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

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

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FOREWORD

Environmental measurements are required to determine the quality of ambient waters and the character of waste effluents. The Environmental Monitoring Systems Laboratory - Cincinnati (EMSL-Cincinnati) conducts research to:

- Develop and evaluate methods to identify and measure the concentration of chemical pollutants in drinking waters, surface waters, groundwaters, wastewaters, sediments, sludges, and solid wastes.

- Investigate and evaluate methods for the identification and measurement of viruses, bacteria and other microbiological organisms in aqueous samples and to determine the response of aquatic organisms to water quality.

- 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 the achievement of data quality objectives in measurements of pollutants in drinking water, surface water, groundwater, wastewater, sediment and solid waste.

- Develop methods and models to detect and quantify responses in aquatic and terrestrial organisms exposed to environmental stressors and to correlate the exposure with effects on biochemical and biological indicators.

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

Thomas A. Clark
Director
Environmental Monitoring Systems
Laboratory - Cincinnati

PREFACE

The Bioassessment and Ecotoxicology Branch, Ecological Monitoring 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 status and/or trends monitoring for 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.

This manual contains field and laboratory fish methods for evaluating the health and biological integrity of fresh, estuarine, and marine waters. The manual is a revision and enlargement of the chapter on fish 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, which were developed by the Bioassessment and Ecotoxicology Branch, Environmental Monitoring Systems Laboratory - Cincinnati, at the request of the Biological Advisory Committee to provide biomonitoring programs with methods for assessing point and nonpoint sources of impacts, status and trends in water quality monitoring.

ABSTRACT

This manual contains biocriteria and describes guidelines and standardized methods for using fish in evaluating the health and biological integrity of surface waters and for protecting the quality of water resources. Included are sections on quality assurance and quality control procedures; safety and health recommendations; fish collection techniques; specimen processing techniques; identification and taxonomic references; fish age, growth, and condition determinations; data recording; length-frequency; length-age conversion; annulus formulation; relative weight index; flesh tainting; fish kill investigation; bioassessment protocols for use in streams and rivers; family-level ichthyoplankton index; fish health and condition assessment; guidelines for fish sampling and tissue preparation for bioaccumulative contaminants; and an extensive bibliography for fisheries.

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SECTION 1

INTRODUCTION

1.1 This manual was prepared to assist biologists and managers in USEPA and other Federal, state, and private water monitoring organizations in the use of fish as indicators of ecosystem health and for evaluating the biological integrity of surface waters and protecting quality water resources. The manual contains biological criteria and laboratory and field methods that will aid in the monitoring and bioassessment of the effects of anthropogenic and environmental stresses on fish populations and communities. It will also facilitate the expansion and refinement of our knowledge of the ecological requirements of fish species in freshwater, estuarine, and marine habitats.

1.2 The manual includes sections on quality assurance and quality control, safety and health, sampling methods and techniques, sample preservation and identification, data analyses, special techniques, bioassessment protocols for use in streams and rivers, a family-level ichthyoplankton index method, fish health and condition assessment procedures, guidelines for fish sampling and tissue preparation for bioaccumulative contaminants, and a fisheries bibliography. Guidelines and procedures for fish kill investigations are provided.

1.3 Fish community evaluation and assessment should measure the overall structure (number of species and individuals within a community) and function (organism interaction in the utilization of food and other biological resources) of various aquatic habitats considered for study. These measurements should include such factors as habitat characteristics and quality, riparian vegetation, and hydraulic characteristics that are expected to influence fish community spatial and temporal variability. One must also distinguish the alterations induced by anthropogenic activities from natural variations which occur in the environment.

1.4 In North America, fish are the focus of economically important sport and commercial fisheries, and are an important source of food for humans. To the general public the size and species composition of a fish community is the most meaningful index of pollution.

1.5 In most aquatic ecosystems, fish are usually the most common vertebrates. Fish communities occupy the upper trophic levels of aquatic food webs, and they are dependent on the same or other trophic level life forms for food. In aquatic communities fish can be one of the most sensitive indicators of water quality assessment and biological integrity in aquatic environments (Angermeier et al., 1991; Fausch et al., 1990; Karr, 1981, 1987, 1990, 1991; Smith, 1971; McKenzie et al., 1992). The literature contains much data on fish species distribution, life histories, ecology, pollution tolerance, and environmental requirements. Fish are directly and indirectly affected by chemical and physical changes in the environment, and the population or community of fish in rivers, streams, lakes, estuaries, and oceans reflects the state of the health of the aquatic environment or watershed as a whole.

Because they are conspicuous, fish populations or fish assemblages are commonly used as environmental indicators or as an index for water quality (Table 1).

1.6 Water quality conditions that significantly affect the lower levels of food webs (e.g., plankton and benthic invertebrates, including macroinvertebrates, USEPA, 1990a) will affect the abundance and species composition of the fish population. In some cases, fish may exhibit signs of being more sensitive to certain pollutants than are the lower animals and plants, and may be adversely affected even when the lower levels of food webs are relatively unharmed.

1.7 Karr (1981, 1987), Karr et al. (1986, 1987), Ohio EPA (1990), and USEPA (1990a,b) have indicated that five major sets of abiotic and biotic factors affect and ascertain biological integrity or water resource integrity (Table 2). To determine anthropogenic or natural impact on aquatic ecosystems, all monitoring or bioassessment programs must survey and evaluate in a methodical and systematic way all five sets of factors. Although a thorough discussion of all these factors is beyond the scope of this document, a discussion of how some of these factors influence the biological integrity of surface waters and several methods and procedures in evaluating these complex set of factors are presented here. For a more comprehensive discussion of all these factors, consult USEPA (1990a, 1990b), Ohio EPA (1990), and the references in Section 12, Fisheries Bibliography.

1.8 Many species of fish have stringent dissolved oxygen and temperature requirements and are intolerant to chemical and physical contaminants resulting from municipal, agricultural, industrial, forestry, and mining activities. Also, fish communities are sensitive to and good indicators of macrohabitat disturbances (Rankin, 1989).

1.9 The discharge of moderate amounts of degradable organic wastes may increase the nutrient levels (eutrophication) in the habitat and result in an increase in the standing crop (total amount of the biomass of organisms of one or more species within a locality) of fish. This increase usually occurs in one or a few species and results in an imbalance in the population. The discharge of large amounts of degradable organic materials may result in depressed oxygen levels which may reduce the number and kinds of fishes present and increase the standing crop of pollution tolerant species. In extreme cases the fishery may be eliminated in the affected area.

1.10 The effects of toxic wastes may range from the elimination of most fish to a reduction in reproductive capacity (fecundity) or resistance to disease and parasitism. Massive and complete fish kills are dramatic signs of abrupt, adverse changes in environmental conditions. Fish, however, can repopulate an area rapidly if the habitat is not destroyed and the water quality improves. The cause of the fish kill may be difficult to detect by examination of the fish community after it has recovered from the effects of the pollutant. Chronic pollution, on the other hand, is more selective in its effects, exerts its influence over a long period of time, and causes recognizable changes in the species composition and relative abundance of the fish.

TABLE 1. ATTRIBUTES OF FISHES AND DESIRABLE COMPONENTS FOR BIOASSESSMENT AND BIOMONITORING PROGRAMS[1]

Goal/Quality	Attribute
Accurate Assessment of Aquatic Ecosystem Integrity	Fish populations and individuals generally remain in the same area during summer seasons.
	Communities are persistent and usually recover rapidly from natural disturbances. Comparable results can be expected from an unperturbed site at various times within a season.
	Fish have larger home ranges and are less affected by natural microhabitat differences than smaller organisms, such as macroinvertebrates. This makes fish extremely useful for assessing regional, macrohabitat, and mesohabitat differences.
	Most fish species have long life spans (3-10+ years) and can reflect both long term and current water resource quality.
	Fish continually inhabit the receiving water and reflect the chemical, physical, and biological histories of the water.
	Fish represent a broad spectrum of community tolerances from very sensitive to highly tolerant, and respond to chemical, physical, and biological degradation in characteristics response patterns.
Visibility	Fish are a highly visible component of the aquatic community, and so are of interest to the public.
	Aquatic resource uses and regulatory language are generally characterized in terms of fish (i.e., fishable and swimmable goals of the Clean Water Act).
Ease of Use and Interpretation	The sampling frequency for trend assessment is less than for short-lived organisms.
	The taxonomy of fishes is well established, allowing professional biologists the ability to reduce laboratory time by identifying many specimens in the field.
	The distribution, life histories, and tolerances to environmental stresses of most North American species are well documented in the literature.

[1]Adapted from Simon (1991).

TABLE 2. FIVE MAJOR CLASSES OF ENVIRONMENTAL FACTORS WHICH INFLUENCE AND DETERMINE THE BIOLOGICAL INTEGRITY OF SURFACE WATERS WITH SOME OF THEIR IMPORTANT CHEMICAL, PHYSICAL, AND BIOLOGICAL COMPONENTS IN LENTIC AND LOTIC SYSTEMS[1]

STREAMS, RIVERS	LAKES, RESERVOIRS, ESTUARIES, OCEANS
1. ENERGY SOURCE	
Nutrient cycling	Nutrients cycling
Organic matter particle size	Organic matter particle size
Primary productivity	Primary productivity
Seasonal cycles	Seasonal cycles
Solar radiation	Solar radiation
2. WATER QUALITY/CHEMICAL VARIABLES	
Adsorption	Adsorption
Alkalinity	Alkalinity
DO	DO
Hardness	Hardness
Metals, other toxic substances	Metals, other toxic substances
Nutrients	Nutrients
Organics	Organics
pH	pH
Solubility	Solubility
Temperature	Temperature
Turbidity	Turbidity
Water cycling	Water cycling
3. HABITAT QUALITY	
Bank stability	Bank stability
Canopy	Shoreline vegetation
Channel morphology (riffles, pools)	Substrate types
Current velocity	Siltation
Gradient	Wave action
Instream cover (woody debris)	Width/depth
Riparian vegetation	Inwater abiotic/biotic cover
Siltation	
Sinuosity	
Substrate types	
Width/depth	

[1]Adapted from Karr (1987, 1991), Karr and Dudley (1981), Karr et al. (1986, 1987), and USEPA (1990a; 1990b).

TABLE 2. FIVE MAJOR CLASSES OF ENVIRONMENTAL FACTORS WHICH INFLUENCE AND DETERMINE THE BIOLOGICAL INTEGRITY OF SURFACE WATERS WITH SOME OF THEIR IMPORTANT CHEMICAL, PHYSICAL, AND BIOLOGICAL COMPONENTS IN LENTIC AND LOTIC SYSTEMS (CONTINUED)

4. FLOW REGIME

STREAMS, RIVERS	LAKES, RESERVOIRS, ESTUARIES, OCEANS
Ground water	Ground water
High/low extremes	High/low extremes
Land use	Land use
Precipitation/runoff	Precipitation/runoff
Water volume	Water volume

5. BIOTIC ASSOCIATIONS

STREAMS, RIVERS	LAKES, RESERVOIRS, ESTUARIES, OCEANS
Feeding	Feeding
Competition	Competition
Disease	Disease
Parasitism	Parasitism
Predation	Predation
Reproduction	Reproduction

1.11 The utilization of biological components (structural and functional) to evaluate the ambient aquatic community of our nations surface water has been discussed and well documented in the literature. Some recent examples are Crowder (1990), Downing et al. (1990), Fausch et al. (1990), Hunsaker and Carpenter (1990), Karr et al. (1986), Karr, (1991), Ohio EPA (1987a, 1987b, 1989, 1990), Plafkin et al. (1989), Shuter (1990), Simon (1991), and USEPA (1990a, 1990b). Structural components of fish communities include diversity, taxa guilds, numbers, and biomass. Functional components of fish communities include the feeding or trophic strategy, reproductive behavior and guild classification, and environmental tolerance to perturbations.

1.12 The principal characteristics of interest in bioassessment studies of fish populations include: (1) species richness (number of species)--presence or absence; relative and absolute abundance of each species, (2) size distribution, (3) habitat guilds--pelagic, littoral, and benthic species, (4) trophic guilds--omnivores, piscivores, and invertivores, (5) growth rate, (6) condition factor, (7) reproductive guilds, egg production and success, (8) general tolerance guilds (indicator taxa)--intolerant, tolerant, and sensitive species, (9) incidence of disease and parasitism (10) fish kills, (11) palatability, and (11) fishability--catchability, desirability, and sustainability. Observations of fish behavior can also be valuable in detecting environmental problems, e.g., ventilation rates, position in the current, and erratic movement. Fish may also be utilized for field and laboratory bioassays (USEPA, 1991a, 1991b, 1992a, 1992b), for tissue analyses to measure the concentrations of metals and pesticides (see Section 10, Guidelines for Fish Sampling and Tissue Preparation for bioaccumulative Contaminants) for histopathologic examination (Hinton and Lauren, 1990), and biomarker studies (Adams, 1990a, 1990b; Anderson, 1990; Jimenez and Stegeman, 1990; Rice, 1990; Schreck, 1990; and Thomas, 1990).

1.13 Fisheries data are useful in enforcement cases and in long-term water quality status and trends monitoring (Tebo, 1965; Ohio EPA, 1990; USEPA, 1991a). Before fishery surveys are initiated, a careful and exhaustive search should be conducted for existing information on the fish populations or communities in question. State and Federal fishery agencies and universities may be potential sources of information. If data are not available and a field study must be conducted, State and other Federal agencies may assist in a survey and may provide needed expertise and specialized equipment for the collection of specific, local fishes. A joint effort is usually more economical and efficient and will promote continued cooperation between agencies and parties involved.

1.14 Fisheries data may have limitations. Even if the species composition of the fish in a specific area is known before and after the discharge of pollutants, the significance of changes in the catch might not be satisfactorily interpreted unless there are adequate data on spawning, seasonal migration, temperature requirements and stream-flow responses, feeding activities, diurnal movements, habitat preferences, and activity patterns. Without adequate data, fish presence or absence cannot be directly correlated with water quality. Furthermore, any existing data of known quality on the water quality requirements of fish would be of value in interpreting field data.

1.15 Federal and state regulations usually require a fish collecting permit because some species of fish are protected by law, and the collection of others is regulated. The state fishery agencies must be contacted before fish can be taken in a field study. Investigators should confirm that they have complied with federal and state regulations before collecting samples of fish. The state should be contacted prior to any fish study to ensure that investigators comply with current regulations.

1.16 The design of fish 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 by investigators involved in fish sampling programs and studies. Useful references include Adams (1990), Angermeier et al. (1991), APHA (1992), Bartell (1990), Edwards and Megrey (1989), Evans et al. (1990), Everhart and Youngs (1981), Fausch et al. (1990), Gammon (1980), Gammon et al. (1990), Hankin and Reeves (1988), Hellawell (1986), Herricks and Schaeffer (1985), Hirsch et al. (1988), Hughes et al. (1986), Johnson and Nielsen (1983), Karr (1981, 1987, 1990, 1991), Karr and Dionne, 1991, Karr and Dudley (1981), Karr et al. (1983, 1986, 1987), Magnuson (1991), Manci (1989), Mangel and Smith (1990), Minshall et al. (1989), Ohio EPA (1986, 1987a, 1987b, 1989, 1990), Omernik (1987), Platts et al. (1983), Robins et al. (1991), Schreck and Moyle (1990), Templeton (1984), Tonn (1990), USEPA (1988), USEPA (1990a, 1990b), (USEPA, 1991c, 1991d, 1991e), Whittier and Paulsen (1992), Wooten (1990), and Yoder (1991).

1.16.1 If fish data are to be useful, they must be acquired according to standardized sampling methods and analyzed with appropriate statistical methods. Two very important qualities of sampling data are accuracy and precision. Accuracy refers to how well the sample represents the whole of the study. In fishery studies, collecting accurate (or unbiased) data may be difficult because studies are poorly designed. Precision refers to repeatability of data. To supplement the statistics in this document, investigators should consult the commonly cited statistical references (Cochran, 1977; Conover, 1980; Green, 1979; Hicks, 1982; Snedecor and Cochran, 1981; Sokal and Rohlf, 1981; Zar, 1984).

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SECTION 2

QUALITY ASSURANCE AND QUALITY CONTROL

2.1 Introduction

2.1.1 Fish studies, like macroinvertebrate studies (USEPA, 1990a), require a strong quality assurance (QA) program and effective quality control (QC) procedures that encompass field and laboratory data collection activities. The term "quality assurance" refers to an integrated system of activities involving planning, quality control, quality assessment, reporting and quality improvement to ensure that a product or service meets defined standards of quality with a stated level of confidence. The term "quality control" refers to the overall system of technical activities whose purpose is to measure and control the quality of a product or service so that it meets the needs of users. The aim is to provide quality that is satisfactory, adequate, dependable, and economical (modified from USEPA, 1974; 1978).

2.1.2 Quality assurance programs have two primary functions in a biomonitoring/bioassessment laboratory. First, the project or program should define the data quality needed for the program's goals in terms of accuracy, precision, representativeness, comparability, and completeness (see Subsection 2.6, Fish Collection). The second function is to provide information on the success with which the measurement data meet these goals.

2.1.3 Quality assurance and quality control (QA/QC) must be a continuous process in the biomonitoring/bioassessment program that includes all aspects of the program, including field collection and preservation, habitat assessment, sample processing, data analysis, and reporting. Otherwise, the data generated may not be reliable and useful for decision making, and the results will be of little use in assessing and establishing the conditions (health, biological integrity, and quality of the water resources) of the water body under study. Without an appropriate program of quality assurance and quality control, data will be of unknown quality, limiting its interpretation and usefulness. Quality must be assured before the results can be accepted with any scientific studies. As described below, quality assurance is accomplished through establishment of thorough investigator training, protocols, guidelines, comprehensive field and laboratory data documentation and management, verification of data reproducibility, and instrument calibration.

2.1.4 To support the operation of a consistent plan, the persons responsible for QA should consult the EPA Quality Assurance manual (USEPA, 1984a; 1984b; 1989; 1992b). All EPA QA programs are implemented and operated under the authority of EPA Order 5360.1. USEPA (1984b) serves as guidance and describes the policy, objectives, and responsibilities of all USEPA programs, regional offices, and laboratories producing data for USEPA to institute a specific QA program. Each office or laboratory that generates data under USEPA's QA/QC program must implement, at a minimum, the prescribed procedures to ensure that precision, accuracy, completeness, comparability, and representativeness of data are known and documented.

2.1.4.1 Information and discussion of statistical tools, data quality objectives, comparison of good laboratory and field practices, and other quality assurance considerations in the context of ecological research are found in USEPA (1992b). Each agency should have a designated QA/QC officer (or a person in charge of the program) responsible for reviewing project plans, SOPs, etc. and auditing the program for improving performance, etc.

2.1.5 The Fish Bioassessment Protocols for Use In Streams and Rivers, Section 8, can be modified to achieve various data quality objectives. A different habitat assessment approach, replicate sampling, more intensive sample enumeration, or modified analytical metrics may be preferred by a particular State over the approaches in this Section. Such refinements can be accommodated, provided they are clearly documented in an USEPA approved QA program and/or project plan.

2.1.6 Components of the QA program (Khalil and Tuckfield, 1992; USEPA, 1984a; 1984b; 1990a; 1991a; 1992a; 1992b) should include the following:

2.1.6.1 Approved methodology and documentation for the collection, preservation, and analysis of data.

2.1.6.2 Documentation and manufacturer's instructions for sampling equipment, flow measuring devices, and other measuring instruments such as pH, DO, and conductivity meters.

2.1.6.3 Methods and documentation to assure that representative samples are collected (See Subsection 2.2, Data Quality Objectives and Subsection 2.8, Standard Operating Procedures).

2.1.6.4 Methods and documentation to assure the precision of sampling and analysis procedures. Collecting precise fish data usually requires extensive sampling as well as careful design.

2.1.6.5 Methods to assure accurate and timely recording, storage, and retrieval of data.

2.1.6.6 Documentation to assure sample evaluation, statistical evaluation, and performance evaluation of laboratory procedures.

2.2 Data Quality Objectives

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 completeness, type, and amount of error associated with a data set. 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 and the rationale behind those statements and levels of data quality. Both qualitative and quantitative descriptors of data quality must be considered to determine whether data are appropriate or adequate for a particular application. However, DQOs are target values and not necessarily

criteria for the acceptance or rejection of data (Table 1). Table 1 is a summary listing QA objectives for precision and completeness. Data quality requirements should be based on prior knowledge of the sampling procedures or measurements system by use of replicate (duplicate) analyses, reference conditions (site-specific or ecoregional), or requirements of the specific project (USEPA, 1989).

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 the 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 examination of the 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 upon the criteria agreed upon in the second stage. It may be necessary to modify the objectives of the study during the development of the DQOs. Details for developing DQOs are described in USEPA (1986; 1989). These documents are available from the Quality Assurance Management Staff, Office of Research and Development, Washington, DC 20460 and the Center For Environment Research Information (CERI), U.S. Environmental Protection Agency, Cincinnati, OH 45268. The CERI information and document ordering phone number is (513) 569-7562. Johnson and Nielsen (1983), Ohio EPA (1989), and Simon (1991) discuss sampling considerations for collecting fish data.

2.2.3 After the 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; 1989; 1992b). These 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 general 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 programs and project plans are in conformance with grant requirements specified in 40 CFR Part 30, and for assisting the states in developing DQOs requirements and Quality Assurance Program Plans (QAPP) that meet state needs (USEPA, 1989).

2.2.5 Regional and state laboratories or monitoring personnel in need of specific guidance in preparing Quality Assurance Project Plans or development of DQOs for bioassessment projects can contact personnel of the Bioassessment and Ecotoxicology Branch in the Ecological Monitoring Research Division, Environmental Monitoring Systems Laboratory-Cincinnati, OH for assistance ((513) 533-8114, FAX (513) 533-8181).

TABLE 1. EXAMPLE OF SUMMARY TABLE FOR DATA QUALITY REQUIREMENTS[1]

Measurement Parameter	Reference	Precision (RPD[2], RSD[3])	Completeness (%)
Benthos	Plafkin et al. (1989)		
No. Individuals		50	95
No. Taxa		15	95
Fish	Karr et al. (1986)		
No. Individuals		25	95
No. Species		15	95
Dissolved Oxygen (mg/L)	ASTM (1992)	5	90
Water Temperature °C	ASTM (1992)	5	90

[1]From USEPA (1992b).
[2]RPD = Relative percent difference.
[3]RSD = Relative standard deviation.

2.3 Facilities And Equipment

2.3.1 Laboratory, field facilities, and equipment must be in place and operating consistently 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 environmental controls (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 (boats, sampling gear, etc.) and supplies necessary to adequately collect, preserve and process fish and other biological samples must be available and in good operating condition. See Section 4, Sample Collection for Analysis of the Structure and Function of Fish Communities, Table 3, General Checklist Of Fish Field Equipment And Supplies.

2.3.3 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 individual 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.

2.4 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 (pH and DO meters, etc.) must be calibrated before each use according to the manufacturer's instructions, and routine checks using National Institute of Standards and Technology standards, 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. All samples collected and field data sheets should also be assigned a unique identification number and label. Data should be documented to allow complete reconstruction, from initial field record through data storage system retrieval.

2.4.3 Sample tracking is important, but 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 a written record (Figures 1 and 2) 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 discussed in four USEPA manuals (USEPA, 1974; 1990b; 1991a; 1992b).

2.4.4 Field and laboratory personnel should keep complete, 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. The field data sheets and sample tags (see Section 4, Sample Collection for Analysis of the Structure and Function of Fish Communities; Section 5, Fish Specimen Processing; Section 8, Fish Bioassessment Protocols For Use In Streams and Rivers) should be filled out as completely and as accurately as possible to provide a record in support of the survey and analysis conclusion. Abbreviations commonly used in documentation (e.g., scientific names) should be standardized to decrease data manipulation error. Field and laboratory data sheets and final reports should be filed. 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 permanently filed in a secure fireproof location.

Project No.	Station I.D.	Month Day Year	Time	Designate: Comp.	Designate: Grab

Tag No.	Station Location	Samplers (Signatures)
4A-20543		

Lab Sample No.	Remarks:						Cyanide	Volatile Organics	Pesticides/PCB's	Extractable Organics	Metals	BOD, Solids	COD, TOC, Nutrients	ANALYSES	Preservative: No ☐ Yes ☐: ____

Figure 1. Example of sample identification tag. From USEPA (1990b) and USEPA (1991a).

2.5 Habitat Assessment

2.5.1 Because the habitat characterization procedures (see Section 4, Sample Collection for Analysis of the Structure and Function of Fish Communities and Section 8, Fish Bioassessment Protocols for Use in Streams and Rivers) are primarily a qualitative evaluation, final conclusions are potentially subject to variability among investigators. This limitation can be minimized however, by ensuring that each investigator is appropriately trained in the habitat evaluation techniques and periodic cross-checks are conducted among investigators to promote consistency. Also, bioassessment laboratories should institute one or two day training courses on habitat characterization and evaluation followed by periodic refresher training. For additional information and discussion on habitat evaluation and a Qualitative Habitat Evaluation Index (QHEI), see Barbour and Stribling (1991), Plafkin et al., (1989), Ohio EPA (1989), Rankin (1989), and USEPA (1990a; 1991b) for additional information and discussion on habitat evaluation and a Qualitative Habitat Evaluation Index (QHEI), regarding rationale, methods, and application for fish bioassessment. Also, see Section 4, Sample Collection for Analysis of the Structure and Function of Fish Communities, Subsection 4.1.5, Habitat Evaluation and Section 8, Fish Bioassessment Protocols For Use In Streams and Rivers, Subsection 8.13.3, Habitat Quality and Assessmemt.

PROJECT NO.	PROJECT LEADER	REMARKS
PROJECT NAME/LOCATION		

SAMPLE TYPES

1. SURFACE WATER
2. GROUND WATER
3. POTABLE WATER
4. WASTEWATER
5. LEACHATE
6. SOIL/SEDIMENT
7. SLUDGE
8. WASTE
9. AIR
10. FISH
11. OTHER _____

SAMPLERS (SIGN)

CIRCLE/ADD parameters desired. List no. of containers submitted

STATION NO.	SAMPLE TYPE	19 __ DATE	TIME	COMP	GRAB	STATION LOCATION/DESCRIPTION	TOTAL CONTAINERS	COD, TOC, NUTRIENTS	BOD, SOLIDS	METALS	EXT ORG/PEST/PCBs	VOA	CYANIDE				TAG NO./REMARKS	LAB USE ONLY

RELINQUISHED BY: (PRINT) / (SIGN)	DATE/TIME	RECEIVED BY: (PRINT) / (SIGN)	RELINQUISHED BY: (PRINT) / (SIGN)	DATE/TIME	RECEIVED BY: (PRINT) / (SIGN)

DISTRIBUTION: White and Pink copies accompany sample shipment to laboratory; Pink copy retained by laboratory; White copy is returned to samplers; Yellow copy retained by samplers.

*U.S. GPO: 1989-732-186

4-20043 (10/89)

Figure 2. Example of a chain-of-custody record form. Modified from USEPA (1990b), USEPA (1991a), and USEPA, Region 4.

2.6 Fish Collection

2.6.1 Ensuring that fish field survey data are representative of the fish assemblage at a particular site requires careful regional analysis and station evaluation. Data comparability is maintained by using similar collection methods and sampling effort in waterbodies (lakes, reservoirs, estuaries, wetlands, streams, rivers, etc.) of similar size . Also, where possible, major habitats in streams (riffle, run, pool) are sampled at each site, and the proportion of each habitat type sampled should be noted.

2.6.2 Precision, accuracy, and completeness should be evaluated in pilot studies along with sampling methods and site size. Variability among replicates from the same site or similar sites should not produce differences exceeding 10 percent at minimally impacted sites and 15 percent at highly impacted sites (Plafkin et al., 1989). Index of Biological Integrity (IBI) differences at the same site should not exceed 4 (Karr et al., 1986).

2.6.3 Data reproducibility may be ensured by having a variety of investigators periodically resample well characterized sites. Investigator precision and accuracy for use of the Index of Biological Integrity (IBI) and the Index of well-being (Iwb) may be determined by having investigators evaluate a standard series of data sets or preserved field collections.

2.6.4 Taxonomists, fishery staff, and aquatic biologists should be capable of identifying fish to the lowest possible level (species, subspecies) and should have at their disposal adequate taxonomic references to perform the level of identification required. See Section 12, Fisheries Bibliography, for a list of selected taxonomic references. Fishery and aquatic biologists should check this list and obtain those references that will be needed for the identification of specimens.

2.6.5 Field identifications are acceptable, but laboratory voucher specimens are always required for new locality records, new species, and any specimens that cannot be identified in the field. All specimens should be retained for laboratory examination if there are any doubts about the correct identification. Biomonitoring laboratories that do not identify fish and other taxa on a regular basis or that have difficulty identifying organisms should have representative specimens of all taxa verified by a specialist who is a recognized authority in that particular taxonomic group. These specimens must be properly labeled as reference or voucher specimens, including the name of the verifying authority, permanently preserved, and stored in the laboratory, or voucher specimens should be offered to regional and state natural history museums for future reference.

2.6.6 Quality control of taxonomic identifications is accomplished by a second qualified individual.

2.7 Qualifications and Training

2.7.1 All personnel need to have adequate education, training, and experience in the areas of their technical expertise, responsibilities, and in quality

assurance (QA). Because no formal academic programs in research QA exist, most QA experience must be acquired through on-the-job training.

2.7.2 At least one professional biologist with training and experience in fish sampling methods and fish identification should be involved directly in the field work or should be involved for at least the first two weeks of the field sampling season (and thereafter if necessary), instructing other less qualified staff in all aspects of the field sampling as well as the laboratory analysis of the samples to ensure data quality. Additionally, the investigators should be familiar with the objectives of each site investigation. Periodic conferences with the sampling crew to assure the sampling effort is being conducted in accordance with the standard operating procedures are also advisable. Statistical expertise should be readily available and consulted during every phase of the project.

2.7.3 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.

2.7.4 Project personnel should have on file an up-to-date resume for each person who is responsible for the collection, analysis, evaluation and reporting of biological data.

2.8 Standard Operating Procedures (SOPs)

2.8.1 Each laboratory should define the precise methods to be used during each step of the collection, analysis, and data evaluation process. These written procedures become the standard operating procedures (SOPs) describing the operation of the laboratory (USEPA, 1991a). Standard operating procedures for a fish laboratory should describe in stepwise fashion, easily understood by the potential user, at least the following:

1. Sampling methodology, including maintenance of electrofishing gear and seines

2. Replication (duplication)

3. Habitat assessment methodology

4. Sampling site and station selections (including reference sites)

5. Details of preservation and labeling of the samples

6. Use of taxonomic keys

7. Use and calibration of measuring instruments (e.g., DO, pH, and conductivity meters, etc.) and QC requirements

8. Sample chain-of-custody and handling procedures

9. Data analysis, evaluation, and handling

2.8.2 The SOPs must include a listing of the taxonomic keys and references that should be used for each level of identification required and for each taxonomic group. Field experience and taxonomic expertise requirements of personnel for the particular level of bioassessment performed must be defined in the preparation of DQOs. It should also provide an outline of the steps to be taken to assure the quality of the data.

2.8.3 The SOPs must stress the need for the traceability of the fish samples. At a minimum it should specify that the fish sample be assigned a unique identification number and be properly labeled with the sample number, sampling location, date, and name of the collector (see Section 5, Specimen Processing Techniques for an example of sample tags). It should describe procedures to ensure that each sample collected, as accurately and precisely as possible, represents the fish community sampled.

2.8.4 The SOPs should be approved by the proper authority and must be easily accessible to all appropriate personnel for referral.

2.8.5 The laboratory SOPs must 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.

2.8.6 Field validation, conducted at a frequency to be determined by each agency, should involve two procedures: (1) collection of replicate samples at various stations to check on the precision and accuracy of the collection effort, and (2) repeat field collections and analyses performed by separate field crews to provide support for the bioassessment. In addition, field crews should occasionally alternate personnel with the same field training to maintain objectivity in the bioassessment study.

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SECTION 3

SAFETY AND HEALTH

3.1 Introduction

3.1.1 Collection and analysis of fish samples can involve significant risks to personal safety and health (drowning, electrical shock, pathogens, etc.). While safety is often not considered an integral part of a fish sampling routine, the biologist must be aware of unsafe working conditions, hazards connected with the operation of sampling gear, boats, and other risks (Berry et al., 1983). Management should assign health and safety responsibilities and establish a program for training in safety, accident reporting, and medical and first aid treatment. The laboratory safety document and standard operating procedures (SOPs) containing necessary and specific safety precautions should be available to all persons involved in fish sample collecting and processing. Field and laboratory safety requirements for biomonitoring laboratories are found also in USEPA (1986) and Ohio EPA (1990).

3.2 General Precautions

3.2.1 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 Field personnel and sampling crew must have mandatory training in Red Cross first aid, cardiopulmonary resuscitation (CPR), boating and water safety, field survey safety (weather conditions, personal safety, and vehicle safety), presurvey safety requirements (equipment design, equipment maintenance, reconnaissance of survey area), and electrofishing safety (Ohio EPA, 1990). It is the responsibility of the group safety officer or field sampling leader to ensure that the necessary safety courses are taken by all field personnel and that all safety policies and procedures are followed.

3.2.3 Operation of fish sampling devices involves potential hazards that must be addressed by the individuals using the equipment. Electrofishing equipment should be operated carefully. Electrofishing should always be done with at least three individuals, and all safety procedures must be followed. Persons using these devices should become familiar with the hazards involved and establish appropriate safety practices prior to using them (Reynolds, 1983; Ohio EPA, 1990). **Note:** Individuals involved in electrofishing must be trained by a person experienced in this method or by attending a certified electrofishing training course (See Section 4, Sample Collection for Analysis of the Structure and Function of Fish Communities, Subsection 4.3 Electrofishing and Ohio EPA, 1990).

3.2.4 Field personnel should be able to swim. Waders should always be worn with a belt to prevent them from filling with water in case of a fall. The

use of a life jacket is advisable at dangerous wading stations if one is not a strong swimmer because of the possibility of sliding into deep water.

3.2.5 Individuals sampling with scuba gear must be certified. The hazards of sampling with scuba gear are sufficiently great that certification is mandatory.

3.2.6 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 can be absorbed through the skin or inhaled may also be present in the water or sediment.

3.2.7 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).

3.2.8 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.

3.2.9 Safety equipment and first aid supplies must be available in the laboratory and in the field at all times. All motor vehicles and boats with motors must have fire extinguishers, boat horns, cushions, and flares or communication devices.

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 must be available and used in accordance with the project safety plan.

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

3.3.3 A properly installed and operating hood must be provided in the laboratory for use when working with carcinogenic chemicals (e.g., formaldehyde) that may produce dangerous fumes.

3.3.4 Communication equipment and posted emergency numbers must 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 must 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, or equivalent, should be suitable for this purpose.

3.4 Field and Laboratory Operations

3.4.1 At least two persons (three persons for electrofishing) must be present during all sample collection activities.

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 must bear the approval of Underwriters Laboratories and must be properly grounded to protect against electric shock.

3.4.4 Use a winch for retrieving large fish nets, trawls, etc., for samples collected with heavy sampling devices, and use care in lifting heavy items to prevent back injury.

3.4.5 Persons working in areas where poisonous snakes may be encountered must 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 more than an hour away, carry a snake bite kit and be familiar with its use. Any person allergic to bee stings or other insect bites must take proper precautions and have any needed medications handy.

3.4.6 Personnel participating in field activities on a regular or infrequent basis should be in sound physical condition and have a physical exam annually or in accordance with Regional or State Safety requirements.

3.4.7 All field personnel should be familiar with the symptoms of hypothermia and know what to do in case symptoms occur. Hypothermia can kill a person at temperatures much above freezing (up to 10°C or 50°F) if he or she is exposed to wind or becomes wet.

3.5 Disease Prevention

3.5.1 Unknown pollutants and pathogens in surface waters and sediments 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 or animal wastes that carry causative agents or pathogens must be immunized against tetanus, hepatitis, typhoid fever, and polio. Field personnel should also protect themselves against the bite of deer or wood ticks because of the potential risk of acquiring pathogens that cause Rocky Mountain spotted fever and Lyme disease.

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SECTION 4

SAMPLE COLLECTION FOR ANALYSIS OF THE STRUCTURE AND FUNCTION OF FISH COMMUNITIES

4.1 General Considerations

4.1.1 A variety of methods, techniques, and equipment exist to sample fish populations and communities in lentic and lotic habitats. In addition, many procedures are available to analyze the fish data collected. Each technique has different assumptions, advantages and disadvantages. It is important to understand the attributes and characteristics of sampling equipment and techniques used in fish biosssessment so that valid conclusions can be drawn from the data. Sampling considerations and design (APHA, 1992; Lagler, 1956; Johnson and Nielson, 1983; Schreck and Moyle, 1990; Section 2, Quality Assurance and Quality Control) are important because aquatic biologists or fisheries scientists spend a major part of their time collecting data and the study results are determined by use of the data with a variety of techniques and equipment for an assortment of studies. Since fish populations are usually nonrandomly distributed and clumped in response to many habitat variables (Allen et al., 1992; Hendricks et al., 1980), the choice of sampling methods and equipment, the habitat and time of sampling, and frequency of sampling will depend on the data quality objectives of the study. For practical considerations, it is often easier to sample at certain places or time of the year (e.g., shallow water areas or during low flow). Therefore, all sampling gear is generally considered selective in sample collection to some degree (Everhart et al., 1975; Gulland, 1980; Henderson, 1980; Lagler, 1956, 1978; Ricker, 1971; Schnick and Moyle, 1990; Yeh, 1977; Zippin, 1956, 1958). Some procedures to reduce sampling bias through better sampling design are found in Armour et al. (1983), Cyr et al. (1992), Gulland (1980); Johnson and Nielsen (1983). The accurate and efficient collection of data can mean the difference between a successful management and research effort and a study that might end with inconclusive or inappropriate data.

4.1.2 In all bioassessment studies key physical, chemical, and biological indicators or parameters to be monitored should be selected carefully for the most direct cause and effect relationships. Some important indicators or parameters of biological integrity for consideration are found in Table 1. For a discussion of these variables and others, see Armour et al. (1983), Lagler (1956, 1978), Orth (1983), Plafkin et al. (1989), Rankin (1989), Ohio EPA (1987a, 1987b, 1989), and Section 8, Fish Bioassessment Protocols for Use In Streams and rivers, Subsection 8.13 Habitat Assessment and Physical/ Chemical Parameters, and references in Section 12, Fisheries Bibliography.

4.1.3 Table 2 is a general list of equipment and supplies needed for the collection of fish samples and biosurvey. The data quality objectives (DQOs), standard operating procedures (SOPs), sampling and analysis methods should determine the type of gear and supplies needed.

4.1.4 Figure 1, A-C are examples of fish field data sheets that can be adapted for field collections. Table 3 contains codes that can be used to

TABLE 1. GENERAL INDICATORS OF BIOLOGICAL/ECOLOGICAL INTEGRITY FOR FISH

Lakes and Reservoirs	Streams and Rivers
Structure and Function Components of Fish Populations and Communities	
Species composition	Species composition
Relative abundance	Relative abundance
Biomass	Biomass
Lengths	Lengths
Weights	Weights
Age and growth	Age and growth
Condition factor	Condition factor
Population numbers	Population numbers
Fecundity	Fecundity
Indices IBI	Indices IBI/Iwb
Health/Condition profile	Health/condition profile
Gross pathology, parasitism, disease incidence	Gross pathology, parasitism, disease incidence
State fish kills	State fish kills
Ice cover period	Pollution indices
Pollution indices	Ichthyoplankton index
Ichthyoplankton index	
Chemical Constituents	
Nutrients (N, P, total, soluble)	Nutrients (N, P, total, soluble)
DO, Alkalinity, conductivity, pH; nutrient dynamics	DO, alkalinity, conductivity, pH; nutrient dynamics
Habitat and Physical Variables	
Temperature	Temperature
Turbidity (secchi)	Suspended solids
Suspended solids	Hydrology
Water depth, area, retention time	Pool/riffle series
Substrate characterization	Substrate characterization
Shoreline development	Embeddedness
	Streambank stability
	Width of riparian zone, percent of stream cover

TABLE 2. GENERAL CHECKLIST OF FISH FIELD EQUIPMENT AND SUPPLIES

Boat(s)	____	Fish survey data forms	____
Motor(s)	____	Habitat survey forms	____
Paddles	____	Clip board with cover	____
Life preservers and flotation cushions	____	Dissecting kit	____
Fire extinguisher, (US Coast Guard approved)	____	Plastic bags, various sizes	____
First aide kit	____	10% Buffered formalin (formaldehyde solution)	____
Running Lights	____	Ethyl alcohol (ethanol) or isopropyl alcohol	____
Air Horn	____	Distilled or deionized water	____
Camera/film	____	Scale envelopes	____
Maps	____	Divider for measuring body proportions	____
Ice chests	____	Magnifier, pocket	____
Ice	____	Microscope, field	____
Blue ice, soft pack	____	Dissecting microscope	____
Dry ice	____	Microscope slides and cover	____
Portable light source	____	Air pump, battery	____
Waterproof notebook	____	Calculator	____
Waterproof pencils, ink pens	____	Marker, permanent black	____
Waterproof labels	____	Fish finder	____
Arm-length insulated water proof gloves	____	Nylon-mesh fish cage	____
Hip boots	____	Sample containers	____
Rain gear	____	Data sheets	____
Feltsole neoprene chest waders	____	Patch kit for wader repair	____
Paper towels	____	Fiberglass hauling tanks	____
Aluminum foil	____	Anesthesia, MS222 (tricanemethane sulfonate)	____
Thermometer	____	Long forceps	____
Water chemistry meters or water test kit	____	Samll envelopes	____
Secchi disk	____	Vials or small bottles	____
Glass jars (4L, 2L, 1L) (chemical samples)	____	Scalpel or knife	____
Hand tallys	____	Divider, fine-pointed, or dial caliper	____
Tape measure (100 yd. or meter)	____	Rule, stainless steel, metric	____
Polaroid glasses	____	Other: _____	
Dip nets	____	_____	
Seines	____	_____	
Gill nets	____	_____	
Trawls	____	_____	
Traps	____	_____	
Hoop nets	____	_____	
Electrofishing gear	____	_____	
Balance (weight scale)	____	_____	
Measuring board (50 cm)	____	_____	
Tubs	____	_____	
Buckets, livewells, coolers	____	_____	

record external anomalies found on fish, and the codes are recorded on the fish field data sheet (Figure 1C).

4.1.5 Habitat Evaluation

4.1.5.1 A general site evaluation of each sampling location should be conducted during the sample processing because the range of habitats (riffles, runs, pools) can have a major effect on the data collected. Figure 2 contains a habitat description sheet for evaluating the surrounding topographical features and physical characteristics of fish sampling locations. The information can be used for calculating a Quality Habitat Evaluation Index (QHEI) described in Ohio EPA (1989) and Rankin (1989). Also, see Hughes et al. (1986; 1987), Hughes and Larsen (1988), Hunt (1992), Omernik (1987), Omernick and Gallant (1988), and Section 8, Fish Bioassessment Protocols For Use In Streams and Rivers.

4.1.6 Regional Reference Site Selection

4.1.6.1 Reference sites should be selected based on the following criteria:

4.1.6.2 Select site using standardized methods.

4.1.6.3 Select site least impacted sites that are typical of the region.

4.1.6.4 Avoid areas below point sources of pollution including known recovery areas (except large rivers).

4.1.6.5 Avoid areas of obvious habitat modification and nonpoint sources of pollution or impacts.

4.1.6.6 Select representative sites distributed by stream size.

4.1.6.7 Site can be maintained by continuing to resample the reference site on a once every ten years basis or less.

4.1.7 Fish Sampling Gear

4.1.7.1 Fish can be collected actively or passively. Active sampling methods include the use of seines, trawls, electrofishing, chemicals, and hook and line. Passive methods involve entanglement (gill nets, trammel nets, tow nets) and entrapment (hoop nets, traps, etc.) devices.

4.1.7.2 The chief limitations in obtaining qualitative and quantitative data on a fish population are gear selectivity and the mobility and rapid recruitment of the fish. Gear selectivity refers to the greater success of a particular type of gear in collecting certain species, or size of fish, or both. All sampling gear is selective to some extent. Two factors that affect gear selectivity are: (1) the habitat or portion of habitat (niches) to be sampled and (2) the actual efficiency of the gear. Another problem is that the efficiency of gear for a particular species in one area does not necessarily apply to the same species in another area. The skill and training

A. FISH FIELD DATA SHEET

Collection No. _____

State or Country: _____ County _____

Locality: _____

_____

Collectors: _____

Date: _____ Time: _____

Water : _____ Temp.: _____ Air: _____

Shore vegetation: _____

Aquatic vegetation: _____

Stream width: _____ Zmean: _____

Amax: _____ Zmean: _____

Shore: _____ Pool current: _____

Bottom: _____ Riffle: _____

Weather: _____

Method of capture: _____ Original preservative: _____

Water Chemistry: Secchi: _____

Depth	T^0	pH	DO	Conductivity	Salinity

COMMENTS: _____

Figure 1. Example of a general fish field data sheet.

A. FISH FIELD DATA SHEET (CONTINUED)

Page _____ Of _____ Collection No. _____

State or Country: _____ County _____

Locality: _____

Collectors: _____

Date: _____ Time: _____

B. FISH FIELD DATA SHEET

Coll. No. _____

State or Country: _____ County _____

Locality: _____

_____

Water: _____

Vegetation: _____

_____

Bottom: _____ Temp.: _____ Air: _____

Shore: _____

Distance from shore or stream width: _____ Current: _____

Depth of capture: _____ Depth of water: _____

Method of capture: _____

Collected by: _____ Date: _____

Orig. preserv.: _____ Time: _____

Weather: _____

_____

Figure 1. Example of a general fish field data sheet.

C. FISH FIELD DATA SHEET

Field Crew: Collector/Recorder ______ **Time of Day:** ______ **Page** ___ **of** ___

River/Stream: ______ **Location:** ______

Date: ______

River Code: ______ **Sampler Type:** ______ **Time Fished:** '___" ___ **Total Seconds** ______

RM: ______ **Depth:** ______ **Observed Flow:** ______

Distance: ______ **Data Source:** ______ **Number of Species:** ______

Anomalies: A-anchor worm; B-black spot; C-leeches; D-deformities; E-eroded fins, F-fungus; L-lesions; M-multiple DELT anomalies, N-blind, P-parasites; Y-popeye; S-emaciated; W-swirled scales; T-tumors; Z-other/. [H-Heavy; L-Light are combined with anomalies A, B, and C]

SPECIES	# WEIGHED	TOTAL COUNTED	WEIGHT (GRAMS)	ANOMALIES

Mass Weighing Convention: Total Weight (g) — 536 (12) — Number Weighed

Figure 1. Example of a general fish field data sheet. Adapted from OHIO EPA (1989).

SITE DESCRIPTION SHEET

Fish **QHEI SCORE:** ☐

Stream_____ RM_____ Date_____ River Code_____

Location_____ Crew:_____

1] SUBSTRATE (Check *ONLY* Two Substrate *TYPE BOXES; Check all types present*); ***SUBSTRATE SCORE:*** ☐

TYPE	POOL	RIFFLE		POOL	RIFFLE
☐☐-BLDER /SLABS[10]	___	___	☐☐-GRAVEL [7]	___	___
☐☐-BOULDER [9]	___	___	☐☐-SAND [6]	___	___
☐☐-COBBLE [8]	___	___	☐☐-BEDROCK[5]	___	___
☐☐-HARDPAN [4]	___	___	☐☐-DETRITUS[3]	___	___
☐☐-MUCK [2]	___	___	☐☐-ARTIFIC.[0]	___	___

TOTAL NUMBER OF SUBSTRATE TYPES: ☐> 4 [2] ☐— <= 4 [0] ☐-COAL FINES [-2]

NOTE: (Ignore sludge that originates from point-sources; score is based on natural substrates)

COMMENTS_____

Substrate Origin (Check all): ☐-LIMESTONE [1] ☐-RIP/RAP [0] ☐-TILLS [1] ☐-HARDPAN [0] ☐-SANDSTONE [0] ☐-SHALE [-1]

Silt Cover (Check One): ☐-SILT HEAVY [-2] ☐-SILT MODERATE [-1] ☐-SILT NORMAL [0] ☐-SILT FREE [1]

Extent Of Embeddness (Check One): ☐—EXTENSIVE [-2] ☐—MODERATE[-1] ☐—LOW[0] ☐—NONE[1]

COVER SCORE: ☐

2] INSTREAM COVER

TYPE (Check *All* That Apply)

☐-UNDERCUT BANKS [1] ☐-DEEP POOLS [2] ☐-OXBOWS [1]
☐-OVERHANGING VEGETATION [1] ☐-ROOTWADS [1] ☐-AQUATIC MACROPHYTES [1]
☐-SHALLOWS (IN SLOW WATER) [1] ☐-BOULDERS [1] ☐-LOGS OR WOODY DEBRIS [1]

AMOUNT(Check *ONLY* One or check 2 and *AVERAGE*)

☐ - EXTENSIVE > 75% [11]
☐ - MODERATE 25-75% [7]
☐ - SPARSE 5-25% [3]
☐ - NEARLY ABSENT < 5%[1]

COMMENTS:_____

CHANNEL: ☐

3] CHANNEL MORPHOLOGY: (Check *ONLY* One PER Category OR check 2 and *AVERAGE*)

SINUOSITY	DEVELOPMENT	CHANNELIZATION	STABILITY	MODIFICATIONS/OTHER	
☐ - HIGH [4]	☐ - EXCELLENT [7]	☐ - NONE [6]	☐ - HIGH [3]	☐ - SNAGGING	☐ - IMPOUND.
☐ - MODERATE [3]	☐ - GOOD [5]	☐ - RECOVERED [4]	☐ - MODERATE [2]	☐ - RELOCATION	☐ - ISLANDS
☐ - LOW [2]	☐ - FAIR [3]	☐ - RECOVERING [3]	☐ - LOW [1]	☐ - CANOPY REMOVAL	☐ - LEVEED
☐ - NONE [1]	☐ - POOR [1]	☐ - RECENT OR NO RECOVERY [1]		☐ - DREDGING	☐ - BANK SHAPING
				☐ - ONE SIDE CHANNEL MODIFICATIONS	

COMMENTS:_____

RIPARIAN: ☐

4] RIPARIAN ZONE AND BANK EROSION - (check ONE box per bank or check 2 and AVERAGE per bank)

"River Right Looking Downstream"

RIPARIAN WIDTH L R (Per Bank)	EROSION/RUNOFF - FLOOD PLAIN QUALITY L R (Most Predominant Per Bank)	FLOOD PLAIN QUALITY L R (Per Bank)	BANK EROSION
☐☐-WIDE>50m [4]	☐☐-FOREST, SWAMP [3]	☐☐-URBAN OR INDUSTRIAL[0]	☐ ☐-NONE OR LITTLE [3]
☐☐-MODERATE 10-50 [3]	☐☐-OPEN PASTURE/ ROWCROP[0]	☐☐-SHRUB OR OLD FIELD[2]	☐ ☐-MODERATE [2]
☐☐-NARROW 5-10m [2]	☐☐- RESID.,PARK,NEW FIELD [1]	☐☐-CONSERV. TILLAGE [1]	☐ ☐-HEAVY OR SEVERE[1]
☐☐-VERY NARROW 1-5m [1]	☐☐-FENCED PASTURE [1]	☐☐-MINING/CONSTRUCTION [0]	
☐☐-NONE[0]			

COMMENTS:_____

POOL: ☐

POOL/GLIDE AND RIFFLE/RUN QUALITY

MAX. DEPTH (Check 1)	MORPHOLOGY (Check 1)	POOL/RUN/RIFFLE CURRENT VELOCITY (Check *All* That Apply)	
☐- >1m [6]	☐-POOL WIDTH > RIFFLE WIDTH [2]	☐-TORRENTIAL[-1]	☐-EDDIES[1]
☐- 0.7-1m [4]	☐-POOL WIDTH = RIFFLE WIDTH [1]	☐-FAST[1]	☐-INTERSTITIAL[-1]
☐- 0.4-0.7m [2]	☐-POOL WIDTH < RIFFLE W. [0]	☐-MODERATE [1]	☐-INTERMITTENT[-2]
☐- < 0.4m [1]		☐-SLOW [1]	
☐—<0.2m [Pool = 0]			

☐-NO POOL[0]

COMMENTS:_____

RIFFLE: ☐

RIFFLE/RUN DEPTH	RIFFLE/RUN SUBSTRATE	RIFFLE/RUN EMBEDDEDNESS	
☐ - GENERALLY >10 cm,MAX>50 [4]	☐-STABLE (e.g.,Cobble, Boulder) [2]	☐-EXTENSIVE [-1]	☐-MODERATE[0]
☐ - GENERALLY >10 cm,MAX<50 [3]	☐-MOD. STABLE (e.g.,Pea Gravel) [1]	☐-LOW. [1]	☐-NONE[2]
☐ - GENERALLY 5-10 cm [1]	☐-UNSTABLE (Gravel,Sand) [0]		
☐ - GENERALLY < 5 cm [Riffle = 0]			

☐-NO RIFFLE[0]

COMMENTS_____

GRADIENT: ☐

6] Gradient (feet/mile): _____ **%POOL:**_____ **%RIFFLE:**_____ **%RUN:**_____

Figure 2. Site description sheet for evaluating the topogeographical features and physical characteristics of fish sampling location. Adapted from Ohio EPA (1989).

