 |
| 6 | 2.1712 | 56 | 5.2264 | 112 | 6.2128 | 230 | 7.2434 |
| 7 | 2.3714 | 57 | 5.2515 | 114 | 6.2380 | 235 | 7.2743 |
| 8 | 2.5465 | 58 | 5.2761 | 116 | 6.2629 | 240 | 7.3045 |
| 9 | 2.7022 | 59 | 5.3004 | 118 | 6.2873 | 245 | 7.3341 |
| 10 | 2.8425 | 60 | 5.3242 | 120 | 6.3113 | 250 | 7.3631 |
| 11 | 2.9701 | 61 | 5.3476 | 122 | 6.3350 | 255 | 7.3915 |
| 12 | 3.0872 | 62 | 5.3707 | 124 | 6.3582 | 260 | 7.4194 |
| 13 | 3.1954 | 63 | 5.3934 | 126 | 6.3811 | 265 | 7.4468 |
| 14 | 3.2960 | 64 | 5.4157 | 128 | 6.4036 | 270 | 7.4736 |
| 15 | 3.3899 | 65 | 5.4378 | 130 | 6.4258 | 275 | 7.5000 |
| 16 | 3.4780 | 66 | 5.4594 | 132 | 6.4476 | 280 | 7.5259 |
| 17 | 3.5611 | 67 | 5.4808 | 134 | 6.4691 | 285 | 7.5513 |
| 18 | 3.6395 | 68 | 5.5018 | 136 | 6.4903 | 290 | 7.5763 |
| 19 | 3.7139 | 69 | 5.5226 | 138 | 6.5112 | 295 | 7.6008 |
| 20 | 3.7846 | 70 | 5.5430 | 140 | 6.5318 | 300 | 7.6250 |
| 21 | 3.8520 | 71 | 5.5632 | 142 | 6.5521 | 310 | 7.6721 |
| 22 | 3.9163 | 72 | 5.5830 | 144 | 6.5721 | 320 | 7.7177 |
| 23 | 3.9779 | 73 | 5.6027 | 146 | 6.5919 | 330 | 7.7620 |
| 24 | 4.0369 | 74 | 5.6220 | 148 | 6.6114 | 340 | 7.8049 |
| 25 | 4.0937 | 75 | 5.6411 | 150 | 6.6306 | 350 | 7.8465 |
| 26 | 4.1482 | 76 | 5.6599 | 152 | 6.6495 | 360 | 7.8870 |
| 27 | 4.2008 | 77 | 5.6785 | 154 | 6.6683 | 370 | 7.9264 |
| 28 | 4.2515 | 78 | 5.6969 | 156 | 6.6867 | 380 | 7.9648 |
| 29 | 4.3004 | 79 | 5.7150 | 158 | 6.7050 | 390 | 8.0022 |
| 30 | 4.3478 | 80 | 5.7329 | 160 | 6.7230 | 400 | 8.0386 |
| 31 | 4.3936 | 81 | 5.7506 | 162 | 6.7408 | 410 | 8.0741 |
| 32 | 4.4381 | 82 | 5.7681 | 164 | 6.7584 | 420 | 8.1087 |
| 33 | 4.4812 | 83 | 5.7853 | 166 | 6.7757 | 430 | 8.1426 |
| 34 | 4.5230 | 84 | 5.8024 | 168 | 6.7929 | 440 | 8.1757 |
| 35 | 4.5637 | 85 | 5.8192 | 170 | 6.8099 | 450 | 8.2080 |
| 36 | 4.6032 | 86 | 5.8359 | 172 | 6.8266 | 460 | 8.2396 |
| 37 | 4.6417 | 87 | 5.8524 | 174 | 6.8432 | 470 | 8.2706 |
| 38 | 4.6792 | 88 | 5.8687 | 176 | 6.8596 | 480 | 8.3009 |
| 39 | 4.7157 | 89 | 5.8848 | 178 | 6.8758 | 490 | 8.3305 |
| 40 | 4.7513 | 90 | 5.9007 | 180 | 6.8918 | 500 | 8.3596 |
| 41 | 4.7861 | 91 | 5.9164 | 182 | 6.9076 | 550 | 8.4968 |
| 42 | 4.8200 | 92 | 5.9320 | 184 | 6.9233 | 600 | 8.6220 |
| 43 | 4.8532 | 93 | 5.9474 | 186 | 6.9388 | 650 | 8.7373 |
| 44 | 4.8856 | 94 | 5.9627 | 188 | 6.9541 | 700 | 8.8440 |
| 45 | 4.9173 | 95 | 5.9778 | 190 | 6.9693 | 750 | 8.9434 |
| 46 | 4.9483 | 96 | 5.9927 | 192 | 6.9843 | 800 | 9.0363 |
| 47 | 4.9787 | 97 | 6.0075 | 194 | 6.9992 | 850 | 9.1236 |
| 48 | 5.0084 | 98 | 6.0221 | 196 | 7.0139 | 900 | 9.2060 |
| 49 | 5.0375 | 99 | 6.0366 | 198 | 7.0284 | 950 | 9.2839 |
| 50 | 5.0661 | 100 | 6.0510 | 200 | 7.0429 | 1000 | 9.3578 |

*The data in this table are reproduced, with permission, from Lloyd and Ghelardi

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## APPENDIX A

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| **PORIFERA** | | | | | | |
| Anheteromeyenia ryderi | X | | Yes | | 3 | |
| Ephydatia fluviatilis | X | | No | 4 | | |
| Ephydatia muelleri | X | | No | | 2 | |
| Eunapius fragilis | X | | Yes | 4 | | |
| Heteromeyenia tubisperma | | X | No | | 3 | |
| Spongilla lacustris | X | | No | | 3 | |
| Trochospongilla horrida | X | | No | | 3 | |
| Trochospongilla pennsylvanica | X | | No | | 2 | |
| **BRYOZOA** | | | | | | |
| Fredericella sultana | | | | | 2 | |
| Hyalinella punctata | X | | No | | 2 | |
| Lophopodella carteri | | | | | | 1 |
| Pectinatella magnifica | | X | | | | 1 |
| Plumatella casmiana | | X | | | 3 | |
| Plumatella emarginata | | X | | 4 | | |
| Plumatella repens | X | | | 4 | | |
| Urnatella gracilis | | | | | 3 | |
| **COELENTERATA** | | | | | | |
| Cordylophora lacustris | | | | | 2 | |
| Craspedacusta sowerbyi | | | | | 2 | |
| **TURBELLARIA** | | | | | | |
| Cura foremanni | | | | | 2 | |
| Dugesia dorotocephala | | | | 4 | | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| tigrina | | 4 | | | | |
| Phagocata | | | | | | |
| gracilis | | | | | 2 | |
| **NEMERTEA** | | | | | | |
| Prostoma | | | | | | |
| graecense | | | | | 3 | |
| **ANNELIDA - POLYCHAETA** | | | | | | |
| **SABELLIDAE** | | | | | | |
| Manayunkia | | | | | | |
| speciosa | | | | | 3 | |
| **ANNELIDA - OLIGOCHAETA** | | | | | | |
| **NAIDIDAE** | | | | | | |
| Amphichaeta | | | | | | |
| americana | | | | | 2 | |
| Chaetogaster | | | | | | |
| diaphanus | | | | | 2 | |
| diastrophus | | | | | 2 | |
| Dero | | | | | | |
| digitata | | | | | 2 | |
| nivea | | | | | 3 | |
| obtusa | | | | | 3 | |
| pectinata | | | | | 2 | |
| Nais | | | | | | |
| barbata | | 4 | | | | |
| behningi | | | | | 3 | |
| bretscheri | | | | | 3 | |
| communis | | 4 | | | | |
| elinguis | | 5 | | | | |
| pardalis | | 4 | | | | |
| simplex | | | | | 3 | |
| variabilis | | 5 | | | | |
| Ophidonais | | | | | | |
| serpentina | | 4 | | | | |
| Pristina | | | | | | |
| aequiseta | | | | | 3 | |
| Slavina | | | | | | |
| appendiculata | | | | | 2 | |
| Specaria | | | | | | |
| josinae | | | | | 2 | |
| Stylaria | | | | | | |
| fossularis | | | | | 3 | |
| lacustris | | | | | 3 | |
| Vejdovskyella | | | | | | |
| comata | | | | | | 1 |
| intermedia | | 4 | | | | |