SITE DESCRIPTION SHEET (CONTINUED)

Is Reach Representative of Stream? (Y/N)_____ If Not: _____

Additional Comments/Pollution Impacts: _____

	GEAR	DISTANC	WATER CLARITY	WATER STAGE
FIRST PASS				
SECOND PASS				
THIRD PASS				

SUBJECTIVE RATING (1-10)

AESTHETIC RATING (1-10)

CANOPY (%OPEN) _____ GRADIENT: Ò-LOW Ò-MODERATE Ò-HIGH PHOTOS: _____

STREAM MEASUREMENTS: AVERAGE WIDTH:_____ AVERAGE DEPTH:_____ MAX. DEPTH _____

LENGTH	WIDTH	DEPTHS ▷										POOL-GLD-RIF-RUN

CROSS-SECTIONS OF STREAM

DRAWING OF STREAM

Figure 2. Site description sheet for evaluating the topogeographical features and physical characteristics of fish sampling location. This part is used to record additional information about the sampling site and adjacent area. Adapted from Ohio EPA (1989).

TABLE 3. CODES UTILIZED TO RECORD EXTERNAL ANOMALIES ON FISH[1]

Anomaly Code	Description
D	Deformities of the head, skeleton, fins, and other body parts.
E	Eroded fins.
L	Lesions, ulcers
T	Tumors
M	Multiple DELT anomalies (e.g. lesions and tumors, etc.) on the same individual fish.
AL	Anchor worm - light infestation: fish with five or fewer attached worms and/or previous attachment sites.
AH	Anchor worm - heavy infestation fish with six or more attached worms and/or previous attachment sites.
BL	Black spot - Light infestation: spots do not cover most of the body with the average distance between spots greater than the diameter of the eye.
BH	Black spot - Heavy infestation: spots cover most of the body and fins with the average distance between spots less than or equal to the diameter of the eye.
CL	Leeches - Light infestation: fish with five or fewer attached leeches and/or previous attachment sites
CH	Leeches - Heavy infestation: fish with six or more attached leeches and/or previous attachment sites.
F	Fungus.
I	Ich
N	Blind - one or both eyes; includes missing and grown over eyes (does not include eyes missing due to popeye disease).
S	Emaciated (poor condition, thin, lacking form).
P	External parasites (other than those already specified).
Y	Popeye disease.
W	Swirled scales.
Z	Other, not included above.

[1]Adapted from Ohio EPA (1989).

of the personnel doing the sampling are also very important in sample collection.

4.1.7.3 Temporal and spatial changes in relative abundance of a species can be assessed under a given set of conditions if that species is readily taken with a particular kind of gear.

4.1.7.4 Passive collection devices usually require little specialized training to operate and can be used to collect data on relative abundance of many species. Passive methods, however, are very selective for some species. Gear type and design used are important in particular habitats to capture specific species or sizes of fish (Carter, 1954; Hubert, 1983; Starrett and Barnickol, 1955). Active methods are generally less selective and more efficient. Although the choice of method depends on the objectives of the fishery investigation and habitat to be sampled, active methods are generally preferred. However, the method selected must provide the information required from the survey or study. The biologist must decide whether he needs information on standing crop, catch per unit effort, qualitative information on the fishery, etc., and choose the sampling technique or techniques accordingly.

4.1.7.5 Sport fish, large specimens, and rare or endangered species should be identified in the field, measured (standard length, total length, body depth), examined for external anomalies, and if possible, released unharmed. If the fish are to be released unharmed, the method and equipment used must be selected appropriately. Some methods (e.g., gill nets) usually kill the fish.

4.2 Active Sampling Techniques

4.2.1 Seines

4.2.1.1 A haul seine is essentially a strip of strong netting hung between a stout cork or float line at the top and a strong, heavily-weighted lead line at the bottom (Figure 3). The wings of the net are often of larger mesh than the middle portion, and the wings may taper so that they are shallower on the ends. The center portion of the net may be formed into a bag to aid in confining the fish. At the ends of the wings, the cork and lead lines are often fastened to a short stout pole or brail. The hauling lines may be attached to the top and bottom of the brail by a short bridle. The quantiative factors of this gear are determined by the total length of the net, the mesh sizes used in its construction (especially in the bag), and whether or not the floatline remains on the surface during operation or under water with the leadline on the bottom. The size of these seines is usually determined by how they are retrived and the species sought.

4.2.1.2 Deepwater or haul seining usually requires a boat. One end of one of the hauling lines is anchored on shore and the boat plays out the line until it reaches the end. The boat then lays out the net parallel to the beach. When all of the net is in the water, the boat brings the end of the second hauling line ashore. The net is then beached as rapidly as possible without allowing the lead line to come off the bottom.

4.2.1.3 Straight seines (without bags) can usually be handled by two people. The method of playing out the seine and bringing it in may be similar to the haul seine or it may be pulled parallel to the shore for some distance before it is beached. The straight seine is generally used in shallow water where one member of the party can wade offshore.

4.2.1.4 Bag and straight seines vary considerably in dimensions and mesh size. The length may vary from 3 to 70 meters, and mesh size and net width vary with the size of the fish, depth of the water, and the habitat to be sampled.

4.2.1.5 Nylon seines are recommended because of the ease of maintenance. Cotton seines should be treated with a fungicide and dried after using to prevent deterioration. Nylon seines should not be left in the sun for prolonged periods of time or they will also deteriorate.

4.2.1.6 Seining is not effective in deep water unless the seine is deep enough to cover the area from surface to bottom. Seining is not effective in areas that have snags, large rocks and boulders, and sunken debris that may tear or foul the net. However, in selected areas seines can be very efficient in sampling fish. Although the results are expressed as number of fish captured per unit area seined, quantitative seining is very difficult. It must be applied consistently along several beaches of a waterbody to achieve a quantitative assessment. The method may be more useful in determining the variety of fish rather than the number of fish inhabiting the water.

4.2.1.7 Choice of seines will depend on the study design, and sampling methods and sizes of seines vary with habitat type.

4.2.1.8 Seining should be performed by at least two investigators, but having more helpers improves sampling effectiveness.

4.2.1.9 In riffles of wadable streams, e.g., the preferred method is the "foot shuffle" using a 3 m minnow seine with 1/4 inch mesh (6 mm) size. This kickset method consists of setting the net in the water perpendicular to the current. Investigators then enter the riffle approximately 3 m upstream from the net and actively disturb the substrate and overturn rocks or other debris. The net is then picked up and carefully examined for the presence of fish. In slower currents, it may be possible to pull the seine downstream, hooking into the bank after a distance of 5 to 10 m.

4.2.1.10 In pools, because larger seines are preferred, depth of water usually precludes effective kicksets. In such situations, pools are actively seined by pulling a 5 m seine with 1/4 inch mesh (6 mm) size through the pool either perpendicular or obliquely to the bank, or, in the case of very quiet water, upstream or downstream and parallel to the bank prior to hooking into shore and examining for fish.

4.2.1.11 Continue seining until two riffles and two pools or, in the absence of discrete habitats, a segment of at least 200 m has been sampled. Distance sampled should not exceed 500 m. Record total time spent collecting.

4.2.1.12 Record all information on field data sheets. Specimens kept for later identification or for voucher specimens should be preserved in 10% formalin solution (see Section 5, Fish Specimen Processing) and kept in separate jars by habitat type with inner and outer waterproof labels. Labels should contain locality data, habitat type, date, collectors names, and study collection numbers from the field sheets for that site.

4.2.2 Trawls

4.2.2.1 Trawls are specialized submarine seines used in large, open water areas of reservoirs, lakes, large rivers, estuaries, and oceans. They may be of considerable size and are towed by boats at speeds sufficient to overtake and enclose the fish. Four basic types are available: (1) the beam trawl used to capture bottom fish, (2) the otter trawl used to capture near-bottom and bottom fish, (3) the mid-water trawl used to collect schooling fish at various depths, and (4) surface tow nets used to collect fish at or near the surface. These trawls can be very effective on selected bottom, mid-water and surface oriented species at specific life history stages.

4.2.2.2 The beam trawls (Figure 4) have a rigid opening and are difficult to operate from a small boat. Otter trawls (Figure 5) have vanes or "otter boards", which are attached to the forward end of each wing and are used to keep the mouth of the net open while it is being towed. The otter boards are approximately rectangular and usually made of wood, with steel strapping. The lower edge is shod with a steel runner to protect the wood when the otter board slides along the bottom. The leading edge of the otter trawl is rounded near the bottom to aid in riding over obstructions. The towing bridle or warp is attached to the board by four heavy chains or short heavy metal rods. The two forward rods are shorter so that, when towed, the board sheers to the outside and down. Thus, the two otters boards sheer in opposite directions and keep the mouth of the trawl open and on the bottom. Floats or corks along the head rope keep the net from sagging, and weights on the lead-line keep the net on the bottom. The entrapped fish are funneled back into the bag of the trawl (codend). The size of the mesh in the codend (bag end of a net) will determine the species and life history stages caught.

4.2.2.3 The midwater trawl resembles an otter trawl with modified boards and vanes for controlling the trawling depth. Such trawls are cumbersome for freshwater and inshore areas, but can be used very effectively in marine and estuarine waters. Surface townets have been used very effectively for emigrant juvenile salmonids in northwest and Alaska estuaries for monitoring year class abundance.

4.2.2.4 A popular, small trawl consists of a 16 to 20 foot (5 to 6 meters) headrope, semiballoon modified shrimp (otter) trawl with 3/4 inch (1.9 cm) bar mesh in the wings and cod end. A 1/4 (0.6 cm) bar mesh liner may be installed in the cod end if smaller fish are desired. This small trawl uses otter boards, the dimensions of which, in inches, are approximately 24 to 30 (61 to 76 cm) x 12 to 18 (30 to 46 cm) x 3/4 to 1/4 inches (0.9 to 3.2 cm), and the trawl can be operated out of a medium-sized boat.

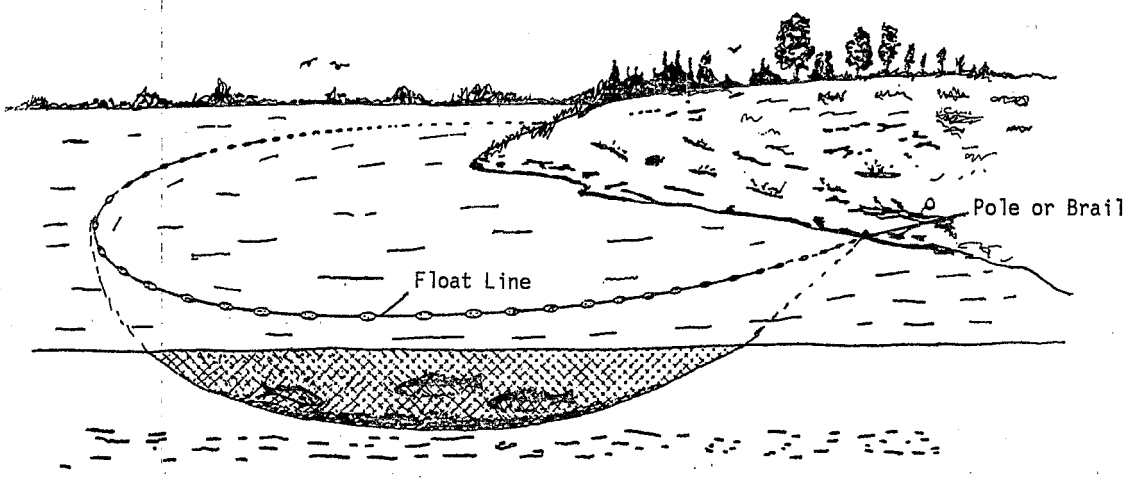

Figure 3. The Common Haul Seine. Modified from Dumont and Sundstrom (1961).

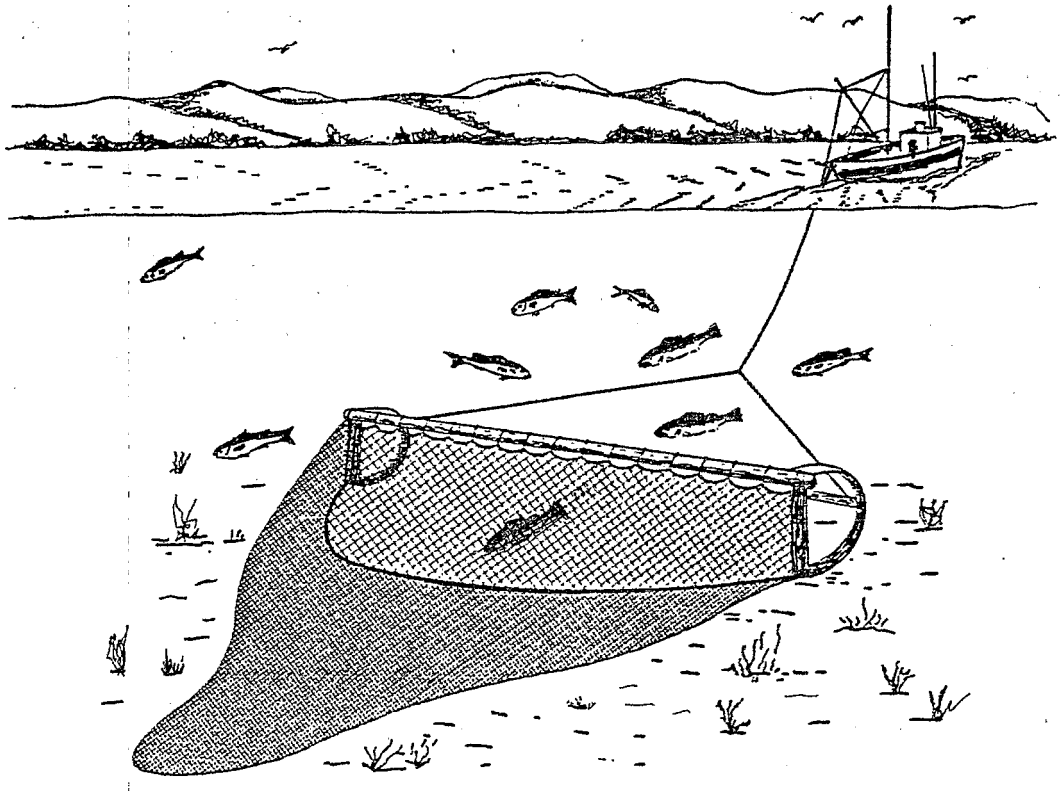

Figure 4. The Beam Trawl. Modified from Dumont and Sundstrom (1961).

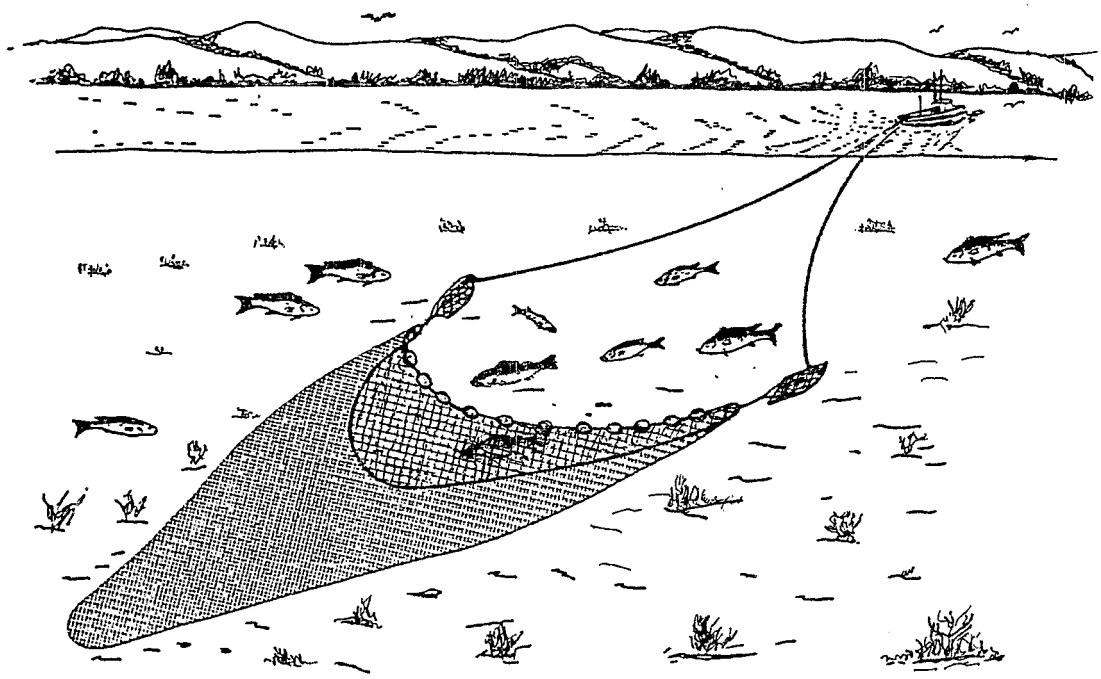

Figure 5. The Otter Trawl. Modified from Dumont and Sundstrom (1961).

4.2.2.5 Trawling data are usually expressed in weight numbers, species, etc.of catch per unit of time.

4.2 2.6 The use of trawls requires experienced personnel. Boats deploying large trawls must be equipped with power winches and large motors. Also, trawls can not be used effectively if the bottom is irregular or harbors snags or other debris. Trawls are used to gain information on a particular species of fish and an overally estimation of fish populations and communities. See Hayes (1983), Massman et al. (1952), Rounsefell and Everhart (1953), and Trent (1967) for further information on trawls.

4.2.2.7 In selected studies a plankton net may be used as a trawl. Larval and young fishes can be collected at the surface and bottom with a 1 meter plankton net by trawling a transect with a predetermined time frame (say ten minutes). A plankton sled can be used to hold a meter plankton net towed at the bottom while a sidearm can be used at the surface (Dovel, 1964). A digital flowmeter can be mounted in the mouth of the net to determine the amount of water strained. Large numbers of plankton can be collected in a short time by using a Miller high-speed sampler. Another sampler type, the bongo net, is a pair of nets held side-by-side in a frame and is towed by a cable that attaches to the frame between the two nets. Bongo nets are good because they can be used off ships at high speed, can be used to sample the horizontal layer of the water column, and can be used to get replicate samples at the same time.

4.2.2.8 The larval stage by some individuals is considered the period from time of hatching until the attainment of the adult fin-ray complement, ossification of spines or rays and the inception of scale development. Mansuetti and Hardy (1967) defined the "larval" stage as the period from the disappearance of the yolk sac until the development of the adult fin-ray complement.

4.2.3 Several companies sell a variety of fish nets, seines, traps, trawls, etc.:

1. Sterling Marine Products, 18 Label Street, Montclair, NJ 07042, Telelphone (201) 783-9800 or Jonesport, Maine 04649, Telephone (207) 497-5635

2. Nylon Net Company, 615 E. Bodley Avenue, P.O. Box 592, Memphis, TN 38101, Telephone (901) 774-1500, FAX (901) 774-8130.

3. Memphis Net and Twine Company, Inc., 2481 Matthews Avenue, P.O. Box 8331, Memphis, TN 38108, Telephone (901) 458-2656, FAX (901) 458-1601.

4. Nichols Net and Twine Company, Inc., R.R.3 Bend Road, East St. Louis, IL 62201, Telephone (618) 876-7700.

4.2.4 Horizontal Ichthyoplankton Tow-Net

4.2.4.1 The larval fish sampler (Figure 6) consists of a modified bridle, frame, and net system with an obstruction-free opening. The tow net is easy to handle, and it is small enough for use on boats 4 m or larger in length. The tow net features a square net frame attached to a 0.5 m diameter cylinder-on-cone plankton net with a bridle. This design eliminates all towing obstructions forward of the net opening; in addition, it significantly reduces currents and vibrations in the water directly preceding the net. See Subsection 4.2.4.2 for the design and construction details of the horizontal ichthyoplankton tow-net. With the aid of a stanchion and winch assembly, one person can easily sample any stratum from near surface to near bottom in lakes and rivers. The cylinder-on-cone net is self-washing while it is being fished, and only the last 20 cm needs to be rinsed to concentrate the sample in the collecting bucket. The system is self closing during deployment and retrieval. During deployment, the towing cable is payed out at approximately the same speed that the vessel is moving forward. This allows the weighted net to rapidly descend, with the net mouth in the vertical plan, while collapsing the net body and thus preventing the net from fishing. When the net has reached the desired fishing depth, the release of the towing cable is stopped and the net begins fishing (Figure 6). Prior to retrieval, the vessel is stopped, and the vertical orientation of the net mouth and rapid lifting causes the net body to collapse, preventing the net from fishing. Nester (1987) and Nester (1992, personal communication) reported that the tow net system is effective in collecting all lentic fish larval species at sampling depths ranging from surface to 10 m and can easily be used at greater depth.

4.2.4.2 The 6.3-mm galvanized steel towing cable (1) is connected to the

center of the fore-bridle (2) with a 76.2-mm heavy duty snap swivel. A 25.4-mm thimble is permanently fixed with 3.2-mm cable clamps in the center of the 3.2-mm galvanized steel cable fore-bridle. The spreader bar (3) is constructed of 9.6-mm cold-rolled steel with two 38.8-mm clevises welded in place at either end. Side cable (4 and 5) of 3.2-mm galvanized steel are connected to the spreader bar clevises and to clevises welded to each corner of the net frame (6). The net frame is constructed of 9.6-mm cold-rolled steel heated, bent to form a 53-cm square, and closed by welding. Corner supports (7) provide additional strength and attachment points for netting. Two flowmeter support brackets (8a and 8b), to which flowmeters (9) are attached, are welded to the net frame and corner supports. Each bracket is bent at two 45° angles, so that the free end is about 5-cm behind the plane of the mouth of the net. Stainless steel support cable (10) is passed through a pair of 116-mm holes drilled 3-cm apart in the free end of the bracket to support the flowmeter. Nylon cord is used to lash the 0.5-m-diameter brass net ring (11) to the net frame and corner supports. The net bucket is secured to the cod end (bag end of a net) of the net with a hose clamp. Cables (12) supporting the 1-kg depressor plate (13) are attached to the lower corners of net frame with 3.2-mm cable clamps.

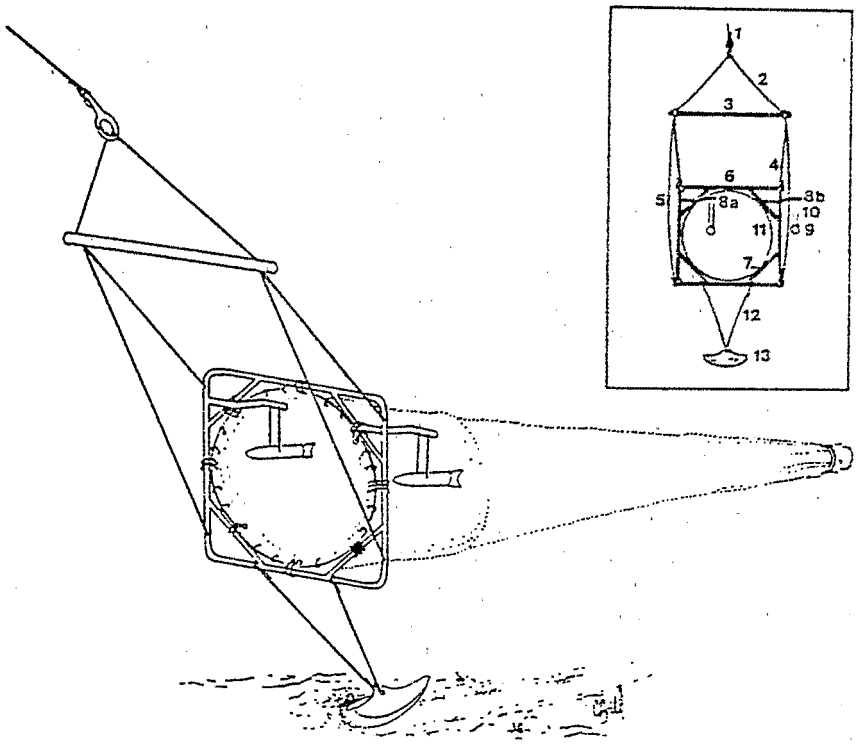

Figure 6. Horizontal Ichthyoplankton Tow-Net. Attitude of the modified bridle, frame, and net of the sampling system and diagram of the construction details. Numbers are referred to in Subsection 4.2.4.2. From Nester (1987).

4.2.4.3 The 0.5-m plankton net, a cylinder-on-cone configuration, is constructed of 0.335-mm mesh to the dimension given by O'Gorman (1984). A net of this type should have superior sustained filtration ability because of the high ratio of open mesh area to mouth area (6.3:1) and because oscillations of the cylindrical portion seemingly help clean the net during a tow (Tranter and Smith, 1968).

4.2.4.4 Commercial ichthyoplankton nets are available with threaded cod ends. Plastic cod end jars can be easily screwed into these and when the sample is finished being collected and preserved, a lid is screwed onto the jar and a new jar added to the net. These allow for rapid sample handling and decreased time. These have been found to be important if there are a lot of samples to be taken and numerous collecting sites.

4.2.4.5 Information on collecting and processing fish eggs and larvae are found in Simon (1989), Snyder (1983), and marine recommendations are provided by Smith and Richardson (1977).

4.3 Electrofishing

4.3.1 Electrofishing is an efficient capture method that can be used to obtain reliable information on fish abundance, length-weight relationships, and age and growth of fish in most streams of order 6 or less (Platts et al., 1983 and Plafkin et al., 1989). **Note:** Individuals involved in electrofishing must have completed a certified training course in electrofishing or have been trained by someone certified and experienced in electrofishing. This subsection provides some general principles and guidelines for understanding electrofishing. Electrofishing is a method for collecting fish using electricity. Either alternating (AC) or direct (DC) electrical current can be used. Most electrofishing in freshwater is done with pulsed DC electrical current equipment. In a boat-rigged shocker (boom shocker) or airboat, one or two people net the fish and another operates the boat and equipment. The fish are nearly always driven into cover as a result of electric stimulus making them difficult to capture. Once driven from cover, the fish are kept within effective range of the electrical field and are immobilized making it possible to pick them up with long-handled dip nets. Electrical dislodgement and immobilization of fish together result in more consistent success under varying conditions than ordinary seining. However, if target assemblage is common species, seining may be just as effective. For a discussion of the general principles and guidelines for electrical fishing, see below and Cowx (1990), Cowx and Lamarque (1990), Cross and Stott (1975), Dauble and Gray (1980), Elson (1950), Friedman (1974), Hartley (1980), Kolz (1989), Kolz and Reynolds (1989a, 1989b), Loeb (1955), Novotny and Priegel (1971, 1974), Ohio EPA (1987a, 1987b, 1989), Reynolds (1983), Sharpe (1964), U.S. Fish and Wildlife Service (1991), Vincent (1971) and Section 12, Fisheries Bibliography, 12.2 Electrofishing.

4.3.2 The decision to use electrofishing equipment (or electrofishers) will depend on size of site, flow, turbidity, and conductivity. If conductivity is below 100μS (micro seimens) or if water is too turbid to locate stunned fish, the investigators should consider other sampling devices (e.g., seines).

4.3.3 A choice of electrofishing equipment will depend on size of stream and access to stream from road. If a site is wadeable and close to the road, use Sportyak-mounted, generator unit or equivalent. If access is problematical, use a back pack unit. **For safety reasons, it is important too always wear waders and lineman's insulated (or Playtex Living) gloves when working with electricity in water.** At least two individuals for safety reasons (see Section 3, Safety and Health) are need when electrofishing. Always wear polarized sunglasses to aid vision.

4.3.4 Electrofishing efficiency can be placed in one of three categories: fish characteristics, habitat characteristics, and operating conditions. For a discussion of these three categories, see Reynolds (1983).

4.3.5 It is also recommended that anyone involved in electrofishing must take a U.S. Fish and Wildlife training course in electrofishing, or they must be trained by someone experienced in electrofishing.

4.3.6 Electrofishing Equipment (Electrofishers)

4.3.6.1 Electrofishing today is done by wading in shallow streams and using electric seines, backpacks, tow barges, longlines, etc. or in deep streams and rivers with electrofishing boats.

4.3.6.2 Typically a flat-bottom boat (usually 12 to 18 ft) is used for electrofishing in waters too deep for wading (Novotny and Priegel, 1974). Paired booms, (length vary according to boat size), protrude in front of the boat and are adjustable for height and spacing by means of lock-in adjustments. The electrode system should also be adjustable, i.e., operating with one or both anodes, varying the number of dropper electrodes, varying the exposure on the dropper electrodes, and alternating the polarity (Figure 7).

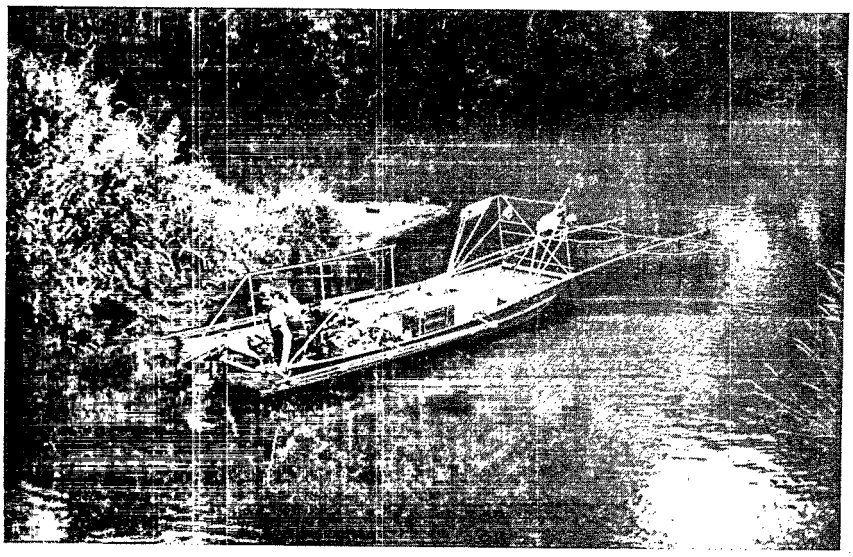

Figure 7. Typical Boom Shocker. Photo courtesy of Wisconsin Department of Natural Resources.

4.3.6.3 The electrofishing power unit may consist of a 240-volt or 500-volt, 2000 watt, heavy-duty generator and an electrical control section consisting of a modified, commercially-available, variable-voltage pulsator. The frequency of the cycles/second or Hz is not a critical factor. For AC electrofishing, 60, 180, and 400 Hz have been used with similar success. An electric control section permits the selection of AC voltage from 50-700 and DC voltage from 25 to 350; furthermore, it permits control of the electrical-field size which is dictated by the variable conductance of dissolved minerals in the water. The power equipment is similar in both boat shocking and stream shocking, but is portable in the latter. The literature indicates that DC electrofishing is the most comprehensive and effective, single method for collecting fish in rivers and streams (Gammon, 1973, 1976; Novotny and Priegel, 1974, and Ohio EPA, 1987b, 1989; Vincent, 1971).

4.3.6.4 Backpack electrofishers are entirely housed in a weatherproof metallic container that is fastened securely to a comfortable pack frame (Blair, 1958; Braem and Ebel, 1961; McCrimmon and Berst, 1963; Reynolds, 1983; Sharpe and Burkhard, 1969). Backpack shocker units can be purchased commercially. The power source is either a 12-volt (deep charge battery or a small 115-volt AC generator). The electrode system is hand-held and must be insulated from the operator by handles 1.5-3.0 m long, preferably made of fiberglass. A horizontal ring or spatula electrode attached to the end of the handle is easiest and most effective to use. Positively activated switches on each electrode handle are an important safety features. Both backpack, tote barge, and boat mounted shockers are available from U.S. manufacturers in a variety of models (see Subsection 4.3.13).

4.3.6.5 Other electrofishing devices include: tote barges/sport yaks (Ohio EPA, 1987b, 1989); longlines (Ohio EPA, 1987b); electric trawls (AC) (Haskell et al., 1955); and (Loeb, 1955); electric seines (Funk, 1947; Holton, 1954; Larimore, 1961; Bayley et al., 1989); and a fly-rod electrofishing device employing alternating polarity current (Lennon, 1961). After reviewing the literature, the user must decide which design is most suitable to the particular needs of the study.

4.3.6.6 Decision on the use of AC, DC, pulsed DC or alternate polarity forms of electricity and selection of the electrode shape, electrode spacing, voltage and proper equipment depends on the resistance, temperature and total dissolved solids in the water. Light-weight conductivity meters are recommended for field use. Lennon (1959) provides a comprehensive table and describes the system or combination of systems that worked best for him. Novotny and Priegel (1974) provide improved designs to increase the effectiveness of boom shockers.

4.3.6.7 Rollefson (1958, 1961) tested and evaluated AC, DC, and pulsated DC, and discussed basic electrofishing principles, wave forms, voltage-current relationships, electrode types and designs and differences between AC and DC and their effects in hard and soft waters. He concluded that pulsated DC was best for power economy and fishing ability when used correctly. Haskell and Adelman (1955) found that slowly pulsating DC worked best in leading fish to the anode. Pratt (1951) also found the DC shocker to be more effective than

the AC shocker. Frankenberger (1960) and Latta and Meyers (1961) used a DC shocker, and Larimore et al. (1950) used AC current in boat shocking. Stubbs (1966) used DC or pulsed DC and had his aluminum boat wired as the negative pole.

4.3.6.8 Fisher (1950) found that brackish water requires much more power (watts) than freshwater even though the voltage drops may be identical. Lennon and Parker (1958) and Seehorn (1968) recommended the use of an electrolyte (salt blocks) when sampling in some soft waters to produce a large enough field with the electric shocker.

4.3.6.9 Novotny and Priegel (1974) provided operational guidelines to increase the effectiveness of boom shockers. They suggest that in the operation of DC or pulsed DC it is important that the electrofishing boat move much more slowly than in using AC. In general, AC operation is preferable at night in shallow clear water where visibility is no problem, and it is not necessary to attract fish from cover. Pulsed DC is effective in deep or turbid water where fish must be drawn from cover and collected by long-handled dip nets.

4.3.7 Areas considered as problems in boat electrofishing are (Novotny and Priegel, 1974):

4.3.7.1 Range limitations (distance at which fish are affected).

4.3.7.2 Water conductivity (difficulty in attaining sufficient current in water of low conductivity.

4.3.7.3 Bottom materials (reduces effectiveness of electrofishing by highly conductive bottom material).

4.3.7.4 Water depth (difficulty capturing immobilized fish at depths beyond 0.9 - 1.2 m) due to visibility, length of dipnet handle, etc.

4.3.7.5 Water clarity and vegetation (these factors restrict visibility).

4.3.7.6 Water temperature (best response depends on the species and water temperature).

4.3.7.7 Fish mortality (much higher with AC electricity than DC or pulsed DC).

4.3.7.8 Fish size (selectivity of size) is not much of a problem with modern electrofishing units.

4.3.7.9 Fish species (selectivity for species-swimming ability).

4.3.7.10 Equipment and operating problems (inadequate lighting, power, voltage controls, instrumentation, electrode design, etc.).

4.3.7.11 Day and night sampling (some species sampled better during the day than night and vice versa) (Sanders, 1991; 1992).

4.3.7.12 Novotny and Priegel (1974) were able to overcome many of the above problems. These same problems are also encountered in stream electrofishing (Novotny and Priegel, 1971).

4.3.8 Safety

4.3.8.1 In order for electricity to flow, electricity needs a complete electrical circuit, moving from the anode to the cathode. Therefore, the only way an individual can get shocked is if they become part of the circuit. During electrofishing, the water becomes the connection that completes the circuit between the anode and the cathode. You must, therefore, be electrically insulated from the water and the electrodes of the electroshocker (electrofisher). Otherwise, you become part of the circuit and will get a shock.

4.3.8.2 Novotny and Priegel (1974) and Ohio EPA (1989, 1990) give a complete description of an electrical safety disconnect system and discuss electrical safety and safety regulations. An 18 foot boat provides a greater margin of safety in rough water, and a safety railing surrounding the front deck and extending along each side of the boat affords protection of the operators against the hazard of falling over-board into the electric field near the boat.

4.3.8.3 Floor mat switches or foot pedals with non-skid surfaces should be permanently installed on the front deck. Thus, each operator must be in position before the system is energized. Likewise, a throw switch should be installed on the rear seat for the outboard motor operator.

4.3.8.4 When metal booms are used an electrical ground wire terminated with a battery clamp should be provided to assure a positive electrical ground for each boom.

4.3.8.5 All electrical circuits should be enclosed in metal conduit with a separate conduit system for the main power (high voltage) circuits, auxiliary power and safety circuits (low voltage). Watertight junction boxes should be used throughout the electrical system.

4.3.8.6 Because the nets used to capture fish must be dipped into the water near the electrodes, it is very important that the net handles be constructed of materials with good electrical insulating properties. Epoxiglass insulating materials used on electricians tools are the best material. Fiberglass covered metal can cause accidents if the fiberglass covering is damaged, allowing contact between the operator and the metal handle. The operator must wear rubber gloves.

4.3.8.7 All leads associated with the generator are carefully insulated. Generally, AC or DC, used in electrofishing provides more than enough voltage and current to shock and electrocute a person.

4.3.9 In a boat shocking operation the following safety precautions should be observed:

4.3.9.1 Wear U.S. Coast Guard approved life jackets.

4.3.9.2 Wear felt sole neoprene waders or hip boots and insulated arm length gloves.

4.3.9.3 Avoid excess fatigue and be constantly alert.

4.3.9.4 Authorize one person to be in charge.

4.3.9.5 Instruct all personnel in the fundamentals of electricity.

4.3.9.6 Thoroughly familiarize all persons with all phases of the equipment and its operation.

4.3.9.7 Make sure that all equipment is in good condition and properly used.

4.3.9.8 Make sure that there is a first aid kit and fire extinguisher on the boat.

4.3.9.9 Know how to administer first aid treatment for electrical shock.

4.3.9.10 Never operate electrofishing equipment if you have any prior heart ailment.

4.3.10 The following things must be done to prevent electrical shock when using electrofishing equipment:

4.3.10.1 Use water-tight, preferably chest or waist-high, waders (neoprene waders with felt-sole boots). If the waders or wading boots become wet inside, stop electrofishing and let them dry out thoroughly before electrofishing again. Wet wading boots can conduct electricity.

4.3.10.2 Use water-tight lineman's insulated gloves that cover up to at least

the elbows. If they get wet inside, stop electrofishing and let them dry out completely before continuing electrofishing.

4.3.10.3 The individual doing the electrofishing must take care not to let the anode come into contact with anyone while the unit is active. In addition, one must make sure and be aware that anyone in or near the water is electrically insulated with wading boots and gloves.

4.3.11 Electrofishing procedures for use in wadable streams

4.3.11.1 The sampling gear should consist of backpack electrofishing equipment supplemented by block netting and seining in habitats where flow, substrate, and structure affect capture of benthic fish species.

4.3.11.2 The investigator(s) should follow project plans, standard operating procedures (SOPs), and safety for electrfishing in wadable streams and rivers.

4.3.11.3 Decision to use electrofishing equipment will depend on size of

site, flow, and turbidity. If flow is too high, site to deep, or water too turbid to locate stunned fish, the investigators may consider use of seine only. This is a safety decision.

4.3.11.4 Once the sampling site has been located, determine the fish sampling reach as a function of mean channel width taken at the site (20-30 channel widths). The sampling site may serve as the midpoint of the sample reach. The investigator should walk the length of the sample site to determine pool depths, habitat composition, barriers, and obstructions which may impede or aid in fish capture. Also, determine if reach requires block nets be placed at upstream and downstream ends of the stream (e.g., where sample reach is a large continuous pool).

4.3.11.5 Set the electrofishing unit to 300 VA and pulsed DC. Based on stream conductivity, select initial voltage setting. Determine that all crewmembers are wearing waders, gloves, are clear of the anode. Start generator, set timer, and depress switch to begin fishing. Starting at the bottom of the most downstream riffle, pool, or other habitat type in the sampling reach, fish in an upstream direction, parallel to the current. Adjust voltage and waveform output according to sampling effectiveness and incidental mortality to specimens. Voltage gradients of 0.1 to 1.0 volts/cm are effective for stunning fish. These gradients can be maintained in freshwater of normal conductivity (100-500 micromhos/cm) by adjusting circuit voltage to produce a current of 3-6 amperes (Reynolds, 1983).

4.3.11.6 With switch depressed, sweep electrodes from side to side in the water in riffles and pools. Sample available cut-bank and snag habitat as well as riffles and pools.

4.3.11.7 Netters follow along behind person operating shocker and net stunned fish which are then deposited in separate buckets or holding tanks based on habitat from which fish are collected. Minnow seines (4 m x 2 m x 0.5 cm) and kick nets (2 m x 2 m x 0.5 cm) may be used to block in riffles, polls, and snags.

4.3.11.8 Depending on the study design, fish may be collected according to time and distance criteria. The collection time should be no less than 45 minutes and no greater than 3 hours for a distance of between 150 - 500 m in order to obtain replicate samples from two riffles and two pools, or in the absence of discrete habitat types, a segment of at least 200 m of stream has been sampled. Homogeneous (or large systems) without clearly defined habitat types should be sampled wherever best fish habitat is found. Distance sampled should not exceed 500 m. Record total time spent collecting.

4.3.11.9 Record all information on field data sheets. Sport fish, large specimens and threatened and endangered species should be identified in the field, measured (standard length, total length, body depth), examined for external anomalies, and released unharmed. All other specimens should be preserved in 10% formalin solution (see Section 5, Specimen Processing Techniques) and kept in separate jars by habitat type with inner and outer waterproof labels. Labels should contain locality data, habitat type, date,

collectors names, and study collection numbers from the field sheets for that site.

4.3.12 Standard operating and safety procedures for commercial shocker boat should be followed in boatable streams and rivers.

4.3.13 Companies that sell a variety of electroshocking equipment (electrofishing boats, boat outfitting electrofishing kits, electrofishing tote barges, backpack electrofishing units, electrofishers, etc.):

1. Coffelt Manufacturing, Inc., P.O. Box 1059, Flagstaff, AZ 86002 or 1311 E. Butler Avenue, Building B, Flagstaff, AZ 86011, Telephone (602) 774-8829

2. Smith-Root, Inc., 14014 N.E. Salmon Creek Avenue, Vancouver, WA 98686, Telephone (206) 573-0202

4.4 Chemical Fishing (Ichthyocides)

4.4.1 Fish toxicants for sampling fish populations are a common practice in impounded waters and streams throughout the United States. Only registered fish chemical toxicants should be used in collection fish populations. The Federal and State rules should be check prior to use because they continually are updated and subjected to change. The decision to use a chemical toxicant should be based not only on the efficacy of the toxicant, but also on its persistence in the environment, toxicity to other animals, and whether it is deleterious to man. Fish toxicants for reclamation are thoroughly reviewed by Lennon et al. (1971), and papers addressing their use in sampling are found throughout the literature. Additional information on sampling fish populations with toxicants is found in APHA (1992), ASTM (1992), Bone (1970), Boccardy and Cooper (1963), Davies and Shelton (1983), Hocutt et al. (1973), Hooper (1960), Marking (1992), Meyer et al. (1976), Platts et al. (1983), Schnick (1974), Schnick and Meyer, (1978), and Section 12, Fisheries Bibliography, Subsection 12.3, Chemical Fishing.

4.4.2 Chemicals used in fish sampling include rotenone, cresol, copper sulfate, antimycin A, and sodium cyanide. The ideal ichythocide indicated by Hendricks et al. (1980) is (1) nonselective; (2) easily, rapidly, and safely used; (3) readily detoxified; and (4) not detected and avoided by fish.

4.4.3 When using an ichthyocide, care must be taken to ensure that it will be used correctly and approval for use must be obtained from proper Federal and State authorities. Hendricks et al. (1980) reported that improper application of rotenone can have disastrous effects downstream.

4.4.4 Rotenone (Derris or Cube roots) has generally been the most acceptable because of its high degradability, freedom from such problems as precipitation (as with copper sulfate), and relative safety for the user.

4.4.5 Pesticides, copper sulfate, cresol, and other chemicals have been used as fish toxicants, but they are toxic to humans, may add taste or odor to the

water, have a slow rate of detoxification, may be toxic to other organisms, and, therefore, should not be used for sampling purposes.

4.4.6 Antimycin A has been registered by the Governments of the United States and Canada as a fish toxicant since 1966. The dry formulation is known as "Fintrol" and has been registered by a commercial company. Field trials have been made and reported by the U.S. Fish and Wildlife Service. Successful usage has been reported over a wide range of water qualities and water temperatures. It is effective on fish at concentrations of 1 part per billion and less but is reported to be relatively harmless to plants, insects, mammals, and birds.

4.4.7 Rotenone is also registered for fishery use by the U.S. Environmental Protection Agency according to the Federal Environmenal Pesticide control Act (Schnick and Meyer, 1978). Rotenone, obtained from the derris root (*Deguelia elliptica*, East Indies) and cube root (*Lonchocarpusw nicour*, South America) in the family Leguminosae, has been used intensively in fisheries work throughout the United States and Canada since 1934 (Krumholz, 1948). Rotenone kills fish by blocking oxygen uptake, and the fish suffocate. The toxicity of Rotenone is a function of the species, size of fish, and water temperature. The pH, dissolved oxygen, and suspended particulate matter in the water can also affect its toxicity. It is effective in a short time period. Also, it has low toxicity to birds and mammals (Hendricks et al., 1980). Davies and Shelton (1983) reports that Rotenone at concentrations of 1.0 to 2.0 mg/L is lethal to zooplankton and many aquatic invertebrates, but the effects is short term. Although toxic to man and warm-blooded animals (132 mg/kg), rotenone has not been considered hazardous in the concentrations used for fish eradication (0.025 to 0.050 ppm active ingredient) (Hooper, 1960), and has been employed in waters used for bathing and in some instances in drinking water supplies (Cohen et al., 1960, 1961). Adding activated carbon in the water treatment process not only effectively removes rotenone, but also removes the solvents, odors, and emulsifiers present in all commercial rotenone formulations.

4.4.8 Rotenone obtained as an emulsion containing approximately 5% active ingredient, is recommended because of the ease of handling. It is a relatively fast acting toxicant. In most cases, the fish will die within 1 to 2 hours after exposure. Rotenone decomposes rapidly in most lakes and ponds and is quickly dispersed in streams. In warm water lakes or streams at summer water temperatures, toxicity lasts 24 hours or less. In cold water lakes toxicity may last for 5 to 30 days. Detoxification is brought about by five principal factors: dissolved oxygen, light, alkalinity, heat, and turbidity. Of these, light and oxygen are the most important factors.

4.4.9 Although the toxicity threshold for rotenone differs slightly among fish species, it has not been widely used as a selective toxicant. It has, however, been used at a concentration of 0.1 ppm of the 5% rotenone emulsion to control gizzard shad (Bowers, 1955). For most species the toxicity of rotenone is greatest between 10°C (50°F) and 23.9°C (75°F), and a 0.5 mg/L of formulation (0.025 mg/L of rotenone) kills most fish species. The toxicity drops as temperature decreases. Formulation of 1.0 to 2.0 mg/L is usually used to insure a complete kill, and blocking nets should be used in the

sampling area to ensure the desired catch. Sensitivity to rotenone varies considerable among species and among life stages within a species (Holden, 1980). The toxicity is affected by temperature, pH, oxygen concentration, and light (Hendricks et al., 1980; Holden, 1980). USEPA (1978) recommends a concentration of 0.1 mg/L for sensitive species, and a concentration of 0.7 mg/L is recommended if bullheads and carp are present.

4.4.10 Chemical sampling is usually employed on a spot basis, e.g., a short reach of river or an imbayment of a lake or reservoir. A concentration of 0.5 ppm active ingredient (1/2 gal. 5% rotenone/acre ft.) will provide good recovery of most species of fish in acidic or slightly alkaline water (Table 4). If bullhead and carp are suspected of being present, a concentration of 0.7 ppm active ingredient is recommended. If the water is turbid and strongly alkaline and resistant species (i.e., carp and bullheads) are present, use 1-2 ppm. However, caution is advised because rotenone dispersed into peripheral water areas may kill fish as long as the concentration is above 0.1 ppm. When rotenone is used in an embayment, some sort of blocking system should be in place to prevent fish in the area from escaping. Block seines or divers have been successfully used in past studies. Chemical blocks can be used but are recommended only when nets or divers cannot be successfully employed.

4.4.11 A very efficient method of applying emulsion products to lake waters and embayments is to pump the emulsion from a drum mounted in the bottom of a boat. The drum should be equipped with an outside tube, mounted on the drum and calibrated to indicate how fast the chemical is being pumped out of the barrel. The emulsion is suctioned out by a venturi pump (Amundson Boat Bailer) clamped on the outboard motor. The flow can be metered by a valve at the drum hose connection. This method gives good dispersion of the chemical and greater boat handling safety since the heavy drum can be mounted in the bottom of the boat rather than above the gunwales as required for gravity flow.

4.4.12 If spraying equipment is used, it will vary according to the size of the job. For small areas of not more than a few acres a portable hand pump ordinarily used for garden spraying or fire fighting is sufficient. Some individuals have successfully used a back-pack fire pump to collect fish samples from small streams or sections of streams. A mixture of one quart rotenone in five gallons of water is applied in small amounts.

4.4.13 A power-driven pump is recommended for a large-scale or long-term sampling program. The capacity of the pump need not be greater than 200 L per minute. Generally, a 1-1/2 h.p. engine is adequate. The power application of rotenone emulsives requires a pressure nozzle, or a spray boom, or both, and sufficient plumbing and hose to connect with the pump. The suction line of the pump should be split by a "y" to attach two intake lines. One line is used to supply the toxicant from the drum, and the other line to supply water from the lake or embayment. The valves are adjusted so that the water and toxicant are drawn into the pumping system in the desired proportion and mixed. A detailed description of spraying equipment can be found in Mackenthun (1969); Mackenthun and Ingram (1967).

4.4.14 A drip method is generally used to dispense rotenone to a flowing

system. Select a 30 to 100 meter reach depending on the depth and width of the stream; measure the depth of the section selected, calculate the area and flow and determine the amount of chemical required (Table 5). Block off the area upstream and downstream with seines. Position containers of liquid rotenone at the upstream end of the stream reach to be sampled. Nozzles on the containers must be metered to deliver the predetermined amount of rotenone to the stream. For additional details concerning the use of a delivery system for the drip method and nomographs for calculating the amount of toxicant refer to Price and Haus (1963) and Davies and Shelton (1983). The toxic effect of rotenone can be eliminated almost immediately with potassium permangate ($KMnO_4$) at 1 mg/L for each 0.05 mg/L of rotenone (Lawrence, 1955, 1956; Davies and Shelton, 1983). In lentic waters, the potassium permangate needed to oxidize rotenone is equal to the amount of rotenone applied plus the chlorine demand of the water. In lotic waters the amount has been estimated as 2.5 mg/L per cubic foot per second during the entire time the rotenone is passing through the neutralization point (Platts et al., 1983). Also, potassium permangate is considered toxic to some fish species at 3 ppm. Potassium permanganate is also hazardous to apply, and nose, throat, and eye protection should be exercised by anyone working with it.

4.4.15 The following company sells aquaculture, quality manufactured drugs, chemicals, biological, scientific supplies, and fish farming equipment:

Argent Laboratories
9702 152nd Avenue Northeast
Richmond, WA 98052, Telephone (206) 885-3377

4.5 Hook and Line

4.5.1 Fish collection by hook and line can be as simple as using a hand-held rod or trolling baited hooks or other lures, or it may take the form of long trot lines or set lines with many baited hooks. In generally, the hook and line method is not acceptable for conducting a fishery survey, because it is too highly selective in the size and species captured and the catch per unit of effort may be low. Although it can only be used as a supporting technique, it may be the best method to obtain a few adult specimens for contaminant analysis, etc., when sampling with other gear is impossible.

4.5.2 A variation of this is "jug fishing" where a short drop line of 2-3 feet with a baited hook is attached to a jug or can and allowed to drift downstream. This is a particularly effective way of sampling catfish.

4.6 Passive Sampling Techniques

4.6.1 Passive sampling devices and techniques (Hubert, 1983) can be used to supplement boat electrofishing data in lakes, reserviors, large rivers, estuaries, marshes, and wetlands. Fyke nets and trap nets are used in shallow water while modified hoop nets and gill nets are used in deep or open waters All passive sampling techniques should be checked and emptied 12 to 24 hours after setting. Data collected by passive sampling techniques can be used to determine relative abundance which are expressed as number/24 hours and weight (kg)/24 hours (Ohio EPA, 1989).

TABLE 4. AMOUNT OF 5% EMULSIFIABLE ROTENONE EQUIVALENT TO 0.5 PPM OR 1.0 PPM PER ACRE-FEET OR POND OR LAKE TO BE SAMPLED

Rotenone (5% Emulsifiable) Application Rates		
Acre-Feet	Pints of 5% Rotenone 0.5 ppm	1.0 ppm
0.25	0.3	0.6
0.50	0.6	1.2
0.75	1.0	2.0
1.00	1.3	2.6
1.25	1.6	3.2
1.50	2.0	4.0
1.75	2.3	4.6
2.00	2.6	5.2
2.25	3.0	6.0
2.50	3.3	6.6
2.75	3.6	7.2
3.00	4.0	8.0
3.25	4.3	8.6
3.50	4.6	9.2
3.75	5.0	10.0
4.00	5.3	10.6
4.25	5.6	11.2
4.50	6.0	12.0
4.75	6.3	12.6
5.00	6.6	13.2
5.25	7.0	14.0
5.50	7.3	14.6
5.75	7.6	15.2
6.00	8.0	16.0

TABLE 5. CUBIC CENTIMETERS (cc) OF LIQUID ROTENONE PER MINUTE FOR GALLONS OF FLOW PER MINUTE

Flow of Stream in Gallons per Minute	Five Percent (5%) Liquid Rotenone Requirements in Cubic Centimeters Per Minute			
	0.5 ppm	1.00 ppm	1.5 ppm	2.0 ppm
10	0.019	0.038	0.057	0.076
20	0.038	0.076	0.114	0.151
30	0.057	0.114	0.170	0.227
40	0.076	0.151	0.227	0.303
50	0.095	0.189	0.284	0.379
60	0.114	0.227	0.341	0.454
70	0.132	0.265	0.397	0.530
80	0.151	0.303	0.454	0.606
90	0.170	0.341	0.511	0.681
100	0.189	0.379	0.568	0.757
200	0.379	0.757	1.136	1.514
300	0.568	1.136	1.703	2.271
400	0.757	1.514	2.271	3.028
500	0.946	1.893	2.839	3.785

4.6.2 Entanglement nets

4.6.2.1 Gill and trammel nets are used extensively to sample fish populations in estuaries, lakes, reservoirs, and larger rivers.

4.6.2.2 A gill net is usually set as an upright, vertical fence of netting and can have either a variable or uniform mesh size. Experimental gill nets made of monofilament may be 37.5 m long and constructed with 7.5 m panels of 15.2 mm, 22.9 mm, 25.4 mm, 40.6 mm, and 50.8 mm bar mesh, and the variable mesh size gill nets are generally preferred. Fish attempt to swim through the net and are caught in the mesh (Figure 8). Because the size of the mesh determines the species and size of the fish to be caught, gill nets are considered selective. The most versatile type is an experimental gill net consisting of five different mesh size sections. Mesh sizes depend on the size range of fish species to be sampled. A range of mesh sizes in an experimental gill net is used to obtain samples of several year classes of a single species, and it will also provide a greater chance to increase the number of species caught. Gill nets made of multifilament or monofilament nylon are recommended. Multifilament nets cost less and are easier to use, but monofilament nets generally capture more fish. The floats and leads usually supplied with the nets can cause net entanglement. To reduce this problem replace the individual floats and float line with a float line made with a core of expanded foam and use a lead-core leadline instead of individual lead weights and lead line. Gill nets are usually set in open waters to sample fishes in large rivers, lakes, and reservoirs. They can be set at the surface, mid-depth, or on the bottom depending on the objectives of the study and target species within the fish community. Gill nets should be anchored and marked well in open water areas with floats on both ends.

4.6.2.3 The trammel net (Figure 9) has a layer of large mesh netting on each side of loosely-hung, smaller gill netting. Small fish are captured in a "bag" of the gill netting that is formed as the smaller-mesh gill netting is pushed through an opening in the larger-mesh netting. Trammel nets are not used as extensively as are gill nets in sampling fish.

4.6.2.4 Trammel nets can be fished in all types of habitats found in rivers such as the Mississippi. If a backwater or quiet stretch of the river is to be fished, the net is set. If the river channel is to be fished, the net is floated or drifted downstream. Trammel nets are very efficient for taking such fish as carp and buffalo. Trammel net float fishing is an excellent method of sampling shovelnose sturgeon and freshwater drum.

4.6.2.5 Stationary gill and trammel nets are fished at right angles to suspected fish movements (e.g., parallel to shore) and at any depth from the surface to the bottom. They may be held in place by poles or anchors. The anchoring method must hold the net in position against any unexpected water movements such as, runoff, tides, or seiches.

4.6.2.6 Drifting gill or trammel nets are also set and fished the same as stationary gear, except that they are not held in place but are allowed to drift with the current. This method requires constant surveillance when fishing. They are generally set for a short period of time. If currents are

too great, stationary gear may be used, but heavy current can cause the net to collapse.

4.6.2.7 Results for both trammel and gill nets are expressed as the number or weight of fish taken per length of net per day (=catch per unit effort).

4.6.2.8 The use of gill nets in estuaries may present special problems, and consideration should be given to tidal currents, predation, optimum fishing time, and types of anchors, floats, and line. When gill net fishing in tidal waters, it is recommended that reversing anchors be used for anchoring if the nets are to be left unattended. Mushroom anchors and concrete blocks will not hold down the nets during tidal cycles and may allow them to move considerable distances if a high tidal cycle is present. The gill nets should be monitored frequently and usually after a tidal cycle change as marine species usually will not survive too long in gill nets. Dead fish tend to attract crabs which tangle in the nets making them difficult to remove. When nets are set in the mouths of creeks, the outgoing tidal cycle generally will be more productive.

4.6.2.9 In freshwater, monofilament gill nets are very effective for lake herring, trout, lake whitefish, yellow perch, walleyes, and northern pike.

4.6.2.10 Necessary equipment for netting includes a pair of "clipper" pliers for removing sharp pectoral and dorsal spines on catfish and bullheads when these fish become tangled in the netting. Also, the gunnels of any boat used in a net fishing operation should be free of rivets, cleats, etc. on which the net can snag.

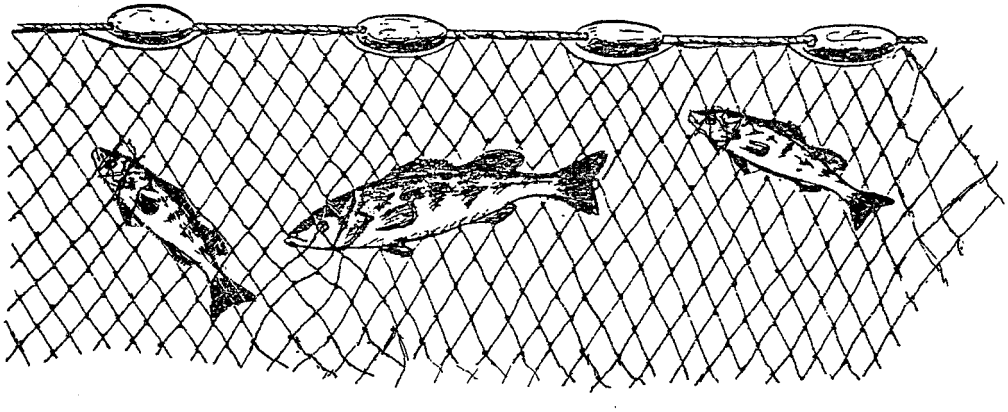

Figure 8. Gill net. Modified from Dumont and Sundstrom (1961).

4.6.3 Entrapment Devices

4.6.3.1 With entrapment devices, the fish enter an enclosed area (which may be baited) through a series of one or more funnels and prevent excapement. They are used to sample reserviors and wide river channels with slow velocity conditions. Entrapment nets are set in structurally complex areas where fish movement and density are anticipated to be highest in order to maximize net catches.

4.6.3.2 The hoop nets (modified hoop nets) and trap nets are the most common types of entrapment devices used in fishery surveys. These traps are small enough to be deployed from a small open boat and are relatively simple to set. They are held in place with anchors or poles and are used in water deep enough to cover the nets, or to a depth up to 4 meters.

4.6.3.3 The hoop net (Figure 10) is constructed by covering hoops or frames with netting. It has one or more internal funnels and does not have wings or a lead. The first two sections can be made square to prevent the net from rolling in the currents.

4.6.3.4 The fyke net (Figure 11) is a hoop net with wings, or a lead, or both attached to the first frame. The second and third frames can each hold funnel throats, which prevent fish from escaping as they enter each section. The opposite (closed) end of the net may be tied with a slip cord to facilitate fish removal.

4.6.3.5 Hoop nets are fished in rivers and other waters where fish move in predictable directions, whereas the fyke net is used when fish movement is more random such as in lakes, impoundments, and estuaries. Hoop and fyke nets can be obtained with hoops from 2 to 6 feet (0.6 to 1.8 meters) in diameter, but any net over 4 feet (1.2 meters) in diameter is too large to be used in a fishery survey.

4.6.3.6 Trap nets use the same principle as hoop nets for capturing fish, but their construction is more complex. Floats and weights instead of hoops give the net its shape. The devices are expensive, require considerable experience, and are usually fished in waters deep enough to cover them.

4.6.3.7 One of the traps which has proven to be quite effective is a 3 x 6 foot frame with a 3 x 50 foot lead consisting of 1/2 inch square mesh of #126 knotless nylon. Traps with 1/4 inch mesh netting have also been used. Trap nets are set with the lead perpendicular to the shoreline. They usually are most effective in depths less than 25 feet with a minimum depth of about 3 feet.

4.6.3.8 One of the most simple types is the minnow trap, usually made of wire mesh or glass, with a single inverted funnel. The bait is suspended in a porous bag. A modification of this type is the slat trap (Figure 12); this employs long wooden slats in a cylindrical trap, and when baited with cheese bait, cottonseed cake, etc., is usually very successfully in sampling catfish in large rivers.

4.6.3.9 Most fish can be sampled by setting trap and hoop nets of varying sizes in a variety of habitats. Hoop and trap nets are made of cotton or nylon, but nets made of nylon have a longer life and are lighter when wet. Protect cotton and nylon nets from decay by using the same methods of treatment mentioned for seines in Subsection 4.2.1.5. The catch is recorded as numbers or weight per unit of effort, usually fish per net day.

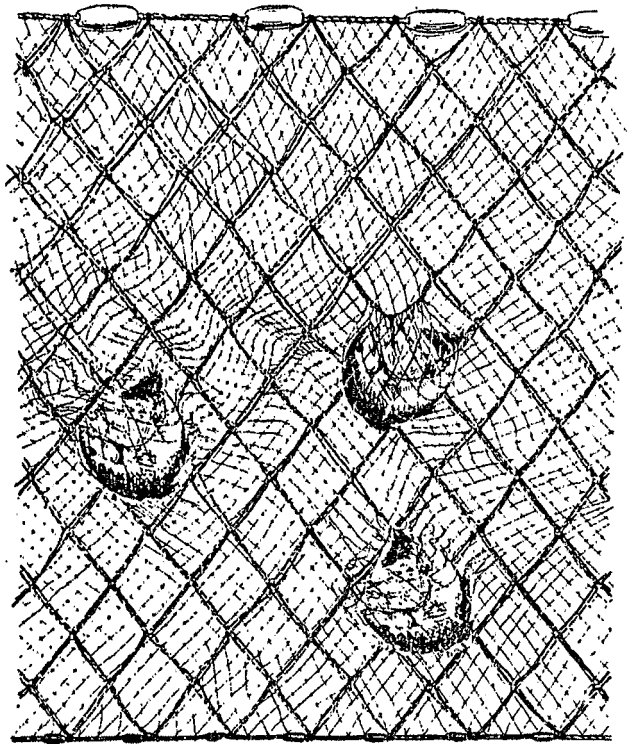

Figure 9. Trammel Net. Modified from Dumont and Sundstrom (1961).

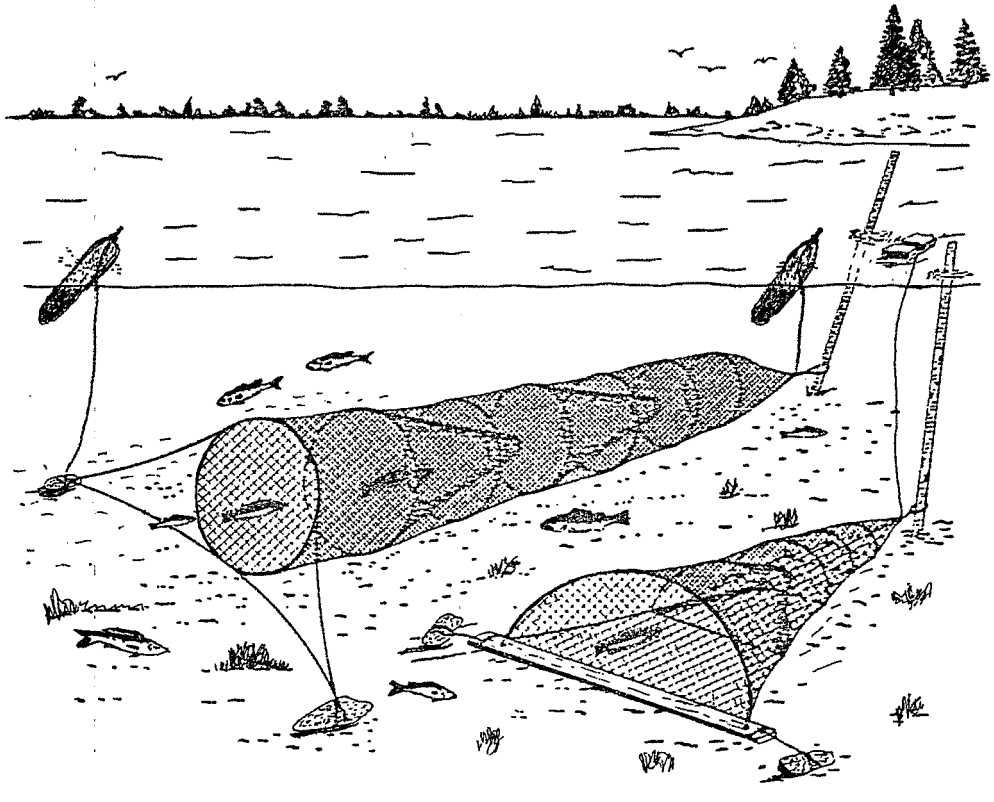

Figure 10. Hoop Nets. Modified from Dumont and Sundstrom (1961).

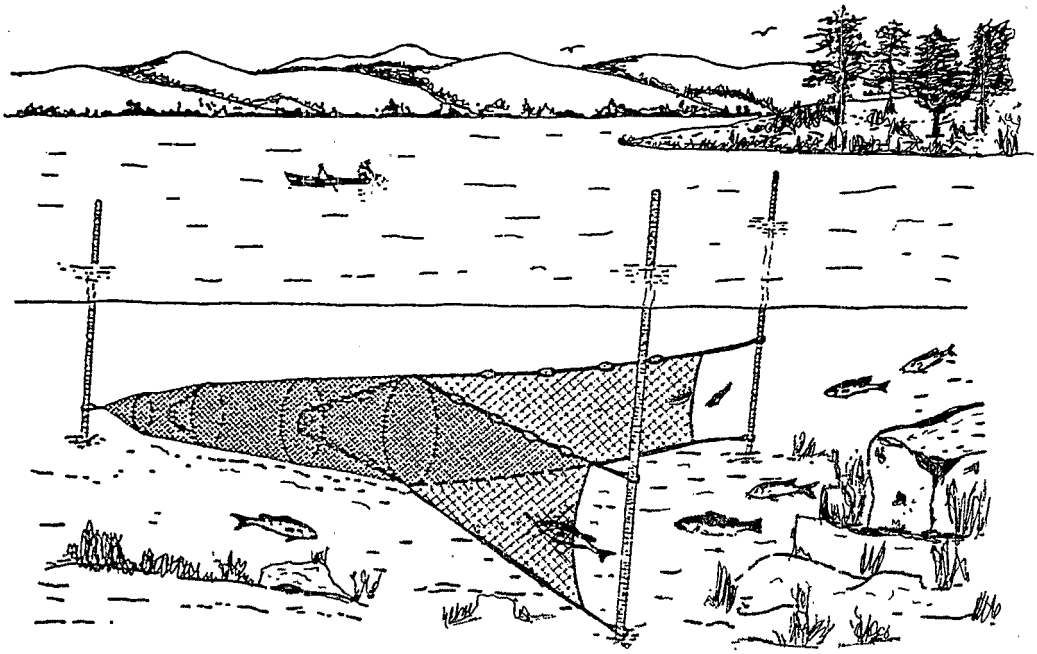

Figure 11. Fyke Net. Modified from Dumont and Sundstrom (1961).

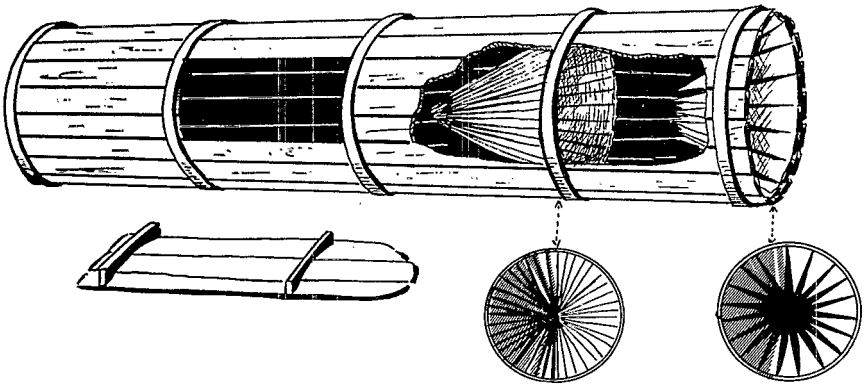

Figure 12. Slat Trap. Modified from Dumont and Sundstrom (1961).

4.7 Pop Nets

4.7.1 Pop nets are useful for sampling fish in shallow riverine waters in heavily vegetated and nonvegetated areas where seining or electroshocking may be difficult (Larson et al., 1986; Dewey et al., 1989). Pop nets are set and retrieved by two individuals and are easily dissembled for easy transport.

4.7.2 Pop nets (Figure 13) are rectangular devices, constructed of lightly tarred 6.4 mm mesh netting. They are 1.8 m wide x 3.1 m long x 1.8 m high when released and enclose an area of 6.5 m^2. The top of the net is attached to a rectangular polyvinylchloride frame filled with foam. The top of the frame should be painted black to reduce the effect of color on fish avoidance or attraction. They are designed to be set from the surface and released with a mechanical device.

4.7.3 The pop net used in nonvegetated areas can simply be a rectangular holding net with its top attached to the buoyant frame and its bottom panel attached to a frame, 19 mm diameter galvanized pipe. After the pop net is tripped, the net and attached frames are picked up and carried to shore as a unit.

4.7.4 The enclosed bottom design pop net cannot be used in vegetated areas. A pop net used in vegetated areas is constructed with an open-bottom. Its bottom is split down the center and attached only along the two long sides of the holding net. The bottom frame is still present but used only to hold the buoyant top frame in position during the setting process and is not attached

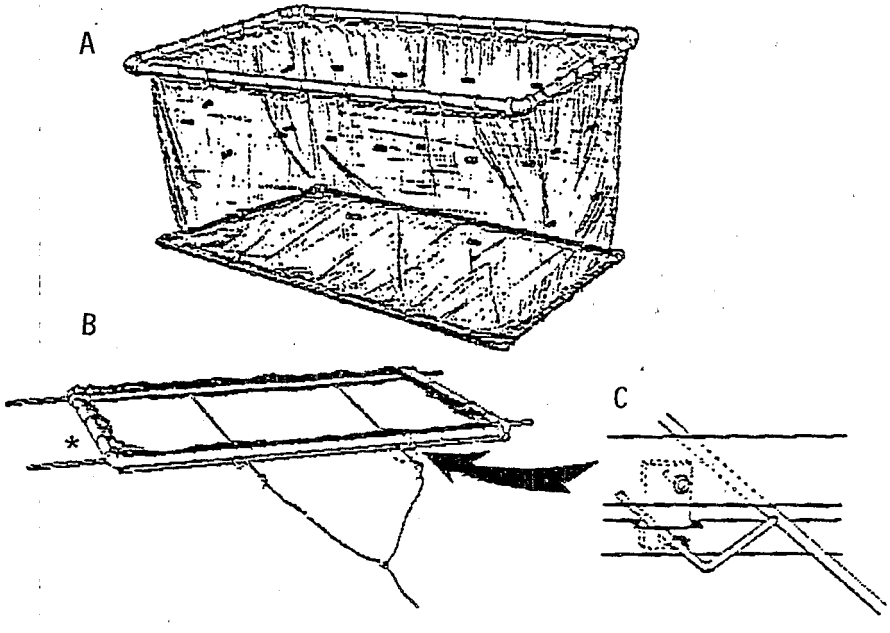

Figure 13. Pop net. A. model for nonvegetative site after release. B. Pop net set for release in vegetated site, show pipes used for bottom closure (*) and position of release mechanism (arrow). C. Release mechanism. From Dewey et al. (1989).

at all to the holding net. Galvanized pipes 3.7 m long, are attached to each of the two split sections and are used to hold the bottom sections to the sides as the pop net is placed over vegetation (Figure 13,B). After the pop net is released, these pipes are used to purse the bottom sections together and thus enclose the catch. The sample is retrieved by carrying the top frame, attached net, and pursing pipes to shore as a unit. The bottom frame is retrieved separately.

4.7.5 Pop nets have two release mechanisms (Figure 13,B and C), each consisting of two devices at opposite sides of the net. At each position, a piece of aluminum flat bar, attached to the top frame, fits into a slot in the bottom frame. An L-shaped extension attached to the trip rod fits through matching holes in the flat bar and in the bottom frame to hold the top and bottom frames together. The trip rods for both mechanisms are joined by a lead core line, to which is tied a 5 cm trip cord. When the trip cord is pulled, both trip rods release simultaneously, allowing the buoyant top frame to rise. In the set position, these release mechanisms hold the upper and lower frames together in a low profile (9.5 cm high), which increases stability in currents.

4.8 Miscellaneous Fish Methods

4.8.1 Underwater Methods

4.8.1.1 Direct observation techniques can be used to study the structure of fish assemblages, spawning, feeding, and movement, etc. For techniques on direct underwater observation which involve the use of divers (snorkeling and scuba) to study fish populations, see Helfman (1983) and Pearsons et al. (1992).

4.8.2 Hydroacoustic Techniques

4.8.2.1 Hydroacoustic assessment techniques are generally applied to methods which use equipment such as sonars or depthsounders. The hydroacoustic techniques use sound from these devices that are actively transmitted and information extracted from the returning echoes to detect fish and make qualitative and quantitative estimates of biomass. For a review, discussion, and guidelines of fishery hydroacoustics, see Thorne (1983).

4.8.2.2 Information on hydroacoustic equipment for fisheries evaluations can be obtained from the following company:

Hydroacoustic Technology, Inc.
715 NE Northlake Way
Seattle, WA 98105, Telephone (206) 633-3383.

4.8.3 Underwater Biotelemetry

4.8.3.1 These techniques are often used to monitor the locations, behavior, and physiology of free-ranging fish, and involves attaching a device that relays biological information. For a review and discussion of telemetry methods, see Winter (1983).

4.8.3.2 Information on a field proven digitally encoded radio telemetry system for fisheries evaluations can be obtained from the following company:

Lotek Engineering, Inc.
115 Pony Drive
Newmarket, Ontario, Canada L3Y 7B5
Telephone (416) 836-6680

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SECTION 5

FISH SPECIMEN PROCESSING

5.1 Introduction

5.1.1 After fish are collected, they must be either examined and identified in the field or if voucher specimens are required, they must be fixed immediately for subsequent identification in the laboratory. If the sampling crew have difficulty identifying any specimens in the field, those specimens must be fixed and later identified in the laboratory. The decision to preserve specimens should depend on study objectives. One set of specimens should be preserved during the study (especially in the early stages) so that a vouchered, archived reference collection of each species from different study areas or ecoregions will be available to investigators. The study team should be become familiar with characteristics of the specimens difficult to identify. For general purposes, formalin is usually used as a fixing agent (ASIH, 1988). This fixative solution helps retain chromatophore patterns which aid in species identification. When using formalin, care must be taken because it is highly allergenic, toxic, and dangerous to human health (carcinogenic) if used improperly.

5.1.2 If specimens are to be kept alive, they should be placed in a live well, container, or bucket and processed upon completion of sampling at each site or when the live well container or bucket are full. To minimize fish mortality in the live well or bucket, water should be changed periodically or aerated with a battery-powered pump. Fish should be handled carefully and released immediately after they are identified to species, examined for external anomalies, and weighed if necessary. Every effort should be made to minimize fish handling and holding times.

5.1.2.1 If a large number of the fish specimens are to be kept alive for later study, see Stickney (1983) for a discussion and guidelines on caring for and handling live fish.

5.2 Fixation and/or Preservation of Fish Samples

5.2.1 Fixation is the process of rapidly killing and chemically stabilizing fish tissues to maintain anatomical form and structure. Preservation is the process by which fixed tissues are maintained in that condition for an indefinite period of time.

5.2.2 Fish and ichtyoplankton should be fixed and preserved (Table 1) in the field in neutral buffered 10% formalin or borax buffered 10% formalin (a 9:1 ambient water dilution of 100% formalin) for 24 hours or longer, depending on size of fish (Haedrich, 1983, Lagler, 1956, Lagler et al., 1962, Humason, 1974, and Knudsen, 1966). The sodium phosphate monobasic and sodium phosphate dibasic, or borax, acts as a buffer which neutralizes the acidic effect of the formaldehyde. This mixture retards shrinkage in fish, prevents the hardening of soft body parts, and prevents decalcification of the tissues (Lagler et al., 1962). Fish should remain in the formalin solution for at least 1-2

weeks to fix the tissue. Fixation may take from a few days with small specimens to a week or more with large forms. Large fish or containers with closely packed fish or temperatures greater than 26.7°C (80°F) require a stronger solution of one part formalin to seven or eight parts water for fixation. Stronger solutions of formalin can cause gaping or distortion of the mouth and gills, thus care should be taken to obtain correct concentrations when making up the formalin solution (Ohio EPA, 1989).

TABLE 1. FORMULATION OF FORMALIN FIXATIVE SOLUTION

37% formaldehyde (100% formalin)	100 mL
Distilled water	900 mL
and	
Sodium phosphate monobasic ($NaH_2PO_4 \cdot H_2O$)	4 g
Sodium phosphate dibasic (Na_2HPO_4)	6.5 g
or	
Add one teaspoon of borax per 1/2 gallon of the formalin	

5.2.3 Since the volume of collected fishes must be taken into account upon fixation, formalin for field use should be stronger than 10%, and even 20% will not hurt. Formaldehyde gas reaches saturation in water at about 37% by weight; this saturated solution is called 100% formalin. Isopropyl alcohol and ethyl alcohol are preservatives, not fixatives. These preservatives do not fix the tissues, a necessary procedure for tissue preparation, staining, etc.

5.2.4 After fixation in the formalin, some scientists transfer the specimens to a preservative for storage. Ethyl alcohol (70-75%) or isopropanol (40-45%) preservation keeps specimens more pliable than formalin and makes working with them easier. Specimens should be rinsed in water to wash off any excess formalin, placed in a 35% alcohol wash for 2-3 weeks, switched to a 50% alcohol wash for 2-3 weeks, and placed in a 70%-75% aqueous solution of ethyl alcohol or 40-45% isopropanol alcohol for permanent preservation and storage (Haedrich, 1983; Ohio EPA, 1989). Fish should be stored in glass or plastic containers or stainless steel vats for large specimens. Metal containers should not be used. It is important that the containers be tightly sealed to prevent evaporation of the preservative.

5.2.5 Specimens are kept in tightly sealed museum jars, along with their field data. The preservatives will always modify the color, and light will further bleach the fish specimens so the various markings and colors of fish

should be documented if the specimens are to be identified later. It is advisable to store specimens in the dark at 18°C to minimize evaporation and bleaching.

5.2.6 Specimens larger than 7.5 cm should be slit on the side at least one-third of the length of the body cavity or injected with a hypodermic syringe to permit the preservative to reach the internal organs. Large and heavy fish (1-2 pounds) should also be injected in the muscles on each side of the backbone with formalin. Fish should be slit on the right side, because the left side is generally used for measurement, scale sampling and photographic records.

5.2.7 Samples for fish tissue contaminant analysis or electrophoresis must be iced, placed in dry ice, or liquid N_2 for temporary storage or shipping. Fish samples for pesticide analysis should be wrapped in aluminum foil, see Section 10, Guidelines for Fish Sampling and Tissue Preparation for Bioaccumulation Contaminants, and placed in a cooler with ice. The sample must be frozen as soon as possible after collection. Fish collected for metals analysis should be placed in plastic bags. All samples should be doubled tagged, with one tag attached outside the foil or plastic bag and one tag inside.

5.2.8 Special preservation techniques must be used for histological, histochemical, or biomarker analyses, and the investigator should be aware of such techniques before collecting tissue samples (Humason, 1974).

5.3 Labelling of Specimens in Field and Laboratory

5.3.1 Each specimen or specimens from a collecting site should be carefully labelled with at least the information asked for in the examples of labels in Figure 1.

5.3.1.1 Collection information should be both on and in the container, a tag, or a paper label. If paper labels are used, they should be made of 100% rag (waterproof) and labelled with India ink or a No. 2 soft lead pencil.

5.4 Species Identification

5.4.1 Many fish can be field identified with certainty. However, the following procedures for fish identification and verification of difficult specimens are recommended by Lowe-McConnell (1978):

1. Assemble and use the best available keys and checklists (see Section 8, Fish Bioassessment Protocols for Use in Stream and Rivers, Subsection 8.14, Selected References for Determining Fish Tolerance, Trophic, Reproductive, and Origin Classifications and Section 12, Fisheries Bibliography, Subsection, 12.5 Fish Identification).

2. Key fish to species level.

3. Maintain a voucher collection in the laboratory for comparison of specimens.

4. Verify difficult species identifications with pictures, published descriptions, known geographic range, museum and lab voucher specimens, or have the specimen identified or verified by a specialist.

FIELD SAMPLE DATA LABEL

Project _____

Date _____ Time _____ Collection No. _____

Location _____

_____

County _____ State/Country _____

Collector(s) _____

Type of sample _____ Preservative(s) _____

Method of collection _____

A. Long Form

FIELD SAMPLE DATA LABEL

Date _____ Collection No. _____

Location _____

_____

Collector(s) _____

Type of sample _____ Preservative(s) _____

B. Short Form

Figure 1. Examples of field sample data labels. A. Long form, B. Short form.

5.4.2 Scientific nomenclature of all specimens should follow the recommendations of the American Fisheries Society (Robins et al., 1990).

5.4.4 Biomonitoring laboratories should maintain a fish reference collection. Unique specimens should also added to the collection. The collection should be archived in a computer data base which cross-references field data and other pertinent information about the study.

5.5 Literature Cited

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SECTION 6

SAMPLE ANALYSIS TECHNIQUES

6.1 Introduction

6.1.1 One of the major concerns of USEPA, other federal, state and private agencies or laboratories is to describe water quality and habitat quality in terms which are easily understood by the nonbiologist. Fish studies frequently include the number of specimens captured per unit area or unit time. Also, the fish can be measured, weighted, aged, and sexed to provide comparative data between populations in different habitats. 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, lake, or water body in the United States. Methods, techniques, and biological criteria used to study fisheries biology and to analyze fisheries data are described in this manual, elsewhere in Bagenal (1978), Lager (1956, 1978), Carlander (1969), Everhart et al. (1975), Gulland (1983), Nielsen and Johnson (1983), Schreck and Moyle (1990), USEPA (1990, 1991), and also in other current literature. To supplement the statistics and data evaluation methods in this section and for additional biometrics, consult the statistical references listed in Section 1, Introduction, Subsection 1.16.1. For other multivariate analyses and other techniques to relate distribution to environmental variables and gradients, confer with Matthews (1985), Matthews and Robison (1988), Mayden (1985; 1988), and McAllister et al. (1986).

6.1.2 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, including fish. A number of data interpretation methods have been developed based on these community characteristics to indicate the health and water quality of the aquatic environment, the degree of habitat degradation, and also to simplify communication problems regarding management decisions.

6.2 Data Recording

6.2.1 The sample records should include collection number, name of water body, date, locality, names of sample collectors, and other pertinent information associated with the sample. Make adequate field notes for each collection. Use water-proof ink and paper to ensure a permanent record. Place the label (Figure 1; also see Section 2, Quality Assurance and Quality Control; Section 5, Fish Specimen Processing) inside the container with the specimens only when fixing or preserving fish for physical examination (**Note: do not place the label with fish if they are to be chemically analyzed.**) and have the label bear the same number or designation as the field notes, including the locality, date, and collector's name. Place a numbered tag on the outside of the container to make it easier to find a particular collection. Place any detailed observations about a collection on the field data sheet (see Section 4, Sample Collection for Analysis of Structure and Function of Fish Communities and Section 8, Fish Bioassessment Protocols for

Use in Streams and Rivers for examples of field data sheets). Record fishery catch data in standard units such as number or weight per area or unit of effort. Use the metric system for length and weight measurements. Designate any chemical analyses to be performed, e.g., toxaphene analysis.

6.3 Fish Identification

6.3.1 Proper identification of fish to species level is mandatory in analysis of the data for water quality interpretation. A list of regional and national references for fish identification is located in Section 8, Fish Bioassessment Protocols for Use in Streams and Rivers; Section 12, Fisheries Bibliography. Assistance in confirming questionable identification is available from State, Federal, and university fishery biologists or ichthyologists. In the Quality Assurance Project Plan (see Section 2, Quality Assurance and Quality Control), key(s) used for fish identification should be specified.

Collection No. _____

Project _____

Location _____

_____

Date _____ Time _____ Mile _____

Sampling Device _____

Collected by _____

Observations _____

_____

Preservation(s) _____

Figure 1. Example of fish sample label information for preserved specimen container.

6.4 Species Composition (Richness)

6.4.1 A list of species can be compiled using any sampling device, technique, or combinations of the two. The method used should not select against one or more species. Also, sampling effort should be thorough enough so that all species are collected from the study area, and the sampling should be

conducted several times during the year to include seasonal species. The calculations for percent species composition in a sample is:

$$= \frac{\text{Number of individuals of a given species}}{\text{Total number of all fish collected}} \times 100.$$

6.5 Length and Weight

6.5.1 Rate of change in length of fish, length frequency distribution, and weight of fish are important attributes of fish populations. These measurements can provide an estimation in growth, standing crop, and production of fish in surface waters.

6.5.1.1 Three length measurements as described by Lagler (1978) are sometimes used in monitoring studies, but total length is used most often. The three length measurements (Figure 2) are standard length, fork length, and total length. Standard length of fish is measured from its most anterior extremity (mouth closed) to the hidden base of the caudal fin rays, where a groove forms naturally when the tail is bent from side to side. Fork length is measured from the most anterior extremity of the fish to the notch in the center of the tail. It is the center of the fin when the tail is not forked. Total length is the greatest length of the fish from the anterior most (mouth closed) and caudal rays squeezed together to give the maximum length measurement. For fish with a forked tail, the two lobes are squeezed together to give a maximum length. If the lobes are unequal, the longer lobe is used.

6.5.1.2 A fish measuring board is commonly used to measure length. Fish measuring boards contain a graduated scale and is usually made of wood or plastic. Lagler (1978) identifies and discusses factors that can cause possible errors and inconsistency in taking length measurements. When taking fish measurements, standard procedures should be written so that the measurements are done the same way if different individuals are involved in this procedure.

6.5.1.3 Measurement of fish weight is taken with an accurate scale that can be used in field studies. Lagler (1978) indicated that precision in weight measurements is not possible because of variation in the amount of stomach contents and the amount of water engulfed at capture of the fish. The weights of live and preserved specimens are not comparable because the percentage of shrinkage is unknown.

6.5.1.4 Additional information on length, weight, and associated structural indices are discussed in Anderson and Gutreuter (1983).

6.6 Age, Growth, and Condition

6.6.1 Changes in water quality can, at times, be detected by studying the age, growth, and condition of fishes taken from a body of water. These studies require extensive knowledge of the life histories of fish and of the area being studied, experience in aging fish, sufficient time and manpower to

adequately sample and analyze the data, and sufficient age, growth, and condition historical data for comparison.

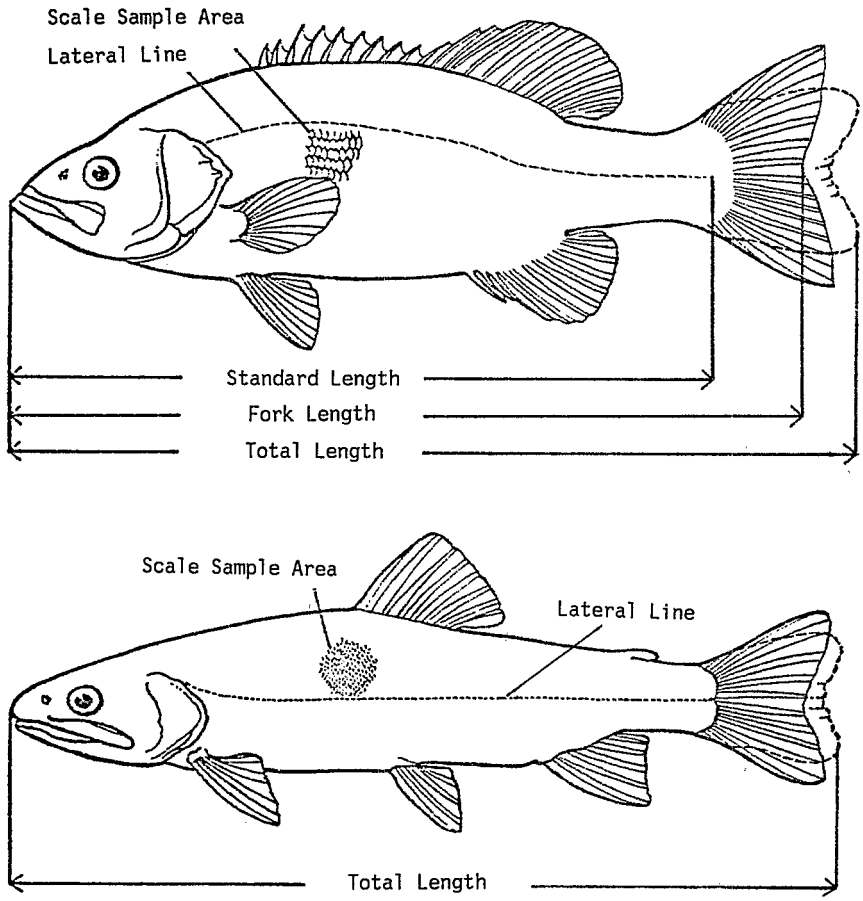

Figure 2. Fish measurements (using a fish measuring board) and scale sampling areas. A. spiny-rayed fish. B. soft-rayed fish. Total length measurement requires compressed tail to give maximum elongation. Modified from Lagler (1956).

6.6.2 A problem in using fish for any type of study is their high mobility. However, Gerking (1959) indicated that many species are relatively sedentary in summer. Depending on the species, there may be no practical way to determine with a first time visit how long an individual fish has been in a given area. Any changes detected in age, growth, or condition are not necessarily attributable to conditions prevailing at the capture site. Some

information on fish movement may be obtained from previous State or Federal studies. Only a carefully planned, long-term study may provide beneficial data, and only if used in conjunction with other biological, physical, and chemical data, e.g,, benthic invertebrates (macroinvertebrates), periphyton, water flow, habitat, and water chemistry.

6.6.3 The methods most commonly used in studying the age and growth of fishes are: (1) length-frequency, (2) annulus formations in hard parts, such as otolith, bone, spine rays, and scales.

6.6.3.1 The knowledge of the age and rate of growth of fish is extremely useful in fishery management. The processes of determining fish age and assessing fish growth rates are different, but they are closely related and are usually done at the same time. Table 1 was compiled by the Institute for Fisheries Research, the University of Michigan, Ann Arbor, Michigan from samples taken of Michigan fish during a period of approximately 30 years. The samples were collected mostly during the summer months but all months of the year are represented. Variations occur among states in sample size according to species and age groups, and some averages are more reliable than others. Busacker et al. (1990) discuss various techniques that are used in the study of fish growth, and they provide guidance to the appropriate uses of specific growth methods.

6.7 Length-Frequency Method

6.7.1 The length-frequency method for making age determinations is based on the assumption that fish increase in size with age. When the number of fish per length is plotted on graph paper for a given species if comparing a population. Peaks generally appear for each age group.

6.7.2 For this method to provide meaningful data it is important that the following criteria be met during sampling: (1) the fish must be collected over a short period; (2) large numbers must be obtained, including fish of all sizes; (3) the affected area and a control (unaffected) area must be sampled simultaneously within the same time frame.

6.7.3 For some studies, the length-frequency method may be of limited value because: (1) it is considered not reliable in aging fish beyond their second or third growing season (2) acquiring a large number of fish generally requires several experienced field biologists utilizing different sampling techniques.

6.8 Length-Age Conversion Method

6.8.1 In certain studies, it may be desirable to know the age of fish of a given length (e.g., selection data are normally in terms of length, but for incorporation in yield equations need to be expressed in terms of age.) Length can be converted to age (Gulland, 1983) by fitting all the observed data of mean length at age to a growth equation, such as the von Bertalanffy equation.

$$l_t = L_\infty \, [1 - e^{-K(t-t_0)}]$$

6.8.2 To calculate age (t) in terms of length (l), divide both sides by L_∞, and subtract from unity, resulting in

$$\frac{L_\infty - l_t}{L_\infty} = e^{-K(t-t_0)}$$

taking natural logs of both sides gives

$$\log_e \frac{L_\infty - l_t}{L_\infty} = -K(t-t_0)$$

therefore,

$$t = \frac{1}{K} \log_e \frac{L_\infty}{L_\infty - l_t} + t_0$$

where:

t = age (present)

l = length of individual specimens (length at time (t))

L_∞ = maximum length expected for a particular species

t_0 = the age at which the fish would be zero size

r = growth rate constant

TABLE 1. AVERAGE TOTAL LENGTHS IN INCHES FOR EACH AGE GROUP OF SEVERAL FISHES IN MICHIGAN[1]

Species	Age Group													
	0	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII
Bluegill	2.3	3.4	4.4	5.5	6.4	7.0	7.5	7.9	8.6	8.8	9.1	9.8	9.7	...
Pumpkinseed	2.8	3.3	4.4	5.2	5.9	6.4	7.9	7.3	7.8	7.4	8.1	9.8	...	...
Black Crappie	3.6	5.1	6.8	8.2	9.0	9.5	10.6	10.9	11.8	12.2	...	...	...	...
Rock bass	1.5	3.1	4.5	5.6	6.5	7.4	8.2	8.9	9.6	9.9	10.1	11.6	11.7	...
Warmouth	....	3.1	4.4	5.2	5.5	6.2	6.7	6.9	6.6	7.5	7.3	...	...	...
Green sunfish	...	3.0	3.9	4.7	5.1	5.7	5.7	5.0	...	...	...	...	...	...
Largemouth bass	3.6	6.1	8.6	10.6	12.2	13.6	15.1	16.7	17.7	18.8	19.8	19.6	20.8	...
Smallmouth bass	3.4	6.1	9.2	11.3	13.3	14.9	15.7	16.8	17.5	18.5	19.2	...	19.2	...
Yellow perch	3.1	4.6	6.1	7.0	8.0	9.0	9.9	10.7	11.3	11.8	12.3	12.3	13.9	13.2
Walleye	7.1	9.5	13.3	15.2	17.2	18.6	19.2	19.6	21.6	21.4	25.2	23.7	26.5	...
Northern pike	10.2	15.6	19.4	22.2	24.6	26.5	28.9	32.7	33.4	38.7	39.6	42.0	48.0	...
Muskellunge	6.8	15.7	19.9	25.4	31.9	34.7	36.8	39.2	41.7	45.3	48.7	47.5	49.7	...
Smelt	...	5.3	6.9	7.7	8.1	8.8	9.6	...	...	...	...	...	...	...
Brook trout	3.0	6.4	9.0	11.5	15.1	18.8	21.3	23.9	...	...	...	...	...	...
Rainbow trout (inland lakes and streams)	2.2	6.3	8.4	10.3	11.0	...	...	...	...	...	...	...	...	...
Steelhead (lake-run rainbow)	...	13.4	17.0	18.7	23.6	25.4	28.1	30.0	30.4	...	...	...	...	...

[1]From Laarman (1964), Length of common Michigan sport fishes at successive ages, Michigan Fisheries No. 7, Department of Fisheries, School of Natural Resources, The University of Michigan, Ann Arbor, MI.

6.9 Annulus Formation Method

6.9.1 This technique is based on the fact that fish are poikilothermic animals and the rate at which their body processes function are affected by the temperature of the water in which they live. Growth is rapid during the warm season and slows greatly or stops in winter. This seasonal change produces a band (annulus) in such hard bony structures as scales, otoliths (ear stones), fin rays and spines, and vertebrae each year the fish lives. Scales (Figure 2) are most commonly used in determining the age and yearly rate of growth because they lengthen throughout the life of the fish at a predictable ratio to the annual increment in body length. The location of the body from where the scales are obtained is important. Each species of fish has a specific body area from which scales should be removed for optimum clarity and ease of identifying the annuli and a size at which scale formation begins (Jearld, 1983; Lagler, 1956; Weatherley, 1972). Coin envelopes are frequently used for holding scales and for recording field data (Figure 3).

Collection No. _____

Species _____

Location _____

Date _____ Time _____ Mile _____

Sampling Device _____

Collected by _____

S.L. _____ T.L._____ Wt. _____

Sex ___ Maturity/and state of organs ____

_____

Annuli _____ Condition _____

Figure 3. Example of recording field data information of scale samples for age and growth studies.