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| **TUBIFICIDAE** | | | | | | |
| Aulodrilus | | | | | | |
| americanus | | | | | 3 | |
| limnobius | | | | | 3 | |
| pigueti | | | | | 3 | |
| pluriseta | | | | | 3 | |
| Bothrioneurum | | | | | | |
| vejdovskyanum | | | | | 2 | |
| Branchiura | | | | | | |
| sowerbyi | | | | 4 | | |
| Ilyodrilus | | | | | | |
| templetoni | | | | | 3 | |
| Isochaetides | | | | | | |
| curvisetosus | | | | | 2 | |
| Limnodrilus | | | | | | |
| cervix | | | | 4 | | |
| claparedianus | | | | 4 | | |
| hoffmeisteri | | | | 5 | | |
| maumeensis | | | | 5 | | |
| udekemianus | | | | 5 | | |
| Potamothrix | | | | | | |
| moldaviensis | | | | | 3 | |
| vejdovskyi | | | | | 3 | |
| Quistadrilus | | | | | | |
| multisetosus | | | | 4 | | |
| Spirosperma | | | | | | |
| carolinensis | | | | | 3 | |
| ferox | | | | | 3 | |
| multisetosus | | | | 4 | | |
| nikolskyi | | | | | 2 | |
| Tubifex | | | | | | |
| tubifex | X | | | 5 | | |
| **ANNELIDA - HIRUDINEA** | | | | | | |
| **ERPOBDELLIDAE** | | | | | | |
| Erpobdella | | | | | | |
| parva | | | | 4 | | |
| punctata | | | | 4 | | |
| Mooreobdella | | | | | | |
| microstoma | | | | 4 | | |
| **HAEMOPIDAE** | | | | | | |
| Haemopis | | | | | | |
| grandis | | | | | 3 | |
| marmorata | | | | | 3 | |
| **GLOSSIPHONIIDAE** | | | | | | |
| Alboglossiphonia | | | | | | |
| heteroclita | | | | | 3 | |
| Gloiobdella | | | | | | |
| elongata | | | | 4 | | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Helobdella | | | | | | |
| stagnalis | | | | 4 | | |
| triserialis | | | | | 3 | |
| Glossiphonia | | | | | | |
| complanata | X | | | 4 | | |
| Placobdella | | | | | | |
| multilineata | | | | | 2 | |
| ornata | | | | | 3 | |
| papillifera | | | | | 3 | |
| parasitica | | | | | 2 | |
| **PISCICOLIDAE** | | | | | | |
| Myzobdella | | | | | | |
| lugubris | | | | | 3 | |
| Piscicola | | | | | | |
| punctata | | | | | 3 | |
| **HYDRACARIA** | | | | | | |
| Albia | | | | | | |
| stationis | | | | | | 1 |
| Arrenurus | | | | | | |
| kenki | | | | | 2 | |
| planus | | | | | 3 | |
| serratus | | | | | 2 | |
| Bandakia | | | | | | |
| anisitsipalpis | | | | | | 0 |
| elongata | | | | | | 0 |
| Euthyas | | | | | | |
| truncata | | | | | 2 | |
| Frontipoda | | | | | | |
| americana | | | | | 2 | |
| Forelia | | | | | | |
| cooki | | | | | 2 | |
| Hydrachna | | | | | | |
| conjecta | | | | | | 1 |
| crenulata | | | | | | 1 |
| magnisculata | | | | | | 1 |
| miliaria | | | | | | 1 |
| rotunda | | | | | | 1 |
| stipata | | | | | | 1 |
| Hydrodromia | | | | | | |
| despiciens | | | | | 2 | |
| Hydryphantes | | | | | | |
| tenuabilis | | | | | 3 | |
| Hygrobates | | | | | | |
| fluviatilis | | | | 4 | | |
| longipalpis | | | | 4 | | |
| neooctoporus | | | | | 2 | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Lebertia | | | | | | |
| quinquemaculosa | | | | | | 1 |
| Limnesia | | | | | | |
| maculata | | | | | 2 | |
| undulata | | | | | 2 | |
| Neumania | | | | | | |
| rotundra | | | | | | 1 |
| Oxus | | | | | | |
| connatus | | | | | | 1 |
| Piona | | | | | | |
| carnea | | | | | | 1 |
| constricta | | | | | 2 | |
| pugilis | | | | | | 1 |
| rotunda | | | | | | 1 |
| Pirata | | | | | | |
| insularis | | | | | 2 | |
| Sperchon | | | | | | |
| crassipalpus | | | | | | 0 |
| glandulosus | | | | 4 | | |
| Sperchonopsis | | | | | | |
| verrucosa | | | | | | 1 |
| Testudacarus | | | | | | |
| minimus | | | | | | 1 |
| Thyas | | | | | | |
| barbigera | | | | | 2 | |
| bruzelii | | | | | 2 | |
| stolli | | | | | 2 | |
| Tiphys | | | | | | |
| americanus | | | | | 3 | |
| simulans | | | | | 3 | |
| Unionicola | | | | | | |
| formosa | | | | | 3 | |
| **ARTHROPODA - CRUSTACEA** | | | | | | |
| **ISOPODA** | | | | | | |
| Asellus | | | | | | |
| attenuatus | | | | | 3 | |
| brevicaudatus | | | | 4 | | |
| communis | | | | | 3 | |
| intermedius | | | | | 3 | |
| militaris | | | | | 3 | |
| racovitzai | | | | | 3 | |
| Lirceus | | | | | | |
| fontinalis | | X | | | 3 | |
| lineatus | | | | | | 1 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| **AMPHIPODA** | | | | | | |
| Crangonyx | | | | | | |
| gracilis | | | | | 3 | |
| obliquus | | | | | | 1 |
| pseudogracilis | X | | | | 3 | |
| serratus | | | | | 2 | |
| Gammarus | | | | | | |
| fasciatus | | | | | 2 | |
| lacustris | | | | | 2 | |
| minus | | | | | 2 | |
| pseudolimnaeus | | X | | | 2 | |
| tigrinus | | | | | 2 | |
| Hyallela | | | | | | |
| azteca | | | | | 2 | |
| Synurella | | | | | | |
| chamberlaini | | | | | 2 | |
| **DECAPODA** | | | | | | |
| Cambarus | | | | | | |
| acuminatus | | | | | 3 | |
| asperimanus | | | | | | 1 |
| bartonii | | | Yes | | 3 | |
| exraneus | | | | | | 1 |
| diogenes | | | Yes | 4 | | |
| floridanus | | | | | 2 | |
| longirostris | | | | | 3 | |
| longulus | | | No | | | 1 |
| Orconectes | | | | | | |
| immunis | | | | | 2 | |
| obscurus | | | Yes | | 2 | |
| propinquus | | | No | | 2 | |
| rusticus | | | | | 3 | |
| virilis | | | | | | 1 |
| Palaemonetes | | | | | | |
| exilipes | | | | 4 | | |
| kadiakensis | | | | | 2 | |
| paludosus | | | | | 2 | |
| Procambarus | | | | | | |
| acutus | | | | 4 | | |
| clarkii | | | | | 3 | |
| **MYSIDACEA** | | | | | | |
| Mysis | | | | | | |
| relicta | | | | | | 1 |

## POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| **INSECTA - DIPTERA** | | | | | | |
| **CHIRONOMIDAE** | | | | | | |
| Ablabesmyia | | | | | | |
| aequifasciata | | | | 4 | | |
| americana | | | | | 2 | |
| annulata | | | Yes | | | 1 |
| aspera | X | | Yes | | 2 | |
| auriensis | | | | | | 1 |
| basalis | | | Yes | | 2 | |
| cinctipes | | | Yes | | 2 | |
| hauberi | | | Yes | | | 1 |
| illinoensis | | | | | | 1 |
| janta | | | | | 3 | |
| mallochi | X | | No | | 2 | |
| monilis | X | | Yes | | 2 | |
| parajanta | | | Yes | | 3 | |
| peleensis | | | | | 2 | |
| philosphagnos | | | Yes | | 2 | |
| rhamphe | | | | | 2 | |
| tarella | | | | | 3 | |
| Arctopelopia | | | | | | |
| flavifrons | | | Yes | | | 0 |
| Boreochlus | | | | | | |
| persimilis | | | Yes | | | 0 |
| Brillia | | | | | | |
| flavifrons | | | | | 2 | |
| par | X | | | | | 1 |
| parva | | | | | | 0 |
| Calopsectra | | | | | | |
| confusa | | | Yes | | | 1 |
| dendyi | | | Yes | | 2 | |
| gregarius | | | | 4 | | |
| neoflavella | | | Yes | | 2 | |
| Cardiocladius | | | | | | |
| obscura | | | Yes | | 2 | |
| platypus | | | Yes | | | 0 |
| Chaetocladis | | | | | | |
| atroviridis | | | | | | 0 |
| ochreatus | | | | | | 0 |

## POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Chironomus | | | | | | |
| anthracinus | | | | | 3 | |
| atrella | | | Yes | 4 | | |
| attenuatus | X | | Yes | 4 | | |
| carus | | | | | 3 | |
| crassicaudatus | | | Yes | | 3 | |
| flavus | | | | | 3 | |
| fulvipilus | | | | 4 | | |
| paganus | | | | | 3 | |
| plumosus | | | Yes | 5 | | |
| riparius | | X | No | 5 | | |
| staegeri | | | Yes | | 3 | |
| stigmaterus | | | | | 3 | |
| tentans | | | | | 3 | |
| tuxis | | | Yes | | | 1 |
| Cladotanytarsus | | | | | | |
| viridiventris | | | No | | 2 | |
| Clinotanypus | | | | | | |
| caliginosus | | | | | | 1 |
| pinguis | | X | Yes | | 3 | |
| Coelotanypus | | | | | | |
| concinnus | | | No | 4 | | |
| scapularis | | | | | 3 | |
| tricolor | | | | | 2 | |
| Corynoneura | | | | | | |
| scutellata | | | | | | 1 |
| taris | | | Yes | | | 1 |
| Cricotopus | | | | | | |
| absurdus | | | | | | 1 |
| bicinctus | X | | No | | 2 | |
| exilis | | | | | | 1 |
| fugax | | | Yes | | | 0 |
| politus | | | | | | 1 |
| remus | | | No | | 2 | |
| sylvestris | | | No | | 2 | |
| tremulus | | | | | 3 | |
| tricinctus | | | | | 2 | |
| trifasciatus | | | | | 3 | |
| Cryptochironomus | | | | | | |
| blarina | | | No | | 2 | |
| fulvus | | | | | 3 | |
| digitatus | | | | | 3 | |
| nais | | | | | 3 | |
| ponderosus | | | | | 3 | |
| psittacinus | | | | | | 1 |
| sorex | | | | | | 1 |

**POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES** (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Cryptotendipes | | | | | | |
| casuarius | | | Yes | | | 1 |
| darbyi | | | | | 2 | |
| emorsus | | | No | | 2 | |
| Demeijerea | | | | | | |
| atrimanus | | | Yes | | 2 | |
| brachialis | | | No | | 2 | |
| Demicryptochironomus | | | | | | |
| vulneratus | | | | | | 1 |
| Diamesa | | | | | | |
| nivoriunda | | | Yes | | | 1 |
| spinacies | | | No | | | 0 |
| Dicrotendipes | | | | | | |
| californicus | | | | | 2 | |
| fumidus | | X | No | | | 1 |
| incurvus | | | Yes | | 3 | |
| leucoscelis | | | Yes | | | 1 |
| lobus | | | Yes | | | 1 |
| modestus | | X | No | | 3 | |
| neomodestus | | | | | 2 | |
| nervosus | | X | No | | 2 | |
| Einfeldia | | | | | | |
| austeni | | | No | | 3 | |
| brunneipennis | | | No | | 3 | |
| natchitocheae | | | | | 3 | |
| Endochironomus | | | | | | |
| nigricans | | | | | 3 | |
| Epiococladius | | | | | | |
| flavens | | | No | | | 0 |
| Eukiefferiella | | | | | | |
| coerulescens | | | | | | 0 |
| Glyptotendipes | | | | | | |
| amplus | | | | | 3 | |
| barbipes | | | | 4 | | |
| lobiferus | | | No | | 3 | |
| meridionalis | | | No | 4 | | |
| paripes | | | No | | 3 | |
| senilis | | | | | | 1 |
| Goeldichironomus | | | | | | |
| holoprasinus | | | No | 5 | | |
| Guttipelopia | | | | | | |
| currani | | | Yes | | | 1 |