6.9.2 Aging can be accomplished by use of a side-field, low-powered microscope, but a microprojector is preferred for determining the rate of growth. Computer assisted microprojectors have been developed for reading scales more rapidly and accurately.

6.9.3 It is important that the investigator realize that not all annuli-like markings are valid. "Spawning-checks", "false annuli", or other annuli-like marks may be present because of disease, body injury, spawning, etc.

6.9.4 The duration of sampling and the number of fish that must be collected are not as critical as the length-frequency method. Sampling can cover a considerable period and only a single method need be used for capturing the fish. Specialized equipment and trained personnel are needed, however, to identify, analyze, and interpret the data.

6.9.5 To determine any changes in the growth rate of a fish population, it is essential to use both the length-frequency and annulus methods and have samples from unaffected localities and/or sufficient background data from the sampling area. Any changes detected may be attributed to a single or a combination of natural or man-associated activities that altered the environment. Some of the most obvious natural modifications are a change in the average annual water temperature, fluctuating water levels, and availability of food. Man may also influence the water temperature and levels, physically alter the environment and fish habitat by damming or dredging activities, surface mining activities, and introducing substances that directly or indirectly affect the well-being of the fish population. It is evident, therefore, that it may be impossible to pin-point what or who was responsible for the change in the growth rate of a fish population except in a small lake.

6.10 Condition Factor (Coefficient of Condition)

6.10.1 The condition of fish can be estimated mathematically or by evaluating physical appearance.

6.10.2 Mathematically, the coefficient of condition is utilized to express the relative degree of well-being, robustness, plumpness or fatness of fish. It is based on a length-weight relationship and is calculated by the formula:

$$\text{Coefficient of Condition} \quad K_{TL} = \frac{W\ 10^5}{L^3}$$

W = weight in grams
L = length in millimeters
10^5 = factor to bring the value of K near unity
TL = designation of measuring system used (fork, standard, or total length)

6.10.2.1 The coefficient of condition is "K" when the metric system is used in expressing the length and weight, and "C" when the English system is used.

6.10.3 The coefficient of condition has been used by ichthyologists and fishery biologists to determine the suitability of the environment for a species. However, it is not recommended for use in short term water quality studies because any non-environmental factors influence the values derived,

e.g., changes due to age, sexual differences, and changes with seasons. These natural fluctuations make it extremely difficult to attribute any change to the quality of the water from which the fish are collected and must be taken into account when designing long term studies and evaluating data.

6.10.4 The observance of the physical appearance or condition of fish will usually indicate the general state of their well being and give some broad indication of the quality of their environment. When fish are captured they should be examined to see if they appear emaciated, are diseased, or contain parasites. The condition of their gills should also be checked. Healthy fish will be active when handled and are reasonably plump. Dissect a few specimens and check the internal organs for disease or parasites. The stomach of fish should also be examined to determine if the fish were actively feeding prior to capture.

6.10.5 For more detailed information on age, growth, and conditions of fish, see Anderson and Gutreuter (1983), Bagenal and Tesch (1978), Calhoun (1966), Carlander (1969), Everhart et al. (1975), Goede (1991), Jearld (1983), Lagler (1956), Lux (1971), Norman (1951), Ricker (1975), Schram et al. (1992), Summerfelt (1987), and Weatherley (1972).

6.11 Relative Weight Index

6.11.1 Usefulness of typical fisheries metrics for evaluating sensitive indicator organisms at the population level provide useful information in comparing subtle differences between sites. The drawbacks to using standard fisheries approaches are the limitations of either state developed or regional expectations and the lack of resolution linked with causes. The assessments require a large sample for site comparison and a large number of reference stations for determining the expected population regression line. The traditional approach to the assessment of condition involves the use of a Fulton-type (Anderson and Guetreuter, 1983) condition factor. This is calculated as:

$$K = W/L^3$$

where W is weight (g) and L is length (mm). These factors are both length and species dependent. Therefore, it is improper to compare fish of different species or fish of the same species at different lengths. Le Cren (1951) developed the relative condition factor:

$$K = W/W' \times 100$$

where W is the observed weight and W′ is the length specific expected weight for fish in the populations under study as predicted by a weight-length regression equation calculated for that population. This approach solved the problem of comparing fish of different lengths and species but, because a different weight-length regression was calculated for each population, interpopulational comparisons were not possible. The relative weight (W_r) index (Wege and Andrson, 1978) enabled interpopulational comparisons by making

the standard weight-length (W_s) regression species-specific rather than population specific or location specific. Relative weight is calculated as:

$$W_r = W/W_s \times 100$$

where W_s is the length-specific standard weight predicted by a weight-length regression constructed to represent the species as a whole.

6.11.2 W_s equations have been defined in most cases to represent populations in better than average conditions (reference conditions) based on the assumption that attempting to produce fish populations that attain only average condition generally does not represent a typical management goal. W_s should be considered a benchmark for comparison of samples and populations. Comparisons are based on the 75th percentile of the weight. An alternative technique, regression-line-percentile (RLP), is based on comparison of $\log_{10}$weight-$\log_{10}$length regression equations for each population whereas the typical W_r equation is based on pooled length-weight data.

6.11.3 Murphy et al. (1991) discussed the development of the index and expounded upon the status and W_r regression equation for 27 species. To calculate W_r properly requires data from representative or reference stations over a broad range for the species of interest. Slopes of less than 3.0 are considered inappropriate for most species because such a slope indicates the species becomes thinner with increased length. Low slopes may also results from including small fish in the regression. Differences of weighing small fishes and the inherent problems of weighing small fishes in the field may preclude development of a single equation for an entire species life history. A minimum applicable length is used to determine the minimum size which should be weighed. For other species the minimum length is a function of the variance:mean ration for $\log_{10}$ weight where it sharply increased.

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SECTION 7

SPECIAL TECHNIQUES

7.1 Flesh Tainting (Flavor Impairment)

7.1.1. Sublethal concentrations of chemicals, such as phenols, benzene, oil, and 2,4-D, are often responsible for imparting an unpleasant taste to fish flesh, even when present in very low concentrations.

7.1.2 Specific methods have been developed by Thomas (1969), APHA (1992), and ASTM (1992) in which untainted fish are placed in cages or exposure tanks upstream and downstream or in the laboratory from suspected waste water sources. The techniques in these references and Subsection 7.1.3 should successfully relate the unacceptable flavor produced in exposed native fish to a particular waste source.

7.1.3 The following procedures are presented as a working guide for fish flesh tainting or flavor impairment.

7.1.3.1 To ensure uniform taste quality before exposure, all fish are held in pollution-free water for a 10 day period. After this period, a minimum of three fish are cleaned and frozen with dry ice as control fish. Test fish are then transferred to the test sites, and a minimum of three fish are placed in each portable cage. The cages are suspended at a depth of 0.6 meter for 48 to 96 hours.

7.1.3.2 After exposure, the fish are filleted, frozen on dry ice, and stored at $0^{o}C$ until tested. The control and exposed samples are shipped to a fish tasting panel, such as is available at the food science and technology departments in many major universities, and treated as follows: (1) The fish are washed, wrapped in aluminum foil, placed on slotted, broiler-type pans, and cooked in a gas oven at $218^{o}C$ ($400^{o}F$) for 23 to 45 minutes depending on the size of the fish; (2) Each sample is boned and the flesh is flaked and mixed to ensure a uniform sample; (3) The samples are served in coded cups to judges. Known and coded references or control samples are included in each test. The judges score the flavor and desirability of each sample on a point scale. The tasting agency will establish a point on the scale designated as the acceptable and desirable level.

7.2 Fish Kill Investigations

7.2.1 Fish kills in natural waters, though unfortunate, can in many instances indicate poor water quality and environmental health leading to investigations which may improve the water quality. Prompt investigations should be organized and conducted so that the resultant data implicates the correct cause. Fish kills tend to be highly controversial, usually involving the general public as well as a number of agencies. Therefore, the investigator(s) can expect his finding to be disputed, quite possibly in a court of law.

7.2.2 Possible Fish Kill Sources

7.2.2.1 Fish mortalities result from a variety of causes, including natural and man-induced. Possible natural fish kills are caused by phenomena such as acute temperature change, storms, ice and snow cover, decomposition of natural organic materials, salinity changes, spawning mortalities, and parasitic, bacterial, and viral epidemics. Man-induced fish kills may be attributed to municipal or industrial wastes, agricultural activities, and water manipulations.

7.2.2.2 Winter kills occur in northern areas where ice on shallow lakes and ponds becomes covered with snow, and the resulting opaqueness stops photosynthesis. The algae and vascular plants die because of insufficient light, and plant decomposition results in oxygen depletion. Oxygen depletion and extreme pH variation can also be caused by the respiration or decay of algae and higher plants during summer months in very warm weather. Fish kills resulting from such causes are often associated with a series of cloudy days that follow a period of hot, dry, sunny days. Fish kills also occur in rivers below high dams immediately following the opening of a gate permitting cold hypolimnionic water to flow into the streams as in the Tennessee Valley Authority (TVA) region.

7.2.2.3 Temperature changes, either natural or the result of a heated water discharge may result in fish kills. Long periods of very warm, dry weather may raise water temperatures above lethal levels for sensitive species. A wind-induced seiche may be hazardous to certain temperature sensitive, deep-lake, cold-water fish, or fish of shallow coastal waters. Lake water inversion during vernal or autumnal turnover may result in toxic materials or oxygen-free water being brought to the surface. Interval seiche movement in which a toxic or low dissolved oxygen hypolimion flows up into a bay or bayou for a limited period of time, and later returns to normal levels may also cause fish kills.

7.2.2.4 Disease, a dense infestation of parasites, infection from bacteria, or viruses, or natural death of weakened fish at spawning time must always be suspected as contributory factors in fish mortalities.

7.2.2.5 Occasionally fish may be killed by toxins released from certain species of living or decaying algae that reached high population densities because of the increased fertility resulting from organic and inorganic pollution.

7.2.2.6 Investigations in Tennessee have shown that the leaking of small amounts of very toxic chemical from spent pesticide-containing barrels used as floats for piers and diving rafts in lakes and reservoirs can produce extensive fish kills (TVA, 1968).

7.2.2.7 Industrial waste discharges and waste discharges from a municipal or domestic type sewerage system may be potential sources of fish kills. These wastes may be subjected to treatment of a municipal treatment plant or may be discharged directly, untreated, to a stream. Generally, the municipality or owner of the sewerage system is held responsible for any discharge in such a

system; consequently, after collecting samples, the owner or a representative of the owner of the sewerage system should be contacted. This may be a sewage treatment plant operator, city engineer, public works supervisor, a subdivision developer, etc. If the cause of the fish kill was the result of an industrial waste discharge to a municipal sewer and thence to a stream, information should be obtained from a municipal official about the industry and the problem. This should be done only in cooperation with a municipal official.

7.2.2.8 Pollution capable of causing fish kills may result from such agricultural operations as pesticide dusting and fertilizer applications, as well as manure or other organic material discharges to a stream. Generally, fish kills related to these factors will be associated with rains and runoff. The source or type of pollution may be difficult or impossible to locate exactly because it may involve a large area. Talking to local residents may help pinpoint the specific problem area. Runoff from fields, drainage ditches, and small streams leading to the kill area are possible sampling places which may be used to trace the causes.

7.2.2.9 Temporary or intermittent activities, such as mosquito spraying, construction activities involving chemicals, other toxic substance, and herbicide containing materials toxic to fish such as arsenic, are also potential causes of fish kills. As with agricultural activities, tracing the cause of these kills is difficult and may require extensive sampling. Accidental spills from ruptured tank cars, pipelines, etc., and dike collapse of industrial pond dikes are frequently sources of fish kills.

7.2.3 Types and Extent of Fish Kills

7.2.3.1 One dead fish in a stream may be called a fish kill. However, in a practical sense some minimal number of dead fish observed plus additional qualifications should be used in reporting and classifying fish kill investigations (USEPA, 1973). These qualifications are based on a stream approximating 200 feet in width and 6 feet in depth. For other size streams, adjustments should be made.

7.2.3.2 Minor fish kills (1-100 dead or dying fish) may be considered "no fish kill" if confined to a small area or stream reach provided this is not a recurring event. For example, fish kill occurring near a waste water outfall in which stream dilution mitigates the effect of the deleterious material. If this is a recurring situation, it could be of major significance and should be investigated.

7.2.3.3 Moderate fish kill (100 - 1000 dead or dying fish) may be considered to have occurred if a number of species and individuals habe been affected in 1-2 km of stream where dilution would have been expected to play a mitigating role. Apparently normal fish may be collected immediately downstream from the observed fish kill area.

7.2.3.4 Major fish kill (1000 - 10,000 fish or more dead or dying fish) may be considered to have occurred in 10-20 km of a stream in which dilution would

have been expected to have a mitigating effect and when many species of fish are affected and dying fish may still be observed downstream.

7.2.4 Preparation for Field Investigation

7.2.4.1 All possible speed must be exercised in conducting the initial phases of any fish kill investigation because fish disintegrate rapidly in hot weather, and the cause of death may disappear or become unidentifiable within a short period of time. Success in solving a fish kill problem is usually related to the speed with which investigators can arrive at the scene after a fish kill begins. The speed of response in the initial investigation is enhanced through the training of qualified personnel who will report immediately the location of observed kills, the time that the kill was first observed, the general kinds of organisms affected, an estimate of the number of dead fish involved, and any unusual phenomena associated with the kill.

7.2.4.2 Because there is always the possibility of legal liability associated with a fish kill, lawyers, judges, and juries may scrutinize the investigation report. Therefore, the investigation must be made with great care. When investigating a fish kill, a specific litigation or case number should be assigned and used on all labels, field data sheets, photographs, and other records related to the fish kill investigation. Table 1 is a general flowchart to help with the coordination of a fish kill investigation.

7.2.5 Legal Aspects

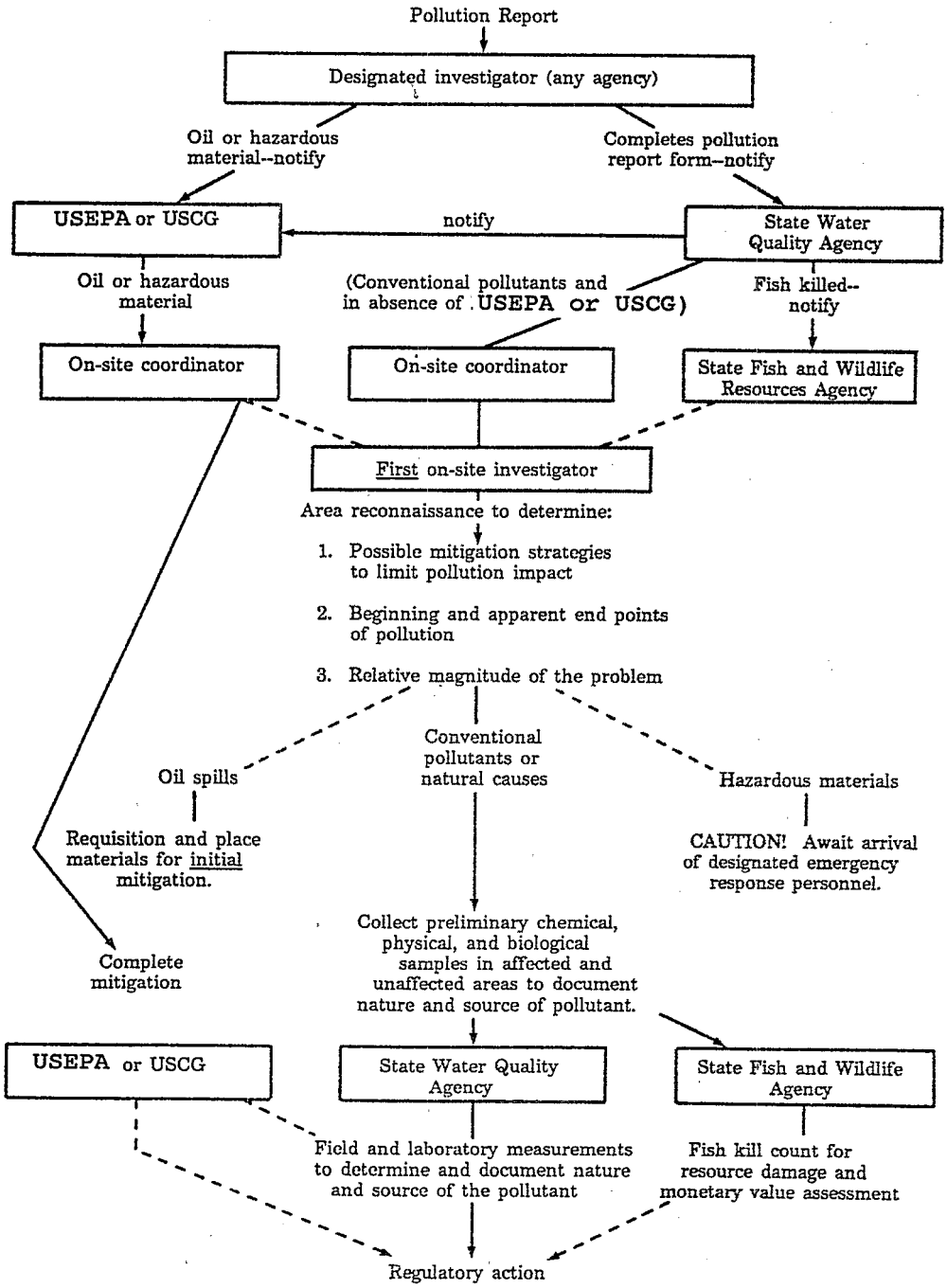
7.2.5.1 A chain-of-custody (see Section 2, Quality Assurance and Quality Control) must be adhered to when any fish kill is investigated and samples collected for analysis and presentation as evidence. If care is not taken to establish the validity of samples collected in the field and transported to a laboratory for analysis, potential evidence for a court action may be lost or ruled invalid.

7.2.5.2 Several types of evidence including oral and hearsay, circumstantial, and graphic may be collected during an investigation. Oral and hearsay evidence should be signed and dated by the individual giving the information. Circumstantial evidence must be carefully documented as to methods of collection, who collected it, and disposition of the evidence. Graphic evidence such as photographs should be accompanied by data listing when taken, how, by whom, the type of camera and film used, and who processed the film.

7.2.5.3 All samples must be handled in a similar orderly procedure and a complete record should be kept on their disposition. Recognized tests should be used and such tests must be approved in detail by USEPA or other recognized authorities. New test methods must be technically defensible. All unused portions of samples must be saved until released by the USEPA attorney working on the case.

7.2.5.4 The investigative team should make every effort to educate the attorney handling the case. The attorney should be aware of the expertise of the team, the methods used, validity of evidence collected, and complete disposition of the evidence.

TABLE 1. FLOWCHART FOR THE COORDINATION OF A FISH KILL INVESTIGATION[1]



[1]Modified from Meyer and Barclay (1990). Abbreviations: U.S. Environmental Protection Agency (USEPA), U.S. Coast Guard (USCG).

7.2.6 Field Investigations

7.2.6.1 The following is a brief discussion of a suggested method of field investigation (Meyer and Barclay (1990) provide guidelines, detailed, and specific procedures for fish kill field investigation). For additional methods, see the following references: AMPA (1992), Amer. Fish. Soc. (1982), ASTM (1992), Burdick (1965), Hill (1983), Smith et al. (1956), Tracy and Bernhardt (1972), U.S. Dept. Interior (1970), USEPA (1973), USEPA (1979a,b), USEPA (1980), and Section 12, Fisheries Bibliography, Subsection 12.7 Fish Kills.

7.2.6.2 Individuals involved in fish kill investigations should have a copy or be familiar with the document, *Field Manual for the Investigation of Fish Kills*, (F.P. Meyer and L.A. Barclay, eds., 1990). This document contains detailed information on the following: planning the investigation (Hunn, 1990), interpreting the fish kill location (Meyer and Herman, 1990), toxic substances effects and diagnosis (Hunn and Schnick, 1990), fish kills due to natural causes (Herman and Meyer, 1990), role of infectious agents in fish kills (Herman, 1990), quality assurance and legal requirements (Schnick, 1990a), where to send samples for analyses (Schnick, 1990b), shipping samples (Barclay, 1990a), writing the fish kill report (Meyer, 1990a), preparing for legal testimony (Barclay, 1990b), specific equipment needed for field assessments (Ardinger, 1990), and case histories of fish kills (Meyer, 1990b).

7.2.6.3 Since the speed with which an investigative team arrives at a fish kill is extremely important, a few advanced preparations are necessary. The public should be aware of whom to contact and where to report fish kills. If possible, a Region- or State-wide network of designated fish kill investigators should be established, each representing an area in which an investigator knows the water, biota, and potential polluters. In preparation for quick action, an investigator must have at his/her immediate disposal: telephone report sheets (Table 2), a checklist of equipment items (Table 3), maps of the area, and a list of cooperating analytical laboratories.

7.2.6.4 Make a reconnaissance of the kill area. Make a decision as to the extent of the kill and if a legitimate kill really has occurred. If a legitimate kill exists take steps to trace or determine the cause. Secure sampling equipment and determine size of investigative team needed. Standard equipment should be taken on all investigations (Table 3), and a standard checklist with space for special equipment will often save embarrassment in the field. The on-site study includes specific field observations (Table 4) that may be made on a fish kill form (Table 5). In addition, specific field observations (Table 4) should be emphasized, and complete weather data should be collected (for the period) prior to and during the fish kill. Water conditions both in and outside the affected area should be noted (i.e., appearance of water, turbidity, algal blooms, oil, unusual appearance, etc.). Stream flow patterns (i.e., high or low flow, stagnant or rapidly moving water, tide moving in or out, etc.) should be noted and recorded. If possible, obtain discharge reading from stream gauge if one is near fish kill area. During the initial steps of the investigation, water chemistry and physical parameters (e.g., pH, dissolved oxygen, temperature, specific conductance, and flow) must be determined immediately upon arrival at the kill

site. While none of these factors may be directly involved in the fish kill, these tests are simply and rapidly performed in the field and can be used as a baseline for isolating the cause(s) of the kill. Make a rough sketch or define the fish kill area on a map so that sampling points, sewer outfalls, etc. can be accurately located on a drawing to be included in a final report. Take close-up and distance photographs of the dead fish in the stream in the polluted area, the stream above the polluted area, and the wastewater discharges. Photographs will often show a marked delineation between the wastewater discharge and the natural flow of water. Pictures taken at a relatively high elevation, (a bridge as opposed to a boat or from a low river bank) will show more and be more effective. Color photographs are also more effective than black and white prints in showing physical conditions of a stream.

7.2.6.5 Certain biological observations should also be made as soon as possible: (1) the presence or absence of plankton blooms, (2) dead or living macroinvertebrates and fish, and (3) the actions of moribund fish. Additional observations are listed in Tables 6, 7, and 8.

7.2.6.6 The location of sampling stations is very important. If there are no obvious reasons for a kill, stations should be selected in and outside the apparent kill area. If there are possible polluters, each should be suspect and sampling stations must be selected within and outside of the area of influence for each possible suspect.

7.2.6.7 In flowing waters, where a pollutant may be discharged as a slug, the investigator should try to estimate the time of kill, determine stream velocity, and collect samples downstream in the vicinity of the slug.

7.2.6.8 Water samples must be collected and processed in a variety of ways depending on the types of analyses required. An updated USEPA methods list for collection and preservation of samples should be at the disposal of the investigator (USEPA, 1979a,b).

7.2.6.9 The collection and preservation of aquatic organisms may require special techniques. For example, it is always best, if possible, to collect moribund fish from the affected area. If none are available, freshly dead fish will have to be utilized. Unaffected fish from outside the kill area must also be collected. All samples should be handled with regard to the type of suspected toxicant and the type of analysis to be performed.

7.2.6.10 Contact personnel from the laboratory or laboratories which will participate in analyzing samples. If possible estimate the following and record on the fish kill general information form (Table 2).

1. The number and size of samples to be submitted.

2. The probable number and types of analyses required.

3. The dates the samples will be received by the laboratory.

4. Method of shipment to the laboratory.

TABLE 2. FISH KILL GENERAL INFORMATION FORM

1. Who is the informant?

Name _____ Phone _____

Address _____

_____

Directions to meeting place _____

_____

Date and Time _____

2. Reporting Source

Agency _____

Address _____

Phone(s) _____

Fish Kill Network _____ Yes _____ NO _____

3. Location of kill (county, town, access point): _____

_____

4. Duration of kill: First noticed - Time _____ Date _____

Is it continuing? (Yes, No). If not, when did it stop _____

5. Extent of Kill: Area covered (miles of stream or size of pond or lake).

_____

6. Approximate number of fish affected _____ Species _____

_____

_____

Size (length, age classes) _____

_____

_____

TABLE 2. FISH KILL GENERAL INFORMATION FORM (CONTINUED)

7. Opinion as to cause _____

_____

_____

_____

8. Recent activities (crop dusting, weather change, etc.) _____

_____

_____

9. Possible sources of pollution _____

_____

_____

10. Measures taken _____

11. Action Requested

Field Investigation _____

Laboratory Analyses _____

12. Assistance to Project

Provided by _____

Personnel _____

_____

Equipment _____

_____

Transportation Facilities _____

_____

_____

_____

TABLE 3. CHECKLIST OF FISH KILL INVESTIGATION EQUIPMENT

General

1. Boat
2. Motor
3. Paddles
4. Life preservers
5. Camera
6. Film
7. Ice chests
8. Wet ice
9. Dry ice
10. Portable light source
11. Waterproof notebook
12. Waterproof pencils
13. Waterproof labels
14. Chain of custody seals
15. Chain of custody forms
16. Arm-length gloves
17. Hip boots
18. Chest waders
19. Paper towels
20. Aluminum foil
21. Thermometer
22. Plastic bags, assorted sizes
23. DO kit
24. pH equipment (probe, colorimeter)
25. Glass jars (chemical samples)
26. Maps
27. Hand tallys
28. Tape measure (100 yd. or meter)
29. Rain gear
30. Polaroid glasses
31. Tamper proof seals

Plankton-Periphyhton

1. Water sampler - Van Dorn
2. Vials, small widemouth jars
3. 6-3-1, Formalin preservative
4. 2-liter jars

Fish

1. Dipnets
2. Seines
3. Nets
4. Electrofishing gear (if available)
5. Weight scale
6. Measuring board
7. Tubs
8. Fish counting forms
9. Dissecting kit
10. Heparinized vials
11. 10% formalin
12. 70-75% ethyl alcohol (ethanol)
13. Scale envelopes

Benthos

1. Ekman grab sampler
2. Ponar grab sampler
3. Surber-type sampler
4. Drift net sampler
5. Dipnets, kick nets
6. Quart and pint widemouth containers
7. 70-75% alcohol
8. 10% formalin
9. Foot tub
10. U.S. Standard 30 sieve
11. Forceps

TABLE 4. FIELD OBSERVATIONS

1. Locate the kill area.

2. Take water samples for chemical analysis and preserve properly.

3. Make chemical and physical field analyses (DO, pH, temperature, flow, weather, etc.).

4. Record observations (odors, flocks, sheens, deposits, etc.).

5. Collect fish for analyses (follow guidelines for various analyses).

6. Collect plankton samples.

7. Collect periphyton.

8. Collect macroinvertebrates (substrate, drifting, and attached).

9. Extensive and pertinent observations:

   * Observe and examine dead and dying fish (see other attachment.

   * Are small fish collected in tributaries--on surface or not?

   * Are the plankters (planktonic organisms) concentrated in the kill area?

   * Are they alive and viable or dead?

   * Is there extensive periphytic growth?

   * Is the benthic community active, over-active, quiescent?

   * Are there many drifting organisms?

   * Record all observations.

10. Repeat the applicable steps above in a non-affected area of the lake or stream.

11. Take numerous pictures of the overall area, specific problem areas, dying fish, algae blooms, water conditions (color, turbidity, etc.).

12. Counts of mortality by species to estimate resource loss.

TABLE 5. FISH KILL INVESTIGATION FORM

Stream, Lake, Other _____ Drainage Basin _____

Stream Mile _____ Tributary to _____

County _____ State _____ Top. Map. _____

Nearest Town _____ Highways _____

Fish Kill Began: Time _____ Date _____ Ended: Time _____ Date _____

Time and Date of First Report _____ Reported by (Name) _____

Address _____ Telephone _____

Investigators: (Name and Agency) _____

_____

Area Affected: Upstream Limits _____
Downstream _____
Miles _____ Acres _____

Weather Conditions: Present _____
Past 48 Hours _____

Photographic Record:

Picture No.	Time and Date	Subject

Field Measurements:

Sample	Upstream of Kill	In Kill Area	Downstream of Kill
Temp.			
pH			
DO			
Conductivity			
Gage Ht./Flow			

Comments and Possible Sources:

TABLE 5. FISH KILL INVESTIGATION FORM (CONTINUED)

Location of Collected Samples

Sample ID. No.	Station Description

Sample ID No:

Sample	Upstream of Kill	In Kill Area	Downstream of Kill
Water Sample*			
Fish (frozen)			
Fish (formalin)			
Fish (fresh)			
Fish Blood:			
Species length			
Species length			
Species length			
Species length			
Sediment			
Algae (frozen)			
Algae (iced)			
Benthos			
Special Analysis			

Approximate # of dead fish of (of each species)/acre, mile, 100 yards, etc.

*Requested Analyses:

TABLE 6. OBSERVATION ON DEAD AND MORIBUND FISH

External

1. External examination for fungus, bacteria, open sores, parasites.
2. Gill examination for color, abnormal morphology, gill lice, slime, collapse of filaments, adhesion of filaments.
3. Eyes opaque, clear, covered by mucus.
4. Fins - anchor lice, extended-folded, bleeding, fungused, frayed.
5. Scales - loose groups, bent, bleeding, missing.
6. Body - bent, twisted, rigid.
7. Mouth - open, normal, hyper-extended in death.

Internal

1. Do they bleed freely?
2. Is the liver clear of spots or open lesions? Is it a light off-brown or tan?
3. Is the air bladder hard, very soft, or partly inflated?
4. Is the stomach full or empty? What is in it?
5. Is the entire intestinal tract empty?
6. Are there internal parasites in the abdominal cavity?
7. Is there watery fluid in the abdomen?
8. Is there discoloration of any of the tissues?
9. Are the muscles pulled away from the ribs or backbone?
10. Are there lesions or spots in the muscles? Describe them.
11. Is the kidney (against the backbone) a normal dark red to purple or unspotted?
12. Are there lesions or watery abscesses (i.e., blisters)?
13. Is the pericardial space free with watery fluid or is it discolored a reddish or yellow color?
14. Are the fish slimy or dry?

TABLE 6. OBSERVATION ON DEAD AND MORIBUND FISH (CONTINUED)

Internal (continued)

15. Are there trailing mucus strings from the gills or fins?

16. Are there large patches of missing scales?

17. Is there bleeding about the fin bases or scale bases?

18. Do the gills look very bright red, dark blue, or purple? Are the gills covered with slime? Are they bleeding or lumpy?

19. Do the gill covers move very rapidly or very slowly?

20. Are the fish unresponsive, roll over in the water, and slowly die? Do they slowly settle to the bottom while upright?

21. Do any rest upside down at the surface and still breathe?

22. Do any cough, flare the gill covers, or flare the fins?

TABLE 7. OBSERVATIONS ON EFFECTED FISH

1. Do the fish swim wildly at the surface? If they do, do they do it continuously or in erratic and irregular bursts of activity?

2. Do they try to leap from the water after racing across the surface?

3. After they race at the surface, do they fall on their side and tremble?

4. How long do they race about?

5. Do they race about, then tremble, turn over, and die , or do they race, rest, then race with increasing periods between bursts of activity?

6. At the end of a run, are the bodies twisted or rigidly bent to one side or the other?

7. As activity decreases, do they rest upright at the surface?

8. As activity decreases, do they rest head-down in the water?

9. As activity decreases, do they rest tail-down in the water?

10. As activity decreases, do they rest tail-down in the water and spin on their long axis?

11. With the slower erratic swimming, do they swim forward, slowly turning over and over, spiraling, or swim forward but describe a long curving arc or circle?

12. Do they swim slowly forward, mouthing at the surface with audible "smacking" sounds?

13. Do they swim slowly forward, ejecting bubbles from the mouth?

14. As swimming slows or ceases, do they settle into the water or do they struggle to stay down and upright?

15. If you can catch them, must you use a net, or can you catch them by hand?

16. Once caught, do they struggle, tremble, lose scales, or go rigid?

17. Are they bleached out, very dark, or blotchy?

18. Are there fuzzy blotches anywhere on the body?

19. Are there open scores?

20. Are the fins and gill covers folded or held rapidly extended from the body?

TABLE 7. OBSERVATIONS ON EFFECTED FISH (CONTINUED)

21. Are they slimy or dry?

22. Are there trailing mucus strings from the gills and fins?

23. Are there large patches of missing scales?

24. Is there bleeding about the fin bases or scale bases?

25. Do the gills look very bright red, dark blue, or purple? Are the gills covered with slime? Are they bleeding or lumpy?

26. Do the gill covers move very rapidly or very slowly?

27. Are the fish unresponsive, roll over in the water, and slowly die?

28. Do they slowly settle to the bottom while upright.

29. Do any rest upside down at the surface and still breathe?

30. Do any cough, flare the gill covers, or flare the fins?

TABLE 8. SYMPTOMS THAT HAVE BEEN RELATED TO CAUSE OF FISH DEATH[1]

SYMPTOM	CAUSATIVE AGENTS
Gasping at surface	Low DO or rotenone
Fish dying in early morning only	Low DO, summer kill
Swimming slowly in circles or only one species affected	Disease
Erratic swimming patterns, contorted bodies, tremors, or convulsions. Other animals involved (i.e., birds,snakes, turtles, etc.)	Pesticides
Fish gills covered with mucus, or clogged	Rotenone, high suspended solids, heavy metals
Small fish kills of various species over a long period of time, altered species composition	Low concentrations of trace metals
Deflated swim bladders and viscera obliterated	Seismic blasts, dynamite, or other explosives.
White film on gills, skin and mouth	Acids, heavy metals, trinitrophenol
Sloughing of gill epithelium	Copper, zinc, lead, detergent, ammonia, quinoline
Gill occlusion	Turbidity, ferric hydroxide precipitate
Bright red gills	Cyanide
Dark gills	Phenolic poisoning, p-cresol, naphthalene, oxygen deficiency
Gill lamellae thickening	Hydrogen sulfide
Distended gill covers	Ammonia, cyanide

[1]Modified from Janet Kuelfer, USEPA, Region 9, San Francisco, CA.

TABLE 8. SYMPTOMS THAT HAVE BEEN RELATED TO CAUSE OF FISH DEATH (CONTINUED)

SYMPTOM	CAUSATIVE AGENTS
Swollen abdomens	Chlorinated hydrocarbon, insecticides
Blue stomachs	Molybdenum
Intestinal epithelium destruction	Hexavalent chromium, pulp mill wastes
Gall bladder distension	Pulp mill wastes
Extreme thinning of stomach wall	Endosulfan
Pin point white spots, fish rubbing against substrate	*Ichthyophonus* sp., *Cryptocaryon* sp. (Ich disease)

5. To whom the laboratory results are to be reported.

6. The date the results are needed.

7.2.7 General Sampling Procedures (also see Meyer and Barclay, 1990). The extent and method of sampling will depend upon location and upon the suspected cause of the kill.

7.2.7.1 For stream and wastewater sampling, sample the following points when the pollution discharge is coming from a well defined outfall:

1. The effluent discharge outfall.

2. The stream at the closet point above the outfall which is not influenced by the waste discharge.

3. The stream, immediately below the outfall.

4. Other points downstream needed to trace the extent of the pollution.

7.2.7.2 The sampling should be extensive enough that when all the data is compiled no question will exist as to the source of the pollution which killed the fish.

1. Streams less than 200 feet wide, not in an industrial area usually can be adequately sampled at one point in a section (Figure 1).

2. Streams 200 feet or wider generally should be sampled two or more places in a section immediately above and below the pollution discharge. Where the pollutional waste has adequately mixed with the stream flow one sample may suffice.

3. A number of samples in a cross section may be required on any size of stream to show that the suspected pollutional discharge is coming from a source located in an industrial or municipal complex (Figure 2).

4. Extensive cross sectional sampling on rivers greater than 2000 feet wide will be required for kills involving suspected agricultural or other types of mass runoff.

5. Sample depth - on streams 5 feet in depth or less, one mid-depth sample per sampling location is sufficient. For streams of greater depths, appropriate sampling judgment should be used since stratification may be present.

7.2.7.3 The number of samples to be collected at a given cross section will depend principally on the size of the stream.

a. Ten 1 L water samples should be collected from the kill area for chemical analyses as well as other 1 L samples from control and other stations. (In flowing waters samples should also be collected in the estimated location of the main slug).

b. Ten pounds including ten individuals of dying fish of each important species frozen with dry ice. An equivalent amount and number of control fish.

c. Five small fish of each important species preserved in formalin.

d. Five dying fish of each significant species placed on wet ice and delivered to a fish disease laboratory within 24 hours. (Some fish disease labs specify fish placed in bags next to wet ice.)

e. A minimum of ten fish should be collected for histochemical analysis. Refer to Section 5, Fish Specimen Processing, on the proper fixation and preservation of fish tissues for histochemistry methods.

f. Five vials containing 5 cc. each of blood from each important species.

g. Ten gallons of water for bioassay.

h. One quart to one gallon of sludge or sediment.

i. Ten cc. of concentrated algae frozen.

j. Ten cc. of concentrated algae chilled.

k. Benthic invertebrate (macroinvertebrates) samples.

7.2.8 Explanation of Figures 1 and 2.

7.2.8.1 Collection point 1 (Figure 1) and points 3 and 4 (Figure 2) should be collected as near to the point of pollutional discharge as possible. These points will vary according to stream flow conditions. The pollution discharges into a slow sluggish stream usually will have a cone of influence upstream of the outfall; whereas, a swift flowing stream usually will not.

7.2.8.2 Collecting an upstream control sample from a bridge within sight of the pollutional discharge would probably be satisfactory in Figure 1 but definitely not in Figure 2.

7.2.8.3 Figures 1 and 2 are given for illustrative purposes only and should be used only as a guide for sampling. Each individual situation must be individually considered to insure adequate, proper sampling. While too many samples are better than too few, effort should be made not to unduly overload the laboratory with samples collected as a result of poor sampling procedures.

7.2.9 Biological Sampling

7.2.9.1 In every investigation of fish kills the paramount item should be the immediate collection of the dying or only recently dead organisms. Sampling and preservation are as follows:

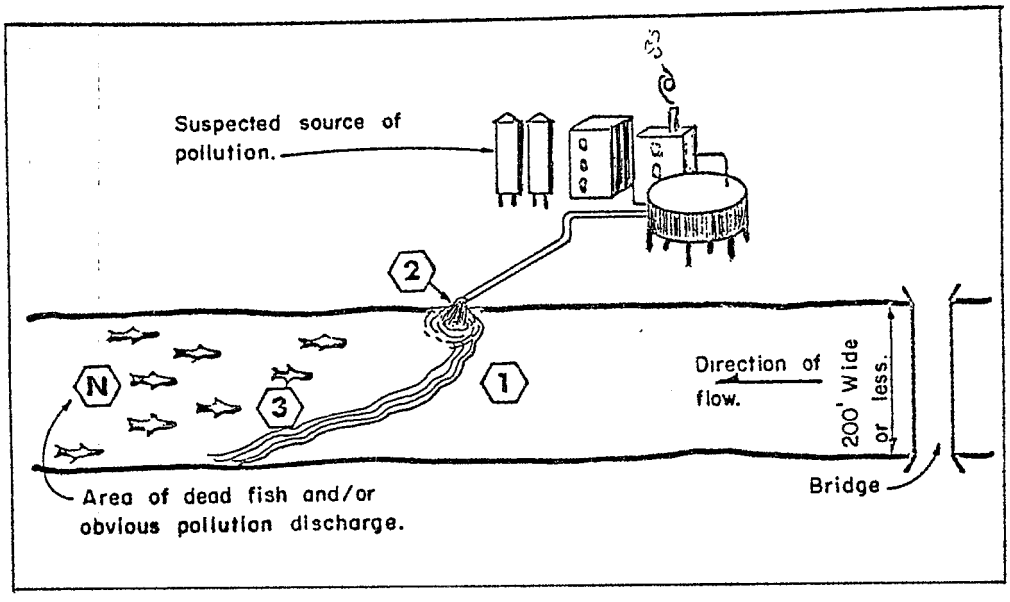


Figure 1. Minimum water sampling point on stream 200 feet or less wide involving an isolated discharge. Modified from USEPA (1973).

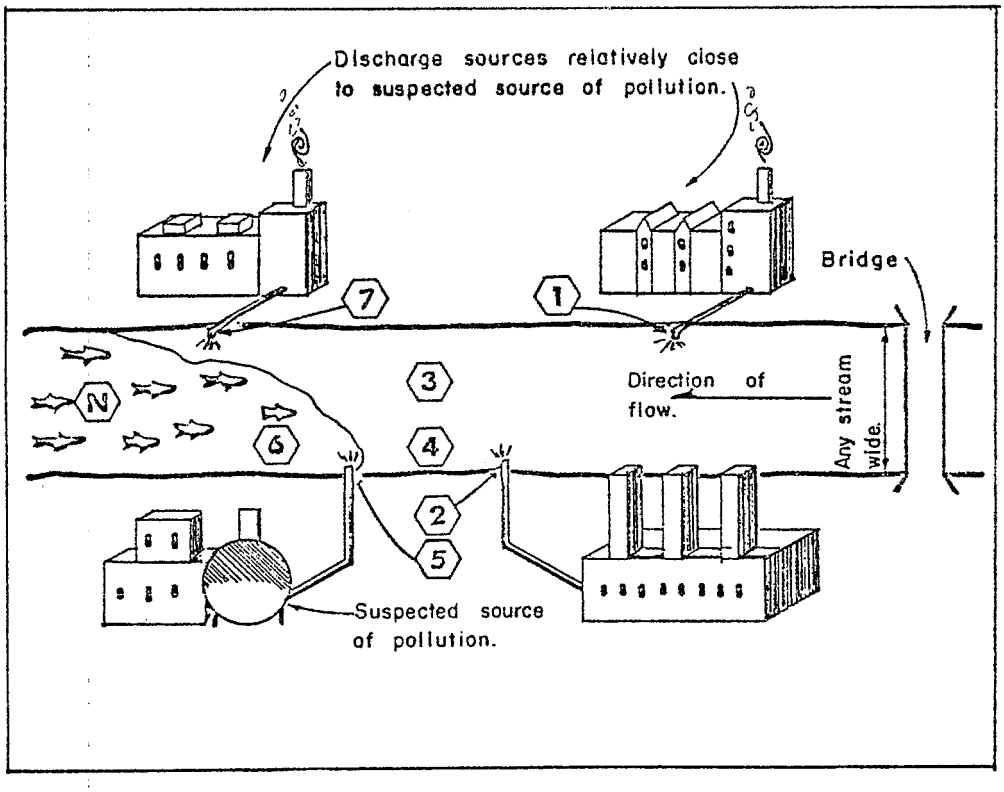


Figure 2. Minimum water sampling points on a stream running through an industrial or municipal complex. Modified from USEPA (1973).

1. Collect 20+ drops of blood in a solvent rinsed vial, seal with aluminum foil, cap, and freeze.

2. Place bleeding specimens, or entire specimens if beyond bleeding stage, in plastic bag and freeze. In case no method of freezing is available, icing for a short period prior to freezing may be acceptable. Labeling of both blood and carcass is important.

3. Controls - live specimens of the affected organisms should be obtained from an area within the same body of water which had not been influenced by the causative agent. Once obtained these specimens should be handled in a like manner.

7.2.8.2 The number of individuals involved and the species affected should be enumerated in some manner. At most these will be estimates. Depending on the given situation such as area or distance involved and personnel available, enumeration of fish kills may be approached in one of the following ways:

1. For large rivers, establish observers at a station or stations (e.g., bridges) and count the dead and/or dying fish for a specified period of time, then project to total time involved.

2. For large rivers and lakes, traverse a measured distance of shoreline, count the number and kinds of dead or dying fish. Project numbers relative to total distance of kill.

3. For lakes and large ponds, count the number and species within measured areas, and then project to total area involved.

4. For smaller streams one may walk the entire stretch involved and count number of dead individuals by species.

7.2.9 Sampling Other Biota

7.2.9.1 Sampling of benthic organisms after the more urgent aspects of the kill investigation has been completed can prove to be valuable relative to the extent and cause of the kill. Benthic invertebrate communities are sampled to determine whether this assemblage, the primary food source of many fishes, has been affected. Also, since this general form of aquatic life is somewhat sedentary by nature, release of deleterious materials to their environment will kill much of the biota. By making a series of collections up and downstream from the affected area, the affected stretch of stream may be delineated when the benthic populations are compared to those organisms from the control area. Also, the causative agent may be realized when the specifics of the benthic population present are analyzed. Other aspects of the biota which should be considered are the aquatic plants. In lakes and ponds floating and rooted plants should be enumerated and identified. The collection of plankton samples (river and lakes) should be taken in order to determine possible toxicity from toxin-producing species and to determine the degree of bloom, which in itself may cause fish kills because of diurnal dissolved oxygen levels. Both aquatic plants and macroinvertebrates may be fixed in a 10% formalin solution and preserved in 70% ethanol.

7.2.9.2 When the material causing a kill is known, some of the above sample collections may not be required. However, if the cause of a kill is unknown, the above samples plus other specific samples, dictated by the type of fishery, may be required.

7.2.9.3 Graphic evidence has a maximum effect on people involved in pollution cases. Two basic types of graphic evidence are: (1) hand drawn maps of the general and specific location of the kill, extent of kill, plankton bloom, location of dead and dying fish, etc. (2) photograph (color and black-and-white) showing dead fish, oil slicks, nasty looking water, sampling location, etc. All graphic evidence should be carefully documented as recommended in Subsection 7.2.5, Legal Aspects, and Meyer and Barclay, 1990).

7.2.9.4 The magnitude of a fish kill should be carefully documented. A recognized method for enumerating the number and species which have been killed should be selected and carefully followed so that data collected will be admissible as evidence. Such methods are found in Meyer and Barclay (1990) and references cited in Subsections 7.2.6.1 and 7.2.6.2.

7.2.10 Bioassays

7.2.10.1 Static bioassay techniques, as outlined in USEPA (1991), may be effectively used to determine acute toxicity of wastes as well as receiving waters. Toxicity testing can be done in-situ using live boxes, a mobile bioassay laboratory, or the samples can be returned to a central laboratory for testing.

7.2.11 Report

7.2.11.1 The final report should contain accurate information and should be well organized to meet the requirements under Legal Aspects (Subsection7.2.5). Essential elements of the report are: (1) introduction, (2) summary, (3) description of the area, (4) description of all sampling methods and analyses, (4) discussion of the magnitude of the fish kill and effects on other aquatic organisms, (5) discussion of other water users in the affected area and (6) conclusion. For additional recommendations, see the references listed in Subsections 7.2.6.1, 7.2.6.2, and Section 12, Fisheries Bibliography, 12.7 Fish Kills.

7.2.12 Case History

7.2.12.1 A lower Mississippi River endrin-caused fish kill is an excellent example of the investigation of a major fish kill Bartsch and Ingram (1966) give the following summary (Table 9).

7.2.12.2 The investigation was designed to consider and eliminate potential fish kill possibilities that were not involved and come to a point focus on the real cause. It was found that the massive kills were not caused by disease, heavy metals, organic phosphorus compounds, lack of dissolved oxygen or unsuitable pH. Blood of dying river fish was found to have concentrations of endrin equal to or greater than laboratory fish killed with this pesticide, while living fish had lesser concentrations. Symptoms of both groups of dying

TABLE 9. SUMMARY OF A LOWER MISSISSIPPI RIVER ENDRIN FISH KILL INVESTIGATION[1,2]

I. Examination of usual environmental factors.

II. Elimination of parasites, bacterial or viral diseases, and botulism as causes of mortalities[1].

III. Consideration of toxic substances: Examination and prognostication of symptoms of dying fish. Necropsy including:

Haematocrits and white cell counts

Brain tissue assay for organic phosphorus insecticide

Kidney tissue study

Tissue analysis for 19 potentially toxic metals

Gas chromatographic analysis of tissues, including blood, for chlorinated hydrocarbon insecticides

IV. Exploration for toxic substances:

Bioassay with Mississippi River water

Bioassay with extracts from river bottom mud

Bioassay with tissue extracts from fish dying in river water and bottom mud extracts

Bioassay with endrin to compare symptoms and tissue extract analyses with those of dying fish in all bioassays.

V. Intensive chemical analysis for pesticides in the natural environment, experimental environment, river fish, and experimental animals.

VI. Surveillance of surface waters for geographic range and intensity of pesticide contamination.

VII. Correlation and interrelation of findings.

[1]Modified from Bartsch and Ingram (1966).
[2]The investigator should be aware of the fact that apparently healthy fish may be harboring pathogenic bacteria in their bloodstreams (see Bullock and Snieszko, 1969). Thus, there may be several factors involved in fish mortalities, all of which may obscure the primary cause or causes.

fish (river and bioassay) in the study (Table 9) were identical. It was concluded from all data obtained that these fish kills were caused by endrin poisoning.

7.3 Instream Flow Incremental Methodology (IFIM)

7.3.1 The IFIM was developed by Bovee (1982) for the U.S. Fish and Wildlife Service and is widely utilized in the United States by the U.S. Fish and Wildlife Service, state agencies, and consulting firms to estimate the effect of change in instream flow on the habitat of stream fish and other aquatic organisms (Baldridge and Amos, 1981; Gan and McMahon, 1990; Gore and Judy, 1981; Hilgert, 1982; Irvine et al., 1987; Mathur et al., 1985; Orth and Maughan, 1982, 1986; Parsons and Hubert, 1988; Waite, 1989; Waite and Barnhart, 1992). This methodology is only discussed here generally, but investigators should consult the authors cited in this Subsection for their application of fisheries bioassessment, management, and related research needs.

7.3.2 The application of the IFIM and its effectiveness have been evaluated and reviewed by several researchers (Bayha, 1978; Conder and Annear, 1987; Gan and McMahon, 1990; Gore and Nestler, 1988; Irvine, et al., 1987; Mathur et al., 1985; Orth and Maughan, 1982; 1986; Shirvell, 1989; Waite, 1989; Waite and Barnhart, 1992). In addition, Wesche and Rechard (1980) reviewed and summarized instream flow methods for fisheries and related research needs.

7.3.3 An important element of the IFIM is the use of physical habitat simulation (PHABSIM) computer models (e.g., IFG-4, HABTAT) that relate changes in discharge or stream channel structure to changes in the availability of physical habitat (Waite and Barnhart, 1992). With PHABSIM the hydraulic and physical variables of a stream or river are simulated for an assigned flow, and the amount of usable habitat (weighted usable area or WUA) can be predicted for a particular life stage of a particular species of fish. The prediction of WUA is based on ecological data and on habitat use by selected species of fish at various developmental life stages. The data are expressed in terms of habitat utilization or probability of use curves (Bovee and Cochnauer, 1977; Raleigh et al., 1984). The habitat utilization curves most commonly used in the IFIM are those for current velocity, substrate particle size, and water depth. According to Parsons and Hubert (1988), the values that are generated by an IFIM study can be misleading if the habitat utilization curves do not adequately reflect the conditions that fish of a life stage need, prefer, or tolerate. In addition, the type of habitat used by stream salmonids varies by species, life stage of the species, and characteristics of the available habitat. Using data found in the literature and additional research, Bovee (1978), Bovee and Cochnauer (1977), and Raleigh et al. (1984) compiled and developed general standard habitat utilization curves which could be broadly applied. Shirvell (1989) found that generic curves were not always accurate. Waite and Barnhart (1992) developed habitat utilization curves for allopatric fry and juveniles of steelhead *Oncorhynchus mykiss* over a range of environmental conditions in a small stream with moderate to high gradient, and also compared these curves with three standard IFIM probability of use curves. Waite and Barnhart (1992) also concluded that applying habitat utilization curves of one stream to generate WUA values

for a different stream should be done only after the investigator has measured and compared other stream characteristics, such as stream width, flow, gradient, depth, substrate particle size, pool:riffle ratio, and seasonal hydrography.

7.3.4 Recent studies by Layher and Brunson (1992) involve modification of the habitat evaluation procedures for determining instream flow requirements in warmwater streams; Olson-Rutz and Marlow (1992) studied the analysis and interpretation of stream channel cross-sectional data and discussed stream channel form and bank stability importance to the biotic community structure of riparian ecosystems.

7.4 Fish Marking and Tagging Techniques (Mark-and-Recapture)

7.4.1 The marking and tagging of fish are important techniques utilized to obtain information necessary for research and management. They are often used to study individual fish or fish populations. Marking or Tagging studies can give investigators data on estimates of biomass, stocking success, migrations, behavior, age, mortality rates, etc. For a review and synthesis of the different types of devices and techniques (e.g., external, internal, electronic, genetic, chemical tags and marks, etc.), consult Lagler (1956, 1978), Wydoski and Emery (1983), Parker et al. (1990).

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SECTION 8

FISH BIOASSESSMENT PROTOCOLS FOR USE IN STREAMS AND RIVERS[1]

8.1 Introduction

8.1.1 Two levels of fish bioassessment analyses are presented. Fish Bioassessment I constitutes a questionnaire approach where local and State fisheries experts are canvassed for existing data and information; Fish Bioassessment II consists of collecting fish at selected sites for biosurvey analyses. The data collected in Fish Bioassessment II is used in the Index of Biotic Integrity (IBI) (Karr et al., 1986) and the Index of well-being (Iwb) or composite index (Gammon, 1976, 1980; Gammon et al., 1981, 1988). This section provides an overview of the IBI and Iwb and their conceptual foundations. Effective use of the Fish Bioassessment II requires information presented in Angemeier and Karr (1986), Karr et al. (1986) and Gammon (1980). Sample field and data sheets are presented for guidance.

8.1.2 Pilot studies based on use of the fish biosurvey (Fish Bioassessment II) have been published. An overview of two of these studies is presented in Plafkin et al. (1989). Other studies by Bramblett and Fausch (1991), Hughes, and Gammon (1987), Ohio EPA (1987b, 1987c. 1990a), Plafkin et al. (1989), Schrader (1989), Simon (1990, 1991), Steedman (1988), Yoder et al. (1981), and those states or agencies cited in Subsection 8.15 have applied the IBI and Iwb, or the modified Iwb, to assess the effects of impacts in habitats of different regions of North America.

8.1.3 Use of Fish in Biosurveys

8.1.3.1 The bioassessment techniques presented here focus on the evaluation of water quality, habitat, and fish community parameters. The fish survey protocols were based largely on Karr's IBI (Karr, 1981; Karr et al., 1986; Miller et al., 1988b), which uses fish community structure to evaluate water quality. The integration of functional and structural compositional metrics, which forms the basis for the IBI is a common element to the fish bioassessment approach.

8.1.3.2 Advantage of Using Fish

8.1.3.2.1 Fish are good indicators of long-term (several years) effects and broad habitat conditions because they are relatively long-lived and contain mobile elements (Karr et al., 1986). In addition, many species are relatively sedentary in summer (Gerking, 1959).

8.1.3.2.2 Fish communities generally include a range of species that are representation of a variety of trophic levels (omnivores, herbivores, insectivores, planktivores, piscivores). They tend to integrate effects of

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[1]Adapted from Plafkin et al. (1989).

lower trophic levels; thus, the fish community structure can present an integrated picture of the environmental health of a stream or river.

8.1.3.2.3 Fish are at the top of the aquatic food chain and are consumed by humans, making them important target assemblage for assessing contamination and habitat alteration.

8.1.3.2.4 Fish are relatively easy to collect and identify to the species level. Most specimens can be sorted and identified in the field and released unharmed.

8.1.3.2.5 Environmental requirements of common fish are comparatively well known.

8.1.3.2.6 Life history information is extensive for most species.

8.1.3.2.7 Information on fish distribution is commonly available.

8.1.3.2.8 Aquatic life uses (water quality standards) are typically characterized in terms of fisheries (coldwater, coolwater, warmwater, sport, forage, commercial).

8.1.3.2.9 Monitoring fish communities provides direct evaluation of "fishability", which emphasizes the importance of fish to anglers and commercial fishermen.

8.1.3.2.10 Fish account for nearly half of the endangered vertebrate species and subspecies in the United States.

8.1.4 Fish Community Consideration

8.1.4.1 Seasonal changes in the relative abundance of the fish community primarily occur during reproductive periods and (for some species) the spring and fall migratory periods. However, because larval fish sampling is not recommended in this method, reproductive period changes in relative abundance are not of primary importance.

8.1.4.2 Generally, the preferred sampling season is mid to late summer and early fall, when stream and riverflows are moderate to low, and less variable than during other seasons. Although some fish species are capable of extensive migration, fish populations and individual fish tend to remain in the same area during summer (Funk, 1957; Gerking, 1959; Cairns and Kaesler, 1971). The Ohio EPA (Rankin, 1987, personal communication) confirmed that few species or individuals of a species in perennial streams migrate long distances. Hill and Grossman (1987) found that the three dominant fish species in a North Carolina stream had home ranges of 13 to 19 m over a period of 18 months. Ross et al. (1985) and Matthews (1986) found that stream fish assemblages were stable and persistent for 10 years, recovering rapidly from droughts and floods indicating that large population fluctuations are unlikely to occur in response to purely natural environmental phenomena. However, comparison of data collected during different seasons is discouraged, as is data collected during or immediately after major flow changes.

8.1.5 Station Siting

8.1.5.1 Fish Bioassessment II includes the collection of biological samples to assess the biotic integrity of a given site. To meaningfully evaluate biological condition, sampling locations must be carefully selected to ensure generally comparable habitats at each station. Unless comparable physical habitat is sampled at all stations, community differences attributable to a degraded habitat will be difficult to separate from those resulting from water quality degradation. The availability of habitats at each sampling location can be established during preliminary reconnaissance. In situations where evaluations at several stations on a waterbody will be compared, the station with the greatest habitat constraints (in terms of productive habitat availability) should be noted. The station with the least number of productive habitats available will often determine the type of habitat to be sampled at all stations of comparison.

8.1.5.2 Locally modified sites, such as small impoundments and bridge areas, should be avoided unless data are needed to assess the effects of these structures. Sampling near the mouths of tributaries entering large waterbodies should also be avoided since these areas will have habitat more typical of the larger waterbody (Karr et al., 1986).

8.1.5.3 Although the specific bioassessment objective is an important consideration in locating sampling stations, all assessments require a site-specific control station or reference data from comparable sites within the same region. A site-specific reference area or site (Ohio EPA, 1990b, 1991) is generally thought to be most representative of "best attainable" conditions for a particular waterbody. However, regional reference conditions may also be desirable to allow evaluation on a larger geographic scale. Where feasible, effects should be bracketed by establishing a series or network of sampling stations at points of increasing distance from the impact source(s). These stations will provide a basis for delineating impact and recovery zones (these zones are not "reference stations").

8.1.5.4 Omernik (1987) and Omernik and Gallant (1988) have provided an ecoregional framework for interpreting spatial patterns in state and national data. The geographical framework is based on regional patterns in land-surface form, soil types, potential natural vegetation, and land use, which vary across the county. The use of ecoregions or similar approaches can provide a geographic framework for more efficient management of aquatic ecosystems and their components (Hughes, 1985; Hughes et al., 1982, 1986, 1987; Hughes and Larsen, 1988; Larsen et al., 1988). One method for evaluating fish community composition is utilizing the ecoregion approach. Another approach includes regional reference sites or control sites. The application of the ecoregion versus the reference site approaches have been documented (e.g., Larson et al., 1986; Ohio EPA, 1987b, 1989, 1990b; Rohm et al, 1987; Whittier et al., 1988), but further studies are still needed to determine the effectiveness of these approaches for other regions of North America. In addition, investigations will be required to (1) delineate areas that differ significantly in their innate biological potential, (2) locate reference sites within each ecoregion that fully support aquatic life uses; and (3) develop biological criteria (e.g., define optimal values for the

metrics recommended) using data generated with the fish bioassessment II protocol.

8.1.6 Importance of Habitat Assessment

8.1.6.1 The procedures for assessing habitat quality presented in this Section are an integral component of the final evaluation of impairment. The matrix used to assess habitat quality is based on key physical characteristics of the waterbody and the surrounding land. All of the habitat parameters evaluated are related to overall aquatic life use and are potential factors which could contribute to a limitation of the aquatic biota in the waterbody.

8.1.6.2 Habitat, as affected by instream and surrounding topographical features, can be a major determinant to aquatic community potential. Both the quality and quantity of available habitat will affect the structure and composition of resident biological communities. The effects of such pertubations can be minimized by sampling similar habitats at all stations being compared. However, when all stations are not physically comparable, habitat characterization is particularly important for proper interpretation of biosurvey results.

8.1.6.3 Where habitat quality is similar, detected impacts can be attributed to water quality factors. However, where habitat quality differs substantially from reference conditions, the question of use attainability and physical habitat alteration/restoration must be addressed. Final conclusions regarding the presence and degree of biological impairment should thus include an evaluation of habitat quality to determine the extent that habitat may be a limiting factor. The habitat characterization matrix included in the fish bioasessment II methods provides an effective means of evaluating and documenting habitat quality at each biosurvey station.

8.1.7 Fish Sampling Methodology (See, Section 4, Sample Collection for Analysis of the Structure and Function of Fish Communities.)

8.1.7.1 Use of Electrofishing, Seining, and Rotenoning

8.1.7.1.1 Although various types of gear are routinely used to sample fish, electrofishers, seines, and rotenone are the most commonly used for collection in freshwater habitats. As detailed earlier each method has advantages and disadvantages (Nielsen and Johnson, 1983; Hendricks et al., 1980). However, electrofishing is recommended for most fish field surveys because of its greater applicability and efficiency. Local conditions may require consideration of seining and/or the use of rotenone as optional collection methods. Advantages and disadvantages of each approach are presented below.

8.1.7.2 Advantages of Electrofishing

1. Electrofishing allows greater standardization of catch per unit of effort.

2. Electrofishing requires less time and manpower than some sampling methods (e.g., use of ichthyocides, like rotenone) (Hendricks et al., 1980).

3. Electrofishing is less selective than seining (although it is selective towards size and species) (Hendricks et al., 1980) (See disadvantage number 2).

4. If properly used, adverse effects on fish are minimized.

5. Electrofishing is appropriate in a variety of habitats.

8.1.7.3 Disadvantages of Electrofishing

1. Sampling efficiency is affected by turbidity, conductivity, aquatic vegetation, depth, etc.

2. Although less selective than seining, electrofishing also is size and species selective. Effects of electrofishing increase with body size. Species specific behavioral and anatomical differences also determine vulnerability to electroshocking (Reynolds, 1983).

3. Electrofishing is a hazardous operation that can injure field personnel if proper safety procedures are ignored.

8.1.7.4 Advantages of Seining

1. Seines are relatively inexpensive.

2. Seines are lightweight and are easily transported and stored.

3. Seine repair and maintenance are minimal and can be accomplished onsite.

4. Seine use is not restricted by water quality parameters.

5. Effects on the fish population are minimal because fish are collected alive and are generally unharmed.

8.1.7.5 Disadvantages of Seining

1. Previous experience and skill, knowledge of fish habitats and behavior, and sampling effort are probably more important in seining than in the use of any other approaches (Hendricks et al., 1980).

2. Seining sample effort and results are more variable than sampling with electrofishing or rotenoning.

3. Seine use is generally restricted to slower water with smooth bottoms, and is most effective in small streams or pools without litter cover or debris.

4. Standardization of unit of effort to ensure data comparability is difficult.

8.1.7.6 Advantages of Using Rotenone

1. The effective use of rotenone is independent of habitat complexity.

2. Rotenoning provides greater standardization of unit of effort than seining.

3. Rotenoning has the potential, if used effectively, to provide more complete censuring of the fish population than seining or electrofishing.

8.1.7.7 Disadvantages of Using Rotenone

1. Use of rotenone is prohibited in many states.

2. Application and detoxification can be time and manpower intensive.

3. Effective use of rotenone is affected by temperature, light, dissolved oxygen, alkalinity, and turbidity (Hendricks et al., 1980).

4. Rotenoning typically has a high environmental impact; concentration miscalculations can produce substantial fish kills downstream of the study site.

8.2 Sampling Representative Habitat

8.2.1 The sampling approach advocated in the Fish Bioassessment II optimizes the conservation of manpower and resources by sampling areas of representative habitat. The fish survey provides a representative estimate of the fish community at all habitats within a site, and a realistic sample of fish likely to be encountered in the water body. When sampling large streams, rivers, or waterbodies with complex habitats, a complete inventory of the entire reach is not necessary for the level of assessment used in the Fish Bioassessment II. The sampling area should be representative of the reach, incorporating riffles, runs, and pools if these habitats are typical of the stream in question. Although a sampling site with two riffles, two runs, and two pools is preferable, at least one of each habitat type should be evaluated. Mid-channel and wetland areas of large rivers, which are difficult to sample effectively, may be avoided. Sampling effort may be concentrated in near-shore habitats where most species will be collected. In doing so, some deep water or wetland species may be under-sampled, however, the data should be adequate for the objective of the Fish Bioassessment II method.

8.3 Fish Sample Processing and Enumeration

8.3.1 To ensure data comparability for assessing biological condition with the Fish Bioassessment II, sample processing and species enumeration must be standardized.

8.3.2 Processing of the fish biosurvey sample includes identification of all individuals to species, weighing (if the Index of well-being (Iwb) or biomass data are desired), and recording the incidence of external anomalies. It is recommended that each fish be identified and counted. Subsamples of abundant species may be weighed if live wells are unavailable. This is especially important for warmwater sites, where handling mortality is highly probable.

The data from the counted and weighed subsample is extrapolated for the total. Ohio EPA (1987a) has reported that subsampling reduced potential error and made the extra time required for individual weighing insignificant. Procedural details for subsampling are presented in Ohio EPA, 1987c. Determination of species trophic status is also necessary for some IBI metrics. It should also be standard practice to collect fish Total Length (TL) and Standard Length (SL) information.

8.4 Fish Environmental Tolerance Characterizations

8.4.1 Use of the Index of Biological Integrity (IBI) in the Fish Bioassessment II requires classification of fish species in terms of environmental tolerance. Responses of individual species to pollution will vary regionally and in accordance with the type of pollutant. The tolerance characterizations of selected midwestern and northwestern fish species are presented in Table 1. Effective use of the tolerance characterization approach requires an appropriate regional tolerance characterization system. Regional modification or substitutions may be based upon regional fish references, historical distribution records, objective assessment of a large statewide database, and toxicological test data. Application of the IBI approach in the southeastern and southwestern United States, and its widespread use by water resource agencies may result in additional modifications. Past modifications have been reported (Subsection 8.8, Miller et al., 1988a) without changing the IBI's basic theoretical foundations.

8.5 Fish Biosurvey and Data Analysis

8.5.1 Bioassessment Technique

8.5.1.1 A biological assessment involves an integrated analysis of the functional and structural components of the aquatic communities. These functional and structural components are evaluated through the use of 12 metrics based on fish. The range of pollution sensitivity exhibited by each metric differs among metrics (Figure 1); some are sensitive across a broad range of biological conditions, others only to part of the range.

8.5.1.2 The 12 IBI metrics used in the Fish Bioassessment II method are based on fish representing different sensitivities (Figure 2). For example, municipal effluents typically affect total abundance and trophic structure (Karr et al., 1986). Unusually low total abundance generally indicates a toxicant effect. However, some nutrient-deficient environments support a limited number of individuals or individual species, and an increase in abundance may indicate organic enrichment. Bottom dwelling species (e.g., darters, sculpins) that depend upon benthic habitats for feeding and reproduction are particularly sensitive to the effects of siltation and benthic oxygen depletion (Kuehne and Barbour, 1983; Ohio EPA, 1987b) and are good indicators of habitat degradation.

8.5.1.3 For the fish biosurvey and habitat assessment, scores are assigned to each metric or parameter based on a decision matrix. In the case of habitat assessment, evaluation of the quality of the parameter is based on visual observation. The score assigned to each habitat parameter is a compilation of

TABLE 1. TOLERANCE DESIGNATIONS, TROPHIC STATUS, AND NORTH AMERICAN ENDEMICITY OF SELECTED FISH SPECIES[a]

	Trophic Level	Tolerance	Origin
WILLAMETTE SPECIES[1]			
Salmonidae			
Chinook salmon	piscivore	intolerant	native
Cutthroat trout	insectivore	intolerant	native
Mountain whitefish	insectivore	intolerant	native
Rainbow trout	insectivore	intolerant	native
Cyprinidae			
Chiselmouth	herbivore	intermediate	native
Common carp	omnivore	tolerant	exotic
Goldfish	omnivore	tolerant	exotic
Leopard dace	insectivore	intermediate	native
Longnose dace	insectivore	intermediate	native
Northern squawfish	piscivore	tolerant	native
Peamouth	insectivore	intermediate	native
Redside shiner	insectivore	intermediate	native
Speckled dace	insectivore	intermediate	native
Catostomidae			
Largescale sucker	omnivore	tolerant	native
Mountain sucker	herbivore	intermediate	native
Ictaluridae			
Brown bullhead	insectivore	tolerant	introduced
Yellow bullhead	insectivore	tolerant	introduced
Percopsidae			
Sand roller	insectivore	intermediate	native
Gasterosteidae			
Threespine stickleback	insectivore	intermediate	native
Centrarchidae			
Bluegill	insectivore	tolerant	introduced
Largemouth bass	piscivore	tolerant	introduced
Smallmouth bass	piscivore	intermediate	introduced
White crappie	insectivore	intermediate	native
Percidae			
Yellow perch	insectivore	intermediate	native

[a]Not necessarily the final designations: designations may vary for different regions.

[1]Classifications for the Willamette River, Oregon were derived from Wydoski and Whitney (1979). Moyle (1976), Scott and Crossman (1973), Simpson and Wallace (1982), Dimick and Merryfield (1945), and Bond (1988, personal communication.)

[2]Classifications for midwestern fishes were taken from Karr et al. (1986) and Ohio EPA (1987b).

Note: The information in this table is on going research and needs further standardization.

TABLE 1. TOLERANCE DESIGNATIONS, TROPHIC STATUS, AND NORTH AMERICAN ENDEMICITY OF SELECTED FISH SPECIES (CONTINUED)

	Trophic Level	Tolerance	Origin
Cottidae			
Paiute sculpin	insectivore	intolerant	native
Prickly sculpin	insectivore	intermediate	native
Reticulate sculpin	insectivore	tolerant	native
Torrent sculpin	insectivore	intolerant	native
MIDWEST SPECIES[2]			
Petromyzontidae			
Silver lamprey	piscivore	intermediate	native
Northern brook lamprey	filterer	intolerant	native
Mountain brook lamprey	filterer	intolerant	native
Ohio lamprey	piscivore	intolerant	native
Least brook lamprey	filterer	intermediate	native
Sea lamprey	piscivore	intermediate	exotic
Polyodontidae			
Paddlefish	filterer	intolerant	native
Acipenseridae			
Lake sturgeon	invertivore	intermediate	native
Shovelnose sturgeon	invertivore	intermediate	native
Lepisosteidae			
Alligator gar	piscivore	intermediate	native
Shortnose gar	piscivore	intermediate	native
Spotted gar	piscivore	intermediate	native
Longnose gar	piscivore	intermediate	native
Amiidae			
Bowfin	piscivore	intermediate	native
Hiodontidae			
Goldeye	insectivore	intolerant	native
Mooneye	insectivore	intolerant	native
Clupeidae			
Skipjack herring	piscivore	intermediate	native
Alewife	invertivore	intermediate	exotic
Gizzard shad	omnivore	intermediate	native
Threadfish shad	omnivore	intermediate	native
Salmonidae			
Brown trout	insectivore	intermediate	exotic
Rainbow trout	insectivore	intermediate	exotic
Brook trout	insectivore	intermediate	native
Lake trout	piscivore	intermediate	native
Coho salmon	piscivore	intermediate	exotic
Chinook salmon	piscivore	intermediate	exotic
Lake herring	piscivore	intermediate	native
Lake whitefish	piscivore	intermediate	native
Osmeridae			
Rainbow smelt	invertivore	intermediate	introduced

TABLE 1. TOLERANCE DESIGNATIONS, TROPHIC STATUS, AND NORTH AMERICAN ENDEMICITY OF SELECTED FISH SPECIES (CONTINUED)

	Trophic Level	Tolerance	Origin
Umbridae			
Central mudminnow	insectivore	tolerant	native
Esocidae			
Grass pickerel	piscivore	intermediate	native
Chain pickerel	piscivore	intermediate	native
Northern pike	piscivore	intermediate	native
Muskellunge	piscivore	intermediate	native
Cyprinidae			
Common carp	omnivore	tolerant	exotic
Goldfish	omnivore	tolerant	exotic
Grass carp	herbivore	intermediate	exotic
Golden shiner	omnivore	tolerant	native
Hornyhead chub	insectivore	intolerant	native
River chub	insectivore	intolerant	native
Silver chub	insectivore	intermediate	native
Bigeye chub	insectivore	intolerant	native
Streamline chub	insectivore	intolerant	native
Gravel chub	insectivore	intermediate	native
Speckled chub	insectivore	intolerant	native
Blacknose dace	generalist	tolerant	native
Longnose dace	insectivore	intolerant	native
Creek chub	generalist	tolerant	native
Tonguetied minnow	insectivore	intolerant	native
Suckermouth minnow	insectivore	intermediate	native
Southern redbelly dace	herbivore	intermediate	native
Redside dace	insectivore	intolerant	native
Pugnose minnow	insectivore	intolerant	native
Emerald shiner	insectivore	intermediate	native
Silver shiner	insectivore	intolerant	native
Roseyface shiner	insectivore	intolerant	native
Redfin shiner	insectivore	intermediate	native
Rosefin shiner	insectivore	intermediate	native
Striped shiner	insectivore	intermediate	native
Common shiner	insectivore	intermediate	native
River shiner	insectivore	intermediate	native
Spottail shiner	insectivore	intermediate	native
Blackchin shiner	insectivore	intolerant	native
Bigeye shiner	insectivore	intolerant	native
Steelcolor shiner	insectivore	intermediate	native
Spotfish shiner	insectivore	intermediate	native
Bigmouth shiner	insectivore	intermediate	native
Sand shiner	insectivore	intermediate	native
Mimic shiner	insectivore	intolerant	native
Ghost shiner	insectivore	intermediate	native
Blacknose shiner	insectivore	intolerant	native
Pugnose shiner	insectivore	intolerant	native

TABLE 1. TOLERANCE DESIGNATIONS, TROPHIC STATUS, AND NORTH AMERICAN ENDEMICITY OF SELECTED FISH SPECIES (CONTINUED)

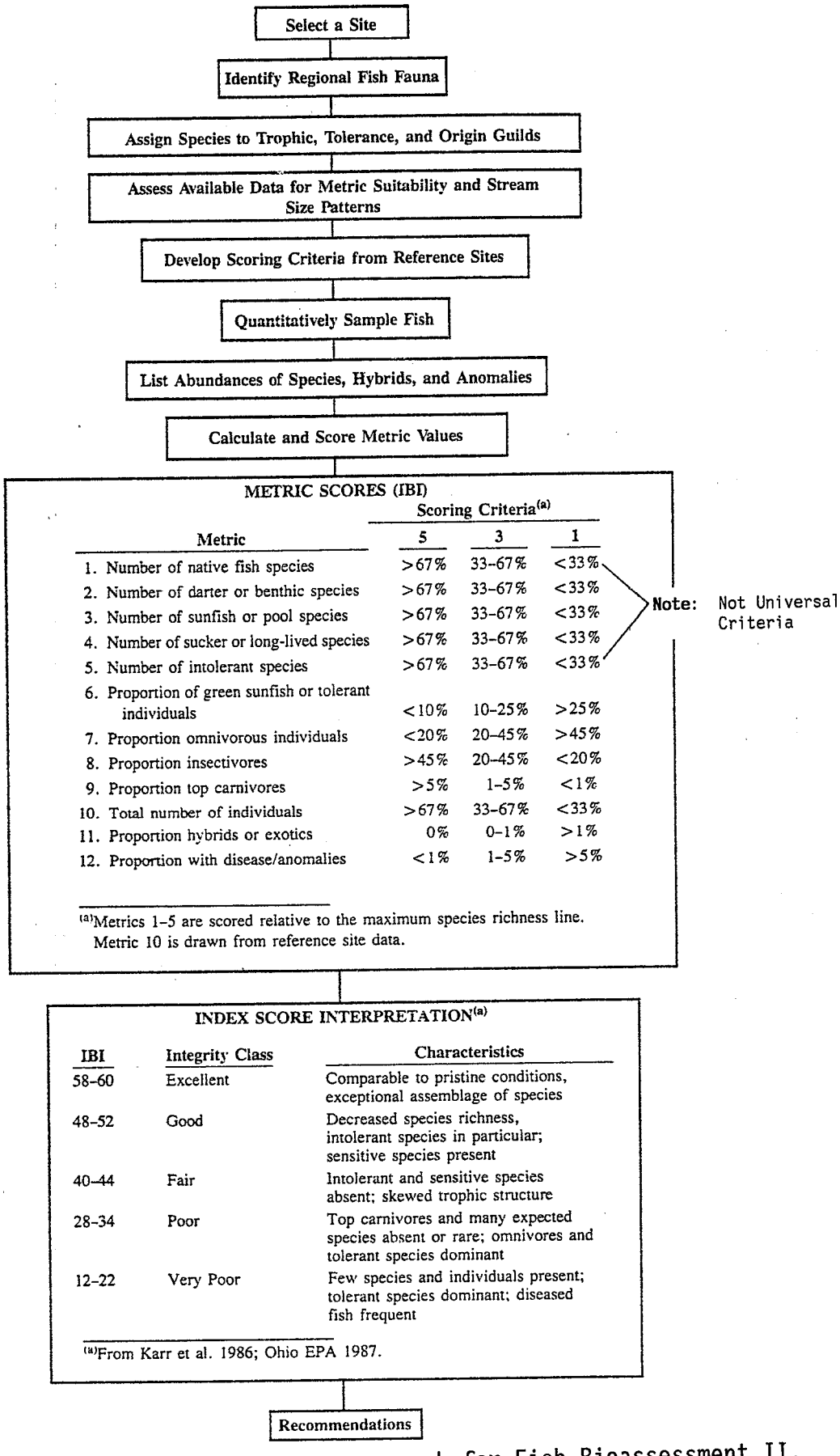
	Trophic Level	Tolerance	Origin
Cyprinidae			
Mississippi silvery minnow	herbivore	intermediate	native
Bullhead minnow	omnivore	intermediate	native
Bluntnose minnow	omnivore	tolerant	native
Fathead minnow	omnivore	tolerant	native
Central stoneroller	herbivore	intolerant	native
Popeye shiner	insectivore	intolerant	native
Silverjaw minnow	insectivore	intermediate	native
Central silvery minnow	herbivore	intolerant	native
Red shiner	omnivore	intermediate	native
Brassy minnow	omnivore	intermediate	native
Catostomidae			
Blue sucker	insectivore	intolerant	native
Bigmouth buffalo	insectivore	intermediate	native
Black buffalo	insectivore	intermediate	native
Smallmouth buffalo	insectivore	intermediate	native
Quilback	omnivore	intermediate	native
River carpsucker	omnivore	intermediate	native
Highfin carpsucker	omnivore	intermediate	native
Silver redhorse	insectivore	intermediate	native
Black redhorse	insectivore	intolerant	native
Golden redhorse	insectivore	intermediate	native
Shorthead redhorse	insectivore	intermediate	native
Greater redhorse	insectivore	intolerant	native
River redhorse	insectivore	intolerant	native
Harelip sucker	invertivore	intolerant	native
Northern hog sucker	insectivore	intolerant	native
White sucker	omnivore	tolerant	native
Longnose sucker	insectivore	intermediate	native
Spotted sucker	insectivore	intermediate	native
Lake chubsucker	insectivore	intermediate	native
Creek chubsucker	insectivore	intermediate	native
Ictaluridae			
Blue catfish	piscivore	intermediate	native
Channel catfish	generalist	intermediate	native
White catfish	insectivore	intermediate	native
Yellow bullhead	insectivore	tolerant	native
Brown bullhead	insectivore	tolerant	native
Black bullhead	insectivore	intermediate	native
Flathead catfish	piscivore	intermediate	native
Stonecat	insectivore	intolerant	native
Mountain madtom	insectivore	intolerant	native
Slender madtom	insectivore	intolerant	native
Freckled madtom	insectivore	intermediate	native
Northern madtom	insectivore	intolerant	native
Scioto madtom	insectivore	intolerant	native

TABLE 1. TOLERANCE DESIGNATIONS, TROPHIC STATUS, AND NORTH AMERICAN ENDEMICITY OF SELECTED FISH SPECIES (CONTINUED)

	Trophic Level	Tolerance	Origin
Ictaluridae			
Brindled madtom	insectivore	intolerant	native
Tadpole madtom	insectivore	intermediate	native
Anguillidae			
American eel	piscivore	intermediate	native
Fundulidae			
Western banded killfish	insectivore	intolerant	native
Eastern banded killfish	insectivore	tolerant	native
Blackstrip topminnow	insectivore	intermediate	native
Poeciliidae			
Mosquitofish	insectivore	intermediate	exotic
Gadidae			
Burbot	piscivore	intermediate	native
Moronidae			
Trout-perch	insectivore	intermediate	native
Aphredoderidae			
Pirate perch	insectivore	intermediate	native
Atherinidae			
Brook silverside	insectivore	intermediate	native
Percichthyidae			
White bass	piscivore	intermediate	exotic
Stripped bass	piscivore	intermediate	exotic
White perch	piscivore	intermediate	exotic
Yellow bass	piscivore	intermediate	exotic
Centrarchidae			
White crappie	invertivore	intermediate	native
Black crappie	invertivore	intermediate	native
Rock bass	piscivore	intermediate	native
Smallmouth bass	piscivore	intermediate	native
Spotted bass	piscivore	intermediate	native
Largemouth bass	piscivore	intermediate	native
Warmouth	invertivore	intermediate	native
Green sunfish	insectivore	tolerant	native
Bluegill	insectivore	intermediate	native
Orangespotted sunfish	insectivore	intermediate	native
Longear sunfish	insectivore	intolerant	native
Redear sunfish	insectivore	intermediate	native
Pumpkinseed	insectivore	intermediate	native
Percidae			
Sauger	piscivore	intermediate	native
Walleye	piscivore	intermediate	native
Yellow perch	piscivore	intermediate	native
Dusky darter	insectivore	intermediate	native
Blackside darter	insectivore	intermediate	native
Longhead darter	insectivore	intolerant	native

TABLE 1. TOLERANCE DESIGNATIONS, TROPHIC STATUS, AND NORTH AMERICAN ENDEMICITY OF SELECTED FISH SPECIES (CONTINUED)

	Trophic Level	Tolerance	Origin
Percidae			
Slenderhead darter	insectivore	intolerant	native
River darter	insectivore	intermediate	native
Channel darter	insectivore	intolerant	native
Gilt darter	insectivore	intolerant	native
Logperch	insectivore	intermediate	native
Crystal darter	insectivore	intolerant	native
Eastern sand darter	insectivore	intolerant	native
Western sand darter	insectivore	intolerant	native
Johnny darter	insectivore	intermediate	native
Greenside darter	insectivore	intermediate	native
Banded darter	insectivore	intolerant	native
Variegate darter	insectivore	intolerant	native
Spotted darter	insectivore	intolerant	native
Bluebreast darter	insectivore	intolerant	native
Tippecanoe darter	insectivore	intolerant	native
Iowa darter	insectivore	intermediate	native
Rainbow darter	insectivore	intermediate	native
Orangethroat darter	insectivore	intermediate	native
Fantail darter	insectivore	intermediate	native
Least darter	insectivore	intermediate	native
Slough darter	insectivore	intermediate	native
Sciaendiae			
Freshwater drum	invertivore	intermediate	native
Cottidae			
Spoonhead sculpin	insectivore	intermediate	native
Mottled sculpin	insectivore	intermediate	native
Slimy sculpin	insectivore	intermediate	native
Deepwater sculpin	insectivore	intermediate	native
Gasterosteidae			
Brook stickleback	insectivore	intermediate	native



METRIC SCORES (IBI)

Metric	Scoring Criteria[a] 5	3	1
1. Number of native fish species	>67%	33–67%	<33%
2. Number of darter or benthic species	>67%	33–67%	<33%
3. Number of sunfish or pool species	>67%	33–67%	<33%
4. Number of sucker or long-lived species	>67%	33–67%	<33%
5. Number of intolerant species	>67%	33–67%	<33%
6. Proportion of green sunfish or tolerant individuals	<10%	10–25%	>25%
7. Proportion omnivorous individuals	<20%	20–45%	>45%
8. Proportion insectivores	>45%	20–45%	<20%
9. Proportion top carnivores	>5%	1–5%	<1%
10. Total number of individuals	>67%	33–67%	<33%
11. Proportion hybrids or exotics	0%	0–1%	>1%
12. Proportion with disease/anomalies	<1%	1–5%	>5%

[a]Metrics 1–5 are scored relative to the maximum species richness line. Metric 10 is drawn from reference site data.

INDEX SCORE INTERPRETATION[a]

IBI	Integrity Class	Characteristics
58–60	Excellent	Comparable to pristine conditions, exceptional assemblage of species
48–52	Good	Decreased species richness, intolerant species in particular; sensitive species present
40–44	Fair	Intolerant and sensitive species absent; skewed trophic structure
28–34	Poor	Top carnivores and many expected species absent or rare; omnivores and tolerant species dominant
12–22	Very Poor	Few species and individuals present; tolerant species dominant; diseased fish frequent

[a]From Karr et al. 1986; Ohio EPA 1987.

Figure 1. Flowchart of biosurvey approach for Fish Bioassessment II.

a range of scores and is weighted in terms of its contribution to the total habitat quality. The scores assigned to the fish metrics are based on computed values of the metrics and a station comparison, wherein the regional or stream reference station serves as the highest attainment criterion or score for the area. Comparison of the total score computed for the metrics orparameters with that of the reference station provides a judgment as to impairment of biological condition.

8.5.1.4 The condition of the aquatic community needs to be evaluated and interpreted within the context of habitat quality in order to determine effects and likely causal factors. A poor habitat in terms of riparian vegetation, bank stability, stream substrate, etc., would not be conducive to supporting a well-developed community structure. The attainment of a higher quality biological condition may be prohibited by the constraints of habitat quality.

8.6 Fish Bioassessment I

8.6.1 The intent of the Fish Bioassessment I is to consist of a questionnaire, to serve as a screening tool, and to maximize the use of existing knowledge of fish communities. **Note**: The Fish Bioassessment I method is not an option for a minimum state bioassessment program. The

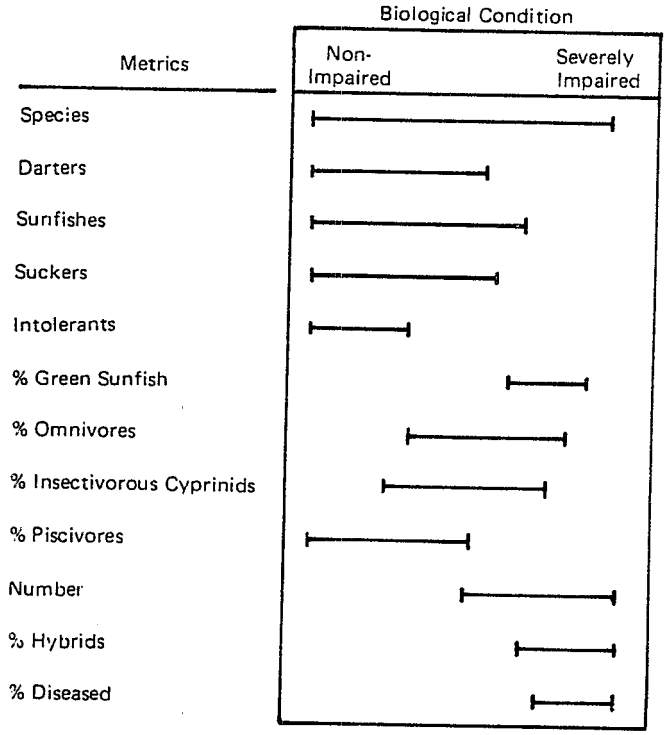


Figure 2. Range of sensitivities of biosurvey for Fish Bioassessment II metrics in assessing biological condition (from Karr et al., 1986).

questionnaire polls State fish biologists and university ichthyologists believed knowledgeable about the fish assemblages in stream reaches of concern. The questionnaire (Figure 3) is modeled after one used in a successful national survey of 1,300 river reaches or segments (Judy et al., 1984). Unlike field surveys, questionnaires can provide information about tainting or fish tissue contamination and historical trends and conditions. Disadvantages of the questionnaire approach include inaccuracy caused by hasty responses, a desire to report conditions as better or worse than they are, and insufficient knowledge. The questionnaire provides a qualitative assessment of a large number of water bodies quickly and inexpensively. Its quality depends on the survey design (the number and location of waterbodies), the questions presented, and the knowledge and cooperation of the respondents.

8.6.2 This section provides guidance on the design and content of the questionnaire survey. Judy et al. (1984) found that State fish and game agencies have a vested interest in assuring the quality of the data, and they generally provide reliable information.

8.6.3 Design of Fish Assemblage Questionnaire Survey

8.6.3.1 Selection of stream reaches requires considerable forethought. If the survey program is statewide or regional in scope, a regional framework is advisable. Regional reference reaches can be selected to serve as benchmarks for comparisons (Hughes et al., 1986). These sites should be characteristic of the water body types and sizes in the region and should be minimally impacted. The definition of minimal impact varies from region to region, but includes those waters that are generally free of point sources, channel modification, and diversions, and have diverse habitats, complex bottom substrate, considerable instream cover, and a wide buffer or natural riparian vegetation.

8.6.3.2 Remaining sites should also be selected carefully. If the questionnaire focuses on larger streams, a 1:1,000,000 scale topographic map should be used for stream reach selection. Reaches of small streams should be selected from the largest scale map possible; reaches selected from 1:250,000 versus 1:24,000 scale topographic maps may omit as much as 10 percent of the permanent streams in humid, densely forested areas. Small, medium, and large streams should be selected based on their importance in the region.

8.6.3.3 The potential respondent (or the agency chief if a number of agency staff are to be questioned) should be contacted initially by telephone to identify appropriate respondents. To ensure maximum response, the questionnaire should be sent at times other than the field season and the beginning and end of the a fiscal year or other seasonally busy time. The questionnaire should be accompanied by a personalized cover letter written on official stationary, and closed by an official title below the signature. A stamped, self-addressed return envelope increases the response rate. Materials mailed first or priority class are effective; special delivery and certified letters are justified in follow-up mailings. Telephone contact is advisable after three follow-up notes.

FISH ASSEMBLAGE QUESTIONNAIRE

INTRODUCTION

This questionnaire is part of an effort to assess the biological health or integrity of the flowing waters of this state. Our principle focus is on the biotic health of the designated waterbody as indicated by its fish community. You were selected to participate in the study because of your expertise in fish biology and your knowledge of the waterbody identified in this questionnaire.

Using the scale below, please circle the rank (at left) corresponding to the explanation (at right) that best describes your impression of the condition of the waterbody. Please complete all statements. If you feel that you cannot complete the questionnaire, check here [ ] and return it. If you are unable to complete the questionnaire but are aware of someone who is familiar with the waterbody, please give this person's name, address, and telephone number in the space provided below.

_____

_____

_____

Waterbody code _____

Waterbody name _____

Waterbody location (also see map)

State _____ County _____ Long/Lat_____

Ecoregion _____

Waterbody size

Stream (<1 cfs, 1-10 cfs, >10 cfs)

(Answer questions 1-4 using the scale below.)

5 Species composition, age classes, and trophic structure comparable to non (or minimally) impacted sites of similar waterbody size in that ecoregion.

4 Species richness somewhat reduced by loss of some intolerant species; young of the year of top carnivores rare; less than optimal abundances, age distributions, and trophic structure for waterbody size and ecoregion.

3 Intolerant species absent, considerably fewer species and individuals than expected for that waterbody size and ecoregion, older age classes of top carnivores rare, trophic structure skewed toward omnivory.

Figure 3. Fish assemblage questionnaire for use with Fish Bioassessment I.

2 Dominated by highly tolerant species, omnivores, and habitat generalists; top carnivores rare or absent; older age classes of all but tolerant species rare; diseased fish and anomalies relatively common for that waterbody size and ecoregion.

1 Few individuals and species present, mostly tolerant species and small individuals, diseased fish and anomalies abundant compared to other similar-sized waterbodies in the ecoregion.

0 No fish

(Circle one number using the scale above.)

1. Rank the current conditions of the reach

   5 4 3 2 1 0

2. Rank the conditions of the reach 10 years ago

   5 4 3 2 1 0

3. Given present trends, how will the reach rank 10 years from now?

   5 4 3 2 1 0

4. If the major human-caused limiting factors were eliminated, how would the reach rank 10 years from now?

   5 4 3 2 1 0

(Complete each subsection by circling the single most appropriate limiting factor and probable cause.)

Subsection 1--Water Quality

Limiting factor		Probable cause	
5	Temperature too high	18	Primarily upstream
6	Temperature too low	19	Within reach
7	Turbidity	20	Point source discharge
8	Salinity	21	Industrial
9	Dissolved oxygen	22	Municipal
10	Gas supersaturation	23	Combined sever
11	pH too acidic	24	Mining
12	pH too basic	25	Dam release
13	Nutrient deficiency	26	Nonpoint source discharge
14	Nutrient surplus	27	Individual sewage
15	Toxic substances	28	Urban runoff
16	Other (specify below) _____	29	Landfill leachate
		30	Construction
		31	Agriculture
17	Not limiting	32	Feedlot
		33	Grazing
		34	Silviculture
		35	Mining
		36	Natural
		37	Unknown
		38	Other (specify below) _____

Figure 3. Fish assemblage questionnaire for use with Fish Bioassessment I (Continued).

Subsection 2--Water Quantity

Limiting factor	Probable source
39 Below optimum flows	45 Dam
40 Above optimum flows	46 Diversion
41 Loss of flushing flows	47 Watershed conversion
42 Excessive flow fluctuation	48 Agriculture
43 Other (specify below) _____	49 Silviculture
	50 Grazing
	51 Urbanization
44 Not limiting	52 Mining
	53 Natural
	54 Unknown
	55 Other (specify below) _____

Subsection 3--Habitat Structure

Limiting factor	Probable cause
56 Excessive siltation	64 Agriculture
57 Insufficient pools	65 Silviculture
58 Insufficient riffles	66 Mining
59 Insufficient shallows	67 Grazing
60 Insufficient concealment	68 Dam
61 Insufficient reproductive habitat	69 Diversion
62 Other (specify below) _____	70 Channelization
	71 Snagging
	72 Other channel modifications
63 Not limiting	73 Natural
	74 Unknown
	75 Other (specify below) _____

Subsection 4--Fish Community

Limiting factor	Probable source
76 Overharvest	84 Fishermen
77 Underharvest	85 Aquarists
78 Fish stocking	86 State agency
79 Non-native species	87 Federal agency
80 Migration barrier	88 Point source
81 Tainting	89 Nonpoint source
82 Other (specify below) _____	90 Natural
	91 Unknown
	92 Other (specify below) _____
83 Not limiting	

Subsection 5--Major Limiting Factor

93 Water quality
94 Water quantity
95 Habitat structure
96 Fish community
97 Other (specify) _____

Your name (please print)_____

Figure 3. Fish assemblage questionnaire for use with Fish Bioassessment I (Continued).

8.6.4 Response Analysis

8.6.4.1 Questionnaire response should provide the following information:

1. The integrity of the fish community

2. The frequency of occurrence of particular limiting factors and causes

3. The frequency of occurrence of particular fish community condition characterizations for the past, present, and future

4. The geographic patterns in these variables

5. The temporal trends in the variables

6. Effect of water body type and size on the spatial and temporal trends and the associated limiting factors

7. The likelihood of improvement and degradation

8. The major limiting factor

8.6.4.2 The questionnaire data are most effectively analyzed by using a microcomputer and an interactive data base management software (e.g., *d*Base III or Revelation). This software reduces data entry errors and facilitates the qualitative analysis of numerous variables. Results can be reported as histograms, pie graphs, or box plots. If such a system is unavailable data can be analyzed and the results plotted by hand.

8.7 **Fish Bioassessment II**

8.7.1 Introduction

8.7.1.1 Fish Bioassessment II involves careful, standardized field collection, species identification and enumeration, and community analyses using biological indices or quantification of the biomass and numbers of key species. The Fish Bioassessment II survey yields an objective, discrete measure of the health of the fish community that usually can be completed onsite by qualified fish biologists (difficult species identifications may require laboratory confirmation). Data provided by the Fish Bioassessment II can allow assessment to use attainment, can be used to develop biological criteria, prioritize sites for further evaluation, provide a reproducible impact assessment, and be used to monitor trends in fish community status. Fish Bioassessment II is based primarily on the Index of Biotic Integrity (IBI) by Karr (1981). A more detailed description of this approach is presented in Karr et al. (1986) and Ohio EPA (1987b). Regional modification and applications are described in Hughes and Gammon (1987), Leonard and Orth (1986), Lyons (1992), Steedman (1988), Wade and Stalcup (1987), Miller et al. (1988a), and Simon (1990, 1991).

8.7.2 Field Survey Methods

8.7.2.1 Fish Bioassessment II involves field evaluation of both physical/chemical and habitat characteristics (see Subsection 8.13, Figures 9, 10, and 11), an impairment assessment (Figure 4), and a fish community biosurvey. Because it provides critical information for evaluating the cause and source of impairment, the habitat and physical characterization are essential to Fish Bioassessment II. The approach for conducting the Fish Bioassessment II site-specific fish community analysis is based on the use of the IBI (Figure 1).

8.7.3 Sample Collection

8.7.3.1 Electrofishing, the most common technique used by agencies that monitor fish communities, and the most widely applicable approach for stream habitats, is the sampling technique recommended for use with the Fish Bioassessment II. However, pilot studies may indicate the need for different or multiple techniques and gear found in this document.

8.7.3.2 The fish community biosurvey data are designed to be representative of the fish community at all station habitats, similar to the "representative qualitative sample" proposed by Hocutt (1981). The sampling station should be representative of the reach, incorporating at least one (preferably two) riffle(s), run(s), and pool(s) if these habitats are typical of the stream in question. Sampling of most species is most effective near shore and cover (Macrophytes, boulders, snags, brush). The biosurvey is not an exhaustive inventory, but it provides a realistic sample of fishes likely to be encountered in the waterbody. Sampling procedures effective for large rivers are described in Gammon (1980), Hughes and Gammon (1987), and Ohio EPA (1987b).

8.7.3.3 Typical sampling station lengths range from 100-200 meters for small streams to 500-1000 meters in rivers, but are best determined by pilot studies. The size of the reference station should be sufficient to produce 100-1000 individuals and 80-90 percent of the species expected from a 50 percent increase in sampling distance. Sample collection is usually done during the day, but night sampling can be more effective if the water is especially clear and there is little cover (Reynolds, 1983; Sanders, 1991; Sanders, 1992). Use of block nets set (with as little wading as possible) at both ends of the reach increases sampling efficiency for large, mobile species sampled in small streams.

8.7.3.4 The community-level assessment of fish assemblages using the Fish Bioassessment II requires that all fish species (not just gamefish) be collected. This reduces the effects of stocking and fishing and acknowledges the growing public interest in nongame species. Small fish that require special gear for their effective collection may be excluded. Exclusion of young-of-the-year fish during collection can have a minor effect on IBI scores (Angermeier and Karr, 1986), but lowers sampling costs and reduces the need for laboratory identification. Karr et al., (1986) recommended exclusion of fish less than 20 mm in length. This recommendation should be considered on a regional basis and is also applicable to large fish requiring special gear for

IMPAIRMENT ASSESSMENT SHEET

1. Detection of impairment: Impairment detected (Complete Items 2-6) No impairment detected (Stop here)

2. Biological impairment indicator:

Fish

___ sensitive species reduced/absent
___ dominance of tolerant species
___ skewed trophic structure
___ abundance reduced/unusually high
___ biomass reduced/unusually high
___ hybrid or exotic abundance unusually high
___ poor size class representation
___ high incidence of anomalies

Other aquatic communities

___ Macroinvertebrates
___ Periphyton
___ Macrophytes

3. Brief description of problem: _____

Year and date of previous surveys: _____

Survey data available in: _____

4. Cause (indicate major cause): organic enrichment toxicants flow sediment temperature poor habitat other _____

5. Estimated areal extent of problem (m^2) and length of stream reach affected (m) where applicable: _____

6. Suspected source(s) of problem

___ point source
___ urban runoff
___ agricultural runoff
___ silvicultural runoff
___ livestock
___ landfill
___ mine
___ dam or diversion
___ channelization or snagging
___ natural
___ other
___ unknown

Comments: _____

Figure 4. Impairment assessment sheet for use with Fish Bioassessment II.

collection (e.g., sturgeon). The intent of the sample (as with the entire Fish Bioassessment II method) is to obtain a representative estimate of the species present, and their abundances, in a reasonable amount of effort.