## POLLUTION TOLLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Harnishia | | | | | | |
| amachaerus | | | Yes | | 2 | |
| boydi | | | Yes | | 3 | |
| collator | | | | | 3 | |
| curtilamellata | | | | | 2 | |
| edwardsi | | | No | | 2 | |
| galeator | | | No | | 2 | |
| tenuicaudata | | | | | 2 | |
| viridulus | | | No | | 2 | |
| Heterotrissocladius | | | | | | |
| marcidus | | | | | | 0 |
| Hydrobaenus | | | | | | |
| pilipes | | | | | 2 | |
| Kiefferulus | | | | | | |
| dux | | | | | 3 | |
| Labrundinia | | | | | | |
| becki | | | Yes | | | 0 |
| floridana | | | | | | 1 |
| johannseni | | | Yes | | 2 | |
| neopilosella | | | Yes | | | 1 |
| pilosella | | | Yes | | 2 | |
| virescens | | | | | 2 | |
| Larsia | | | | | | |
| lurida | | | | | 2 | |
| Lauterborniella | | | | | | |
| agrayloides | | | No | | | 0 |
| varipennis | | | Yes | | 3 | |
| Leptochironomus | | | | | | |
| nigrovittatus | | | | | 2 | |
| Macropelopia | | | | | | |
| decedens | | | Yes | | | 1 |
| Metriocnemus | | | | | | |
| abdomino-flavatus | | | Yes | 4 | | |
| hamatus | | | Yes | | | 0 |
| knabi | | | Yes | 4 | | |
| lundbecki | X | | | | | 1 |
| Micropsectra | | | | | | |
| deflecta | | | | | | 1 |
| dives | | | Yes | | 2 | |
| dubia | | | No | | | 1 |
| nigripila | | | | | 3 | |
| polita | | | No | | | 0 |
| Microtendipes | | | | | | |
| pedellus | | X | Yes | | | 1 |

**POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES** (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Monopelopia | | | | | | |
| boliekae | | | | | | 0 |
| tillandsia | | | Yes | 4 | | |
| Nanocladius | | | | | | |
| alternantherae | | | No | | 2 | |
| balticus | | | | | | 1 |
| distinctus | | | | | 3 | |
| minimus | | | | | | 1 |
| parvulus | | | | | | 1 |
| Natarsia | | | | | | |
| fastuosa | | | No | | | 0 |
| Nilodorum | | | | | | |
| devineyae | | | | | 2 | |
| Nilotanypus | | | | | | |
| americanus | | | Yes | | 3 | |
| Nilothauma | | | | | | |
| babyi | | | | | | 1 |
| bicornis | | | | | 2 | |
| Odontomesa | | | | | | |
| fulva | | | No | | | 0 |
| Omisus | | | | | | |
| pica | | | Yes | | | 1 |
| Orthocladius | | | | | | |
| annectens | | | Yes | | 2 | |
| obumbratus | | | Yes | | | 1 |
| Pagastiella | | | | | | |
| orophila | | | Yes | | 3 | |
| ostansa | | | | | 2 | |
| Parachironomus | | | | | | |
| abortivus | | | | | 3 | |
| alatus | | | Yes | | 2 | |
| carinatus | | | No | | 3 | |
| directus | | | No | | 2 | |
| hirtalatus | | | | | 3 | |
| loganae | | | Yes | | | 1 |
| monochromus | | | No | | 3 | |
| pectinatellae | | | | | 3 | |
| potamogeti | | | | | | 1 |
| schneideri | | | Yes | | 3 | |
| sublettei | | | | | 2 | |
| tenuicaudatus | | | | | 2 | |
| Paracladopelma | | | | | | |
| nais | | | | | 3 | |
| undine | | | Yes | | | 1 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Paralauterborniella | | | | | | |
| elachista | | | No | | 3 | |
| nigrohalteralis | | | | | 3 | |
| subcincta | | | No | | 2 | |
| Paramerina | | | | | | |
| anomala | | | Yes | | | 0 |
| smithae | | | No | | | 0 |
| Parametriocnemus | | | | | | |
| lundbeckii | | | | | | 0 |
| Paratendipes | | | | | | |
| albimanus | X | | No | | | 1 |
| subaequalis | | | | | 2 | |
| thermophilus | | | No | | | 0 |
| Parochlus | | | | | | |
| kiefferi | | | No | | | 0 |
| Pedionomus | | | | | | |
| beckae | | | | | 2 | |
| Pentaneura | | | | | | |
| americana | | | | | 2 | |
| carneosa | | | | | 2 | |
| comosa | | | No | | | 0 |
| flavifrons | | | | 4 | | |
| inconspicua | | | Yes | | 2 | |
| inculta | | | | | 2 | |
| melanops | | | | 4 | | |
| ornata | | | Yes | | 2 | |
| Phaenopsectra | | | | | | |
| profusa | | | | | 2 | |
| Polypedilum | | | | | | |
| angulum | | | | | 1 | |
| apicatum | | | No | | | 1 |
| aviceps | | | | | | 1 |
| braseniae | | | Yes | | | 1 |
| convictum | | | | | 2 | |
| digitifer | | | No | | 2 | |
| fallax | | X | Yes | | 3 | |
| halterale | | X | No | | 3 | |
| illinoense | X | | Yes | | 3 | |
| labeculosum | | | No | | | 0 |
| laetum | | | | | 3 | |
| nubeculosum | | | | | 2 | |
| obtusum | | | Yes | | | 1 |
| scalaenum | X | | Yes | | 2 | |

**POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES** (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| simulans | | | No | | 3 | |
| sordens | | | | | | 1 |
| trigonum | | | No | | 2 | |
| tritum | | | | | 3 | |
| vibex | | | | | | 1 |
| Procladius | | | | | | |
| adumbratus | | | No | | 2 | |
| bellus | | X | No | | 3 | |
| culiciformis | | | Yes | | 2 | |
| denticulatus | | | No | 4 | | |
| riparius | | | No | | 2 | |
| Prodiamesa | | | | | | |
| olivacea | | | Yes | | | 0 |
| Psectrocladius | | | | | | |
| elatus | | | | | 2 | |
| julia | | | | | 3 | |
| niger | | | | | 3 | |
| vernalis | | | Yes | | 2 | |
| Psectrotanypus | | | | | | |
| dyari | | | | 4 | | |
| venustus | | | No | | | 1 |
| Pseudochironomus | | | | | | |
| fulviventris | | | | | 2 | |
| julia | | | | | 2 | |
| richardsoni | | | No | | 2 | |
| Psilotanypus | | | | | | |
| bellus | | | | 4 | | |
| Rheocricotopus | | | | | | |
| robacki | | | | | 3 | |
| Rheotanytarsus | | | | | | |
| exiguus | | X | Yes | | 3 | |
| Robackia | | | | | | |
| claviger | | | | | 3 | |
| demeijerei | | | | | 3 | |
| Sergentia | | | | | | |
| coracina | | | No | | | 0 |
| Smittia | | | | | | |
| aterrima | | | Yes | | | 1 |
| Stempellina | | | | | | |
| johannseni | | | | | 2 | |
| Stenochironomus | | | | | | |
| hilaris | | | No | | | 1 |
| macateei | | | No | | | 1 |
| Stictochironomus | | | | | | |
| devinctus | | X | Yes | | | 0 |
| varius | | | No | | 2 | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Tanypus | | | | | | |
| carinatus | | | No | | 3 | |
| clavatus | | | No | | 2 | |
| grodhausi | | | | | 3 | |
| neopunctipennis | | | | | 3 | |
| parastellatus | | | No | | | 1 |
| punctipennis | | X | No | 4 | | |
| stellatus | | X | No | | 3 | |
| Tanytarsus | | | | | | |
| buckleyi | | | No | | 2 | |
| dissimilis | | | | | | 1 |
| gracilentus | | | | | | 1 |
| neoflavellus | | | | | 3 | |
| quadratus | | | No | | 2 | |
| recens | | | No | | 2 | |
| Thalassomyia | | | | | | |
| bureni | | | No | | 2 | |
| Thienemanniella | | | | | | |
| xena | | | | | | 0 |
| Thienemannimyia | | | | | | |
| barberi | | | No | | | 0 |
| senata | | | Yes | | | 0 |
| Tribelos | | | | | | |
| fuscicornis | | | Yes | | | 1 |
| jucundus | | X | Yes | | | 1 |
| Trichocladius | | | | | | |
| robacki | | | | | | 1 |
| Xenochironomus | | | | | | |
| rogersi | | | Yes | | | 1 |
| scopula | | X | No | | 2 | |
| taenionotus | | | | | 2 | |
| xenolabis | | X | No | | 2 | |
| Zavrelimyia | | | | | | |
| carneosa | | | | | | 0 |
| **OTHER DIPTERA** | | | | | | |
| Anopheles | | | | | | |
| crucians | | | | | 3 | |
| punctipennis | | | | | 2 | |
| Antocha | | | | | | |
| saxicola | | | No | | | 1 |
| Atherix | | | | | | |
| variegata | | X | | | 2 | |
| Bezzia | | | | | | |
| glabra | | | | 4 | | |

**POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)**

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Blepharicera | | | | | | |
| tenuipes | | | | | | 0 |
| Brachydeutera | | | | | | |
| argentata | | | | 4 | | |
| Cnephia | | | | | | |
| dacotensis | | | | | | 0 |
| mutata | | | | | 2 | |
| pecuarum | | | | | 2 | |
| Chaoborus | | | | | | |
| albatus | | | | | 3 | |
| americanus | | | | | 2 | |
| flavicans | | | | | 2 | |
| punctipennis | | | | | 3 | |
| Culex | | | | | | |
| attratus | | | | | 3 | |
| erraticus | | | | | 3 | |
| pipiens | | | | 4 | | |
| restuans | | | | | 3 | |
| Eristalis | | | | | | |
| aeneus | | | | 5 | | |
| bastardii | | | | 5 | | |
| brousii | | | | 5 | | |
| Mansonia | | | | | | |
| titillans | | | | | 3 | |
| Metasyrphus | | | | | | |
| americanus | | | | 4 | | |
| Odontomyia | | | | | | |
| cincta | | | | | 3 | |
| Palpomyia | | | | | | |
| tibialis | | | | | 3 | |
| Prosimulium | | | | | | |
| fuscum | | | | | 3 | |
| gibsoni | | | | | 3 | |
| johannseni | | | | | | 1 |
| magnum | | | | | | 1 |
| mixtum | | | | | 3 | |
| mysticum | | | | | | 1 |
| rhizophorum | | | | | | 1 |
| Protoplasa | | | | | | |
| fitchii | | | | | 3 | |
| Pseudolimnophila | | | | | | |
| luteipennis | | | | | | 1 |
| Psychoda | | | | | | |
| alternata | | | Yes | 5 | | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Simulium | | | | | | |
| aurium | | | | 4 | | |
| clarkei | | | | | 2 | |
| corbis | | | | | | 1 |
| croxtoni | | | | | | 1 |
| decorum | | | | | 2 | |
| euryadminiculum | | | | | | 1 |
| fibrinflatum | | | | | 3 | |
| jenningsi | | | | | 2 | |
| johannseni | | | | | | 0 |
| latipes | | | | | | 1 |
| luggeri | | | | | 3 | |
| meridionale | | | | | | 1 |
| pictipes | | | | | 2 | |
| rugglesi | | | | | 3 | |
| tuberosum | | | | | 2 | |
| venustum | | | | | 2 | |
| verecundum | | | | | 3 | |
| vittatum | | | | | 3 | |
| Sphaeromais | | | | | | |
| longipennis | | | | | 3 | |
| Stegopterna | | | | | | |
| mutata | | | | | 3 | |
| Stilobezzia | | | | | | |
| antennalis | | | | 4 | | |
| Stratiomys | | | | | | |
| discalis | | | | 4 | | |
| meigenii | | | | 4 | | |
| Tabanus | | | | | | |
| atratus | | | | | 3 | |
| benedictus | | | | 4 | | |
| giganteus | | | | | | 1 |
| lineola | | | | 4 | | |
| stygius | | | | | 2 | |
| variegatus | | | | | | 1 |
| Telmatoscopus | | | | | | |
| albipunctatus | | | | 4 | | |
| Tipula | | | | | | |
| abdominalis | X | | Yes | | | 1 |
| caloptera | | | | | | 1 |
| Toxorhynchites | | | | | | |
| rutilus | | | | | 3 | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| **INSECTA - TRICOPTERA** | | | | | | |
| Agarodes | | | | | | |
| distinctum | | | | | 2 | |
| Agrypnia | | | | | | |
| vestita | | | | | | 1 |
| Amiocentrus | | | | | | |
| aspilus | | | No | | | 0 |
| Anisocentropus | | | | | | |
| pyraloides | | | Yes | | | 0 |
| Apatania | | | | | | |
| incerta | | | | | | 0 |
| Aphropsyche | | | | | | |
| doringa | | | | | | 0 |
| Arctopsyche | | | | | | |
| grandis | | X | | | | 1 |
| irrorata | | | Yes | | | 0 |
| ladogensis | | | | | | 0 |
| Asynarchus | | | | | | |
| montanus | | | | | 3 | |
| Brachycentrus | | | | | | |
| americanus | | | No | | | 1 |
| incanus | | | | | | 0 |
| lateralis | | | No | | | 0 |
| numerosus | | | | | | 1 |
| occidentalis | | | | | | 1 |
| Ceraclea | | | | | | |
| ancylus | | | | | 2 | |
| cancellata | | | | | | 0 |
| diluta | | | | | | 1 |
| flava | | | No | | | 1 |
| maculata | | | | | | 1 |
| neffi | | | | | | 1 |
| nepha | | | | | 2 | |
| punctata | | | | | | 0 |
| slossonae | | | | | 2 | |
| tarsipunctata | | | No | | | 1 |
| transversa | | X | | | | 1 |
| Ceratopsyche | | | | | | |
| alhedra | | | | | 2 | |
| alternans | | | | | 2 | |
| bronta | | | | | 3 | |
| bifida | | | | | 3 | |
| morosa | | | | | | 1 |
| slossonae | | | | | 2 | |
| sparna | | | | | | 1 |
| walkeri | | | | | | 1 |
| vexa | | | | | 2 | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Chimarra | | | | | | |
| atterrima | | X | Yes | | | 1 |
| feria | | | | | | 1 |
| obscura | | | | | 2 | |
| perigua | | | | | | 1 |
| socia | | | | | | 0 |
| Culophila | | | | | | |
| thoracica | | | | | | 0 |
| Cyrnellus | | | | | | |
| fraternus | | X | No | | 3 | |
| Diplectrona | | | | | | |
| metaqui | | | | | | 0 |
| modesta | | | Yes | | | 1 |
| Dolophilodes | | | | | | |
| distinctus | | | | | | 0 |
| Fattigia | | | | | | |
| pele | | | Yes | | | 1 |
| Frenesia | | | | | | |
| missa | | | | | | 0 |
| Glyphopsyche | | | | | | |
| irrorata | | | | | | 1 |
| Goera | | | | | | |
| calcarata | | | | | | 0 |
| fuscula | | | | | | 0 |
| stylata | | | | | | 0 |
| Helicopsyche | | | | | | |
| borealis | | X | No | | 2 | |
| Hesperophylax | | | | | | |
| designatus | | | | | 2 | |
| Heteroplectron | | | | | | |
| americanum | | | | | | 0 |
| Hydatophylax | | | | | | |
| argus | | | | | | 1 |
| Hydropsyche | | | | | | |
| aerata | | | | | 2 | |
| arinale | | | | | 3 | |
| betteni | X | | Yes | | 3 | |
| bidens | | | | | 2 | |
| cuanis | | | | | 3 | |
| demora | | | | | 3 | |
| depravata | | | | | 3 | |
| dicantha | | | | | | 1 |
| frisoni | | X | No | | 3 | |
| incommoda | | | | | 2 | |
| leonardi | | | | | | 0 |

**POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES** (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| orris | | X | No | | 3 | |
| phalerata | | | | | | 1 |
| placoda | | | | | 2 | |
| scalaris | | | | | | 1 |
| simulans | | X | No | | 2 | |
| venularis | | | | | | 1 |
| Hydroptilia | | | | | | |
| waubesiana | | | | | 2 | |
| Ironoquia | | | | | | |
| punctatissima | | | | | 2 | |
| Leptocerus | | | | | | |
| americanus | | | | | | 1 |
| Leuchotrichia | | | | | | |
| pictipes | | | | | | 1 |
| Limnephilus | | | | | | |
| rhombicus | | | No | | | 1 |
| submonilifer | | | No | | | 1 |
| Lype | | | | | | |
| diversa | | | Yes | | | 1 |
| Macronemum | | | | | | |
| carolina | | X | | | | 0 |
| zebratum | | | | | 2 | |
| Matrioptila | | | | | | |
| jeanae | | | | | | 0 |
| Micrasema | | | | | | |
| kluane | | | | | | 1 |
| rusticum | | | | | | 1 |
| wataga | | | | | | 1 |
| Molanna | | | | | | |
| blenda | | | Yes | | | 1 |
| Mystacides | | | | | | |
| sepulchralis | X | | No | | | 1 |
| Nectopsyche | | | | | | |
| albida | | | | | | 1 |
| dorsalis | | | | | 2 | |
| exquisita | | | | | | 0 |
| pavida | | | | | 2 | |
| Nemotaulius | | | | | | |
| hostilis | | | | | 2 | |
| Neureclipsis | | | | | | |
| crepuscularis | | X | Yes | | | 1 |
| Oligostomis | | | | | | |
| ocelligera | | | | | | 1 |
| Onocosmoecus | | | | | | |
| quadrinotatus | | | | | | 1 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Oropsyche howellae | | | | | | 0 |
| Palaeagapetus celsus | | | | | | 0 |
| Parapsyche apicalis | | | | | | 0 |
| Phylocentropus placidus | | X | Yes | | 2 | |
| Potamyia flava | | X | Yes | | 3 | |
| Pseudogoera singularis | | | | | | 0 |
| Pseudostenophylax uniformis | | | | | | 0 |
| Psilotreta indecisa | | | | | | 0 |
| Psychoglypha subborealis | | | | | | 0 |
| Psychomyia flavida | | | | | | 1 |
| Pycnopsyche gentilis | | | Yes | | 2 | |
| guttifer | X | | Yes | | 2 | |
| lepida | X | | | | 2 | |
| Rhyacophila acutilaba | | | | | | 0 |
| amicis | | | Yes | | | 0 |
| atrata | | | Yes | | | 0 |
| brunnea | | | | | | 0 |
| carolina | | | Yes | | 2 | |
| carpenteri | | | | | | 0 |
| fuscula | | | | | | 1 |
| glaberrima | | | Yes | | 3 | |
| invaria | | | Yes | | | 1 |
| ledra | | | | | | 1 |
| lobifera | | | | | 2 | |
| melita | | | | | | 0 |
| mycta | | | | | | 0 |
| nigrita | | | Yes | | 2 | |
| torva | | | Yes | | 2 | |
| vibox | | | | | | 1 |
| vulphipes | | | | | | 1 |

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Symphitopsyche | | | | | | |
| bifida | X | | No | | 3 | |
| bronta | | | | | 2 | |
| macleodi | | | | | | 0 |
| morosa | | | | | 2 | |
| riola | | | | | 2 | |
| sparna | | | Yes | | 3 | |
| Trentonius | | | | | | |
| distinctus | | | | | 2 | |
| Wormaldia | | | | | | |
| moestus | | | | | | 0 |
| **INSECTA - EPHEMEROPTERA** | | | | | | |
| Ameletus | | | | | | |
| lineatus | | | | | | 0 |
| Ametropus | | | | | | |
| albrighti | | | | | | 0 |
| Arthroplea | | | | | | |
| bipunctata | | | No | | | 1 |
| Attenella | | | | | | |
| attenuata | | | | | 2 | |
| Baetis | | | | | | |
| australis | | | | | | 0 |
| bicaudatus | | | No | | | 0 |
| brunneicolor | | | | | 2 | |
| flavistriga | | | | | 2 | |
| frondalis | | | | | 3 | |
| hageni | | | | | 3 | |
| intercalaris | | | | | 3 | |
| longipalpus | | | | | 3 | |
| macdunnoughi | | | | | 3 | |
| propinquus | | | | | 3 | |
| pygmaeus | | | | | 2 | |
| spiethi | | | Yes | | | 0 |
| tricaudatus | | | No | | 2 | |
| vagans | | | No | | 2 | |
| Baetisca | | | | | | |
| bajkovi | | | No | | 2 | |
| carolina | | | | | | 0 |
| escambiensis | | | No | | | 0 |
| gibbera | | | Yes | | | 0 |
| lacustris | | | | | 3 | |
| laurentina | | | | | 2 | |
| obesa | | | Yes | | | 1 |
| rogersi | | | Yes | | | 0 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Brachycercus | | | | | | |
| lacustris | X | | | | | 1 |
| maculatus | | | Yes | | 2 | |
| Caenis | | | | | | |
| amica | | | | | 3 | |
| anceps | | | | | | 0 |
| diminuta | | | Yes | 4 | | |
| forcipata | | | | | | 1 |
| hilaris | | | Yes | | | 1 |
| latipennis | | | | | | 0 |
| simulans | | | No | | | 1 |
| Callibaetis | | | | | | |
| coloradensis | | | No | | | 0 |
| floridanus | | | Yes | 4 | | |
| pretiosus | | | No | | | 0 |
| Centroptilum | | | | | | |
| viridocularis | | | | | | 0 |
| Choroterpes | | | | | | |
| basalis | | | | | | 1 |
| hubbelli | | | | | | 1 |
| Cloeon | | | | | | |
| alamance | | | | | | 1 |
| rubropictum | | | No | | | 0 |
| Dannella | | | | | | |
| lita | | | | | 2 | |
| simplex | | | | | | 1 |
| Dolania | | | | | | |
| americana | | X | Yes | | | 0 |
| Drunella | | | | | | |
| cornutella | | | | | | 0 |
| Epeorus | | | | | | |
| albertae | | | | | 2 | |
| longimanus | | | No | | | 1 |
| vitreus | | | No | | | 1 |
| Ephemera | | | | | | |
| blanda | | | | | 2 | |
| guttulata | | | | | | 1 |
| simulans | | X | No | | | 1 |
| varia | | | | | | 0 |
| Ephemerella | | | | | | |
| allegheniensis | | | | | | 0 |
| aestiva | | | | | | 0 |
| attenuata | | | | | | 0 |
| aurivilli | | | | | | 0 |
| berneri | | | | | | 0 |
| bicolor | X | | No | | 2 | |