8.7.3.5 Sampling effort among stations is standardized as much as possible. Regardless of the gear used, the collection method, site length (or area), and work hours expended must be comparable to allow comparison of fish community status among sites. Major habitat types (riffle, run, and pool) sampled at each site and the proportion of each habitat type sampled should also be comparable. Generally 1 to 2 hours of actual sampling time are required, but this varies considerably with the gear used and the size and complexity of the site.

8.7.3.6 Atypical conditions, such as high flow, excessive turbidity or turbulence, heavy rain, drifting leaves, or other unusual conditions that affect sampling efficiency, should be avoided.

8.7.3.7 Glare, a frequent problem, is reduced by wearing polarized glasses during sample collection.

8.7.3.8 At least four individuals (one with the electrofisher, two fish netters, and one for holding container of collected fish) are necessary for effective electrofishing, and electrofishing efficiency is increased by having experienced netters involved.

8.7.4 Sample Processing

8.7.4.1 A field collection data sheet (Figure 5) is completed for each sample. Sampling duration and area or distance sampled are recorded in order to determine level of effort. Species may be separated into adults and juveniles by size and coloration; then total numbers and weights and the incidence of external anomalies are recorded for each group. Reference specimens of each species from each site are preserved in 10 percent formaldehyde (see Section 5, Fish Specimen Processing), the jar labeled, and the collection placed with the State ichthyological museum to confirm identifications and to constitute a biological record. This is especially important for uncommon species, for species requiring laboratory identification, and for documenting new distribution records. If retained in a live well, most fish can be identified, counted, and weighted in the field by trained personnel and returned to the stream alive. In warmwater sites, where handling mortality is highly probable, each fish is identified and counted, but for abundant species, subsampling may be considered. When subsampling is employed, the subsample is extrapolated to obtain a final value. Subsampling for weight is a simple, straightforward procedure, but failure to examine all fish to determine frequency of anomalies (which may occur in about 1 percent of all specimens) can bias results. The trade off between handling mortality and data bias must be considered on a case-by-case basis. If a site is to be sampled repeatedly over several months (i.e., monitoring), the effect of sampling mortality may outweigh data bias. Holding fish in live boxes in shaded, circulating water will substantially reduce handling mortality. More information on field methods is presented in Karr et al. (1986) and Ohio EPA (1987a, 1987b, 1989).

Collection No. _____ Page ____ of ____

FISH FIELD COLLECTION DATA SHEET

State or Country _____ County _____ Date _____
Locality _____
_____
Water _____ Sampling duration (min.) _____
Vegetation _____
_____
Bottom _____ Temp _____ Air _____
Shore _____
Distance from shore or stream width _____
Habitat complexity/quality (excellent good fair poor very poor
Sampling distance (m) _____ Sampling area (m^2) _____
Depth of capture _____
Method of capture _____
Collected by _____ Date _____
Orig. preservation _____ number of individuals____ number of anomalis*____
Weather _____ Flow (flood bankfull moderate low)
Gear/crew performance _____
Comments _____

Genus/Species	Adults		Juveniles		Anomalies(*)
	No.	Wt.	No.	Wt.	No.

*Discoloration, deformities, eroded fins, excessive mucus, excessive external parasites, fungus, poor condition, reddening, tumors, and ulcers.

Figure 5. Fish field collection data sheet for use with Fish Bioassessment II.

8.7.5 Data Analysis Techniques

8.7.5.1 Based on observations made in the assessment of habitat, water quality, physical characteristics, and the fish biosurvey, the investigator concludes whether impairment is detected. If impairment is detected, the probable cause and source is estimated and recorded on an Impairment Assessment Sheet (Figure 4). A preliminary judgment on the presence of biological impairment is particularly important if the Fish Bioassessment I is not used prior to the Fish Bioassessment II.

8.7.5.2 Data can be analyzed using the Index of Biotic Integrity (IBI) (or individual IBI metrics), the Index of well-being (Iwb) (Gammon, 1976, 1980), and multivariate statistical techniques to determine community similarities. Detrended correspondence analysis (DCA) is a useful multivariate analysis technique for revealing regional community patterns and patterns among multiple sites (Matthews et al., 1992). It also demonstrates assemblages with compositions differing from others in the region or reach. The reader may consult Gauch (1982) and Hill (1979) for descriptions of, and software for, DCA. Data analyses and reporting, including parts of the IBI, can be computer generated. Computerization reduces the time needed to produce a report and increases staff capability to examine data patterns and implications. The Illinois EPA has developed software to assist the professional aquatic biologists in calculating IBI values in Illinois streams (Bickers et al. 1988). Use of this software outside Illinois or the particular ecoregion without modification is not recommended. However, hand calculation in the initial use of the IBI promotes understanding of the approach and provides insight into local inconsistencies.

8.7.5.3 The IBI is a broadly-based index firmly grounded in fisheries community ecology (Karr, 1981; Karr et al., 1986). The IBI incorporates zoogeographic, ecosystem, community, population, and individual perspectives. It can accommodate natural differences in the distribution and abundance of species that result from differences in waterbody size, type, and region of occurrence (Miller et al., 1988a). Use of the IBI allows national comparisons of biological integrity without the traditional bias for small coldwater streams (e.g., a salmon river in Alaska and a minnow stream in Georgia both could be rated excellent if they were comparable to the best streams expected in their respective regions).

8.7.5.4 Karr et al. (1986) provided a consistent theoretical framework for analyzing fish community data. The IBI uses 12 biological metrics to assess integrity based on the fish community's taxonomic and trophic composition and the abundance and condition of fish. Such multiple-parameter indices are necessary for making objective evaluations of complex systems. The IBI was designed to evaluate the quality of small mid-western streams but has been modified for use in many regions of the country and in large rivers (Subsection 8.8).

8.7.5.5 The metrics attempt to quantify an ichthyologist's best professional judgment of the quality of the fish community. The IBI utilizes professional judgment, but in a prescribed manner, and it includes quantitative standards for discriminating fish community condition. Judgment is involved in choosing

the most appropriate population or community element that is representative of each metric and in setting the scoring criteria. This process can be easily and clearly modified, as opposed to judgments that occur after results are calculated. Each metric is scored against criteria based on expectation developed from appropriate regional reference sites. Metric values approximating, deviating slightly from, or deviating greatly from values occurring at the reference sites are scored as 5, 3, or 1, respectively. The scores of the 12 metrics are added for each station to give an IBI of 60 (excellent) to 12 (very poor). Trophic and tolerance classifications of midwestern and northwestern fish species are listed in Table 1. Additional classifications can be derived from information in State and regional fish texts or by objectively assessing a large statewide database. Use of the IBI in the southern and southwestern United States and its widespread use by water resource agencies may result in further modifications. Past modifications have occurred (Subsection 8.8; Miller et al., 1988a) without changing the IBI's basic theoretical foundations. Sample calculations of the IBI are given in Plafkin et al. (1989).

8.7.6 The steps in calculating the IBI (Figure 1) are explained below:

8.7.6.1 Assign species to trophic guilds; identify and assign species tolerances. Where published data are lacking, assignments are made based on knowledge of closely related species and morphology.

8.7.6.2 Develop scoring criteria for each IBI metric. Maximum species richness (or density) lines are developed from a reference database.

8.7.6.3 Conduct field study and identify fish; note anomalies, eroded fins, poor condition, excessive mucous, fungus, external parasites, reddening, lesions, and tumors. Complete field data sheets (Figure 5).

8.7.6.4 Enumerate and tabulate number of fish species and relative abundances.

8.7.6.5 Summarize site information for each IBI metric.

8.7.6.6 Rate each IBI metric and calculate total IBI score.

8.7.6.7 Translate total IBI score to one of the five integrity classes.

8.7.6.8 Interpret data in the context of the habitat assessment (for a discussion of Integration of Habitat, Water Quality, and Biosurvey data, see Plafkin et al., 1989). Individual metric analysis may be necessary to ascertain specific trends.

8.7.7 The Index of Biotic Integrity (IBI) is based on an integrated analysis of the metrics. However, individual IBI metrics may serve as separate variables to aid in data interpretation. Comparison of commonly-occurring and dominant species are revealing, especially when related to their ecological requirements and tolerances. Larsen et al. (1986) and Rohm et al. (1987) provide examples of such regional characterizations of common and abundant species. The Index of well-being (Iwb), (Gammon, 1980; Hughes and Gammon,

1987) incorporates two abundance and two diversity estimates in approximately equal fashion, thereby representing fish assemblage quality more realistically than a single diversity or abundance measure. The Iwb is calculated as

$$Iwb = 0.5 \ln N + 0.5 \ln B + H'_N + H'_B$$

where N equals the number of individuals caught per kilometer, B equals the biomass of individuals caught per kilometer, and H′ is the Shannon diversity index. Ohio EPA (1987b) and Gammon (1989, personal communication) found that subtracting highly tolerant species from the number and biomass variables, or modified Index of well-being (Iwb), increases sensitivity of the index in degraded environments (Ohio EPA, 1987b; Yoder et al., 1981). The modified Iwb has the same computational formula as the proposed Iwb by Gammon (1976). The main difference is that any of 13 highly tolerant species, exotics, and hybrids are deleted from the numbers and biomass components of the Iwb. The tolerant and exotic species, however, are included in the two Shannon index calculations. This modification eliminated the undesired effect caused by high abundance of tolerant species, but retains the desired influence of the Shannon indices (Ohio EPA, 1987b).

8.7.8 If the size of a particular fish population (e.g., trout or bass species) is of concern, it can be estimated with known confidence limits by several methods. One of the most popular approaches is the removal method (Seber, 1982; Seber and LeCren, 1967; Seber and Whale, 1970). The approach assumes a closed population, equal probability of capture for all fish, and a constant probability of capture from sample to sample (equal sampling effort and conditions). The removal method is applicable to situations in which the total catch is large relative to the total population. If subsequent samples produce equal or greater numbers than previous samples, the population must be resampled. Population size in the two sample cases is estimated by

$$N = C_1^1/(C_1 - C_2)$$

where C_1 and C_2 are the number of fish captured in the first and second samples, respectively. In the three sample cases, population size is estimated by

$$N = \frac{6X^2 - 3XY - Y^2 + 6XY - 3X^2)^{1/2}}{18(X - Y)}$$

where $X = 2C_1 + C_2$, and $Y = C2_1 + C_3$.

8.7.9 Many methods are available to calculate population statistics from removal data including regression, maximum likelihood, and maximum weighted-likelihood. Pubic-domain software is available to assist in calculating these and other fisheries population statistics (Van Deventer and Platts, 1989).

8.8 Description of IBI Metrics

8.8.1 The IBI serves as an integrated analysis because individual metrics may differ in their relative sensitivity to various levels of biological condition. A description and brief rationale for each of the 12 IBI metrics

is outlined below. The original metrics described by Karr (1981) for Illinois streams (underlined) are followed by substitutes used in or proposed for different geographic regions and stream sizes. Because of zoogeographic differences, dissimilar families or species are evaluated in different regions, with regional substitutes occupying the same general habitat or niche. The sources for each substitute is footnoted below. Table 2 presents an overview of the IBI metric variations for six areas of the United States and Canada and their sources. Scoring criteria for the 12 original IBI metrics (Karr, 1986) are included in Figure 1).

8.8.2 These metrics assess the species richness component of diversity and the health of the major taxonomic groups and habitat guilds of fishes. Two of the metrics assess community composition in terms of tolerant or intolerant species. Scoring for the first five of these metrics or their substitute metrics requires development of species-waterbody size relationships for different zoogeographic regions. Development of this relationship requires data sufficient to plot the number of species collected from regional reference sites of various stream sizes against a measure of stream size (watershed area, stream order) of those sites. A line is then drawn with slope fit by eye to include 95 percent of the points. Finally the area under the line is trisected into areas that are scored as 5, 3, or 1 (Figure 6). A detailed description of these methods can be found in Fausch et al. (1984), Ohio EPA (1987b), and Karr et al. (1986).

8.8.2.1 Metric 1. Total number of fish species (1,4,5). Substitutes: Total number of native fish species (2,8), and salmonid age classes (6). This number decreases with increased degradation; hybrids and introduced species are not included. In coldwater streams supporting few fish species, the age classes of the species found represent the suitability of the system for spawning and rearing. The number of species is strongly affected by stream size at small stream sites, but not at large river sites (Karr et al., 1986; Ohio EPA, 1987b). Thus, scoring depends on developing species/waterbody size relationships.

8.8.2.2 Metric 2. Number and identity (Page, 1983) of darter species (1). Substitutes: Number and identity of sculpin species (2,4), benthic insectivore species (3,4) salmonid yearlings (individuals) (6); number of sculpins (individuals) (4); percent round-bodied suckers (5), sculpin, and darter species (8). These species are sensitive to degradation resulting from siltation and benthic oxygen depletion because they feed and reproduce in benthic habitats (Kuehne and Barbour, 1983; Ohio EPA, 1987b). Many smaller species live within the rubble interstices, are weak swimmers, and spend their entire lives in an area of 100-400 m^2 (Hill and Grossman, 1987; Matthews, 1986). Darters are appropriate in most Mississippi Basin streams; sculpins and yearling trout occupy the same niche in western streams. Benthic insectivores and sculpins or darters are used in small Atlantic slope streams that have few sculpins or darters and round-bodied suckers are suitable in large midwestern rivers. Scoring requires development of species/waterbody size relationships.

8.8.2.3 Metric 3. Number and identity of sunfish species (1). Substitutes: Number and identity of cyprinid species (2,4), water column species (3,4),

TABLE 2. REGIONAL VARIATIONS OF IBI METRICS[1]

Variations in IBI Metrics	Midwest	New England	Ontario	Central Appalachia	Colorado Front Range	Western Oregon	Sacramento-San Joaquin
1. Total Number of Species	X	X		X	X		X
# native fish species			X			X	
# salmonid age classes[2]						X	X
2. Number of Darter Species	X			X	X		
# sculpin species						X	
# benthic insectivore species		X					
# darter and sculpin species			X				
#salmonid yearlings (individuals)[2]						X	X
% round-bodied suckers	X						
#sculpins (individuals)							X
3. Number of Sunfish Species	X				X		
# cyprinid species						X	
# water column species		X					
# sunfish and trout species			X				
# salmonid species							X
# headwater species	X						

[1]Taken from Karr et al. (1986), Hughes and Gammon (1987), Miller et al. (1988a), Miller et al. (1988b), Ohio EPA (1987b), and Steedman (1988).

[2]Metric suggested by Moyle (1976) or Hughes (1985) as a provisional replacement metric in small western salmonid streams.

[3]An optional metric found to be valuable by Hughes and Gammon (1987).

Note: X = metric used in the region. Many of these variations are applicable elsewhere.

TABLE 2. REGIONAL VARIATIONS OF IBI METRICS (CONTINUED)

Variations in IBI Metrics	Midwest	New England	Ontario	Central Appalachia	Colorado Front Range	Western Oregon	Sacramento-San Joaquin
4. Number of Sucker Species	X	X				X	
# adult trout species[2]						X	X
# minnow species	X				X		
# sucker and catfish species			X				
5. Number of Intolerant Species	X	X			X	X	
# sensitive species	X						
# amphibian species							X
presence of brook trout			X				
6. % Green Sunfish	X						
% common carp						X	
% white sucker		X			X		
% tolerant species	X						
% creek chub				X			
% dace species			X				
7. % Omnivores	X	X	X	X	X	X	
% yearling salmonids[2]						X	X
8. % Insectivorous Cyprinids	X						
% insectivores		X				X	
% specialized insectivores				X	X		
# juvenile trout							X
% insectivorous species	X						

TABLE 2. REGIONAL VARIATIONS OF IBI METRICS (CONTINUED)

Variations in IBI Metrics	Midwest	New England	Ontario	Central Appalachia	Colorado Front Range	Western Oregon	Sacramento-San Joaquin
9. % Top Carnivores	X	X	X				
% catchable salmonids						X	
% catchable wild trout							X
% pioneering species	X						
Density catchable wild trout							X
10. Number of Individuals	X		X	X	X	X	X
Density of individuals		X					
11. % Hybrids	X	X					
% introduced species					X	X	
% simple lithophils	X						
% simple lithophilic species	X						
% native species							X
% native wild individuals							X
12. % Diseased Individuals	X	X	X	X	X	X	
13. Total Fish Biomass[3]						X	

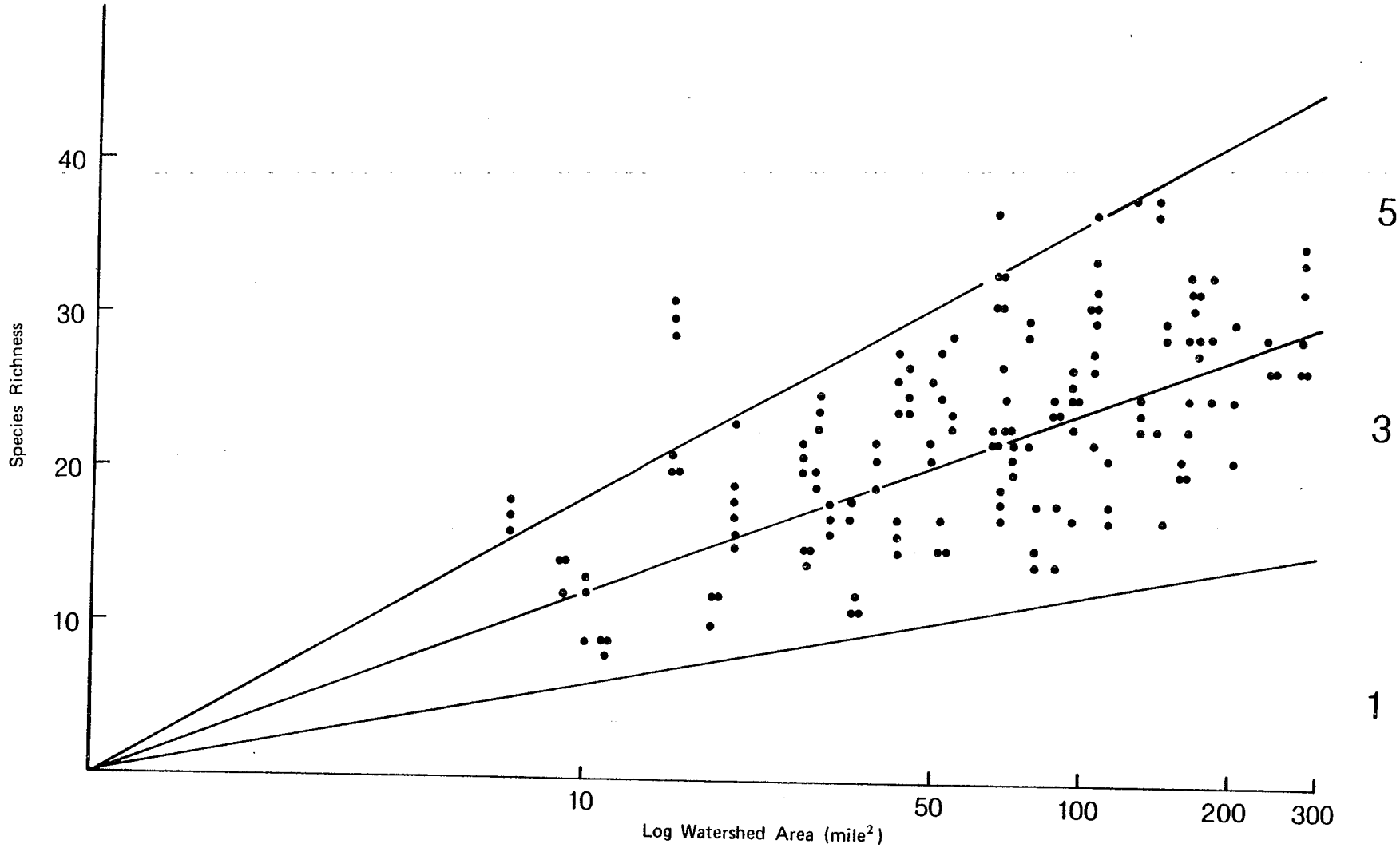


Figure 6. Total number of fish species versus watershed area for Ohio regional reference sites.

salmonid species (4), headwater species (5), and sunfish and trout species (8). These pool species decrease with increased degradation of pools and instream cover (Gammon et al., 1981; Angermeier, 1983; Platts et al., 1983). Most of these fishes feed on drifting and surface invertebrates and are active swimmers. The sunfishes and salmonids are important sport species. The sunfish metric works for most Mississippi Basin streams, but where sunfish are absent or rare, other groups are used. Cyprinid species are used in coolwater western streams; water column species occupy the same niche in northeastern streams; salmonids are suitable in coldwater streams; headwater species serve for midwestern headwater streams and trout and sunfish species are used in southern Ontario streams. Karr et al. (1986) and Ohio EPA (1987b) found the number of sunfish species to be dependent on stream size in small streams, but Ohio EPA (1987b) found no relationship between stream size and sunfish species in medium to large streams, nor between stream size and headwater species in small streams. Scoring of this metric requires development of species/waterbody size relationships.

8.8.2.4 Metric 4. Number and identity of sucker species (1). Substitutes: Number of adult trout species (6), number of minnow species (5); and number of sucker and catfish (8). These species are sensitive to physical and chemical habitat degradation and commonly comprise most of the fish biomass in streams. All but the minnows are long-lived species and provide a multiyear integration of physical/chemical conditions. Suckers are common in medium and large streams; minnows dominate small streams in the Mississippi Basin; and trout occupy the same niche in coldwater streams. The richness of these species is a function of stream size in small and medium sized streams, but not in large rivers. Scoring of this metric requires development of species/waterbody size relationships.

8.8.2.5 Metric 5. Number and identity of intolerant species (1). Substitutes: Number and identity of sensitive species (5), amphibian species (4); and presence of brook trout (8). This metric distinguishes high and moderate quality sites using species that are intolerant of various chemical and physical perturbations. Intolerant species are typically the first species to disappear following disturbance. Species classified as intolerant or sensitive should only represent the 5-10 percent most susceptible species, otherwise this becomes a less discriminating metric. Candidate species are determined by examining regional fishery books for species that were once widespread but have become restricted to only the highest quality streams. Ohio EPA (1987b) uses number of sensitive species (which includes highly intolerant and moderately intolerant species) for head-water sites because highly intolerant species are generally not expected in such habitats. Moyle (1976) suggested using amphibians in northern California streams because of their sensitivity to silvicultural impacts. This also may be a promising metric in appalachian streams which may naturally support few fish species. Steedman (1988) found that the presence of brook trout had the greatest correlation with IBI score in Ontario streams. The number of sensitive and intolerant species increases with stream size in small and medium sized streams but is unaffected by size of large rivers. Scoring of this metric requires development of species/waterbody size relationships.

8.8.2.6 Metric 6. Proportion of tolerant individuals as green sunfish (1). Substitutes: Proportion of individuals as common carp (2,4), white sucker (3,4), tolerant species (5), creek chub (7), and dace (8). This metric is the reverse of Metric 5. It distinguishes low from moderate quality waters. These species show increased distribution or abundance despite the historical degradation of surface waters, and they shift from incidental to dominant in disturbed sites. Green sunfish are appropriate in small Midwestern streams; creek chubs were suggested for central Appalachian streams; common carp were suitable for a coolwater Oregon river; white sucker were selected in the northeast and Colorado where green sunfish are rare to absent; and dace (*Rhinichthys* species) were used in southern Ontario. To avoid weighing the metric on a single species, Karr et al. (1986) and Ohio EPA (1987b) suggest using a small number of highly tolerant species. Scoring of this metric may require development of expectations based on waterbody size.

8.8.3 Trophic Composition Metrics

8.8.3.1 These three metrics assess the quality of the energy base and trophic dynamics of the community. Traditional process studies, such as community production and respiration, are time consuming to conduct and the results are equivocal; distinctly different situations can yield similar results. The trophic composition metrics offer a means to evaluate the shift toward more generalized foraging that typically occurs with increased degradation of the physicochemical habitat.

8.8.3.2 Metric 7. Proportion of individuals as omnivores (1,2,3,4,5,8). Substitutes: Proportion of individuals as yearlings (4).

8.8.3.2.1 The percent of omnivores in the community increases as the physical and chemical habitat deteriorates. Omnivores are defined as species that consistently feed on substantial proportions of plant and animal material. Ohio EPA (1987b) excludes sensitive filter feeding species such as paddlefish and lamprey ammocoetes and opportunistic feeders like channel catfish. Where omnivorous species are nonexistent, such as in trout streams, the proportion of the community composed of yearlings, which initially feed omnivorously, may be substituted.

8.8.3.3 Metric 8. Proportion of individuals which are insectivorous cyprinids (1). Substitutes: Proportion of individuals as insectivore (2,3,5), specialized insectivores (4), and insectivorous species (5);and number of juvenile trout (4).

8.8.3.3.1 Insectivores or invertivores are the dominant trophic guild of most North American surface waters. As the invertebrate food source decreases in abundance and diversity due to physical/chemical habitat deterioration, there is a shift from insectivorous to omnivorous fish species. Generalized insectivores and opportunistic species, such as blacknose dace and creek chub were excluded from this metric by Ohio EPA (1987b). This metric evaluates the midrange of biotic integrity.

8.8.3.4 Metric 9. Proportion of individuals as top carnivores (1,3,8).

Substitutes: Proportion of individuals as catchable salmonids (2), catchable wild trout (4), and pioneering species (5).

8.8.3.4.1 The top carnivore metric discriminates between systems with high and moderate integrity. Top carnivores are species that feed as adults predominantly on fish, other vertebrates, or crayfish. Occasional piscivores, such as creek chub and channel catfish, are not included. In trout streams, where true piscivores are uncommon, the percent of large salmonids is substituted for percent piscivores. These species often represent popular sport fish such as bass, pike, walleye, and trout. Pioneering species are used by Ohio EPA (1987b) in headwater streams typically lacking piscivores.

8.8.4 Fish Abundance and Condition Metrics

8.8.4.1 The last three metrics indirectly evaluate population recruitment, mortality, condition, and abundance. Typically, these parameters vary continuously and are time consuming to estimate accurately. Instead of such direct estimates, the final results of the population parameters are evaluated. Indirect estimation is less variable and much more rapidly determined.

8.8.4.2 Metric 10. Number of individuals in sample (1,2,4,5,8). Substitutes: Density of individuals (3,4).

8.8.4.2.1 This metric evaluates population abundance and varies with region and stream size for small streams. It is expressed as catch per unit effort, either by area, distance, or time sampled. Generally sites with lower integrity support fewer individuals, but in some nutrient-poor regions, enrichment increases the number of individuals. Steedman (1988) addressed this situation by scoring catch per minute of sampling greater than 25 fish as a three, and less than 4 fish as a one. Unusually low numbers generally indicate toxicity, making this metric most useful at the low end of the biological integrity scale. Hughes and Gammon (1987) suggest that in larger streams, where sizes of fish may vary in orders of magnitude, total fish biomass may be an appropriate substitute or additional metric.

8.8.4.3 Metric 11. Proportion of individuals as hybrids (1). Substitutes: Proportion of individuals as introduced species (2,4), simple lithophils (5); and number of simple lithophilic species (5).

8.8.4.3.1 This metric is an estimate of reproductive isolation or the suitability of the habitat for reproduction. Generally as environmental degradation increases, the percent of hybrids and introduced species also increases, but the proportion of simple lithophils decreases. However, minnow hybrids are found in some high quality streams, hybrids are often absent from highly impacted sites, and hybridization is rare and difficult for many to detect. Thus, Ohio EPA (1987b) substitutes simple lithophils for hybrids. Simple lithophils spawn where their eggs can develop in the interstices of sand, gravel, and cobble substrates without parental care. Hughes and Gammon (1987) and Miller et al. (1988a) propose using percent introduced individuals. This metric is a direct measure of the loss of species segregation between

midwestern and western fishes that existed before the introduction of midwestern species into western rivers.

8.8.4.4 Metric 12. Proportion of individuals with disease, tumors, fin damage, and skeletal anomalies (1).

8.8.4.4.1 This metric depicts the health and condition of individual fish. These conditions occur infrequently or are absent from minimally impacted reference sites but occur frequently below point sources and in areas where toxic chemicals are concentrated. They are excellent measures of the subacute effects of chemical pollution and the aesthetic value of game and nongame fish.

8.8.4.5 Metric 13. Total fish biomass (optional). Hughes and Gammon (1987) suggest that in larger areas where sizes of fish may vary in orders of magnitude this additional metric may be appropriate.

8.8.4.5.1 Because the IBI is an adaptable index, the choice of metrics and scoring criteria is best developed on a regional basis through use of available publications (Karr et al., 1986; Ohio EPA, 1987b; Miller et al., 1988a). Several steps in the IBI process are common to all regions. The fish species must be listed and assigned to trophic and tolerance guilds. Scoring criteria are developed through use of high quality historical data and data from minimally-impacted regional reference sites. The development of reference sites have been accomplished for much of the country, but continued refinements are expected as more fish community ecology data become available. Once scoring criteria have been established, a fish sample is evaluated by listing the species and their abundances (Figure 5), calculating values for each metric and comparing these values with the scoring criteria. Individual metric scores are added to calculate the total IBI score (Figure 7). Hughes and Gammon (1987) and Miller et al. (1988a) suggest that scores lying at the extremes of scoring criteria can be modified by a plus or minus; a combination of three pluses or three minuses results in a two point increase or decrease in IBI. Ohio EPA (1987b) scores proportional metrics as 1 when the number of species and individuals in samples are fewer than 6 and 75, respectively, when their expectations are of higher numbers.

8.9 Guidance for Use of Field Data Sheets

8.9.1 This subsection provides guidance for use of the bioassessment field and laboratory data sheets. The guidance sheets give brief descriptions of the information required for each data sheet.

8.9.2 Guidance for Header Information (Figure 8)

8.9.2.1 Water body Name: Name of stream or drain.

8.9.2.2 Location: Township, range, section, county where problem area is located. For streams or drains; road crossings or outfall locations should be referenced where applicable.

8.9.3 Reach/Milepoint: Indicate station reach/milepoint.

8.9.4 Latitude/Longitude: Indicate station latitude/longitude.

8.9.5 County/State: Name of county and state where station is located.

8.9.6 Aquatic Ecoregion: Name of ecoregion.

8.9.7 Station: Agency name or number for station.

8.9.8 Investigators: List field personnel involved.

8.9.9 Date: Date of survey.

8.9.10 Agency: Agency name or affiliation (academic, private consulting)

8.9.11 Hydrologic Unit Code: Indicate the USGS cataloging unit number in which the station is located.

8.9.12 Form Completed By: List personnel completing form.

8.9.13 Reason for Survey: The reason why this survey was conducted.

Station No. _____

Site _____

Metrics[a]	Scoring Criteria[b] 5 (%)	3 (%)	1 (%)	Metric Value	Metric Score
1. Number of Native Fish Species	>67	33-67	<33		
2. Number of Darter or Benthic Species	>67	33-67	<33		
3. Number of Sunfish or Pool Species	>67	33-67	<33		
4. Number of Sucker or Long-Lived Species	>67	33-67	<33		
5. Number of Intolerant Species	>67	33-67	<33		
6. % Green Sunfish or Tolerant Individuals	<10	10-25	>25		
7. % Omnivores	<20	20-45	>45		
8. % Insectivores or Invertivores	>45	20-45	<20		
9. % Top Carnivores	>5	1-5	<1		
10. Total Number of Individuals	>67	33-67	<33		
11. % Hybrids or Exotics	0	0-1	>1		
12. % Anomalies	<1	1-5	>5		

Scorer _____ IBI Score _____

Comments: _____

(a) Karr's original metrics or commonly used substitutes. See Figure 4 for other possibilities.

(b) Karr's original scoring criteria or commonly used substitutes. These may require refinement in other ecoregions.

Figure 7. Data summary sheet for Fish Bioassessment II.

Waterbody Name _____ Location _____

Reach/Milepoint _____ Latitude/Longitude _____

County _____ State _____ Aquatic Ecoregion _____

Station Number _____ Investigators _____

Date _____ Time _____ Agency _____

Hydrologic Unit Code _____ Form Completed by _____

Reason for Survey _____

_____

_____

_____

Figure 8. Header information used for documentation and identification for sampling stations.

8.10 Guidance for Impairment Assessment Sheet (Figure 4)

8.10.1 Detection of Impairment: Circle the one that applies.

8.10.2 Biological Impairment Indicator: Circle those that apply, as indicated by the benthos, fish, and other aquatic biota.

8.10.3 Brief Description of Problem: Briefly explain the biological nature of the problem, based on field investigation and sampling. List the year and date of previous biological data and reports, and where the information can be found (state file, BIOS).

8.10.4 Cause: Circle those that apply. Indicate which problem appears to be the major cause of the stream impairment.

8.10.5 Estimated Areal Extent of Problem: Record estimated downstream extent of impact (in m) and multiply by approximate stream width (in m) to estimate areal width.

8.10.6 Suspected Source(s) of Problem: Check those that are suspected. Briefly explain why you suspect a specific source, and reference other surveys or studies done to document the problem and its source. Give title of applicable report, author(s) and year published or completed. Use back of sheet if necessary.

8.11 Guidance for Field Collection Data Sheet for Fish Bioassessment II (Figure 5)

8.11.1 Drainage: Give name of stream or river and its basin site descriptor, and unique site code.

8.11.2 Date: Enter day, month, and year of collection.

8.11.3 Sampling Duration: Record length of time in minutes actually collecting fish. If replicates are taken, record them separately.

8.11.4 Sampling Distance: Measure, with a tape or calibrated range finder, the length in meters of reach sampled.

8.11.5 Sampling Area: Multiply the length or reach sampled by the average width sampled. Express in meters squared.

8.11.6 Crew: Indicate crew chief and crew members.

8.11.7 Habitat Complexity/Quality: Circle the descriptor that best describes subjective evaluation of the physicochemical habitat.

8.11.8 Weather: Record air temperature, estimated wind velocity, percent cloud cover, and precipitation.

8.11.9 Flow: Circle most appropriate descriptor.

8.11.10 Information on Gear Used: Specify type, model, and number of electrofisher, or the mesh size and length of seine, or concentration of fish toxicant.

8.11.11 Gear/Crew Performance: Indicate effectiveness of crew in sampling the site. Note problems with equipment, staff, or site obstacles, such as extensive cover, high velocity current, excessive turbidity, floating debris, deep muck or pools, or weather conditions. Electrofishing should be conducted only during normal water flow and clarity conditions. Abnormally turbid conditions are to be avoided as are elevated flow and current because these conditions affect sampling efficiency. Also, if weather conditions are bad (rain or high winds, lightning, etc.), electrofishing should be suspended immediately or at the discretion of field personnel (Ohio EPA, 1990c).

8.11.12 Comments: Record any additional qualitative site data: sketch map or take photographs, note the presence of springs, the evidence of fishing activity, and/or potential or current impacts, the weather conditions (such as evidence of recent high flows or unusually hot or cold weather immediately preceding the survey), the biota observed (insect hatches, potential vertebrate predators, the fish nesting and grazing sites, fish reproductive conditions, or the fish seen but not captured.

8.11.13 Fish (preserved): Indicate if specimens were preserved for permanent collection or further examination.

8.11.14 Number of Individuals; Number of Anomalies: Give total numbers of fish and anomalies for the sample.

8.11.15 Genus/Species: Enter scientific name or unique standard abbreviation for each species captured.

8.11.16 Adults (Number, Weight): Enter the number of adults of each species and their total weight in grams. Individual or batch weight, depending on the species' size and abundance. Species weight can also be determined by weighing a subsample of individuals (20-30 fish spanning the size range collected) and extrapolating for the total number of that species.

8.11.17 Juveniles (Number, Weight): Record the number of juveniles of each species and their total weight as above. Juveniles and adults are distinguished subjectively by coloration and size; the objective is to determine whether both age classes are present.

8.11.18 Anomalies (Number): Indicate the number of fish by individual or species, that are diseased, deformed, damaged, or heavily parasitized. These are determined through careful external examination by a field-trained fish biologist.

8.12 Guidance For Data Summary Sheet for Fish Bioassessment II (Figure 7)

8.12.1 Station Number: Indicate station number.

8.12.2 Station Location: Record brief description of sampling site relative to established landmarks (i.e., roads, bridges).

8.12.3 Metrics: List metrics used to conduct IBI calculations. Use either Karr's original metrics or a published (or well supported) substitute approach. Precede metric selection with analysis of reference site data or a high quality historical database from a representative, large river basin.

8.12.4 Scoring Criteria: List published scoring criteria or use substitutes where necessary. Analyze reference site data or historical data from a representative large river basin before selecting criteria.

8.12.5 Metric Value: Record metric values (number or percent) for the station. Metric values are obtained by comparing the collection data (Figure 5) with the tolerance and trophic guilds previously listed (Table 1). For taxonomic metrics the numbers of different species are added. the total number of individuals is recorded from the field collection data sheet. Proportional metrics are determined by adding the number of individuals in each category and dividing this total by the total number of individuals.

8.12.6 Metric Score: Score each metric by comparing the metric value for the station with the previously chosen scoring criteria. Marginal values can be given a plus or minus (see IBI score below).

8.12.7 Scorer: Enter the scorer's name.

8.12.8 IBI Score: The metric scores (and the pluses and minuses if used) are added to give the IBI score. Three pluses or three minuses may increase or decrease the IBI score by two points.

8.12.9 Comments: Metrics producing contrary results or suggestions for improvement are entered here.

8.13 Habitat Assessment and Physical/Chemical Parameters

8.13.1 An evaluation of habitat quality is critical to any assessment of ecological integrity. The habitat quality evaluation can be accomplished by characterizing selected physical/chemical parameters and by systematic habitat assessment. Through this approach, key parameters can be identified to provide a consistent assessment of habitat quality. This evaluation of habitat quality is relevant to all levels of rapid bioassessment.

8.13.2 Physical Characteristics and Water Quality

8.13.2.1 Both physical characteristics and water quality parameters are pertinent to characterization of the stream habitat. An example of the data sheet used to characterize the physical characteristics and water quality of a site is shown in Figure 9. The information requested includes measurements made routinely during biological surveys. This phase of the survey is broken into two sections: Physical Characterization and Water Quality (Figure 9). These subsections are discussed separately below.

PHYSICAL CHARACTERIZATION/WATER QUALITY
FIELD DATA SHEET

PHYSICAL CHARACTERIZATION

RIPARIAN ZONE/INSTREAM FEATURES

Predominant Surrounding Land Use:

Forest Field/Pasture Agricultural Residential Commercial Industrial Other _____

Local Watershed Erosion: None Moderate Heavy

Local Watershed NPS Pollution: No evidence Some Potential Sources Obvious Sources

Estimated Stream Width _____ m Estimated Stream Depth: Riffle _____ m Run _____ m Pool _____ m

High Water Mark _____ m Velocity _____ Dam Present: Yes ___ No ___ Channelized: Yes ___ No ___

Canopy Cover: Open Partly Open Partly Shaded Shaded

SEDIMENT/SUBSTRATE:

Sediment Odors: Normal Sewage Petroleum Chemical Anaerobic None Other _____

Sediment Oils: Absent Slight Moderate Profuse

Sediment Deposits: Sludge Sawdust Paper Fiber Sand Relict Shells Other _____

Are the undersides of stones which are not deeply embedded black? Yes No

Inorganic Substrate Components

Substrate Type	Diameter	Percent Composition in Sampling Area
Bedrock		
Boulder	>256-mm (10 in.)	
Cobble	64-256-mm (2.5-10 in.)	
Gravel	2-64-mm (0.1-2.5 in.)	
Sand	0.06-2.00-mm (gritty)	
Silt	.004-.06-mm	
Clay	<.004-mm (slick)	

Organic Substrate Components

Substrate Type	Characteristic	Percent Composition in Sampling Area
Detritus	Sticks, Wood, Coarse Plant Materials (CPOM)	
Muck-Mud	Black, Very Fine Organic (FPOM)	
Marl	Grey, Shell Fragments	

WATER QUALITY

Temperature _____ C Dissolved Oxygen _____ pH _____ Conductivity _____ Other _____

Instrument(s) Used _____

Stream Type: Coldwater Warmwater

Water Odors: Normal Sewage Petroleum Chemical None Other _____

Water Surface Oils: Slick Sheen Globs Flecks None

Turbidity: Clear Slightly Turbid Turbid Opaque Water Color _____

WEATHER CONDITIONS

PHOTOGRAPH NUMBER

OBSERVATIONS AND/OR SKETCH

Figure 9. Physical characterization/water quality field data sheet for use with Fish Bioassessment II.

8.13.2.2 Physical Characterization

8.13.2.2.1 Physical characterization parameters include estimations of general land use and physical stream characteristics such as width, depth, flow, and substrate. The evaluation begins with the riparian zone (stream bank and drainage area) and proceeds instream to sediment/substrate descriptions. Such information will provide insight as to what organisms may be present or are expected to be present, and the presence of stream impacts. The information requested in the Physical Characterization section of the Field Data Sheet (Figure 9) is briefly discussed below.

8.13.2.2.2 Predominant Surrounding land Use: Observe the prevalent land-use type in the vicinity (noting any other land uses in the area which, although not predominant, may potentially affect water quality).

8.13.2.2.3 Local Watershed Erosion--The existing or potential detachment of soil within the local watershed (the portion of the watershed that drains directly into the stream) and its movement into a stream is noted. Erosion can be rated through visual observation of the watershed and stream characteristics. (Note any turbidity observed during water quality assessment below.)

8.13.2.2.4 Local Watershed Nonpoint-Source Pollution--This item refers to problems and potential problems other then siltation. Nonpoint source pollution is defined as diffuse agricultural and urban runoff. Other compromising factors in a watershed that may affect water quality or impacts on the stream are feedlots, wetlands, septic systems, dams, and impoundments, and/or mine seepage.

8.13.2.2.5 Estimated Stream Width (m): Estimate the distance from shore to shore at a transect representative of the stream width in the area.

8.13.2.2.6 Estimated Stream Depth (m): riffle, run, and pool. Estimate the vertical distance from water surface to stream bottom at a representative depth at each of the three habitat types.

8.13.2.2.7 High Water Mark (m): Estimate the vertical distance from the stream bank to the peak overflow level, as indicated by debris hanging in bank or floodplain vegetation, and deposition of silt or soil. In instances where bank overflow is rare, a high water mark may not be evident.

8.13.2.2.8 Velocity: Record an estimate of stream velocity in a representative run area.

8.13.2.2.9 Dam Present: Indicate the presence or absence of a dam upstream or downstream of the sampling station. If a dam is present, include specific information relating to alteration of flow.

8.13.2.2.10 Channelized: Indicate whether or not the area around the sampling station is channelized.

8.13.2.2.11 Canopy Cover: Note the general proportion of open to shaded area which best describes the amount of cover at the sampling station.

8.13.2.2.12 Sediment Odors: Disturb sediment and note any odors described (or include any other odors not listed) which are associated with sediment in the area of the sampling station.

8.13.2.2.13 Sediment Oils: Note the term which best describes the relative amount of any sediment oils observed in the sampling area.

8.13.2.2.14 Sediment Deposits: Note those deposits described (or include any other deposit not listed) which are present in the sampling area. Also indicate whether or not the undersides of rocks which are not deeply embedded are black in color (which generally indicates low dissolved oxygen or anaerobic conditions).

8.13.2.2.15 Inorganic Substrate Components: Visually estimate the relative proportion of each of the seven substrate particle types listed that are present in the sampling area.

8.13.2.2.16 Organic Substrate Components: Indicate relative abundance of each of the three substrate types listed.

8.13.2.3 Water Quality

8.13.2.3.1 Information requested in this Subsection (Figure 9) is standard to many aquatic studies and allows for some comparison between sites. Additionally, conditions that may significantly affect aquatic biota are documented. It is important to document recent and current weather conditions because of the potential impact that weather may have on water quality. To complete this phase of the bioassessment, a photograph may be helpful in both identifying station location and documenting habitat conditions. Any observations or data not requested but deemed important by the field observer should be recorded. This section is identical for all protocols and the specific data requested are described below.

8.13.2.3.2 Temperature (^{o}C), Dissolved Oxygen, pH, Conductivity: Measure and record values for each of the water quality parameters indicated, using the appropriate calibrated water quality instrument(s). Note the type of instrument and unit number used.

8.13.2.3.3 Stream Type: Note the appropriate stream designation according to State water quality standards.

8.13.2.3.4 Water Odors: Note those odors described (or include any other odors not listed) that are associated with the water in the sampling area.

8.13.2.3.5 Water Surface Oils: Note the term that best describes the relative amount of any oils present on the water surface.

8.13.2.3.6 Turbidity: Note the term which, based upon visual observation, best describes the amount of material suspended in the water column.

8.13.3 Habitat Quality and Assessment

8.13.3.1 The habitat assessment matrices (Figures 10 and 11) are taken from Barbour and Stribling (1991). The habitat assessment matrix originally published by Plafkin et al (1989) was based on the Stream Classification Guidelines for Wisconsin developed by Ball (1982) and Methods of Evaluating Stream, Riparian, and Biotic Conditions developed by Platts et al. (1983). Also, see Subsection 8.16 for an example of a specific qualitative habitat evaluation index field sheet (Figure 12) constructed for use by Ohio EPA. Because this habitat assessment approach is intended to support biosurvey analysis, the various habitat parameters are weighted to emphasize the most biologically significant parameters. All parameters are evaluated for each station studied. The ratings are then totaled and compared to a reference to provide a final habitat ranking. Scores increase as habitat quality increases. To ensure consistency in the evaluation procedure, descriptions of the physical parameters and relative criteria are included in the rating form.

8.13.3.2 There is a great variability among streams; however, some generalizations concerning similarities among stream types can be made relative to gradient (Barbour and Stribling, 1991). Four generic stream categories using gradient for establishing the framework can be identified: montane, piedmont, valley/plains, and coastal plains. For these four categories, two sets of parameters for assessing habitat quality have been developed. For higher gradient streams there tends to be an increased prevalence of riffles and runs. The matrix for "riffle/run prevalence" was constructed (Barbour and Stribing, 1991) for use in montane and piedmont streams (Figure 10). That for "glide/pool prevalence" (Figure 11) is for use in valley/plains and coastal plains streams.

8.13.3.3 Reference conditions are used to normalize the assessment to the "best attainable" situation. This approach is critical to the assessment because stream characteristics will vary dramatically across different regions. Other habitat assessment approaches may be used; or a more rigorously quantitative approach to measuring the habitat parameters may be used. However, the importance of a holistic habitat assessment to enhance the interpretation of biological data cannot be overemphasized. A more detailed discussion of the relationship between habitat quality and biological condition is presented in Plafkin et al. (1989) and Barbour and Stribling (1991).

8.13.3.4 Habitat parameters (Tabel 3) pertinent to the assessment of habitat quality are separated into three principal categories: primary, secondary, and tertiary. Primary parameters are those that characterize the stream "microscale" habitat and have the greatest direct influence on the structure of the indigenous communities. The primary parameters, which include characterization of the bottom substrate and available cover, estimation of embeddedness, estimation of the flow or velocity and depth regime, and canopy cover have the widest score range (0-20) to reflect their contribution to habitat quality. The secondary parameters measure the "macroscale" habitat such as channel morphology characteristics. These parameters evaluate: channel alteration, bottom scouring and deposition, and pool/riffle, run/bend ratio, and lower bank channel capacity and have a range of 0-15. Tertiary

HABITAT ASSESSMENT FIELD DATA SHEET
RIFFLE/RUN PREVALENCE

Habitat Parameter	Category: Optimal	Sub-Optimal	Marginal	Poor
1. Bottom substrate/ instream cover	Greater than 50% mix of rubble, gravel, submerged logs, undercut banks, or other stable habitat. 16–20	30–50% mix of rubble, gravel, or other stable habitat. Adequate habitat. 11–15	10–30% mix of rubble, gravel, or other stable habitat. Habitat availability less than desirable. 6–10	Less than 10% rubble, gravel, or other stable habitat. Lack of habitat is obvious. 0–5
2. Embeddedness	Gravel, cobble, and boulder particles are between 0–25% surrounded by fine sediment. 16–20	Gravel, cobble, and boulder particles are between 25–50% surrounded by fine sediment. 11–15	Gravel, cobble, and boulder particles are between 50–75% surrounded by fine sediment. 6–10	Gravel, cobble, and boulder particles are over 75% surrounded by fine sediment. 0–5
3. ≤0.15 cms (5 cfs)→ Flow at rep. low	Cold >0.05 cms (2 cfs) Warm >0.15 cms (5 cfs) 16–20	0.03–0.05 cms (1–2 cfs) 0.05–0.15 cms (2–5 cfs) 11–15	0.01–0.03 cms (.5–1 cfs) 0.03–0.05 cms (1–cfs) 6–10	<0.01 cms (.5 cfs) <0.03 cms (1 cfs) 0–5
OR >0.15 cms (5 cfs)→ velocity/depth	Slow (<0.3 m/s), deep (>0.5 m); slow, shallow (<0.5 m); fast (>0.3 m/s), deep; fast, shallow habitats all present. 16–20	Only 3 of the 4 habitat categories present (missing riffles or runs receive lower score than missing pools). 11–15	Only 2 of the 4 habitat categories present (missing riffles or runs receive lower score). 6–10	Dominated by 1 velocity/depth category (usually pools). 0–5
4. Canopy cover (shading)	A mixture of conditions where some areas of water surface fully exposed to sunlight, and other receiving various degrees of filtered light. 16–20	Covered by sparse canopy; entire water surface receiving filtered light. 11–15	Completely covered by dense canopy; water surface completely shaded OR nearly full sunlight reaching water surface. Shading limited to <3 hours per day. 6–10	Lack of canopy, full sunlight reaching water surface. 0–5
5. Channel alteration	Little or no enlargement of islands or point bars, and/or no channelization. 12–15	Some new increase in bar formation, mostly from coarse gravel; and/ or some channelization present. 8–11	Moderate deposition of new gravel, coarse sand on old and new bars; and or embankments on both banks. 4–7	Heavy deposits of fine material, increased bar development; and/or extensive channelization. 0–3
6. Bottom scouring and deposition	Less than 5% of the bottom affected by scouring and/or deposition. 12–15	5–30% affected. Scour at constrictions and where grades steepen. Some deposition in pools. 8–11	30–50% affected. Deposits and/or scour at obstructions, constrictions, and bends. Filling of pools prevalent. 4–7	More than 50% of the bottom changing frequently. Pools almost absent due to deposition. Only large rocks in riffle exposed. 0–3
7. Pool/riffle, run/bend ratio (distance between riffles divided by stream width)	Ratio: 5–7. Variety of habitat. Repeat pattern of sequence relatively frequent. 12–15	7–15. Infrequent repeat pattern. Variety of macrohabitat less than optimal. 8–11	15–25. Occasional riffle or bend. Bottom contours provide some habitat. 4–7	>25. Essentially a straight stream. Generally all flat water or shallow riffle. Poor habitat. 0–3
8. Lower bank channel capacity	Overbank (lower) flows rare. Lower bank W/D ratio <7. (Channel width divided by depth or height of lower bank.) 12–15	Overbank (lower) flows occasional. W/D ratio 8–15. 8–11	Overbank (lower) flows common. W/D ratio 15–25. 4–7	Peak flows not contained or contained through channelization. W/D ratio >25. 0–3
9. Upper bank stability	Upper bank stable. No evidence of erosion or bank failure. Side slopes generally <30°. Little potential for future problems. 9–10	Moderately stable. Infrequent, small areas of erosion mostly healed over. Side slopes up to 40° on one bank. Slight potential in extreme floods. 6–8	Moderately unstable. Moderate frequency and size of erosional areas. Side slopes up to 60° on some banks. High erosion potential during extreme high flow. 3–5	Unstable. Many eroded areas. "Raw" areas frequent along straight sections and bends. Side slopes >60° common. 0–2
10. Bank vegetative protection	Over 90% of the streambank surfaces covered by vegetation. 9–10	70–89% of the streambank surfaces covered by vegetation. 6–8	50–79% of the streambank surfaces covered by vegetation. 3–5	Less than 50% of the streambank surfaces covered by vegetation. 0–2
OR Grazing or other disruptive pressure	Vegetative disruption minimal or not evident. Almost all potential plant biomass at present stage of development remains. 9–10	Disruption evident but not affecting community vigor. Vegetative use is moderate, and at least one-half of the potential plant biomass remains. 6–8	Disruption obvious; some patches of bare soil or closely cropped vegetation present. Less than one-half of the potential plant biomass remains. 3–5	Disruption of streambank vegetation is very high. Vegetation has been removed to 2 inches or less in average stubble height. 0–2
11. Streamside cover	Dominant vegetation is shrub. 9–10	Dominant vegetation is of tree form. 6–8	Dominant vegetation is grass or forbes. 3–5	Over 50% of the streambank has no vegetation and dominant material is soil, rock, bridge materials, culverts, or mine tailings. 0–2
12. Riparian vegetative zone width (least buffered side)	>18 meters. 9–10	Between 12 and 18 meters. 6–8	Between 6 and 12 meters. 3–5	<6 meters. 0–2
Column Totals _____ Score _____	____	____	____	____

Figure 10. Habitat assessment field data sheet, riffle/run prevalence. From Barbour and Stribling (1991).

HABITAT ASSESSMENT FIELD DATA SHEET
GLIDE/POOL PREVALENCE

Habitat Parameter	Category: Optimal	Sub-Optimal	Marginal	Poor
1. Bottom substrate/ instream cover	Greater than 50% mix of rubble, gravel, submerged logs, undercut banks, or other stable habitat. 16–20	30–50% mix of rubble, gravel, or other stable habitat. Adequate habitat. 11–15	10–30% mix of rubble, gravel, or other stable habitat. Habitat availability less than desirable. 6–10	Less than 10% rubble, gravel, or other stable habitat. Lack of habitat is obvious. 0–5
2. Pool substrate characterization	Mixture of substrate materials with gravel and firm sand prevalent; root mats and submerged vegetation common. 16–20	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present. 11–15	All mud or clay or channelized with sand bottom; little or no root mat, no submerged vegetation. 6–10	Hard-pan clay or bedrock; no root mat or vegetation. 0–5
3. Pool variability	Even mix of deep/ shallow/large/small pools present. 16–20	Majority of pools large and deep; very few shallow. 11–15	Shallow pools much more prevalent than deep pools. 6–10	Majority of pools small and shallow or pools absent. 0–5
4. Canopy cover (shading)	A mixture of conditions where some areas of water surface fully exposed to sunlight, and other receiving various degrees of filtered light. 16–20	Covered by sparse canopy; entire water surface receiving filtered light. 11–15	Completely covered by dense canopy; water surface completely shaded OR nearly full sunlight reaching water surface. Shading limited to <3 hours per day. 6–10	Lack of canopy, full sunlight reaching water surface. 0–5
5. Channel alteration	Little or no enlargement of islands or point bars, and/or no channelization. 12–15	Some new increase in bar formation, mostly from coarse gravel; and/ or some channelization present. 8–11	Moderate deposition of new gravel, coarse sand on old and new bars; and/or embankments on both banks. 4–7	Heavy deposits of fine material, increased bar development; and/or extensive channelization. 0–3
6. Deposition	Less than 5% of bottom affected; minor accumulation of coarse sand and pebbles at snags and submerged vegetation. 12–15	5–30% affected; moderate accumulation of sand at snags and submerged vegetation. 8–11	5–30% affected; major deposition of sand at snags and submerged vegetation; pools shallow, heavily silted. 4–7	Channelized; mud, silt, and/or sand in braided or nonbraided channels; pools almost absent due to deposition. 0–3
7. Channel sinuosity	Instream channel length 3 to 4 times straight line distance. 12–15	Instream channel length 2 to 3 times straight line distance. 8–11	Instream channel length 1 to 2 times straight line distance. 4–7	Channel straight; channelized waterway. 0–3
8. Lower bank channel capacity	Overbank (lower) flows rare. Lower bank W/D ratio <7. 12–15	Overbank (lower) flows occasional. W/D ratio 8–15. 8–11	Overbank (lower) flows common. W/D ratio 15–25. 4–7	Peak flows not contained or contained through channelization. W/D ratio >25. 0–3
9. Upper bank stability	Upper bank stable. No evidence of erosion or bank failure. Side slopes generally <30°. Little potential for future problems. 9–10	Moderately stable. Infrequent, small areas of erosion mostly healed over. Side slopes up to 40° on one bank. Slight potential in extreme floods. 6–8	Moderately unstable. Moderate frequency and size of erosional areas. Side slopes up to 60° on some banks. High erosion potential during extreme high flow. 3–5	Unstable. Many eroded areas. "Raw" areas frequent along straight sections and bends. Side slopes >60° common. 0–2
10. Bank vegetative protection	Over 90% of the streambank surfaces covered by vegetation. 9–10	70–89% of the streambank surfaces covered by vegetation. 6–8	50–79% of the streambank surfaces covered by vegetation. 3–5	Less than 50% of the streambank surfaces covered by vegetation. 0–2
OR Grazing or other disruptive pressure	Vegetative disruption minimal or not evident. Almost all potential plant biomass at present stage of development remains. 9–10	Disruption evident but not affecting community vigor. Vegetative use is moderate, and at least one-half of the potential plant biomass remains. 6–8	Disruption obvious; some patches of bare soil or closely cropped vegetation present. Less than one-half of the potential plant biomass remains. 3–5	Disruption of streambank vegetation is very high. Vegetation has been removed to 2 inches or less in average stubble height. 0–2
11. Streamside cover	Dominant vegetation is shrub. 9–10	Dominant vegetation is of tree form. 6–8	Dominant vegetation is grass or forbes. 3–5	Over 50% of the streambank has no vegetation and dominant material is soil, rock, bridge materials, culverts, or mine tailings. 0–2
12. Riparian vegetative zone width (least buffered side)	>18 meters. 9–10	Between 12 and 18 meters. 6–8	Between 6 and 12 meters. 3–5	<6 meters. 0–2
Column Totals	___	___	___	___

_____ Score _____

Figure 11. Habitat assessment field data sheet, glide/pool prevalence. From Barbour and Stribling (1991).

QHEI Score:

Stream_____ RM_____ Date_____ River Code_____

Location_____ Scorers Name:_____

1] SUBSTRATE (Check *ONLY* Two Substrate *TYPE BOXES; Estimate % or note every type present*);

TYPE	POOL	RIFFLE		POOL	RIFFLE
☐ ☐-BLDR /SLABS[10]	____	____	☐ ☐-GRAVEL [7]	____	____
☐ ☐-BOULDER [9]	____	____	☐ ☐-SAND [6]	____	____
☐ ☐-COBBLE [8]	____	____	☐ ☐-BEDROCK[5]	____	____
☐ ☐-HARDPAN [4]	____	____	☐ ☐-DETRITUS[3]	____	____
☐ ☐-MUCK [2]	____	____	☐ ☐-ARTIFICIAL[0]	____	____
☐ ☐-SILT [2]	____	____			

NOTE: (Ignore sludge that originates from point-sources; score on natural substrates)

NUMBER OF SUBSTRATE TYPES: ☐-5 or More [2] ☐-4 or Less [0]

COMMENTS_____

SUBSTRATE ORIGIN
Check ONE (OR 2 & AVERAGE)
☐ -LIMESTONE [1]
☐ -TILLS [1]
☐ -WETLANDS[0]
☐ -HARDPAN [0]
☐ -SANDSTONE [0]
☐ -RIP/RAP [0]
☐ -LACUSTRINE [0]
☐ -SHALE [-1]
☐-COAL FINES [-2]

SUBSTRATE QUALITY
Check ONE (OR 2 & AVERAGE)

SILT:
☐ -SILT HEAVY [-2]
☐ -SILT MODERATE [-1]
☐ -SILT NORMAL [0]
☐ -SILT FREE [1]

EMBEDDEDNESS:
☐ -EXTENSIVE [-2]
☐ -MODERATE [-1]
☐ -NORMAL [0]
☐ -NONE [1]

Substrate ☐ Max 20

2] INSTREAM COVER

TYPE: (Check *All* That Apply)

☐ -UNDERCUT BANKS [1]
☐ -OVERHANGING VEGETATION [1]
☐ -SHALLOWS (IN SLOW WATER) [1]
☐ -ROOTMATS [1]
☐ -DEEP POOLS> 70 cm [2]
☐ -ROOTWADS [1]
☐ -BOULDERS [1]
☐ -OXBOWS [1]
☐ -AQUATIC MACROPHYTES [1]
☐ -LOGS OR WOODY DEBRIS [1]

COMMENTS:_____

AMOUNT: (Check *ONLY* One or check 2 and *AVERAGE*)
☐ - EXTENSIVE > 75% [11]
☐ - MODERATE 25-75% [7]
☐ - SPARSE 5-25% [3]
☐ - NEARLY ABSENT < 5%[1]

Cover ☐ Max 20

3] CHANNEL MORPHOLOGY: (Check *ONLY* One PER Category OR check 2 and *AVERAGE*)

SINUOSITY	DEVELOPMENT	CHANNELIZATION	STABILITY	MODIFICATIONS/OTHER
☐ - HIGH [4]	☐ - EXCELLENT [7]	☐ - NONE [6]	☐ - HIGH [3]	☐ - SNAGGING ☐ - IMPOUND.
☐ - MODERATE [3]	☐ - GOOD [5]	☐ - RECOVERED [4]	☐ - MODERATE [2]	☐ - RELOCATION ☐ - ISLANDS
☐ - LOW [2]	☐ - FAIR [3]	☐ - RECOVERING [3]	☐ - LOW [1]	☐ - CANOPY REMOVAL ☐ - LEVEED
☐ - NONE [1]	☐ - POOR [1]	☐ - RECENT OR NO RECOVERY [1]		☐ - DREDGING ☐ - BANK SHAPING
				☐ - ONE SIDE CHANNEL MODIFICATIONS

Channel ☐ Max 20

COMMENTS:_____

4]. RIPARIAN ZONE AND BANK EROSION - (check ONE box per bank or check 2 and AVERAGE per bank) ★River Right Looking Downstream★

RIPARIAN WIDTH
L R (Per Bank)
☐ ☐ -WIDE > 50m [4]
☐ ☐ - MODERATE 10-50m [3]
☐ ☐ -NARROW 5-10 m [2]
☐ ☐ - VERY NARROW <5 m[1]
☐ ☐ - NONE [0]

FLOOD PLAIN QUALITY (*PAST 100 FOOT RIPARIAN*)
L R (Most Predominant Per Bank)
☐ ☐-FOREST, SWAMP [3]
☐ ☐-SHRUB OR OLD FIELD [2]
☐ ☐-RESIDENTIAL,PARK,NEW FIELD [1]
☐ ☐ -FENCED PASTURE [1]
☐ ☐-CONSERVATION TILLAGE [1]
☐ ☐ -URBAN OR INDUSTRIAL [0]
☐ ☐ -OPEN PASTURE,ROWCROP [0]
☐ ☐ -MINING/CONSTRUCTION [0]

BANK EROSION
L R (Per Bank)
☐ ☐ -NONE/LITTLE [3]
☐ ☐ -MODERATE [2]
☐ ☐ -HEAVY/SEVERE[1]

Riparian ☐ Max 10

COMMENTS:_____

5.]POOL/GLIDE AND RIFFLE/RUN QUALITY

MAX. DEPTH
(Check 1 ONLY!)
☐ - >1m [6]
☐ - 0.7-1m [4]
☐ - 0.4-0.7m [2]
☐ - 0.2- 0.4m [1]
☐ - < 0.2m [POOL=0]

MORPHOLOGY
(Check 1 or 2 & AVERAGE)
☐'-POOL WIDTH > RIFFLE WIDTH [2]
☐ -POOL WIDTH = RIFFLE WIDTH [1]
☐'-POOL WIDTH < RIFFLE W. [0]

CURRENT VELOCITY [POOL & RIFFLES!]
(Check *All* That Apply)
☐'-EDDIES[1]
☐'-FAST[1]
☐'-MODERATE [1]
☐'-SLOW [1]
☐'-TORRENTIAL[-1]
☐'-INTERSTITIAL[-1]
☐'-INTERMITTENT[-2]

Pool/Glide ☐ Max 12

COMMENTS:_____

CHECK ONE OR CHECK 2 AND AVERAGE

RIFFLE /RUN DEPTH
☐ - GENERALLY >10 cm;MAX > 50 [4]
☐ - GENERALLY >10 cm; MAX < 50[3]
☐ - GENERALLY 5-10 cm[1]
☐ - GENERALLY < 5 cm [RIFFLE=0]

RIFFLE/RUN SUBSTRATE
☐-STABLE (e.g.,Cobble, Boulder) [2]
☐-MOD. STABLE (e.g.,Large Gravel) [1]
☐-UNSTABLE (Fine Gravel,Sand) [0]

RIFFLE/RUN EMBEDDEDNESS.
☐ - NONE [2]
☐ - LOW [1]
☐ - MODERATE [0]
☐ - EXTENSIVE [-1]
☐ - NO RIFFLE [Metric=0]

Riffle/Run ☐ Max 8

COMMENTS:_____

6] GRADIENT (ft/mi): _____ DRAINAGE AREA (sq.mi.):_____ %POOL: ____ %GLIDE: ____ %RIFFLE: ____ %RUN: ____

Gradient ☐ Max 10

Figure 12. Example of Ohio EPA (1991) qualitative habitat evaluation index field sheet.

Is Sampling Reach Representative of Stream? (Y/N)____ If Not, Explain:_____

Additional Comments/Pollution Impacts: _____

	Gear	Distance	Water Clarity	Water Stage
First Sampling Pass:	_____	_____	_____	_____
Second Sampling Pass:	_____	_____	_____	_____
Third Sampling Pass:	_____	_____	_____	_____

Subjective Rating (1-10) Aesthetic Rating (1-10)

CANOPY (% OPEN) _____ GRADIENT: ☐-LOW ☐-MODERATE ☐-HIGH PHOTOS: _____

STREAM MEASUREMENTS: AVERAGE WIDTH: _____ AVERAGE DEPTH: _____ MAXIMUM DEPTH: _____

DRAWING OF STREAM:

FLOW⇨

Figure 12. Example of Ohio EPA (1991) qualitative habitat evaluation index field sheet (continued).

parameters evaluate riparian and bank structure and comprise four parameters: upper bank stability, bank vegetative stability, streamside cover, and width of riparian vegetative zone. These tertiary parameters are most often ignored in biosurveys. The tertiary parameters have a score range of 0-10.

8.13.3.5 Habitat evaluations (Table 3) are first made on instream habitat, followed by channel morphology, and finally on structural features of the bank and riparian vegetation. Stream segment length or area assessed will vary with each site. Generally, primary parameters are evaluated within the first riffle/pool sequence, or the immediate sampling area such as in the case of fish sampling. Secondary and tertiary parameters are evaluated over a larger stream area, primarily in an upstream direction where conditions will have the greater impact on the community being studied. The actual habitat assessment process involves rating each of the nine parameters as either: excellent, good, fair, or poor based on the criteria included on the Habitat Assessment Field Data Sheet (Figures 10 and 11).

8.13.3.6 A total habitat score is obtained for each biological station and compared to a site-specific control or regional reference station. The ratio between the score for the station of interest and the score for the control or regional reference provides a percent comparability measure for each station Table 3). The station is then classified on the basis of its similarity to expected conditions (as represented by the control or reference station), and its inferred potential to support an acceptable level of biological comminity health.

8.13.3.7 The use of a percent comparability evaluation (Table 3) allows for regional and stream-size differences which affect flow or velocity, substrate, and channel morphology. Some regions are characterized by streams having a lower channel gradient. Such streams are typically shallower, have a greater pool/riffle or run/bend ratio, and less stable substrate than streams with a steep channel gradient. Although some low gradient streams do not provide the diversity of habitat or fauna afforded by steeper gradient streams, they are characteristic of certain regions. Use of the matrix presented as Figure 14 can allow more direct evaluation of low gradient streams relative to regional expectations.

8.13.3.8 Listed below is a general explanation for each of the twelve habitat parameters to be evaluated for riffle/run prevalent streams (higher gradient, Figure 10).

8.13.3.9 Primary Parameters-Substrate and Instream Cover

8.13.3.9.1 The primary instream habitat characteristics directly pertinent to the support of aquatic communities consist of substrate type and stability, availability of refugia, and migration/passage potential. These primary habitat parameters are weighted with the highest weighting reflective of their degree of importance to the biological communities.

1. Bottom Substrate/Instream Cover--This refers to the availability of habitat for support ofaquatic organisms. A variety of substrate materials and habitat types is desirable. The presence of rock and gravel in flowing

TABLE 3. NINE HABITAT PARAMETERS AND ASSESSMENT CATEGORY

Condition/Parameter	Condition			
	Excellent	Good	Fair	Poor
PRIMARY-SUBSTRATE AND INSTREAM COVER				
1. Bottom substrate/instream cover	16-20	11-15	6-10	0-5
2. Embeddedness	16-20	11-15	6-10	0-5
3. Flow/velocity/depth	16-20	11-15	6-10	0-5
4. Canopy cover (shading)	16-20	11-15	6-10	0-5
SECONDARY-CHANNEL MORPHOLOGY				
5. Channel alteration	12-15	8-11	4-7	0-3
6. Bottom scouring and deposition	12-15	8-11	4-7	0-3
7. Pool/riffle, run/bend ratio	12-15	8-11	4-7	0-3
8. Lower bank channel capacity	12-15	8-11	4-7	0-3
TERTIARY-RIPARIAN AND BANK STRUCTURE				
9. Upper Bank stability	9-10	6-8	3-5	0-2
10. Bank vegetative stability (grazing/ disruptive pressure)	9-10	6-8	3-5	0-2
11. Streamside cover	9-10	6-8	3-5	0-2
12. Riparian vegetative zone width	9-10	6-8	3-5	0-2

Assessment Category	Percent of Comparability
Comparable to Reference	≥90%
Supporting	75-89%
Partially Supporting	60-74%
Non-Supporting	≤59%

streams is generally considered the most desirable habitat. However, other forms of habitat may provide the niches required for community support. For example, logs, tree roots, submerged or emergent vegetation, undercut banks, etc., will provide excellent habitat for a variety of organisms, particularly fish. Bottom substrate is evaluated and rated by observation.

2. Embeddedness--The degree to which boulders, rubble, or gravel are surrounded by fine sediment indicates suitability of the stream substrate as habitat for benthic macroinvertebrates and for fish spawning and egg incubation. Embeddedness is evaluated by visual observation of the degree to which larger particles are surrounded by sediment. In some western areas of the United States, embeddedness is regarded as the stability of cobble substrate by measuring the depth of burial of large particles (cobble, boulders).

3. Stream Flow and/or Stream Velocity--Stream flow relates to the ability of a stream to provide and maintain a stable aquatic environment. Stream flow (water quantity and gradient) is most critical to the support of aquatic communities when the representative low flow is $\leq$0.15 cms (5 cfs). In these small streams, flow should be estimated in a straight stretch of run area where banks are parallel and bottom contour is relatively flat. Even where a few stations may have flows in excess of 0.15 cms, flow may still be the predominate constraint. Therefore, the evaluation is based on flow rather than velocity.

4. Canopy Cover (Shading)--Shading, as provided by canopy cover, is important for the control of water temperature, its effect on biological processes in general, and as a factor in photosynthetic activity and primary production. A diversity of shade conditions is considered optimal, that is, with some areas of the sampling station receiving direct sunlight, others, complete shade, and other, filtered light.

8.13.3.10 In larger streams and rivers (> 0. 15 cms), velocity, in conjunction with depth, has a more direct influence than flow on the structure of benthic communities (Osborne and Hendricks, 1983) and fish communities (Oswood and Barber, 1982). The quality of the aquatic habitat can, therefore, be evaluated in terms of a velocity, and depth relationship. As patterned after Oswood and Barber (1982), four general categories of velocity and depth are optimal for benthic and fish communities: (1) slow (<0.3 m/s), shallow (<0.5 m); (2) slow (<0.3 m/s), deep (>0.5 m); (3) fast (>0.3 m/s), deep (>0.5 m); and (4) fast (>0.3 m/s), shallow (<0.5 m). Habitat quality is reduced in the absence of one or more of these four categories.

8.13.3.11 Secondary Parameters-Channel Morphology

8.13.3.11.1 Channel morphology is determined by the flow regime of the stream, local geology, land surface form, soil, and human activities (Platts et al. 1983). The sediment movement along the channel, as influenced by the tractive forces of flowing water and the sinuosity of the channel, also affects habitat conditions.

5. Channel Alteration--The character of sediment deposit from upstream is an indication of the severity of watershed and bank erosion and stability of the stream system. The growth or appearance of sediment bars tends to increase in depth and length with continued watershed disturbance. Channel alteration also results in deposition, which may occur on the inside of bends, below channel constrictions, and where stream gradient flattens out. Channelization (e.g., straightening, construction of concrete embankments) decreases stream sinuosity, thereby increasing stream velocity and the potential for scouring.

6. Bottom Scouring and Deposition--These parameters relate to the destruction of instream habitat resulting from the problems described above. Characteristics to observe are scoured substrate and degree of siltation in pools and riffles. Scouring result from high velocity flows. The potential for scouring is increased by channelization. Deposition and scouring result from the transport of sediment or other particulates and may be an indication of large scale watershed erosion. Deposition and scouring is rated by estimating the percentage of an evaluated reach that is scoured or silted (i.e., 50-ft silted in a 100-ft stream length equals 50 percent).

7. Pool/Riffle, Run/Bend Ratio--These parameters assume that a stream with riffles or bends provides more diverse habitat than a straight (run) or uniform depth stream. Bends are included because low gradient streams may not have riffle areas, but excellent habitat can be provided by the cutting action of water at bends. The ratio is calculated by dividing the average distance between riffles or bends by the average stream width. If a stream contains riffles and bends, the dominant feature with the best habitat should be used.

8. Lower bank channel capacity--This parameter is designed to allow evaluation of the ability of a stream channel to contain normal peak flows. since the lower bank is that over which water initially escapes, it is the focus of this individual parameter.

8.13.3.12 Tertiary Parameters-Riparian and Bank Structure

8.13.3.12.1 Well-vegetated banks are usually stable regardless of bank undercutting; undercutting actually provides excellent cover for fish (Platts et al., 1983). The ability of vegetation and other materials on the streambanks to prevent or inhibit erosion is an important determinant of the stability of the stream channel and instream habitat for indigenous organisms. Because riparian and bank structure indirectly affect the instream habitat features, they are weighted less than the primary or secondary parameters.

8.13.3.12.2 Tertiary parameters are evaluated by observation of both upper and lower bank characteristics. The upper bank is the land area from the break in the general slope of the surrounding land to the normal high water line. The upper bank is normally vegetated and covered by water only during extreme high water conditions. Land forms vary from wide, flat floodplains to narrow, steep slopes. The lower bank is the intermittently submerged portion

of the stream cross section from the normal high water line to the lower water line. The lower channel defines the stream width.

9. Upper Bank Stability--Bank stability is rated by observing existing or potential detachment of soil from the upper and lower stream bank and its potential movement into the stream. Steeper banks are generally more susceptible to erosion and failure, and may not support stable vegetation. Streams with poor banks will often have poor instream habitat. Adjustments should be made in areas with clay banks where steep, bare areas may not be as susceptible to erosion as other soil types.

10. Bank Vegetative Stability (Grazing/Disruptive Pressure)--Vegetative stability is evaluated here as it relates to reduction of erosion and biological contribution to the aquatic ecosystem. Bank soil is generally held in place by plant root systems. Erosional protection may also be provided by boulder, cobble, or gravel material. Areas of higher vegetative coverage receive higher ratings (Ball, 1982; Platts et al., 1983). An estimate of the density of bank vegetation (or proportion of boulder, cobble, or gravel material) covering the bank provides an indication of bank stability and potential instream sedimentation. Vegetative stability is best rated in areas of little riparian zone disturbance. Areas exposed to grazing pressures or other disruption should be evaluated under the second set of conditions. Grazing or other disruptive pressure is evaluated in terms of the potential plant biomass at the site in any given season.

11. Streamside Cover--Streamside cover vegetation is evaluated in terms of provision of stream-shading; and escape cover or refuge for fish. A rating is obtained by visually determining the dominant vegetation type covering the exposed stream bottom, bank, and top of bank. Platts (1974) found that streamside cover consisting primarily of shrub had a higher fish standing crop than similar-size streams having tree or grass streamside cover. Riparian vegetation dominated by shrubs and trees provides the course particulate organic matter (CPOM) source in allochthonous systems.

12. Riparian Vegetative Zone Width (Least Buffered Side)--The riparian buffer zone is rated by its width on the side with the nearest disturbance or human influence. Increasing buffer zone width is positively correlated with shade. Vegetated buffer zones are also effective in removal of particulate pollutants from storm runoff, can reduce runoff velocity and volume, and can aid in the recharging of groundwater.

8.13.3.12 The matrix constructed for lower gradient streams likely to be encountered is coastal plains and prairie regions (Figure 11; Barbour and Stribing, 1991) differs from Figure 10 by two parameters. The following two parameters (numbers 2 and 3) have been added to emphasize the increased importance of pools as habitat in these streams.

2. Pool Substrate Characterization--diversity and variability in substrate

particle size are rated higher than uniform particle sizes in pool substrates.

3. Pool Variability--This parameter rates the mixture of pool sizes within a stream reach. Variability in pool sizes will support a healthy fisheries and a more diverse benthic macroinvertebrate assemblage.

8.13.3.13 Additional Habitat Assessment Considerations

8.13.3.13.1 Two additional variables are important and should be considered by the investigator: (1) seasonal aspects of habitat evaluation; and (2) the length of the stream reach to be evaluated for habitat quality. To properly address both of these considerations, the major objective of the habitat assessment should be identified. If the habitat assessment is being conducted in relation to the biological collections, all field assessments and collections should be performed concurrently, and the sampling domain (site boundaries) should be critically established. On the other hand, if the purpose of the habitat assessment is to characterize or classify a stream or watershed, a different sampling regime or criterion might be established.

8.13.13.2 With regard to seasonality, it is important to understand that the habitat quality may change depending on the time of the assessment. However, the primary habitat parameters amy change most dramatically, having the greatest influence on the communities under study. This particular habitat assessment approach is designed as a tool for evaluating the potential biological condition of the communities. With this in mind, the actual sampling site where the resident communities are being collected is of central importance in the habitat evaluation. The sampling site should be evaluated for the primary habitat parameters.

8.13.13.3 The stream reach upstream of the site should be included in the evaluation of the secondary and tertiary parameters. The actual delineation of the length of the reach will depend on the objectives of the study. For nonpoint source assessment, the reach may be much as a half mile; for point source evaluations, the reach may be only a few hundred yards. In the assessment of the fish community, a downstream reach amy be incorporated onto the habitat evaluation for the primary and secondary parameters.

8.14 Selected References for Determining Fish Tolerance, Trophic, Reproductive, and Origin Classifications (Also, See Section 12, Fisheries Bibliography)

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8.15 Agencies Currently Using or Evaluating Use of the IBI and Iwb for Water Quality Investigations

1. Alabama Geological Survey

2. Illinois Environmental Protection Agency

3. Iowa Conservation Commission

4. Kansas Department of Wildlife and Parks

5. Kansas Department of Health and Environment

6. Kentucky Cabinet for Natural Resources and Environmental Protection

7. Nebraska Department of Environmental Control

8. North Carolina Division of Environmental Management

9. Ohio Environmental Protection Agency

10. Oklahoma State Department of Health

11. Tennessee Valley Authority

12. U.S. EPA Region I

13. U.S. EPA Region II

14. U.S. EPA Region V

15. Vermont Department of Environmental Conservation

16. Wisconsin Department of Natural Resources

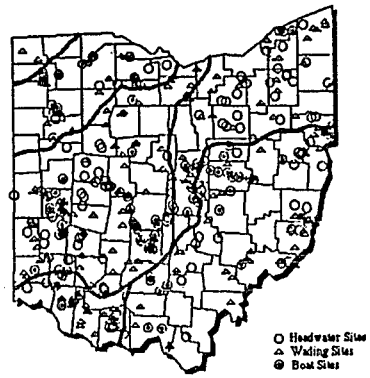
17. Indiana Department of Environmental Management

18. Arizona Department of Game and Fish

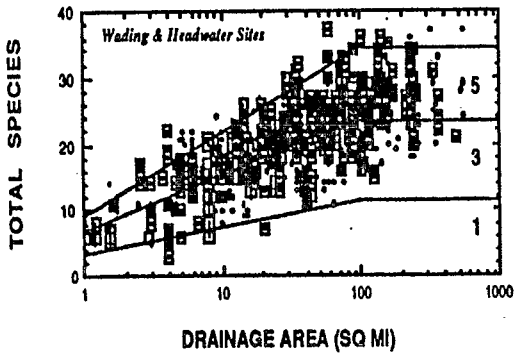
8.16 Ohio EPA Fish Index of Biotic Integrity (IBI), Modified Index of Well-Being (Iwb), and Qualitative Habitat Evaluation Index (QHEI)

8.16.1 The principal methods for determining the overall fish community health and well-being used by the Ohio EPA are the Index of Well-Being (Iwb) developed by Gammon (1976), and modified by Ohio EPA (see Ohio EPA, 1987b, 1991), the Index of Biotic Integrity (IBI) developed by Karr (1981), and the qualitative habitat evaluation index (QHEI) developed by Rankin (1989). The Iwb is based on structural attributes of the fish community, and the IBI incorporates functional characteristics. The fish technique used by Ohio EPA to obtain fish relative abundance and distribution data is pulsed direct current (D.C.) electrofishing. Depending on the type of habitat sampled, six sampling methods currently being used are: (1) boat-mounted electrofishing - straight electrode array (2) boat-mounted electrofishing - circular electrode array, (3) boat longline - riffle method; (4) Sportyak generator unit (5) longline generator unit, and (6) Backpack electrofishing - battery unit. Fish data collected with these devices are used for the purpose of calculating the Index of Biotic Integrity (IBI) and Modified Index of Well-Being (Iwb) scores from which aquatic life use attainment and water quality are determined. Figure 13 is a flowchart of the biosurvey approach for fish bioassessment used

I. Reference Sites - Select & Sample



II. Calibrate multi-metric indices (IBI, ICI)

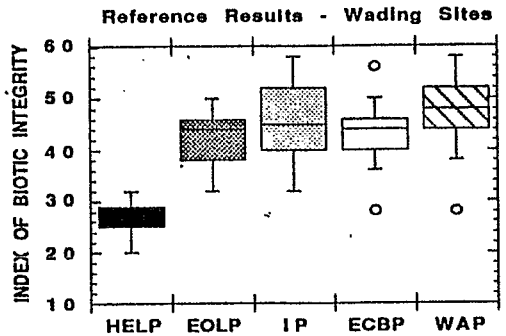


III. Fully calibrated index - differentiate site types for fish; statewide for invertebrates.

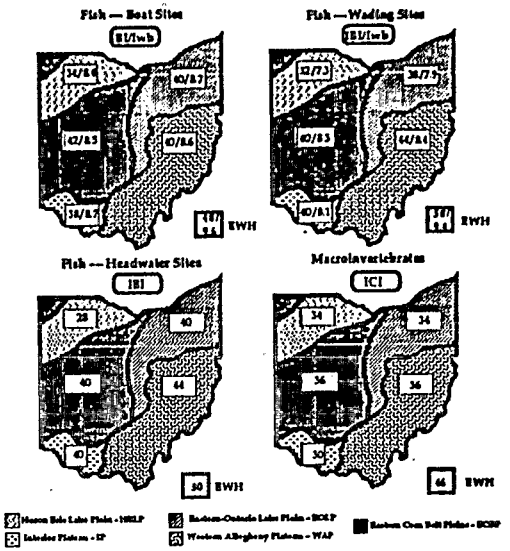
IBI - calibrated for use in Ohio for Wading Sites.

Category	IBI Metric	Metric Score 5	3	1
	# of Species	Varies with drainage area		
Species	# of Darters	Varies with drainage area		
Comp-	# of Sunfish	>3	2-3	<2
osition	# of Suckers	Varies with drainage area		
	# of Intolerants			
	<100 Sq. Mi.	>5	3-5	<3
	>100 Sq. Mi.	Varies with drainage area		
	% Tolerants	Varies with drainage area		
Trophic	% Omnivores	>19	19-34	>34
Comp-	% Insectivores			
osition	<30 Sq. Mi.	Varies with drainage area		
	>30 Sq. Mi.	>55	26-55	<26
	% Top Carnivores	>5	1-5	<1
Fish	# of Individuals	>750	200-750	<200
Condition	% Simple Litho.	>36	18-36	<18
	% DELTs	<0.1	0.1-1.3	<200

IV. Evaluate reference site score distribution-examine for ecoregion differences.



V. Derive numerical biocriteria for each aquatic life use designation as defined in the Ohio WQS.



VI. Use biocriteria in ambient assessments.

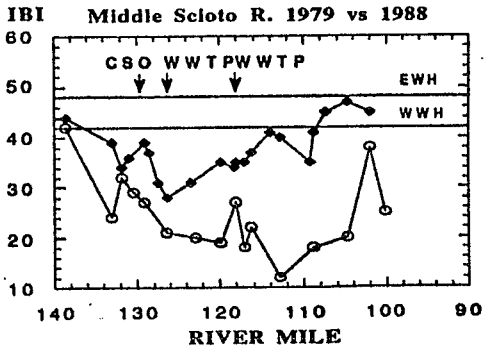


Figure 13. Flowchart of biosurvey approach for fish bioassessment used by Ohio EPA (1991).

by Ohio EPA. Figure 14 is an example of a fish data sheet constructed for immediate entry into a computer data base.

8.16.2 Ohio EPA (1989) also collects data for a general qualitative habitat evaluation (Figure 12) for calculating the Qualitative Habitat Evaluation Index (QHEI) developed by Rankin (1989). The QHEI is designed to provide an empirical, quantified evaluation of the general lotic macrohabitat characteristics that are important to fish communities. A detailed analysis of the development and use of the QHEI is found in Rankin (1989).

8.16.3 For details of specific Ohio EPA field and laboratory methods for fish bioassessment (e.g., sampling site selection, fish sampling procedures, field counting and weighing procedures, handling preserved specimens, data handling and analysis), one should consult Ohio EPA (1987a, 1987b, 1989, 1990b).

Fish Data Sheet

Field Crew: Collector/Recorder ______ Time of Day: ______ Page ___ of ___
River/Stream: ______ Location: ______
Date: ______
River Code: ______ Sampler Type: ______ Time Fished: '___" ___ Total Seconds ______
RM: ______ Depth: ______ Observed Flow: ______
Distance: ______ Data Source: ______ Number of Species: ______

Anomalies: A-anchor worm; B-black spot; C-leeches; D-deformities; E-eroded fins, F-fungus; L-lesions; M-multiple DELT anomalies, N-blind, P-parasites; Y-popeye; S-emaciated; W-swirled scales; T-tumors; Z-other/. [H-Heavy; L-Light are combined with anomalies A, B, and C]

SPECIES	# WEIGHED	TOTAL COUNTED	WEIGHT (GRAMS)					ANOMALIES					

Mass Weighing Convention: Total Weight (g) — 536 (12) — Number Weighed

Figure 14. Example of Ohio EPA (1991) field data sheet constructed for immediate entry into a computer data base.

Page ____ of ____

Anomalies: A-anchor worm; B-black spot; C-leeches; D-deformities; E-eroded fins, F-fungus; L-lesions; M-multiple DELT anomalies, N-blind, P-parasites; Y-popeye; S-emaciated; W-swirled scales; T-tumors; Z-other/. [H-Heavy; L-Light are combined with anomalies A, B, and C]

SPECIES	# WEIGHED	TOTAL COUNTED	WEIGHT (GRAMS)					ANOMALIES					

Mass Weighing Convention: Total Weight (g) — 536 (12) — Number Weighed

Figure 14. Example of Ohio EPA (1991) field data sheet constructed for immediate entry into a computer data base (continued).

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SECTION 9

FAMILY-LEVEL ICHTHYOPLANKTON INDEX METHODS[1]

9.1 Introduction

9.1.1 The early life history stages of fishes are recognized as the most sensitive and vulnerable life stage (Blaxter, 1974; Moser et al., 1984; Weis and Weis, 1989). The ability to document status and trends without identifying most taxa to species has caused some doubt as to the relevance of resolution abilities of using ichthyoplankton in bioassessment studies.

9.1.2 Although there are some reluctance to conduct further ichthyoplankton studies detailed enough to answer water quality questions, investigators have continued to gather important and useful knowledge on the early life stages of fishes. A recent explosion in the amount and types of literature includes documentation of nursery habitats (Goodyear et al., 1982), ecological early life history notes (Simon and Wallus, 1989; Wallus, 1986; Wallus and Buchanan, 1989), taxonomic studies of regionally important systems (Auer, 1982; Holland and Huston, 1983; Simon, 1990; Wallus et al., 1989), toxicological studies using early life history stages (Norberg and Mount, 1983; Birge et al., 1985; Simon, 1988), and effects of environmental pollution (Weis and Weis, 1989).

9.1.3 The purpose of the family-level ichthyoplankton index methods is to present guidelines and an index for the use of ichthyoplankton in bioassessment studies and for determining water quality. The use of a qualitative collection method with a family-level taxonomic approach will facilitate use without complicating logistics and level of effort. The family-level index is based on three components: taxonomy, reproductive guild, and abundance and deformity. Water quality managers, in addition, could use this information to document reproduction, nursery habitats, and backwater habitats not conventionally surveyed during routine adult fish or marcoinvertebrate collection. The format and structure of the ichthyoplankton index (I^2) is modeled after the index of biotic integrity (IBI) using a family-level approach. Since the proponents of the IBI recommend against use of larval and juvenile stages in they analyses (Angermeier and Karr 1986; Karr et al., 1986), the I^2 can be an additional use of data collected during a routine adult sampling event. Current knowledge on the identification of most freshwater faunas are limited, however, a listing of appropriate references is included in Table 1.

9.1.4 The loss of habitat through the accumulation of toxic chemicals in the sediment, reduction of dissolved oxygen, and increase in siltation, is perhaps the greatest obstacle to the protection of environmental quality the environmentalist must face. Degradation by conventional nonpoint sources of pollution have yet to be addressed, rather efforts have concentrated on point sources. USEPA has spent two decades quantifying the effluent quality of point source dischargers. With toxicity endpoints established in industrial

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[1]Adapted from Simon (1989).

TABLE 1. TAXONOMIC LITERATURE USEFUL FOR IDENTIFICATION OF LARVAL AND EARLY JUVENILE NORTH AMERICAN FRESHWATER FISH (ALSO SEE SECTION 12, BIBLIOGRAPHY, SUBSECTION 12.4.2 LARVAL AND IMMATURE FISHES)

Author(s) and Publication Date	Region
Auer, 1982	Great Lakes Basin, emphasis Lake Michigan
Colton and Marak, 1969	Northeast Coast, Black Island to Cape Sable
Drewry, 1979	Great Lakes Region
Elliott and Jimanez, 1981	Beverly Salem Harbor Area, Massachusetts
Fish, 1932	Lake Erie
Fritzsche, 1978	Mid-Atlantic Bight (Chaetodontidae through Ophidiidae)
Hardy, 1978a	Mid-Atlantic Bight (Aphredoderidae through Rachycentridae)
Hardy, 1978b	Mid-Atlantic Bight (Anguillidae throuygh Syngnathidae)
Holland and Huston, 1983	Upper Mississippi River
Hogue et al., 1976	Tennessee River
Johnson, 1978	Mid-Atlantic Bight (Carangidae through Ephippidae)
Jones et al., 1987	Mid-Atlantic Bight (Acipenseridae through Ictaluridae)
Lippson and Moran, 1974	Potomac River Estuary
Mansueti and Hardy, 1967	Chesapeake Bay Region
Martin and Drewry, 1978	Mid-Atlantic Bight (Stromateidae through Ogcocephalidae)
May and Gasaway, 1967	Oklahoma, Canton Reservoir
McGowen, 1984	South Carolina, Robinson Impoundment
McGowen, 1989	North Carolina Piedmont Impoundment

TABLE 1. TAXONOMIC LITERATURE USEFUL FOR IDENTIFICATION OF LARVAL AND EARLY JUVENILE NORTH AMERICAN FRESHWATER FISH (CONTINUED) (ALSO SEE SECTION 12, BIBLIOGRAPHY, SUBSECTION 12.4.2 LARVAL AND IMMATURE FISHES)

Author(s) and Publication Date	Region
Scotton et al., 1973	Delaware Bay Region
Snyder, 1981	Upper Colorado River System, Colorado
Sturm, 1988	Alaska
Taber, 1969	Oklahoma and Texas, Lake Texoma
Wallus et al., 1989	Ohio River basin, emphasis on Tennessee and Cumberland drainages
Wang, 1981	Sacramento-San Joaquin Estuary and Moss Landing Harbor Elkhorn Slough, CA
Wang and Kernehan, 1979	Delaware Estuary

and municipal permits, attention must be focused on instream degradation through chronic exposure to ambient residents.

9.1.5 The effort to combine a community approach for addressing these issues has been accomplished in adult fish (Karr, 1981; Karr et al., 1986), macroinvertebrates (Plafkin et al., 1989), and now with ichthyoplankton here in this section. Karr and colleagues have described in detail the rationale for this overall approach. The reader is referred to their documentation for further reading rather than repeating their rationale (Karr et al. 1986). In this Section details are provided for the scoring and information of an ichthyoplankton index using a community based approach.

9.1.6 The need to look at various trophic levels in the analysis of environmental degradation, through biological integrity, is difficult to explore in insects due to taxonomic and limited ecological information. In fishes, ontogenetic shifts during development not only is apparent in morphological changes (Fuiman and Corazza, 1979), but also niche shifts (George and Hadley, 1979; Brandt, 1986). The early life stages of fishes often documents the use of habitats by endangered or rare species when the adults can frequently not be found. The protection of these important habitats require further consideration in protection of species diversity.

9.1.7 The I^2 is an additional tool which can be concurrently conducted using IBI type techniques, and the method may prove useful in both lotic and lentic habitats. The difficulty in assessing lentic habitats is the inability of species to recolonize closed systems. Field evaluations of both habitat types are necessary prior to further evaluation of the method.

9.1.8 The implications of data quality depends on the calibration of the metrics and collection of a representative sample (Davis and Simon, 1988). Every effort should be made to incorporate quality assurance checks into standard operating procedures and data analysis. Further refinement of techniques and interpretation will become apparent with increases in knowledge of a balance aquatic environment especially as recruitment success and early life history states of fishes are influenced.

9.1.9 Interpretation of the I^2 follows that previously established by the IBI. The use of a three tiered scoring criteria, 5, 3, and 1, are assigned to each metric depending on whether it approximates, deviates somewhat from, or deviates strongly from the value expected at the least impacted ecoregion reference site. The sampling site is then assigned to one of six quality classes based on the sum total of the eleven metric ratings. The highest score, 55, indicates a site without perturbation and deviations decline proportionally. The qualitative ratings and descriptions of Karr (1981) range from excellent to very poor (Table 2). These similar integrity classes and attributes have been appropriately scaled for the I^2 bases on those of Karr et al. (1986).

9.1.10 Finally, although the level of discernment of taxa to a species level would be highly desired, the taxonomic literature is unable to support this level currently. The family level of discernment will reduce confusion among novices using the techniques, provided a high level of reproducability, and

TABLE 2. TOTAL ICHTHYOPLANKTON INDEX (I^2) SCORES, INTEGRITY CLASSES, AND ATTRIBUTES (MODIFIED FROM KARR, 1981)

Total I^2 Score (Sum Of 11 Metrics)	Integrity Class	Attributes
53-55	Excellent	Comparable to the best situations without human disturbance; all regionally expected taxa for habitat, stream size, and ecoregion, including the most intolerant forms; balanced guild structure and reproduction.
44-48	Good	Species richness somewhat below expectations, especially due to loss of the most intolerant forms; some taxa are present with less than optimal abundances; guild structure indicates signs of some stress.
37-40	Fair	Signs of additional deterioration include loss of intolerant forms, skewed dominance, and guild structure. Reduction in simple lithophils and in mean generation time.
26-31	Poor	Dominated by r-strategists, tolerant forms and pioneer species. Increase in guild A.1, and in deformities or teratogenic fish.
11-20	Very Poor	Few fish present, lack of successful reproduction in any guild, deformed or teratogenicity frequently observed.
	No Fish	Repeated sampling finds no fish.

subsequently data quality assurance through accuracy. As an increase in the ecological requirements and taxonomic literature become available, a more sensitive analyses will be possible. Stimulation of single species and comparative larval descriptions and species reproductive characterization should receive higher priority among researchers in the field.

9.2 Methods and Materials

9.2.1 Sampling and Requirements

9.2.1.1 The objectives of the I^2 are to provide a rapid screening method using a single collection event to determine effects of water quality on reproduction and the early life stages of fishes. Collection of a representative sample of ichthyoplankton requires a variety of gear types, and geographical, spatial and temporal considerations. The greater the stream complexity, the greater the distance needed to be sampled; e.g., a second order stream should be surveyed approximately 100 m, while a good rule of thumb is fifteen times the river width or two habitat cycles (Gammon et al., 1981; Karr et al., 1986). Reproduction by fishes occurs within a smaller habitat scale than adult species occurrence. Fishes may rely on a broader area for foraging and etching out an existence, however, only specialized "select"habitats are utilized for reproduction and serve as a nursery habitat. Because of patchy distribution of eggs and larvae a large enough area needs to be investigated to determine local use of a particular stream reach.

9.2.2 Gear Types

9.2.2.1 The more complex the environment the more numerous and sophisticated are equipment needs. The most typical equipment used for collection of larval fishes include, plankton nets; seines, dip nets, and sweep nets; light traps; and push nets and benthic sleds. Snyder (1983) provides documentation on rationale and use of most of the above equipment. Light traps can be constructed for lentic (Faber, 1981; 1982), and lotic waters (Muth and Haynes, 1984), and information on the use of the equipment can be determined from references contained therein. Push nets and benthic sleds are described by Tuberville (1979) and Burch (1983). Also, see Section 4, Sample Collection for Analysis of the Structure and Function of Fish Communities.

9.2.3 Geographical Considerations

9.2.3.1 Landscape differences have long been recognized, and methods to differentiate between various scales have been attempted using zoogeographical realms, biomes, and most recently ecoregions. The ecoregions concept is the most consistent means if evaluating community composition for a water quality based approach. Omernik (1987) defined the conterminous United States into a series of smaller discrete units. Aquatic biological characterization using this approach has been completed for adult fish and macroinvertebrates in several States including Ohio (Larsen et al., 1986; Ohio EPA, 1987), Arkansas (Bennett et al., 1987; Geise and Keith, 1988), North Carolina (Penrose and Overton, 1988), and Vermont (Langdon, 1988).

9.2.4 Spatial Considerations

9.2.4.1 Riffles of rapid flow areas are not the most likely places to encounter larval or juvenile fishes, rather the head of a pool, side margin of a channel and backwater areas are preferred. A representative larval sample should be collected from all available habitats within a stream reach. For example, a large river sample should consist of various depth fractions from the main channel, main channel border, side border and backwaters. Low flow areas will reveal higher diversity of taxa while the remaining large river species will be collected while drifting in the main channel (Simon, 1986a). These diverse areas should be pooled for an overall evaluation of the site while each component habitats, "relative value", can be quantitatively assessed for its contribution to the whole. Creeks, stream, and small rivers will require fewer areas to comprise a representative sample, however, any reduced flow or eddy area will be in need of sampling within a given location. Ideal habitats include those with submerged and emergent aquatic macrophytes, overhanging bank vegetation and roots.

9.2.5 Temporal Considerations

9.2.5.1 Numerous reports and journal articles have documented spawning temperature requirements of various faunas. In order to collect a representative sample from a particular location, familiarity with the reproductive literature and selection of appropriate sample times are necessary. For example, in the midwest the earliest spawning fishes initiate spawning under the ice, with larval emergence and hatching immediately after ice-out during late March and early April. The last species to initiate spawning are usually finished by mid-July with a majority of species spawning during June (Simon, 1986a). Ichthyoplankton and early juvenile sampling should be initiated in the midwest, no sooner than mid-June and no later than the end of September to ensure collection of a representative sample.

9.2.5.2 The use of different gear types will facilitate collection of families which are earlier spawning, e.g. percids, cottids, salmonids, and catostomids. Due to north to south temperature clines, and east to west rainfall differences, species will cue on spawning earlier in the south and west and later in the north and east for the same species. Sampling needs to be adjusted accordingly.

9.2.5.3 Equally important is diel differences in specimen collection. Numerous studies have documented significant differences between dusk and sunset, daylight, and night sampling. The general pattern is the more turbid the water body the less likely diel affects will be a problem. When one decides to sample, is not as important as it is for them to be consistent. Safety considerations and study objectives may not deem night sampling necessary. However, light trap use, set up using an automatic timing device may enable night time sampling without the inconvenience and danger. This method has successfully been used by Alabama Power on the Tallapoosa River.