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| carolina | | | | | 2 | |
| catawba | | | | | 2 | |
| coloradensis | | | No | | | 1 |
| cornuta | | | | | | 1 |
| coxalis | | | | | | 0 |
| crenula | | | | | | 0 |
| deficiens | | X | No | | 3 | |
| doddsi | | | No | | | 1 |
| dorothea | | | | | | 1 |
| excrucians | | | | | | 1 |
| flavilinea | | | No | | | 0 |
| frisoni | | | | | | 0 |
| funeralis | | | | | | 1 |
| grandis | | | | | | 0 |
| hecuba | | | No | | 2 | |
| inermis | | | No | | | 0 |
| invaria | | | | | | 1 |
| lita | X | | | | | 0 |
| longicornis | | | | | | 0 |
| needhami | | | No | | | 1 |
| rotunda | | | | | | 1 |
| septentrionalis | | | | | | 0 |
| serrata | | | | | | 1 |
| serratoides | | | | | | 0 |
| simplex | | X | Yes | | | 1 |
| spiculosa | | | | | | 0 |
| subvaria | | | No | | | 1 |
| temporalis | | | | | 2 | |
| teresa | | | No | | 3 | |
| tibialis | | | No | | 2 | |
| trilineata | | | Yes | | | 1 |
| versimilis | | | | | | 1 |
| walkeri | | | | | | 1 |
| wayah | | | | | | 0 |
| Ephoron | | | | | | |
| album | | | No | | | 1 |
| Eurylophella | | | | | | |
| aestiva | | | | | 3 | |
| bicolor | | | | | | 1 |
| funeralis | | | | | | 0 |
| lutulenta | | | | | 3 | |
| temporalis | | | | | 3 | |
| Habrophlebia | | | | | | |
| vibrans | | | | | | 0 |
| Habrophlebiodes | | | | | | |
| americana | X | | | | 2 | |
| brunneipennis | | | | | | 1 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Heptagenia | | | | | | |
| criddlei | | | No | | 2 | |
| diabasia | | | | | 2 | |
| flavescens | | | | | 2 | |
| hebe | | | No | | 2 | |
| lucidipennis | | | | | 2 | |
| maculipennis | | | | | 2 | |
| pulla | | | No | | | 0 |
| Heterocloeon | | | | | | |
| curiosum | | | | | | 1 |
| Hexagenia | | | | | | |
| atrocaudata | | X | No | | | 0 |
| bilineata | | | No | | 2 | |
| limbata | | X | No | | | 1 |
| munda | | | No | | 2 | |
| rigida | | | No | | 2 | |
| Homoeoneuria | | | | | | |
| dolani | X | | | | | 0 |
| Isonychia | | | | | | |
| bicolor | | | | | 2 | |
| pictipes | | | | | | 1 |
| sadleri | | | No | | | 0 |
| Leptophlebia | | | | | | |
| bradleyi | | | Yes | | | 0 |
| cupida | | | No | | | 0 |
| intermedia | | | Yes | | 2 | |
| nebulosa | | | No | 4 | | |
| nervosa | | | | | 2 | |
| Leucocuta | | | | | | |
| hebe | | | | | | 1 |
| Litobrancha | | | | | | |
| recurvata | | | | | 3 | |
| Neoephemera | | | | | | |
| purpurea | | | | | | 0 |
| *youngi* | | X | Yes | | | 1 |
| Nixe | | | | | | |
| lucidipennis | | | | | | 1 |
| Paraleptophlebia | | | | | | |
| bicornuta | | | Yes | | 2 | |
| bradleyi | | | | | 3 | |
| debilis | | | No | | | 0 |
| heteronea | | | No | | | 1 |
| mollis | | | No | | | 1 |
| praepedita | | | No | | | 0 |
| volitans | | | Yes | | 3 | |

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Pentagenia | | | | | | |
| vittigera | | X | No | | 2 | |
| Potamanthus | | | | | | |
| distinctus | | | | | | 1 |
| rufous | | | | | | 1 |
| Pseudiron | | | | | | |
| centralis | | | | | 3 | |
| Pseudocloeon | | | | | | |
| carolina | | | No | | | 1 |
| dubium | | | | | 2 | |
| myrsum | | | | | 2 | |
| parvulum | | | No | | | 1 |
| punctiventris | | | | | 2 | |
| Rhithrogenia | | | | | | |
| hageni | | | No | | 2 | |
| impersonata | | | | | | 0 |
| jejuna | | | | | | 0 |
| pellucida | | | | | | 0 |
| robusta | | | No | | | 1 |
| undulata | | | | | | 0 |
| Serratella | | | | | | |
| deficiens | | | | | | 1 |
| sordida | | | | | | 1 |
| Siphlonurus | | | | | | |
| alternatus | | | No | | | 0 |
| Siphloplecton | | | | | | |
| speciosum | | | Yes | | | 0 |
| Stenacron | | | | | | |
| candidum | | | | | | 0 |
| carolina | | | | | | 0 |
| floridense | | | Yes | | | 0 |
| interpunctatum | X | | Yes | | 2 | |
| pallidum | | | No | | 2 | |
| Stenonema | | | | | | |
| annexum | | | | | | 0 |
| ares | | | No | | | 1 |
| bipunctatum | | X | No | | 2 | |
| carlsoni | | | | | | 0 |
| exiguum | | | Yes | | 3 | |
| femoratum | | X | No | | 3 | |
| fuscum | | | | | 2 | |
| integrum | | | No | | 3 | |
| ithaca | | | | | | 0 |
| luteum | | | | | | 0 |
| mediopunctatum | | | | | | 1 |
| modestum | | | | | | 1 |

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| nepotellum | | | No | | 2 | |
| pudicum | | | | | | 1 |
| pulchellum | | | No | | 2 | |
| quinquespinum | | | No | | | 1 |
| rubromaculatum | | | No | | | 1 |
| rubrum | | | Yes | | 2 | |
| smithae | | | Yes | | 2 | |
| terminatum | | | No | | 2 | |
| tripunctatum | | X | No | | 3 | |
| vicarium | X | | No | | | 1 |
| Tortopus | | | | | | |
| incertus | | | | | | 1 |
| Tricorythodes | | | | | | |
| albilineatus | | | Yes | | | 1 |
| minutus | | | No | | 2 | |
| **INSECTA - PLECOPTERA** | | | | | | |
| Acroneuria | | | | | | |
| abnormis | | X | Yes | | 2 | |
| arida | | | No | | | 1 |
| carolinensis | | | | | | 1 |
| evoluta | | X | No | | | 1 |
| georgiana | | | | | | 0 |
| internata | | X | No | | 2 | |
| lycorias | | | Yes | | | 1 |
| perplexa | | | | | 2 | |
| ruralis | X | | No | | | 1 |
| xanthenes | | | | | | 1 |
| Agnetina | | | | | | |
| capitata | | | | | | 1 |
| Allocapnia | | | | | | |
| granulata | | | No | | | 0 |
| nivicola | | | No | | | 0 |
| recta | | | No | | | 0 |
| rickeri | | | No | | | 0 |
| vivipara | | | No | | | 1 |
| Amphinemura | | | | | | |
| delosa | | | Yes | | 2 | |
| linda | | | | | | 0 |
| Atoperla | | | | | | |
| ephyre | | | | | | 0 |
| Attaneuria | | | | | | |
| ruralis | | | | | | 1 |
| Brachyptera | | | | | | |
| fasciata | | | | | | 1 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Clioperla | | | | | | |
| clio | | | | | | 1 |
| Diploperla | | | | | | |
| duplicata | | | No | | | 0 |
| Eccoptura | | | | | | |
| xanthenes | | | | | | 1 |
| Hastaperla | | | | | | |
| brevis | | | Yes | | | 1 |
| Hesperoperla | | | | | | |
| pacifica | | | No | | 2 | |
| Hydroperla | | | | | | |
| crosbyi | | | No | | | 0 |
| Isogenoides | | | | | | |
| frontalis | | | | | | 0 |
| olivaceus | | | | | | 0 |
| Isogenus | | | | | | |
| bilobatus | | | | | | 1 |
| decisus | | | | | 2 | |
| subvarians | | | | | 2 | |
| Isoperla | | | | | | |
| bilineata | | | No | | 2 | |
| clio | | | No | | | 1 |
| cotta | | | | | | 1 |
| decepta | | | No | | | 0 |
| dicala | | | | | | 1 |
| frisoni | | | Yes | | | 1 |
| fulva | | | No | | 2 | |
| holochlora | | | | | 2 | |
| lata | | | | | | 1 |
| marlynia | | | No | | | 1 |
| mohri | | | No | | | 0 |
| namata | | | | | 2 | |
| nana | | | No | | 2 | |
| orata | | | No | | | 1 |
| richardsoni | | | No | | | 1 |
| signata | | | No | | | 1 |
| similis | | | | | 2 | |
| slossonae | | | | | | 1 |
| transmarina | | | Yes | | | 1 |
| Leuctra | | | | | | |
| ferruginea | | | | | | 0 |
| sibleyi | | | | | | 0 |
| tenella | | | | | | 0 |
| tenuis | | | | | | 0 |
| Nemocapnia | | | | | | |
| carolina | | X | | | | 0 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Nemoura | | | | | | |
| trispinosa | | | | | | 1 |
| Neoperla | | | | | | |
| clymene | | X | No | | | 1 |
| stewarti | | | | | | 1 |
| Oemopteryx | | | | | | |
| glacialis | | | | | | 1 |
| Paracapnia | | | | | | |
| angulata | | | | | | 1 |
| Paragnetina | | | | | | |
| immarginata | | X | No | | | 1 |
| media | | X | No | | | 1 |
| Perlesta | | | | | | |
| frisoni | | | | | | 1 |
| placida | | | No | | 2 | |
| Perlinella | | | | | | |
| drymo | | X | | | | 1 |
| ephyre | | | | | | 1 |
| Phasganophora | | | | | | |
| capitata | | X | No | | | 1 |
| Prostoia | | | | | | |
| completa | | | | | | 1 |
| similis | | | | | | 1 |
| Shipsa | | | | | | |
| rotunda | | | | | | 1 |
| Soyedina | | | | | | |
| vallicularia | | | | | | 0 |
| Strophopteryx | | | | | | |
| fasciata | | | No | | | 1 |
| Sweltsa | | | | | | |
| mediana | | | Yes | | | 0 |
| Taeniopteryx | | | | | | |
| burksi | | | No | | 2 | |
| maura | | | Yes | | 2 | |
| metequi | | | | | 2 | |
| nivalis | | X | No | | | 1 |
| parvula | | | | | | 1 |
| Zapada | | | | | | |
| cinctipes | | | No | | | 1 |
| Zealeuctra | | | | | | |
| claasseni | | | No | | | 0 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| **INSECTA - ODONATA** | | | | | | |
| Aeshna | | | | | | |
| umbrosa | | | No | | 2 | |
| Amphiagrion | | | | | | |
| saucium | | | | 5 | | |
| Anax | | | | | | |
| junius | | | No | | 3 | |
| Argia | | | | | | |
| apicalis | | | No | | 3 | |
| moesta | X | | No | 4 | | |
| translata | | | No | | 2 | |
| Basiaeschna | | | | | | |
| janata | | | | | 3 | |
| Boyeria | | | | | | |
| grafiana | | | | | 3 | |
| vinosa | X | | Yes | | 2 | |
| Calopteryx | | | | | | |
| aequabilis | | | | | 3 | |
| maculata | | | | | 3 | |
| Cannacria | | | | | | |
| gravida | | | | | 2 | |
| Chromagrion | | | | | | |
| conditum | | | | | 2 | |
| Coenagrion | | | | | | |
| resolutum | | | | 4 | | |
| Cordulegaster | | | | | | |
| erroneus | | | | | 2 | |
| fasciatus | | | | | | 1 |
| maculatus | | | | | 2 | |
| sayi | | | | | | 0 |
| Dromogomphus | | | | | | |
| spinosus | X | | No | | 2 | |
| spoliatus | | | No | | | 1 |
| Didymops | | | | | | |
| transversa | | | | | 2 | |
| Enallagma | | | | | | |
| antennatum | | | No | | 3 | |
| civile | | | | 4 | | |
| ebrium | | | | 4 | | |
| hageni | | | | 4 | | |
| signatum | | | Yes | | 2 | |
| Epitheca | | | | | | |
| cynosura | | | | | 2 | |
| princeps | | | | | 2 | |
| semiaquea | | | | | | 1 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Erythrodiplax | | | | | | |
| berenice | | | | | 2 | |
| connata | | | | | 2 | |
| Gomphus | | | | | | |
| externus | | | No | | 2 | |
| pallidus | | | | | 2 | |
| plagiatus | | | No | | 2 | |
| spiniceps | | | No | | 2 | |
| vastus | | | No | | 2 | |
| Hagenius | | | | | | |
| brevistylus | | | | | | 1 |
| Hetaerina | | | | | | |
| americana | | | | | 3 | |
| titia | | | No | | | 0 |
| Hylogomphus | | | | | | |
| brevis | | | | | 2 | |
| Ischnura | | | | | | |
| posita | | | No | | 3 | |
| verticalis | | | No | 4 | | |
| Lanthus | | | | | | |
| albistylus | | | No | | | 1 |
| parvulus | | | | | 3 | |
| Leucorrhinia | | | | | | |
| intacta | | | | 4 | | |
| Libellula | | | | | | |
| deplanata | | | | | 2 | |
| lydia | | | | | 2 | |
| pulchella | | | | 4 | | |
| Macromia | | | | | | |
| georgiana | | | | | | 1 |
| illinoiensis | | | | | | 1 |
| taeniolata | | | | | 2 | |
| Neurocordulia | | | | | | |
| molesta | | | | | 2 | |
| obsoleta | | | No | | | 1 |
| yamaskanensis | | | | | | 0 |
| Pachydiplax | | | | | | |
| longipennis | | | No | | 3 | |
| Plathemis | | | | | | |
| lydia | | | | 4 | | |
| Progomphus | | | | | | |
| obscurus | | | No | | 3 | |
| Stylogomphus | | | | | | |
| albistylus | | | | | | 0 |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| **INSECTA - NEUROPTERA** | | | | | | |
| Climacia | | | | | | |
| areolaris | | X | No | | | 1 |
| **INSECTA - MEGALOPTERA** | | | | | | |
| Chauliodes | | | | | | |
| pectinicornis | | | | | 2 | |
| rastricornis | | | | | 2 | |
| Corydalus | | | | | | |
| cornutus | | X | Yes | | 3 | |
| Nigronia | | | | | | |
| fasciatus | | | | | | 0 |
| serricornis | | | | | | 1 |
| Sialis | | | | | | |
| infumata | X | | Yes | 4 | | |
| **INSECTA - HEMIPTERA** | | | | | | |
| Belostoma | | | | | | |
| fluminea | X | | Yes | 4 | | |
| Benacus | | | | | | |
| griseus | | | | | 2 | |
| Callicorixa | | | | | | |
| audeni | | | Yes | | 2 | |
| Hydrometra | | | | | | |
| martini | | | | 4 | | |
| Limnogonus | | | | | | |
| hesione | | X | No | | 3 | |
| Merragata | | | | | | |
| hebroides | | | | | 3 | |
| Mesovelia | | | | | | |
| mulsanti | | | | | 3 | |
| Nepa | | | | | | |
| apiculata | | | | | | 1 |
| Rhagovelia | | | | | | |
| obesa | X | | | | 3 | |
| **INSECTA - COLEOPTERA** | | | | | | |
| Anchytarsus | | | | | | |
| bicolor | | | | | | 1 |
| Ancyronyx | | | | | | |
| variegatus | | | Yes | | 2 | |
| Anodocheilus | | | | | | |
| exiguus | | | | | 2 | |
| Bidessus | | | | | | |
| fuscatus | | | | | 2 | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Copelatus glyphicus | | | | | 2 | |
| Cybister fimbriolatus | | | | | 3 | |
| Derallus altus | | | | | 2 | |
| Dibolocelus ovatus | | | | | 3 | |
| Dineutus americanus | | | | 4 | | |
| Dubiraphia bivittata | | | | 4 | | |
| minima | | | | | 3 | |
| quadrinotata | X | | Yes | | 3 | |
| vittata | X | | | | 2 | |
| Dytiscus hybridus | | | | | 2 | |
| Ectopria nervosa | | X | | | | 0 |
| Gonielmis dietrichi | | | | | 2 | |
| Graphoderus liberus | | | | | 2 | |
| Gyrinus floridensis | | | | 4 | | |
| Haliplus fasciatus | | | | | 3 | |
| Helichus lithophilus | | | | | 3 | |
| striatus | | | | | 3 | |
| Helochares maculicollis | | | | | 2 | |
| Hoperius planatus | | | | | | 1 |
| Hydrochara obtusata | | | | | | 1 |
| Hydrophilus triangularis | | | | | | 1 |
| Hyogrotus farctus | | | | | 2 | |
| Laccobius agilis | | | | | 2 | |
| Laccophilus maculosus | | | | 4 | | |
| Laccornis difformis | | | | | 2 | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Macronychus glabratus | X | | Yes | | 2 | |
| Matus ovatus | | | | | 2 | |
| Microcylloepus pusillus | | | | | | 1 |
| Optioservus fastiditus | | | | | 2 | |
| ovalis | | | No | | 2 | |
| trivittatus | | | | | | 1 |
| Oulimnius latiusculus | | | | | | 0 |
| Pelonomus obscurus | | | | | 2 | |
| Peltodytes muticus | | | | | 3 | |
| sexmaculatus | | | | | 3 | |
| Promoresia elegans | | | | | | 0 |
| tardella | | | | | | 0 |
| Psephenus herricki | | X | No | | | 1 |
| Ptilodactyla augustata | | | | | | 0 |
| serricollis | | | | | | 0 |
| Sperchopsis tesselatus | | | | | 2 | |
| Stenelmis crenata | | X | Yes | | | 1 |
| decorata | | X | No | 4 | | |
| sexlineata | | X | No | | 3 | |
| Tropisternus dorsalis | | | | | 3 | |
| lateralis | | | | 4 | | |
| natator | | | | 4 | | |
| **MOLLUSCA - GASTROPODA** | | | | | | |
| Amnicola emarginata | | | | | | 1 |
| limosa | | | No | | | 1 |
| Aplexa hypnorum | | | No | | 2 | |
| Bithynia tentaculata | | | | 4 | | |

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Tolerance to Organic Wastes* Facultative | Tolerance to Organic Wastes* Intolerant |
|---|---|---|---|---|---|---|
| Campeloma | | | | | | |
| decisum | | | Yes | | 3 | |
| integrum | | | | | 2 | |
| rufum | | | | | 2 | |
| subsolidum | | | | | 3 | |
| Elimia | | | | | | |
| livescens | | | No | | 3 | |
| virginica | | | No | 4 | | |
| Ferrissia | | | | | | |
| fusca | | | | | 3 | |
| rivularis | | | No | | | 1 |
| tarda | | | No | | 3 | |
| Fossaria | | | | | | |
| modicella | | | | 4 | | |
| obrussa | | | | | 3 | |
| Gyraulus | | | | | | |
| arcticus | | | | | 3 | |
| Helisoma | | | | | | |
| anceps | | | No | | 3 | |
| trivolvis | | | No | 4 | | |
| Lioplax | | | | | | |
| subcarinata | | | | | | 1 |
| Lymnaea | | | | | | |
| appressa | | | | | | 1 |
| humilis | | | | | 3 | |
| ovata | | | | 5 | | |
| peregrina | | | | | 3 | |
| stagnalis | | | No | | 2 | |
| Neoplanorbis | | | | | | |
| carinatus | | | | | | 1 |
| Physa | | | | | | |
| fontinalis | | | | | 2 | |
| halei | | | | 4 | | |
| Physella | | | | | | |
| acuta | | | | | 2 | |
| anatina | | | | 4 | | |
| cubensis | | | | 4 | | |
| gyrina | | | | 4 | | |
| heterostropha | | | No | 4 | | |
| integra | | | No | 4 | | |
| Planorbis | | | | | | |
| trivolvis | | | | 4 | | |
| Planobula | | | | | | |
| armigera | | | | 4 | | |

## POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Pleurocera | | | | | | |
| acuta | | | No | | 2 | |
| lewisi | | | | | 2 | |
| Pseudosuccinea | | | | | | |
| columella | | | | | 3 | |
| Radix | | | | | | |
| auricularia | | | | | 3 | |
| Stagnicola | | | | | | |
| caperata | | | | | 3 | |
| catascopium | | | | 4 | | |
| palustris | | | No | | 2 | |
| Valvata | | | | | | |
| bicarinata | | | No | | | 1 |
| piscinalis | | | No | | 2 | |
| sincera | | | No | | 3 | |
| tricarinata | | | No | 4 | | |
| Viviparus | | | | | | |
| contectoides | | | | | | 1 |
| subpurpureus | | | | | | 1 |
| **MOLLUSCA - BIVALVIA** | | | | | | |
| Alasmidonta | | | | | | |
| triangulata | | | | | | 0 |
| undulata | | | | | 2 | |
| Amblema | | | | | | |
| plicata | | | Yes | | 2 | |
| Anodonta | | | | | | |
| cataracta | | | | | | 1 |
| gibbosus | | | | | 3 | |
| grandis | | | | | 2 | |
| imbecillus | | | | | 2 | |
| implicata | | | | | | 1 |
| undulata | | | | | 2 | |
| Corbicula | | | | | | |
| manilensis | | | | | 3 | |
| Cyclonaias | | | | | | |
| tuberculata | | | | | 2 | |
| Elliptio | | | | | | |
| complanata | | | | | 2 | |
| congaraea | | | | | | 1 |
| icterina | | | | | | 1 |
| shepardiana | | | | | | 0 |
| waccamawensis | | | | | | 0 |

POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Eupera | | | | | | |
| cubensis | | | | | 3 | |
| Lampsilis | | | | | | |
| cariosa | | | | | | 0 |
| luteola | | | | | 2 | |
| ochracea | | | | | | 0 |
| parvus | | | | | 2 | |
| teres | | | | | 2 | |
| Lasmigona | | | | | | |
| complanata | | | | | 2 | |
| costata | | | | | 2 | |
| Leptodea | | | | | | |
| fragilis | | | | | | 0 |
| Margaritifera | | | | | | |
| margaritifera | | | | | | 0 |
| Musculium | | | | | | |
| partumeium | | | 4 | | | |
| securis | | | | | 2 | |
| transversum | | | | | 3 | |
| Obliquaria | | | | | | |
| reflexa | | | | | | 0 |
| Pisidium | | | | | | |
| abditum | | | 4 | | | |
| amnicum | | | | | 2 | |
| casertanum | | | 4 | | | |
| complanatum | | | 4 | | | |
| compressum | | | 4 | | | |
| crystalense | | | | | 2 | |
| fallax | | | | | 2 | |
| henslowanum | | | | | 2 | |
| idahoense | | | 4 | | | |
| subtruncatum | | | | | 2 | |
| Proptera | | | | | | |
| alata | | | | | | 1 |
| Quadrula | | | | | | |
| lachrymosa | | | | | 2 | |
| pustulosa | | | | | 2 | |
| rubiginosa | | | | | 2 | |
| Rangia | | | | | | |
| cuneata | | | | | 3 | |

**POLLUTION TOLERANCE OF SELECTED MACROINVERTEBRATES** (Continued)

| Taxa | Heavy Metals Tolerant | Heavy Metals Sensitive | Acid Tolerant | Tolerance to Organic Wastes* Tolerant | Facultative | Intolerant |
|---|---|---|---|---|---|---|
| Sphaerium | | | | | | |
| corneum | | | | | 3 | |
| lilycashense | | | | | 3 | |
| notatum | | | | 4 | | |
| rhomboideum | | | | | 3 | |
| solidula | | | | | | 1 |
| sulcatum | | | | | 3 | |
| stamineum | | | | | 3 | |
| striatinum | | | | | 3 | |
| transversum | | | | 4 | | |
| Strophitus | | | | | | |
| edentulus | | | | | 2 | |
| Truncilla | | | | | | |
| donaciformis | | | | | | 1 |
| Uniomerus | | | | | | |
| tetralasmus | | | | | | 1 |

*Ranking from 0 to 5 with 0 being the least tolerant.

**References used in determining tolerances**


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## APPENDIX B

### Hilsenhoff's Family Level Pollution Tolerance Values for Aquatic Arthropods[1]

| Order | Family | Tolerance Value |
|---|---|---|
| Plecoptera | Capniidae | 1 |
| | Chloroperlidae | 1 |
| | Leuctridae | 0 |
| | Nemouridae | 2 |
| | Perlidae | 1 |
| | Perlodidae | 2 |
| | Pteronarcyidae | 0 |
| | Taeniopterygidae | 2 |
| Ephemeroptera | Baetidae | 4 |
| | Baetiscidae | 3 |
| | Caenidae | 7 |
| | Ephemerellidae | 1 |
| | Ephemeridae | 4 |
| | Heptageniidae | 4 |
| | Leptophlebiidae | 2 |
| | Metretopodidae | 2 |
| | Oligoneuriidae | 2 |
| | Polymitarcyidae | 2 |
| | Potomanthidae | 4 |
| | Siphlonuridae | 7 |
| | Tricorythidae | 4 |
| Odonata | Aeshnidae | 3 |
| | Calopterygidae | 5 |
| | Coenagrionidae | 9 |
| | Cordulegastridae | 3 |
| | Corduliidae | 5 |
| | Gomphidae | 1 |
| | Lestidae | 9 |
| | Libellulidae | 9 |
| | Macromiidae | 3 |
| Trichoptera | Brachycentridae | 1 |
| | Glossosomatidae | 0 |
| | Helicopsychidae | 3 |
| | Hydropsychidae | 4 |
| | Hydroptilidae | 4 |
| | Lepidostomatidae | 1 |
| | Leptoceridae | 4 |

[1]From Hilsenhoff, 1988. Rapid field assessment of organic pollution with a family-level biotic index. J.N. Am. Benthol. Soc. 7(1):65-68.

| Order | Family | Tolerance Value |
|---|---|---|
| Trichoptera (cont.) | Limnephilidae | 4 |
| | Molannidae | 6 |
| | Odontoceridae | 0 |
| | Philopotamidae | 3 |
| | Phryganeidae | 4 |
| | Polycentropodidae | 6 |
| | Psychomyiidae | 2 |
| | Rhyacophilidae | 0 |
| | Sericostomatidae | 3 |
| Megaloptera | Corydalidae | 0 |
| | Sialidae | 4 |
| Lepidoptera | Pyralidae | 5 |
| Coleoptera | Dryopidae | 5 |
| | Elmidae | 4 |
| | Psephenidae | 4 |
| Diptera | Athericidae | 2 |
| | Blephariceridae | 0 |
| | Ceratopogonidae | 6 |
| | Blood-red Chironomidae (Chironomini) | 8 |
| | Other (including pink) Chironomidae | 6 |
| | Dolochopodidae | 4 |
| | Empididae | 6 |
| | Ephydridae | 6 |
| | Psychodidae | 10 |
| | Simuliidae | 6 |
| | Muscidae | 6 |
| | Syrphidae | 10 |
| | Tabanidae | 6 |
| | Tipulidae | 3 |
| Amphipoda | Gammaridae | 4 |
| | Talitridae | 8 |
| Isopoda | Asellidae | 8 |

# APPENDIX C

## EXAMPLES OF MACROINVERTEBRATE BENCH SHEETS

# MACROINVERTEBRATE DATA SHEET

Type of Sampler_____ Sample No._____
Collection Depth_____ Date_____
Substrate Type_____ Location_____
Remarks_____

Station #_____
Identification by_____ Collector_____
Enter Family and/or Genus and Species Name on Blank Line.

| Organisms | No. | A. | I. |
|---|---|---|---|
| Diptera | | | |
| Chironomidae | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| | | | |
| Other | | | |
| | | | |
| Trichoptera | | | |
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| Plecoptera | | | |
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| Ephemeroptera | | | |
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| Odonata | | | |
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| Hemiptera | | | |
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| | No. | A. | I. |
|---|---|---|---|
| Coleoptera | | | |
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| Neuroptera and Megaloptera | | | |
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| | | | |
| Crustacea | | | |
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| Oligochaeta | | | |
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| Hirudinea | | | |
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| Bivalvia | | | |
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| Gastropoda | | | |
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| Bryozoa | | | |
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| Coelenterata | | | |
| | | | |
| Other | | | |
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A = Adult, I = Immature
Total No. Organisms_____ Total No. Taxa_____

MARINE MACROINVERTEBRATES

Name of water body_____ Sample No._____
Collected by_____ Station No._____
Sorted by_____ Date collected_____
Identified by_____

| Group* | Number of Orders | Number of Families | Number of Genera | Number of Species | Total Individuals |
|---|---|---|---|---|---|
| Porifera | | | | | |
| Hydrozoa | | | | | |
| Scyphozoa | | | | | |
| Anthozoa | | | | | |
| Ctenophora | | | | | |
| Turbellaria | | | | | |
| Rhynchocoela | | | | | |
| Echiura | | | | | |
| Priapulida | | | | | |
| Sipuncula | | | | | |
| Pogonophera | | | | | |
| Polychaeta | | | | | |
| Oligochaeta | | | | | |
| Hirudinea | | | | | |
| Monoplacophora | | | | | |
| Polyplacophora | | | | | |
| Aplacophora | | | | | |
| Bivalvia | | | | | |
| Gastropoda | | | | | |
| Scaphopoda | | | | | |
| Cephalopoda | | | | | |
| Merostomata | | | | | |
| Pycnogonida | | | | | |
| Ostracoda | | | | | |
| Cirripedia | | | | | |
| Leptostraca | | | | | |
| Stomatopoda | | | | | |
| Cumacea | | | | | |
| Tanaidacea | | | | | |
| Isopoda | | | | | |
| Amphipoda | | | | | |
| Decapoda | | | | | |
| Phoronida | | | | | |
| Bryozoa | | | | | |
| Entoprocta | | | | | |
| Brachiopoda | | | | | |
| Cinoidea | | | | | |
| Stelleroidea | | | | | |
| Echinoidea | | | | | |
| Holothuroidea | | | | | |
| Enteropneusta | | | | | |
| Pterobranchia | | | | | |
| Chaetognatha | | | | | |
| Urochordata | | | | | |
| Cephalochorsata | | | | | |

*Use separate sheet for taxa names when identified beyond group.

# APPENDIX D

## EXAMPLE OF MACROINVERTEBRATE SUMMARY SHEET

MACROINVERTEBRATE LABORATORY - Summary of Data

Water Body_____ Sampler_____

Bottom Type_____

Location_____ Depth to Sampler_____

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Organisms: | | | | | | | | | |
| Diptera | | | | | | | | | |
| Chironomidae | | | | | | | | | |
| Other | | | | | | | | | |
| Trichoptera | | | | | | | | | |
| Plecoptera | | | | | | | | | |
| Ephemeroptera | | | | | | | | | |
| Odonata | | | | | | | | | |
| Anisoptera | | | | | | | | | |
| Zygoptera | | | | | | | | | |
| Neuroptera | | | | | | | | | |
| Hemiptera | | | | | | | | | |
| Coleoptera | | | | | | | | | |
| Lepidoptera | | | | | | | | | |
| Crustacea | | | | | | | | | |
| Amphipoda | | | | | | | | | |
| Isopoda | | | | | | | | | |
| Annelida | | | | | | | | | |
| Oligochaeta | | | | | | | | | |
| Hirudinea | | | | | | | | | |
| Turbellaria | | | | | | | | | |
| Mollusca | | | | | | | | | |
| Pelecypoda | | | | | | | | | |
| Gastropoda | | | | | | | | | |
| Bryozoa | | | | | | | | | |
| Coelenterata | | | | | | | | | |
| | | | | | | | | | |
| T. Individuals | | | | | | | | | |
| T. Species | | | | | | | | | |

X = organisms present, not counted F - fragmented E - exuvia

Species Present:

SUMMARY OF MACROINVERTEBRATE DATA

STATION (LOCATION):

| ORGANISM: Date | | | | | | | | | | |
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| Total Individuals | | | | | | | | | | |
| Total Taxa | | | | | | | | | | |

## APPENDIX E

## LIST OF EQUIPMENT AND SUPPLIES

Listed below are equipment and supplies needed for the collection and analysis of macroinvertebrate samples. The data quality objectives and sampling and analysis methods should determine the type of equipment and supplies needed. The source numbers refer to the companies that are listed at the end of the table. Mention of these sources or products does not constitute endorsement by the U.S. Environmental Protection Agency.

| Item | Unit | Source |
|---|---|---|
| Boat, flat bottom, 14-16 ft snatch-block meter wheel and trailer, 18 hp outboard motor. life jackets, other accessories | 1 | (7,15) |
| Boat crane kit and winch | 1 | (3,15) |
| Boat, inflatable with oar set | 1 | (1,15) |
| Cable fastening tools | | (4,15) |
| Cable clamps, 1/8 " | 25 | |
| Nicro-press clamps, 1/8 " | 100 | |
| Nicro-press tool, 1/8 " | 1 | |
| Wire cutter, Felco | 1 | |
| Wire thimbles, 1/8 " | 25 | |
| Cable, 1/8 ", galvanized steel | 1000 ft | (3,15) |
| Large capacity metal wash tub | 1 | |
| Sample wash bucket (sieve) | 1 | (8,14) |
| Core sampler, hand held | 1 | (3,8,14) |
| Box corer | 1 | (14) |
| K-B corer | 1 | (8) |
| Wide-barrel gravity corer | 1 | (14) |
| Phleger corer | 1 | (8,14) |
| Ballchek single or multiple corer | 1 | (8,14) |
| Ewing portable piston corer | 1 | (14) |
| Hardboard multiplate sampler | 10 | (3,8) |
| Ceramic multiplate sampler | 10 | (14) |
| Trawl net | 1 | (8) |
| Dredge | 1 | (3,8,14) |
| Rectangular box sediment sampler | 1 | (14) |
| Drift net, stream | 6 | (8,14) |
| Triple-net drift sampler | 2 | (14) |
| Stream bottom sampler, Surber type | 2 | (3,8,14) |
| Portable invertebrate box sampler | 2 | (13) |
| Stream-bed fauna sampler, Hess type | 2 | (14) |
| Hess stream bottom sampler | 2 | (8) |
| Grab sampler, Ponar | 1 | (3,8,14) |

| | | |
|---|---|---|
| Wildco box corer | 1 | (8) |
| Grab sampler, Ekman | 1 | (3,8,14) |
| Grab sampler, Petersen | 1 | (3,8,14) |
| Grab sampler, Smith-McIntyre | 1 | (14) |
| Grab sampler, Van Veen | 1 | (14) |
| Grab sampler, Orange Peel | 1 | (14) |
| Grab sediment sampler, Shipek | 1 | (8) |
| Basket, bar B-Q, tumbler (#740-0035) | 12 | (9,11) |
| Sieves, US standard No. 30 | 2 | (5) |
| Flow meter, mechanical | 1 | (3) |
| Mounting media, CMCP-9/9AF with stain | 4 oz | No longer available |
| Mounting medium, CMCP-9 | 4 oz | (6) |
| Mounting medium, CMCP-10 | 4 oz | (6) |
| Fuchsin basic, C.I. dye | 25 g | (6) |
| Mounting medium, Aquamount | 4 oz | (12) |
| Refrigerated circulator | 1 | (5) |
| Water pump, epoxy-coated | 2 | (1) |
| Holding tank, constant temp | 1 | (10) |
| Balance, top-loading | 1 | (5) |
| Counter, 12-unit, 2X6 | 1 | (3) |
| Counter, hand tally | 2 | (3) |
| Waders, with suspenders | 1 pr | (1,15) |
| Boots, hip | 1 pr | (1,15) |
| Raincoat | 1 | (3,15) |
| Magni-focuser, 2X | 1 | (5) |
| Microscope, field | 1 | (3) |
| Magnifier, illuminated + base | 1 | (3) |
| Magnifier, pocket, 5X, 10X, and 15X | 1 | (3) |
| Microscope, compound, with phase and bright-field, trinocular, 10X and 15X eyepieces, 4X, 10X, 20X, 45X and 100X objectives | 1 | (5) |
| Microscope, stereoscopic, with stand | 1 | (2) |
| Microscope slide dispenser | 1 | (1) |
| Microscope slides and cover slips, 12 and 15 mm circles | 10 gross | (1) |
| Photographic system, photostar | 1 | (5) |
| Camera, photomicrographic, with 50 mm lens | 1 | (1,15) |
| Stirrer, magnetic | 1 | (5) |
| Aquarium, 10 gal., with cover, air pump and filter | 1 | (1,15) |
| Aquatic dip net, Model 412D | 2 | (3) |
| Jars, screw cap, specimen | 5 dz | (1) |
| Bottles, Wide mouth, 32 oz | 1 case | (1) |
| Specimen jars, wide mouth, 4 oz | 48 | (1) |
| Specimen jars, wide mouth, 6 oz | 48 | (1) |
| Vials, specimen, 1 oz | 10 gross | (1) |
| Petri dish, ruled grid | 4 | (1) |
| Petri dish, compartmented | 1 case | (1) |
| Watch glasses | 10 | (1) |

| | | |
|---|---|---|
| Vacuum oven | 1 | (5) |
| Sounding lead and calibrated line | 1 | (3) |
| Forceps, watchman's, stainless | 1 pr | (1) |
| Forceps, microdissection | 2 pr | (1) |
| Dissecting set, basic | 1 | (1) |
| Water test kit, limnology | 1 | (1) |
| Thermometer, digital | 1 | (1) |
| Wash bottle, wide mouth, 500 mL | 4 | (1) |
| Wash bottle, polyethylene, 4 oz | 2 | (1) |
| Dropper bottle, polystop, 30 mL | 2 | (2) |
| Desiccator, polypropylene | 1 | (1) |
| Clip board with cover | 2 | (3,15) |
| Calculator, scientific | 1 | (3,15) |
| Marker, permanent, black | 2 | (3,15) |
| Pen set, slim pack, Koh-i-noor | 1 | (3,15) |
| Heavy paper tags with string | 1000 | (1,15) |
| Ice chest, insulated, 48 qt | 2 | (3,15) |
| Blue ice, soft pack | 10 | (3,15) |
| Plastic bags | 100 | (3,15) |
| Formalin, 10 percent | 4 L | (2) |
| Ethyl alcohol | 20 L | (2) |
| Trays, polypropylene, sorting | 6 | (5) |

**Sources of equipment and supplies:**

1. Carolina Biological Supply Co.
   2700 York Rd.
   Burlington, NC 27215

2. Fisher Scientific
   50 Fadem Rd.
   Springfield, NJ 07081

3. Forestry Suppliers, Inc.
   205 West Rankin Street
   Jackson, MS 39284-8397

4. Industrial Rope Supply
   5250 River Rd.
   Cincinnati, OH 45233

5. Curtin Matheson Scientific, Inc.
   9999 Veterans Memorial Drive
   Houston, TX 77038-2499

6. Polyscience
   400 Valley Rd.
   Warrington, PA 18976

7. MonArk Boat Company
   Monticello, AK 71655

8. Wildlife Supply Company
   301 Case Street
   Saginaw, MI 48602

9. Tenaco
   2007 NE 27th Ave.
   Gainesville, FL 32609

10. Frigid Units, Inc.
    3214 Sylvania Ave.
    Toledo, OH 43613

11. W.C. Bradly Enterprises, Inc.
    P.O. Box 1240
    Columbus, GA 31993

12. Gallard-Schlesinger Chemical Mfg. Corp.
    584 Mineola Avenue
    Carle Place, NY 11514

13. Ellis-Rutter Associates
    P.O. Box 401
    Punta Gorda, FL 33950

14. Kahl Scientific Instrument Corp.
    P.O. Box 1166
    El Cajon, CA 92022-1166

15. Locally



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