9.2.5.4 Since much of the North American fauna is incompletely described (Simon, 1986b), use of the index is limited to a family approach until the taxonomic literature facilitates species specific recognition. The eleven I^2 metrics are based on three broad categories. Metrics are organized into taxonomic composition, reproductive guild, and abundance, generation time and

deformity categories. No single metric is always a reliable indicator of degradation, however, relative sensitivity is determined by region, scale, and application.

9.2.5.5 The metrics will react differentially based on the type of perturbation. For example, if contaminated sediments are suspected, the proportion of lithophils and number of sensitive families should decline depending on the magnitude of the impact, while equitability and perhaps deformity should increase.

9.2.5.6 The remainder of this section provides information, justification and rationale behind each of the I^2 metrics (Table 3). Additional refinement may be necessary to meet the objectives of the investigators study.

9.2.5.7 Taxonomic Composition. This category is useful for assessing family diversity and community richness. The current level of taxonomy requires that discussion be limited to a family level but future use of the index may make this a species specific approach. Expectations should be determined for various stream size and calibrated by equipment based on information presented in Fausch et al. (1984). Taxa diversity has been determined to be the best sole indicator of "good" water quality. Sensitive families such as percids, cottids. ictalurids, and others listed in Table 4, are useful for determining the extent of impact to sediments and nursery habitats. Finally, dominance of tolerant species increase proportionally to environmental degradation.

9.2.6 **Metric 1. Total Number of Families.** The fluctuation in number of families of an ecoregion increased with stream order. If the same order stream, in the same ecoregion, with similar habitat cycles were sampled, then reduction in numbers of families would correspond to environmental degradation. A number of investigators have determined number of taxa is the single most important metric which highly correlates with more pristine water quality (Ohio EPA, 1987; Davis and Lubin, 1989; Plafkin et al., 1989).

9.2.7 **Metric 2. Number of Sensitive Families.** Certain families of freshwater fish are sensitive to degradation, particularly as a result of reproduction requirements and early life ecology (Table 4). Families such as Percidae, Cottidae, and Salmonidae are intolerant to siltation and low dissolved oxygen. Sediment contamination due to toxins and low dissolved oxygen inhibits most benthic families (e.g., Ictaluridae). Reduction in habitat quality (e.g., channelization, thermal inputs, reservoir flooding) reduces Catostomidae, Centrarchidae, Cyprinidae, and Fundulidae. Sensitive families should be restricted to those most sensitive to low dissolved oxygen, toxic chemicals, siltation, and reduced flow. Karr et al. (1986) suggested that species sensitive to habitat degradation, especially siltation, are most likely to be identified as intolerant.

9.2.8 **Metric 3. Equitability/Dominance.** As water quality declines certain taxa tend to become increasingly abundant (Karr et al., 1986). Also, species defined as r-strategists tend to inundate the environment with early life phases (MacArthur, 1957; MacArthur and Wilson, 1967). The strategy to produce large numbers of young are indicative of "pioneer" species which are attempting to colonize perturbed areas. In habitats with least impacted

TABLE 3. METRICS USED TO ASSESS ICHTHYOPLANKTON COMMUNITIES FROM FRESHWATERS OF NORTH AMERICA

Category	Metric	Scoring Criteria 5	3	1
Taxonomic Composition				
1.	Total Number of Families	Drainage Size and Ecoregion Dependent		
2.	Number of Sensitive Families	Drainage Size and Ecoregion Dependent		
3.	Equitability/Dominance	>0.8-1.0	>0.6-0.8	0-< 0.6
4.	Family Biotic Index	0-4.5	>4.5-7.5	>7.5-10
Reproductive Guild				
5.	% Non-guarding Guild A.1 and A.2	Drainage Size and Ecoregion Dependent		
6.	% Guarding Guild B.1 and B.2	Drainage Size and Ecoregion Dependent		
7.	% Bearers Guild C.1 and C.2	Drainage Size and Ecoregion Dependent		
8.	% Simple Lithophil Mode Reprod.	Drainage Size and Ecoregion Dependent		
Abundance, Generation Time, and Deformity				
9.	Catch per Unit Effort	Drainage Size and Ecoregion Dependent		
10.	Mean Generation Time	Drainage Size and Ecoregion Dependent		
11.	% Deformity or Teratogenicity	<1%	>2-5%	>5%

TABLE 4. SENSITIVITIES, MEAN GENERATION TIME, AND REPRODUCTIVE GUILD CHARACTERISTICS OF 34 NORTH AMERICAN FRESHWATER FISH FAMILIES

Family	Sensitivity	Generation Time[1]	FBI[2]	Reproductive Guild
Petromyzontidae	Moderate	Short/Moderate	3	A.1
Acipensideridae	Moderate	Long	2	A.1
Polyodontidae	Intolerant	Long	2	A.1
Lepisosteidae	Tolerant	Moderate	4	A.1
Amiidae	Tolerant	Moderate	8	B.2
Anguillidae	-	Moderate	3	A.1
Clupeidae	Moderate	Short	6	A.1
Hiodontidae	Intolerant	Short/Moderate	4	A.1
Salmonidae	Intolerant	Moderate/Long	1	A.1
Osmeridae	Moderate	Short	5	A.1
Umbridae	Tolerant	Short	9	A.1
Esocidae	Moderate	Moderate	6	A.1
Characidae	Moderate	Short	5	A.1
Cyprinidae	Moderate	Short	6	A.1, A.2, B.1, B.2
Catostomidae	Intolerant	Moderate	4	A.1, A.2
Cobitidae	Intolerant	Short	4	A.1
Ictaluridae	Intolerant	Moderate	3	B.2
Claridae	Tolerant	Moderate	10	A.2
Amblyopsidae	Intolerant	Short	4	C.1
Aphredoderidae	Tolerant	Short	8	C.1
Percopsidae	Moderate	Short	7	A.1
Gadidae	Moderately	Moderate/Long	5	A.1
Oryzintidae	Tolerant	Short	7	C.2
Cyprinodontidae	Intolerant	Short	2	A.1, A.2
Fundulidae	Intolerant	Short	5	A.1, A.2
Poeciliidae	Tolerant	Short	8	C.2
Atherinidae	Moderate	Short	3	A.1
Gasterosteidae	Tolerant	Short	9	B.2
Moronidae	Intolerant	Moderate	6	A.1
Centrarchidae	Intolerant	Moderate	5	B.1
Elassomatidae	Intolerant	Short	3	B.2
Percidae	Intolerant	Short	0	A.1, A.2, B.1, B.2
Sciaenidae	Moderate	Moderate	4	A.1
Cichlidae	Tolerant	Moderate	7	B.2
Cottidae	Intolerant	Short	0	B.2

[1]Classified as short, moderate, and long appropriately scored 1, 3, 5, respectively. A community mean is calculated by summing scores and dividing by total number of families.

[2]Scored from 0 to 10. The higher the score the greater the tolerance to organic enrichment. FBI = Family Biotic Index.

environments, taxa tend to be equally distributed and more moderately abundant. The Shannon diversity index and the measure of evenness are used to determine quality environments which have balanced communities. These single unit measures are not adequate in themselves to extrapolate excellent quality, but they do determine increasing levels of disturbance. Equitability (Lloyd and Ghelardi, 1964) is determined by comparing the number of families in the sample with the expected number of families from a community which conforms to the MacArthur broken stick model. MacArthurs' broken stick model is normally higher than real diversity and is the ecologically maximum diversity attainable (Washington, 1984). Equitability is measured by:

$$e = s'/s$$

where:

s = number of taxa in the sample,
s'= the tabulated value based on the Shannon diversity index

The diversity index is the $\bar{d}$ formulation of Lloyd, Zar, and Karr (1968). The diversity index is:

$$\bar{d} = C/N\ (N \log_{10} N - E\ n_i \log_{10} n_i)$$

where:

C = 3.321928,
N = total number of individuals in the ith taxa,
n_i = total number of individuals in the ith taxa.

An example calculation and reproduction of Lloyd and Ghelardi's (1964) table are included in Table 5 and are taken from USEPA (1973, 1990). As a side note, if solely ichthyoplankton data sets are to be used excluding juveniles, the following families need to be omitted: Clupeodae. Scianenidae, and Osmeridae.

9.2.9 **Metric 4. Family Biotic Index.** Discussions with other ichthyoplanktologists studying the ecological and taxonomic early life stages of fishes suggest varying degrees of sensitivity exists between organic pollution and perturbations such as sediment, degradation, siltation, low dissolved oxygen, toxic chemicals, and flow reduction (Table 4). The calculation of the Family Biotic Index (FBI) is modeled after Hilsenhoff's (1988) modified biotic index which summarizes tolerances to organic pollution. Tolerance values range between 0 to 10 for families and increase as water quality decreases. The formula for calculating the Family Biotic Index is:

$$FBI = e\ x_i t_i / N$$

where:

x_i = total number of individuals within a taxon,
t_i = tolerance value of a taxon,
N = total number of organisms in the sample.

TABLE 5. THE DIVERSITY OF SPECIES, $\bar{d}$, CHARACTERISTIC OF MACARTHUR'S MODEL FOR VARIOUS NUMBERS OF HYPOTHETICAL SPECIES, S' (From Lloyd and Ghelardi, 1964)

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

	Number of individuals in each taxa (n_i)	$n_i \log_{10} n_i$*
	41	66.1241
	5	3.4949
	18	22.5949
	3	1.4314
	1	.0000
	22	29.5333
	1	.0000
	2	.6021
	12	12.9502
	4	2.4082
Total	109	139.1391

Total number of taxa. n = 10
Total number of individuals. N = 109

$N \log_{10} N = 222.0795$ (from Table 5)
$\Sigma n_i \log_{10} n_i = 139.1391$
$= 3.321928 - \frac{}{109}(222.0795 - 139.1391)$
$= 0.030476 \times 82.9404$
$= 2.5$

*From Table 5, in Macroinvertebrates, USEPA (1973) or Table 23, Section 7, Data Evaluation, USEPA (1990).

9.2.10 **Reproductive Guild.** Reproductive requirements of fishes coupled with early life history strategies enable a diversification of the ways habitats are used. Balon (1975, 1981) divided reproductive modes of fishes in order of evolutionary trends. Species are divided into nonguarders (guild A), guarders (guild B), and bearers (guild C). The increase in evolutionary sophistication from guilds A to C, generally conforms to levels of increased diversification and reduction in niche overlap in complex environments (Table 6). Guild dynamics are determined by three metrics in this category. The destruction of diverse habitats not only reduce utilization of these habitats for reproduction by adults, but also destroys nursery habitats for larval and juvenile phases.

9.2.11 **Metric 5. Proportion of Non-guarding Guild A.1 and A.2.** The nonguarding guild includes mostly r-stragegists which provide little parental investment into each egg, usually possess early reproduction, small body size, many small offspring, single production, and exhibit a type III mortality (MacArthur, 1957; MacArthur and Wilson, 1967). Balon (1975) described the nonguarding guild as broadcast spawners, usually without much developmental specialization, and although may construct some nests always abandons them post-reproduction. These species are often "pioneer" species and frequently are dominant only in stressed and dominant only in stressed areas which are periodically disturbed.

9.2.12 **Metric 6. Proportion of Guarding Guild B.1 and B.2.** The guarding guild typically include k-strategists as defined by MarArthur (1957) and MacArthur and Wilson (1967). This strategy favors slower development, greater competitive ability, delayed reproduction, larger body size, repeated reproduction, fewer larger progeny, and exhibits types I and II mortality. The guarding guild (Balon, 1975) is a solely ethological aspects of guild with profound ecomorphological consequences. Better protected from enemies, guarded eggs need not be numerous to assure survival of the species. As a consequence, eggs can be larger and result in more viable offspring with less food specialization. Spawning sites with low oxygen content can be used because the guarding parents clean the eggs and produce a flow of water around them by fin-fanning and oral ventilation. Fishes that do not build complicated structures, nests, but that deposit their eggs on top of a selected object, are also included in this section. The evolutionary progression has been from (1) an exclusively parental male, (2) shared parental care by the male and female, to (3) a division of roles with the female as the direct parent and the male as the guardian to (4) polygyny (Barlow, 1974).

9.2.13 **Metric 7. Proportion of Bearers Guild C.1 and C.2.** This group is divided into external and internal brooders (Balon, 1975). External brooders carry their developing eggs on the surface of their bodies or in externally filled body cavities or special organs. These include transfer, forehead, mouth, gill-chamber. skin and pouch brooders. Internal brooders have eggs fertilized internally before they are expelled from the body cavity. Special organs are developed to facilitate sperm transfer. Mating does not necessarily coincide with fertilization. After fertilization eggs can be expelled and incubated externally or retained in the body cavity of the

TABLE 6. CLASSIFICATION OF REPRODUCTION STYLES FOR FISHES IN ORDER OF EVOLUTIONARY TRENDS (FROM BALON, 1981)

	Ethological Section	A. Nonguarders
	Ecological Group	A.1. Open Substratum Spawners
Guild		Selected key features of early ontogeny
A.1.1	Pelagic spawners (pelagophils)	Numerous buoyant eggs, none or poorly developed embryonic respiratory organs, little pigment, no photophobia
A.1.2	Rock and gravel spawners with pelagic larvae (lithopelagophils)	Adhesive chorion at first, some eggs soon buoyant, after hatching free embryos pelagic by positive buoyancy or active movement, no photophobia, limited embryonic respiratory structures*
A.1.3	Rock and gravel spawners with benthic larvae (lithophils)	Early hatched embryo photophobic, hide under stones, moderately developed embryonic respiratory structures, pigment appears late
A.1.4	Nonobligatory plant spawners (phytolithophils)	Adhesive eggs on submerged items, late hatching, cement glands in free embryos, photophobic, moderately develop respiratory structures
A.1.5	Obligatory plant spawners (phytophils)	Adhesive egg envelope sticks to submerged live or dead plants, late hatching, cement glands, not photophobic, extremely will developed embryonic respiratory structures
A.1.6	Sand spawners (psammophils)	Adhesive eggs in running water on sand or fine roots over sand, free embryos without cement glands, phototropic, freely developed respiratory structures, large pectorals, large neuromast rods (cupulae)

*See the final amendment in Balon (1981), page 389.

TABLE 6. CLASSIFICATION OF REPRODUCTION STYLES FOR FISHES IN ORDER OF EVOLUTIONARY TRENDS (FROM BALON, 1981) (CONTINUED)

Ethological Section		A. Nonguarders
Ecological Group		A.2. Brood hiders
Guild		Selected key features of early ontogeny
A.1.7	Terrestrial spawners (aerophils)	Small adhesive eggs scattered out of water in damp sod, not photophobic, moderately developed respiratory structures
A.2.1	Beach spawners (aeropsammophils)	Spawning above the water line of high tides, zygotes in damp sand hatch upon vibration of waves, pelagic afterward
A.2.2	Annual fishes (xerophils)	In cleavage phase blastomeres disperse and rest in 1st facultative diapause, two more resting intervals obligate--eggs and embryos capable of survival for many months in dry mud
A.2.3	Rock and gravel spawners (lithophils)	Zygotes buried in gravel depressions called redds or in rock interstices, large and dense yolk, extensive respiratory plexuses for exogenous and carotenoids for endogenous respiration, early hatched free embryos photophobic, large emerging alevins
A.2.4	Cave spawners (speleophils)	A few large adhesive eggs, most hide in crevices, extensive embryonic respiratory structures, large emerging larvae
A.2.5	Spawners in live invertebrates (ostracophils)	Zygotes deposited via female's ovipositor in body cavities of mussels, crabs, ascidians or sponges(?), large dense yolk, lobes or spines and photophobia to prevent expulsion of free embryos, large embryonic respiratory plexuses and carotenoids, probable biochemical mechanism for immunosuppression

TABLE 6. CLASSIFICATION OF REPRODUCTION STYLES FOR FISHES IN ORDER OF EVOLUTIONARY TRENDS (FROM BALON, 1981) (CONTINUED)

Ethological Section	A. Nonguarders
Ecological Group	A.2. Brood hiders
Guild	Selected key features of early ontogeny
B.1.1 Pelagic spawners (pelagophils)	Nonadhesive, positively buoyant eggs, guarded at the surface of hypoxic waters, extensive embryonic respiratory structures
B.1.2 Above water spawners (aerophils)	Adhesive eggs, embryos with cement glands, male in water splashes the clutch periodically
B.1.3 Rock spawners (lithophils)	Strongly adhesive eggs, oval or cylindrical, attached at one pole by fibers in clusters, most have pelagic free embryos and larvae
B.1.4 Plant spawners (phytophils)	Adhesive eggs each to variety of aquatic plants, free embryos without cement glands swim instantly after prolonged embryonic period

Ethological Section	B. Guarders
Ecological Group	B.2 Nest spawners
Guild	Selected key features of early ontogeny
B.2.1 Froth nesters (aphrophils)	Eggs deposited in a cluster of mucous bubbles, embryos with cement glands and well developed respiratory structures
B.2.2 Miscellaneous substrate and material nester (polyphils)	Adhesive eggs attached singly or in clusters on any available substratum, dense yolk with high carotenoid contents, embryonic respiratory structures well developed, feeding of young on parental mucus common

TABLE 6. CLASSIFICATION OF REPRODUCTION STYLES FOR FISHES IN ORDER OF EVOLUTIONARY TRENDS (FROM BALON, 1981) (CONTINUED)

	Ethological Section	A. Nonguarders
	Ecological Group	B.2. Nest spawners
Guild		Selected key features of early ontogeny
B.2.3	Rock and gravel nesters (lithophils)	Eggs in spherical or elliptical envelopes always adhesive, free embryos photophobic or with cement glands swing tail-up in respiratory motions, moderate to well developed embryonic respiratory structures, many young feed first on the mucus of parents
B.2.4	Gluemaking nesters (ariadnophils)	Male guards intensively eggs deposited in nest bind together by a viscid thread spinned from a kidney secretion, eggs and embryos ventilated by male in spite of well developed respiratory structure
B.2.5	Plant material nesters (phytophils)	Adhesive eggs attached to plants, free embryos hang on plants by cement glands, respiratory structures well developed in embryos assisted by fanning parents
B.2.6	Sand nesters (psammophils)	Thick adhesive chorion with sand grains gradually washed off or bouncing buoyant eggs, free embryo leans on large pectorals, embryonic respiratory structures feebly developed
B.2.7	Hole nesters (speleophils)	At least two modes prevail in this guild: cavity roof top nesters have moderately developed embryonic respiratory structures. While bottom burrow nesters have such structures developed strongly
B.2.8	Anemone nesters (actiniariophils)	Adhesive eggs in cluster guarded at the base of sea anemone, parent coats the eggs with mucus against nematocysts, free embryo phototropic, planktonic, early juveniles select host anemone

TABLE 6. CLASSIFICATION OF REPRODUCTION STYLES FOR FISHES IN ORDER OF EVOLUTIONARY TRENDS (FROM BALON, 1981) (CONTINUED)

Ethological Section	B. Bearers
Ecological Group	C.1 External bearers
Guild	Selected key features of early ontogeny
C.1.1 Transfer brooders	Eggs carried for some time before deposition: in cupped pelvic fins, in a cluster hanging from genital pore, inside the body cavity (earlier ovoviviparous), after deposition most similar to nonguarding phytophils (A.1.4)
C.1.2 Auxiliary brooders	Adhesive eggs carried in clusters or balls on the spongy skin of ventrum, back, under pectoral fins or on a hook in the superoccipital region, or encircled within coils of female's body, embryonic respiratory circulation and pigments well developed
C.1.3 Mouth brooders	Eggs incubated in buccal cavity after internal synchronous or asynchronous, or buccal fertilization assisted by egg dummies, large spherical or oval eggs with dense yolk are rotated (churning) in the cavity or densely packed when well developed embryonic respiratory structures had to be assisted by endogenous oxydative metabolism of carotenoids, large young released
C.1.4 Gill-chamber brooders	Eggs of North American cavefishes are incubated in gill cavities
C.1.5 Pouch brooders	Eggs incubated in an external marsupium: an enlarged and everted lower lip, fin pouch, or membraneous or bony plate covered ventral pouch, well developed embryonic respiratory structures and pigments, low number of zygotes

TABLE 6. CLASSIFICATION OF REPRODUCTION STYLES FOR FISHES IN ORDER OF EVOLUTIONARY TRENDS (FROM BALON, 1981) (CONTINUED)

Ethological Section	B. Bearers
Ecological Group	C.2 Internal bearers
Guild	Selected key features of early ontogeny
C.2.1 Facultative internal bearers	Eggs are sometimes fertilized internally by accident via close apposition of gonopores in normally oviparous fishes, and may be retained within the female's reproductive system to complete some of the early stages of embryonic development, rarely beyond the cleavage phase: weight decreases during embryonic development (examples**: *Galeus polli*, *Rivulus marmoratus*, *Oryzias latipes*)
C.2.2 Obligate lecithotrophic livebearers	Eggs fertilized internally, incubate in the reproductive system of female until the end of embryonic phase or beyond, no maternal-embryonic nutrient transfer: as in oviparous fishes yolk is the sole source of nourishment and most of the respiratory needs; some specialization for intrauterine respiration, excretion and osmoregulation: decrease in weight during embryonic development (examples: *Torpedo ocellata*, *Poeciliopsis monaclia*, *Poecilia reticulata*, *Xenopoecilus poptae*, *Schastes marinus*)
C.2.3 Matrotrophous oophages and adelphophages	Of many eggs released from an ovary only one or at most a few embryos develop into alevins and juveniles+, feeding on other less developed yoked ova present and/or periodically ovulated (oophagy), and in more specialized forms, preying

**Note differences in the earier paper (Balon, 1975)
+Terminology as in Balon (1981).

TABLE 6. CLASSIFICATION OF REPRODUCTION STYLES FOR FISHES IN ORDER OF EVOLUTIONARY TRENDS (FROM BALON, 1981) (CONTINUED)

Ethological Section	B. Bearers
Ecological Group	C.2 Internal bearers
Guild	Selected key features of early ontogeny
C.2.3 Matrotrophous oophages and adelphopages (continued)	on less developed sibling embryos (adelphophagy): specialization for intrauterine respiration, secretion and osmoregulation similar to the previous guild: large gain in weight during intrauterine development (examples: *Lumma cornubica*, *Eugamphodus temus*, *Latimeria chalumnae*?)
C.2.4 Viviparous trophoderms	Internally fertilized eggs develop into embryos, alevins or juveniles whose partial or entire nutrition and gaseous exchange is supplied by the mother via secretory histotrophes ingested or absorbed by the fetus via epithelial absorptive structures (placental analogues) or a yolk sac placenta: small to moderate gain in weight during embryonic development (examples: *Galeus canis*, *Myliobatis bovina*, *Mustelus canis*, *Sphyrna tiburo*, *Zoarces viviparus*, *Ameca splendens*, *Poeciliopsis turneri*, *Heterandria formosa*, *Anableps dowi*, *Embiotoca lateralis*, *Clinus superciliosus*)

female, after which full-grown juveniles are born (Hoar, 1969; Balon, 1975, 1981).

9.2.14 **Metric 8. Proportion of Simple Lithophil Mode of Reproduction.** This metric is used by Ohio EPA (1987) as a substitute in the adult IBI for hybrids. Simple lithophils spawn where their eggs can develop in the interstices of sand, gravel, and cobble substrates without parental care. Generally, as the level of environmental degradation of simple lithophils decreases. This is important in determining impacts from chronic levels of exposure in sediments, and settling out of toxins in pools or backwater habitats.

9.2.14.1 Abundance, Generation Time, and Deformity. Impacts to individuals often are a compounding problem effecting community analyses. Reduction in numbers of individuals, lowering of community mean generation time, and increases in observed deformity and teratogenicity correspond with environmental degradation. Loss of longer-lived species which require specialized habitats (e.g., *Acipenser fulvescens* and *Atractosteus spatula*) during reproduction and nursery are increasing at an alarming rate. Mean generation time is a function of the time to first reproduction. This metric may need further research before it can be utilized since it is proposed as a community metric rather than as an individual metric as it was conceived.

9.2.15 **Metric 9. Catch per Unit Effort.** Population abundance varies with ecoregion, stream size, and gear type used. It may be expressed as the catch per unit effort, either by area, distance, or time sampled. Sites with lower biological integrity will have reduced numbers of individuals, however, rapidly flowing riffles should be excluded from comparison with pools and run habitats (see spatial considerations). Organic enrichment usually increases the number of individuals. Steedman (1988) addressed this situation by scoring catch per minute of sampling. Unusually low numbers generally indicate toxicity which is readily apparent at low levels of biological integrity.

9.2.16 **Metric 10. Mean Generation Time.** Mean generation time is the average age of parenthood, or the average age at which all offspring are born. A longer-lived k-strategists species often spend several years before reaching reproductive maturity, e.g., Salmonidae, Polyodontidae and Acipenseridae. Vulnerability of these organisms to perturbations may have significant impact to future recruitment during the larval and juvenile stages of development. Mean generation time is an average value for a family based on life strategy of representative taxa. Mean generation time is calculated as:

$$\overline{T} = (a + w)/2$$

where:

a = age at first reproduction
w = age at last reproduction

9.2.16.1 The community mean generation time is the sum of all generation times for all families collected, divided by the total number of families.

9.2.17 **Metric 11. Proportion of Deformity or Teratogenicity.** Toxicological literature suggests that increased exposure to metals and organic chemical compounds increases the proportion of teratogenicity among fathead minnows (Birge et al., 1985; Simon, 1988). Additional effects have been documented in a recent literature review by Weis and Weis (1989), as well as, exposure to radiation (Lanthrop, personal communication). Teratogenic effects include edematous yolk sacs, post caudal swellings, clear blood, reduced heart beat, lack of fusiform shape, enlarged craniums, square eyes, or improper development of the mandible (Simon, 1988). An increase in deformities or teratogenicity is a result of increased exposure to toxic chemicals or radiation. In reference and complex effluent testing using the fathead minnow embryo-larval survival and teratogenicity test, one very infrequently observed any teratogenicity in control samples. When deformities were observed they were always less than 1% (Simon, personal communication).

9.2.17.1 Improperly preserved specimens will exhibit signs of deformity. Birchfield (1987) determined that cranial anomalies were induced in centrarchids and clupeids by fixing them in low concentrations of formalin (<105), exposing them to high temperatures, or vigorously shaking the fixed specimens. No cranial anomalies were found in larval fish fixed in formalin solutions greater than 10% or in Bouin's fluid.

9.3 Taxonomic Considerations

9.3.1 The ability to differentiate families or larval fishes requires a basic understanding of the morphometric and meristic characteristics which are included in most taxonomic studies (Figures 1 and 2). Extensive literature exists on specific families of larval or larval fishes and alternative measurements, but certain standard measurements and counts continue to be the main ones reported in the literature. The following explanation of how to construct the character in question and the appropriate position to measure or count the character is defined by Simon (1987) and Simon et al. (1987).

9.3.2 Characteristics are subdivided into morphometric, measurable structures, and meristic, countable structures. Standard length and total length are measured from the tip of the snout to the posterior portion of the notochord and to the tip of the caudal finfold, respectively. Morphometric measurements include head length--from the snout to pectoral fin origin; snout length--from tip of the snout to anterior margin of eye; eye diameter--anterior to posterior margin; preanal length--snout to posterior margin of anus; body depth--vertical distance at anus; greatest body depth (also referred to as shoulder depth or head depth)--largest vertical distance (usually anterior dorsal finfold) or measured at origin of pectoral fin; mid-postanal depth--vertical distance measured from dorsal to ventral margin of body at anterior apex of the mean of the postanal myomeres; caudal peduncle depth--vertical distance at anterior apex of penultimate myomere; head width--measured dorsally at the posterior margin of eyes; yolk sac length and depth--measured horizontally and vertically, respectively at the greatest distance on the yolk sac.

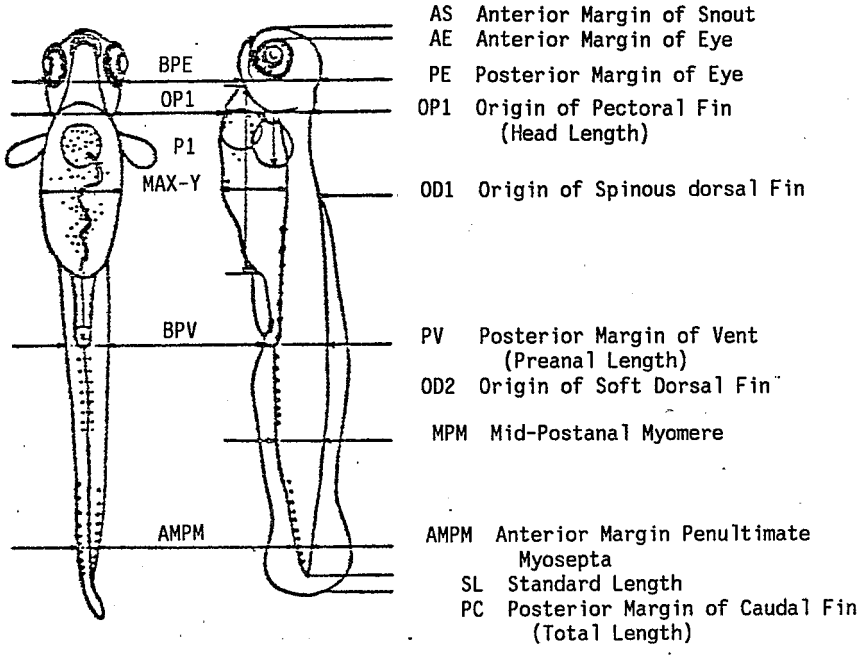

Figure 1. Morphometric characteristics for larval fishes. The yolk sac (Y) is included in width and depth measurements, but fin folds are not. "B" means immediately behind, but not including, the eye or vent. Location of width and depth measures at OD can only be approximated before the dorsal fin begins to form. Fin length is measured along the plane of the fin from the origin to the most distal margin. From Simon et al. (1987).

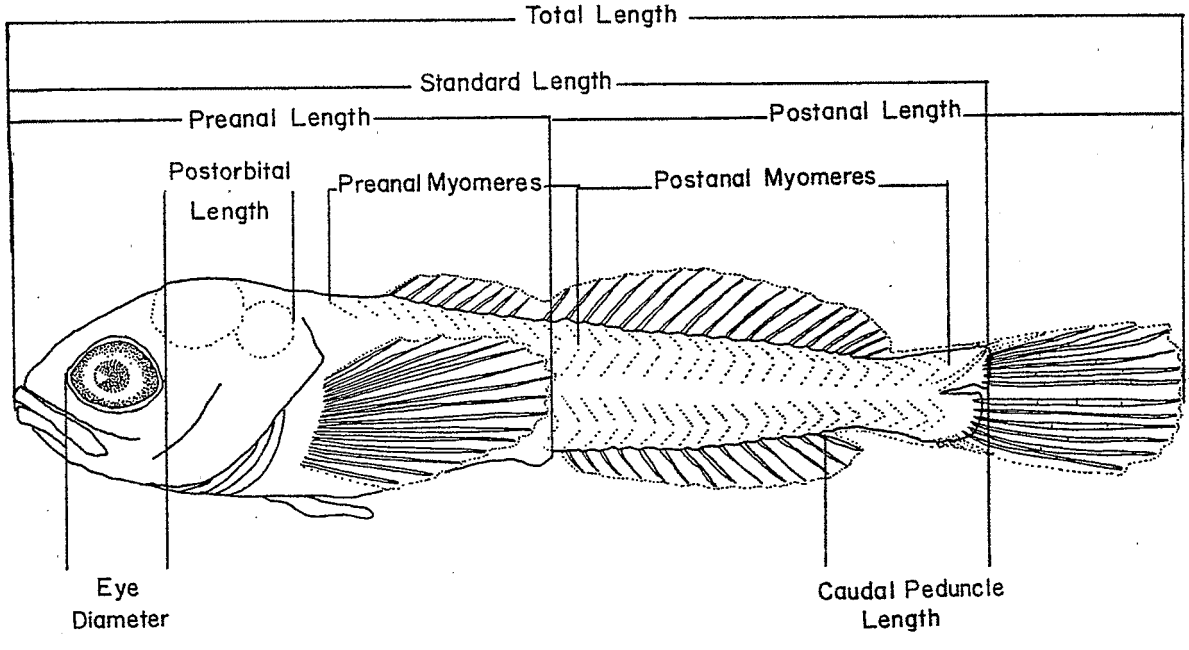

Figure 2. Diagrammatic representation of morphology of a teleost larva. From Auer (1982).

9.3.3 Meristic measurements include the enumeration of all fin rays following methods in Hubbs and Lagler (1958); head canal pores (Hubbs and Cannon, 1935); preanal myomeres--those anterior to a vertical line drawn from the posterior portion of the anus, including those bisected by the line, while postanal myomeres include an urostylar element.

9.4 Provisional Key to the Families of North American Freshwater Fishes

(Adequate information is not available for all early life phases. Families omitted from this key include Amblyopsidae, Cichlidae, Cyprinodontidae, Poeciliidae, Umbridae, Cobitidae, Claridae, Oryziatidaw, and Elassomatidae). Also see Section 12, Fisheries Bibliography, Subsection 12.5.2, Larval and Immature Fishes.

KEY TO THE FAMILIES OF NORTH AMERICAN FRESHWATER FISHES

1a. Body tubular, elongate, eel-like.....2

1b. Body not eel-like; usually with a single gill opening; stomodeum or functional jaws present.....3

2a. Body tubular, elongate, eel-like; seven gill openings; oral sucking disc without jaws; lacking paired fins and distinct eyes.....Petromyzontidae

2b. Body eel-like; usually with a single gill opening; stomodeum, or functional jaws present; eye large; processing paired fins...Anguillidae

3a. Barbels present on chin; mandibular barbels at corners of mouth; usually hatching with some incipient fin rays present; yolk large usually with complex vitellin veins.....Icataluridae

3b. Chin barbels and mandibular barbels absent; if barbels are present limited to ventral portion of snout or single on chin.....4

4a. Adhesive disc present on snout; caudal fin heterocercal.....5

4b. Adhesive disc absent on snout.....6

5a. Adhesive disc papillose; preanal myomeres number x; snout elongate with remnant of adhesive disc until 20 mm total length (TL); dorsal and anal finfolds originating posteriorly, finfold with dark triangular areas near future dorsal, anal, and caudal Fins.....Lepisosteidae

5b. Adhesive disc smooth; preanal myomeres number x; without elongate snout, dorsal finfold originating anterior pectoral fin; gular plate present; body robust.....Amiidae

6a. Larval 10-11 mm TL at hatching; preanal length 60-65% TL; yolk sac large, oval, vascularized; barbels developing on ventral extension of snout; head small.....7

6b. Larvae < 10 mm TL at hatching; preanal length greater than 60-65% TL; large, oil globule; without barbels on ventral surface of snout.....8

7a. Decreasing preanal length at increasing length, 65% TL becomes 60% TL > 11 mm; moderate dorsal finfold originates immediately behind head; dorsal finfold origin length 25% TL; late protolarvae with four barbels; dorsal fin origin posterior to vent; posterior margin of operculum not extending past base of pectoral fin; scutes developing at juvenile stages.....Acipenseridae

7b. Decreasing preanal length at increasing length, 60% TL becomes 50% TL at > 11 mm; dorsal finfold originates at mid-preanal; dorsal finfold origin length 35% TL; late protolarvae with two barbels; dorsal fin origin anterior anus; posterior margin of operculum extending past base or pectoral fin; no scutes developing at juvenile stages.....Polyodontidae

8a. Preanal length greater than 65% TL.....9

8b. Preanal length 60% TL or less.....19

9a. Preanal length greater than 75% TL.....10

9b. Preanal length between 65-75% TL.....13

10a. Preanal length 76-89% TL; total myomeres greater than 45.....12

10b. Preanal length usually less than 75-79% TL; total myomeres less than 45 11

11a. Preanal myomeres > 27; mouth subterminal; body elongate, with usually one to several rows of dorsal pigment.....Catosomidae

11b. Preanal myomeres > ; mouth superior, body elongate usually without pigmentation dorsally.....Clupeidae

12a. Postanal myomeres 13-17; yolk sac small, round and far forward..... .....Osmeridae

12b. Postanal myomeres < 10; yolk sac larger, elongate or oval, situated posteriorly.....Clupeidae

13a. Preanal myomeres greater than or equal to 40.....14

13b. Preanal myomeres less than 40.....15

14a. Postanal myomeres 14015; preanal length 72-75% TL; adipose fin present; swim bladder visibly present.....Osmeridae

14b. Postanal myomeres 15-22; preanal length 67-72% TL; adipose fin absent; swim bladder not visible.....Esocidae

15a. Yolk sac long, bilobed with the anterior portion thick and oval, posterior section thick and tubular, preanal length 58-74% TL.....16

15b. Yolk sac not bilobed, either elongate or oval; if bilobed usually with both sections of equal portion; preanal length 68-75% TL.....17

16a. Larvae densely pigmented, evenly over body, with a dark stripe over gut; usually less than 27 preanal myomeres; body robust.....Cyprinidae

16b. Pigmentation limited to dorsum, usually on cranium and sometimes mid-dorsally in two to four distinct rows; body elongate....Catostomidae

17a. Preanal myomeres < 31, postanal myomeres less than 41.....Catostomidae

17b. Preanal myomeres ≥ 31.....18

18a. Postanal myomeres < 41; larvae large, at 7 mm still possess yolk; preanal length 62-64% TL.....Hiodontidae

18b. Postanal myomeres ≥ 41; preanal length 67-74% TL.....Cyprinidae

19a. Preanal length ≥ 48% TL.....20

19b. Preanal length < 48% TL.....27

20a. Preanal ≥ 56% TL.....21

20b. Preanal 48-55% TL.....23

21a. Preanal myomeres < 26; preanal length 56-58% TL; larvae large, yolk sac present until 7-10mm TL.....Hiodontidae

21b. Preanal myomeres < 26; preanal length < 56% TL; yolk sac larvae < 7 mm TL.....22

22a. Preanal myomeres 8-12; postanal myomeres 9-15.....Moronidae

22b. Preanal myomeres 15-26; postanal myomeres 18-26.....Percidae

23a. Preanal myomeres ≥ 15.....Percidae

23b. Preanal myomeres < 15.....24

24a. Total myomeres ≤ 26.....Moronidae

24b. Total myomeres > 26.....25

25a. Preanal myomeres 14-16; preanal length > 50% TL.....Gasterosteidae

25b. Preanal myomeres <.....26

26a. Postanal myomeres < 19; gut massive, uncoiled; pectoral fins proportional.....Centrarchidae

26b. Postanal myomeres ≥ 19; large pectoral fins.....27

27a. Preanal length < 35%; preanal myomeres 6-7; postanal myomeres 28-31.....Atherinidae

27b. Preanal length > 35%.....28

28a. Postanal myomeres approximately 40; preanal length 39-44% TL.....Gadidae

28b. Postanal myomeres much less than 40; preanal length 44% TL.....29

29a. Postanal myomeres ≤ 11; posterior oil globule in yolk sac.....30

29b. Postanal myomeres > 20; mouth terminal to superior; preanal length 45% TL.....30

30a. Postanal myomeres > 30; mouth terminal to superior; preanal length 45% TL.....Fundulidae

30b. Postanal myomeres < 20; mouth subterminal to inferior; preanal length 45% TL.....Percopsidae

9.5 Fish Larvae Sampling Precision

9.5.1 When investigators collect larval fish samples, the accuracy of the sampling methods and equipment must be carefully considered. Using literature data, Cyr et al. (1992) demonstrated that past sampling designs have been inadequate for the comparison of larval fish abundance across sites or time periods. Therefore, Cyr et al. (1992) developed a general model based on published data to predict the variance in larval fish abundance among replicate samples and provided guidelines for estimating the number of larval fish samples necessary to obtain acceptable or desired levels of precision at a collecting site. For studies that include large aquatic habitats of many sites as well as changes in abundance through time, they concluded that investigators must consider patterns of spatial and temporal variation when sampling larval fish populations. They also indicated that in arriving at an efficient allocation of sampling effort, that each scale of variation must be considered. Furthermore, careful consideration of precision in the context of data quality objectives (DQOs) (See Section 2, Quality Assurance and Quality Control) will improve the qualitative or quantitative evaluations of ichthyoplanktonic population studies.

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SECTION 10

FISH HEALTH AND CONDITION ASSESSMENT METHODS[1]

10.1 Introduction

10.1.1 The fish health and condition assessment methods provide relatively simple and rapid indication of how well fish live in their environment. They are manifestations of biochemical and physiological alterations expressed at the organism level. Goede and Barton (1990) and Goede (1992) review various types of condition indices that can be used to assess stress in fish, and they also describe an empirical necropsy-based system of organ and tissue indices that provides a fish health and condition profile of fish populations. External aspects, blood parameters, and the normal appearances of internal vital organs are assumed to indicate that a fish population is in harmony with its environment, or if the fish have been challenged, that the animals have not been stressed enough to cause obvious structural changes. When the necropsy system is applied in the field, departure from normal growth, bioenergetic state, and general homeostasis can be detected, as well as the presence of infectious agents in fish. Advantages of these methods over physiological monitoring or community analyses are that they are simple to use, requires little training, and does not need costly, sophisticated equipment. The fish health and condition assessment could be used routinely in research, culture, management, and regulatory programs to establish a data base for evaluating whether a fish population is coping successfully with its environment.

10.1.2 Novotny and Beeman (1990) evaluated the fish health and condition assessment methods on juvenile chinook salmon (*Oncorhynchus tshawytscha*) that were reared in net pens in the Columbia River, Washington, and they found the procedures were efficient in assessing the condition of fish held under various rearing conditions. They, furthermore, concluded that the simplicity of the methods makes them useful for monitoring fish in culture facilities and fish from wild stocks. These methods are meant to be used by investigators who routinely work in the field and for determining the general health and condition of a group of fish.

10.1.3 It is important that the investigator be able to use the minimum of equipment needed for these methods and to be able to recognize gross appearance or differences of systems in tissues and organs. The investigator does not specifically have to be able to diagnose the cause or causes of the condition. If a departure from normal condition is evident in a significant proportion of the fish population, it is appropriate that a specialist be called to help determine the cause of the variation.

10.1.4 A list of equipment and materials for the fish health and condition assessment is found in Table 1.

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[1]Adapted from Goede and Barton (1990) and Goede (1992).

TABLE 1. EQUIPMENT AND MATERIALS FOR FISH HEALTH AND CONDITION ASSESSMENT

- Microhematocrit Centrifuge
- Microhematocrit tubes[a,b]
- Critoseal clay to seal hematocrit tubes
- Microhematocrit tube reader
- 1.0 percent sodium or ammonia heparin solution
- Hand held serum protein refractometer
- Lens paper
- Bunsen Burner to sharpen hematocrit tubes
- Sharp/blunt scissors
- Dissecting forceps (preferably a small "mouse tooth type")
- MS-222 or comparable anesthetic[c]
- Metric scale to weigh individual fish
- Fish measuring board
- Hand held magnifying glasses for small fish
- Buckets and tubs to handle fish
- Calculators with standard deviation button

Heart puncture:

[a]Using capillary tubes: Sharpen capillary tubes and re-heparinize sharpened end at least 1/3 to 1/2 of tube.

[b]Heparin:

Use 0.1 gm of heparin to 10 mL distilled water. Fill capillary tube 1/3 to 1/2, then drain back into heparin solution. This solution can be reused again for rest of tubes. Remove all heparin from tubes and dry tubes overnight.

[c]MS-222 Mixture:

To incapacitate but not kill. A solution in excess of 50 mg/L (ppm) MS-222 is recommended. Use 4 times this amount for lethal dosage.

10.2 Sampling and Collection of Fish

10.2.1 The desired sample size for this procedure is 20 fish of the same species. When working with free-ranging populations, it is not always easy to obtain fish. In the field, the samples often are collected from fish captured in routine netting or electrofishing operations. In some sampling situations 20 fish of the same species might be difficult to collect. In this circumstance the investigator must work with what is caught.

10.2.2 The composition of the fish sampled (e.g., age class, length grouping, etc.) depends upon the data quality objectives (DQOs) of the investigation and upon what fish are available (see Section 2, Quality Assurance and Quality Control).

10.3 Handling of Fish

10.3.1 The ideal collection is taken alive and handled carefully until they can be anesthetized. The fish should be immobilized shortly after capture with an appropriate anesthetic, e.g., tricaine MS-222 (see Table 1).

10.4 Sampling and Reading of Blood

10.4.1 Blood should be collected by cardiac puncture with a sharpened, heparinized microhematocrit tube. If blood is needed for purposes in addition to those of this procedure, a larger volume can be sampled with a syringe and needle from the caudal vasculature. The microhematocrit tube can then be filled from that volume with the syringe. The tube, once filled, is plugged on one end using a commercial clay, prepared and sold for that purpose. It is advised that you place the filled tubes upright in a rack with numbered holes to await placement into a centrifuge. Every effort should be made to keep the tubes in order so that they can be accurately matched to the fish from which they were taken. The tubes are then placed in the numbered slots of a microhematocrit centrifuge and spun for five minutes. A typical microhematocrit centrifuge develops approximately 13,000 G. Erythrocytes (red blood cells) have been shown to "swell" when exposed to carbon dioxide. Thus, it is important that the tubes be spun within one hour of sampling. Once the tubes have been centrifuged they can be transported and read in a more convenient location but they should be read within two hours and definitely before the plasma begins to coagulate. Once the blood fractions have been separated by centrifuging, you can remove the tubes and place them again in the numbered rack. Always keep them in the order in which they were collected so they can be matched with the individual fish from which they were collected. The tubes can be kept until later or one can proceed to read the hematocrit, leucocrit, and plasma protein.

10.4.2 Hematocrit is the packed red cell volume of the blood and is expressed as a percentage of the total column. It is obtained by placing the centrifuged tubes on a microhematocrit reader. These are available in several styles and costs but the simple plastic reader cards containing a nomograph are preferred. The tube is placed on the card so that the bottom of the red (erythrocytes) portion of the column is at the zero line and the meniscus of the clear plasma portion of the column is on one hundred percent. The

location of the top of the red portion indicates the volume percentage of red blood cells or hematocrit.

10.4.3 There is usually a small "buffy or gray" zone just above the red zone. This is composed of the leucocytes or white blood cells and is used to estimate the leucocrit or percent leucocytes in the packed column. The card reader can be used to read this, and a small magnifying glass is helpful.

10.4.4 Next, the protein content of the plasma is determined. This is done by carefully breaking the hematocrit tube just above the "buffy" zone to obtain only the clear plasma fraction. Be sure that there are no small glass fragments on the broken end and then express the clear plasma onto the glass surface of the hand-held protein refractometer. Read the weight/volume percent of protein. The refractometer must be calibrated before use. To do this, place a few drops of distilled water on the prism surface and adjust the boundary line to the "w" or "wt" mark with the adjusting screw. Some instruments have a thumbscrew and some require a small screwdriver. The investigator should consult the manual supplied with the unit in question. The instrument should be cleaned between readings with lens paper to avoid scratching the surface. The surface should be cleaned with water and dried with lens paper after every use.

10.5 Length and Weight Measurements

10.5.1 The lengths and weights can be measured immediately after the blood samples have been collected for hematocrit determinations.

10.5.2 The total length of each fish should be determined in millimeters and the weight in grams. This is fairly straight forward but might be pointed out that the length and weight were initially included in the procedure to see if there was any correlation between fish size and the other parameters.

10.5.3 If it is desired to obtain an accurate estimate of size of the fish in the population, more lengths and weights should be taken through non-lethal sampling. The computer program, discussed later, will accommodate 60 fish.

10.6 External Examination

10.6.1 When the fish (Figure 1. External features of a composite fish) are laid out in front of you it is the best time to make general observations about the fish. Record general remarks about fins, skin, and other external features before you begin the specific observation of particular organs and systems. Important conditions to note are deformities, scale loss, and external parasites. These observations are carried as remarks in the data base. It must be noted here that primary observations included in this procedure were intended to permit some inference with respect to health and condition of the fish. This is only one aspect of "quality". Observations relative to esthetics are included as remarks only. Fish species (e.g., Catostomidae, Cyprinidae) develop cornified epithelial tubercles and engage in nuptial bouts. If external lesions or scars are observed in some specimens, the possibility of external anomalies related to spawning behavior should be noted.

10.6.2 Begin the observations as outlined in the classification system (Table 2). Be sure to record all observations using the abbreviations or codes listed on the classification scheme. This is necessary for subsequent entry into the computer program (see AUSUM PROGRAM USE, page 270). If the observation does not seem to fit any of the listed categories, list it as OT which indicates "other". If you use this category be sure to describe it in the remarks column. It is much easier for the recorder if you proceed routinely in the same order laid out on the fish necropsy (postmortem examination) worksheet (Figure 2). There are many systematic approaches to the order of the procedures, but Goede (1992) has found it more efficient to "open" all of the fish first with the use of sharp/blunt scissors by making a ventral cut from the anal vent forward to the pectoral girdle, cutting closely to one side of the pelvic girdle. A short distance of the "hind gut" is opened with this first cut to permit later observation. Do not insert the scissors so far that the internal organs are damaged. The fish are opened and laid down in front, in proper order, to wait the final inspection.

10.6.3 Take into consideration the circumstances of the collection. If the fish were collected dead, you must be aware of the often subtle differences this can make in appearance of organs and tissues while still permitting valid observation within the context of this procedure. A photographic, colored atlas (Goede, 1988) of necropsy classification categories has been prepared and may be obtained from Ronald W. Goede, Utah Division of Wildlife Resources, Fisheries Experiment Station, 1465 West 200 North, Logan, Ut. 84321-6233. The cost of the atlas is $80.00.

10.7 External Organs

10.7.1 Eyes

10.7.1.1. Normal (N) - no aberrations in evidence. Good "clear" eyes.

10.7.1.2 Exopthalmia (E1 or E2) - Swollen, protruding eye. More commonly referred to as "popeye". It is coded as E1 or E2. This refers to the presence of exopthalmia in one eye or two eyes.

10.7.1.3 Hemorrhagic (H1 or H2) - Refers to bleeding in the eye. "Blind" (B1 or B2) - This is a very graphic category and you need not know whether the eye is functionally blind. It generally refers to opaque eyes, and the opacity is not important here.

10.7.1.4 "Missing" (M1 or M2) - An eye is actually missing from the fish.

10.7.1.5 "Other" (OT) - Any manifestations which do not "fit" the above. Describe in the remarks column.

10.7.2 Gills

10.7.2.1 Normal (N) - no apparent aberrations in gills. Be very careful in this observation. The gill can easily be effected by the manner in which the fish is handled during and after collecting.

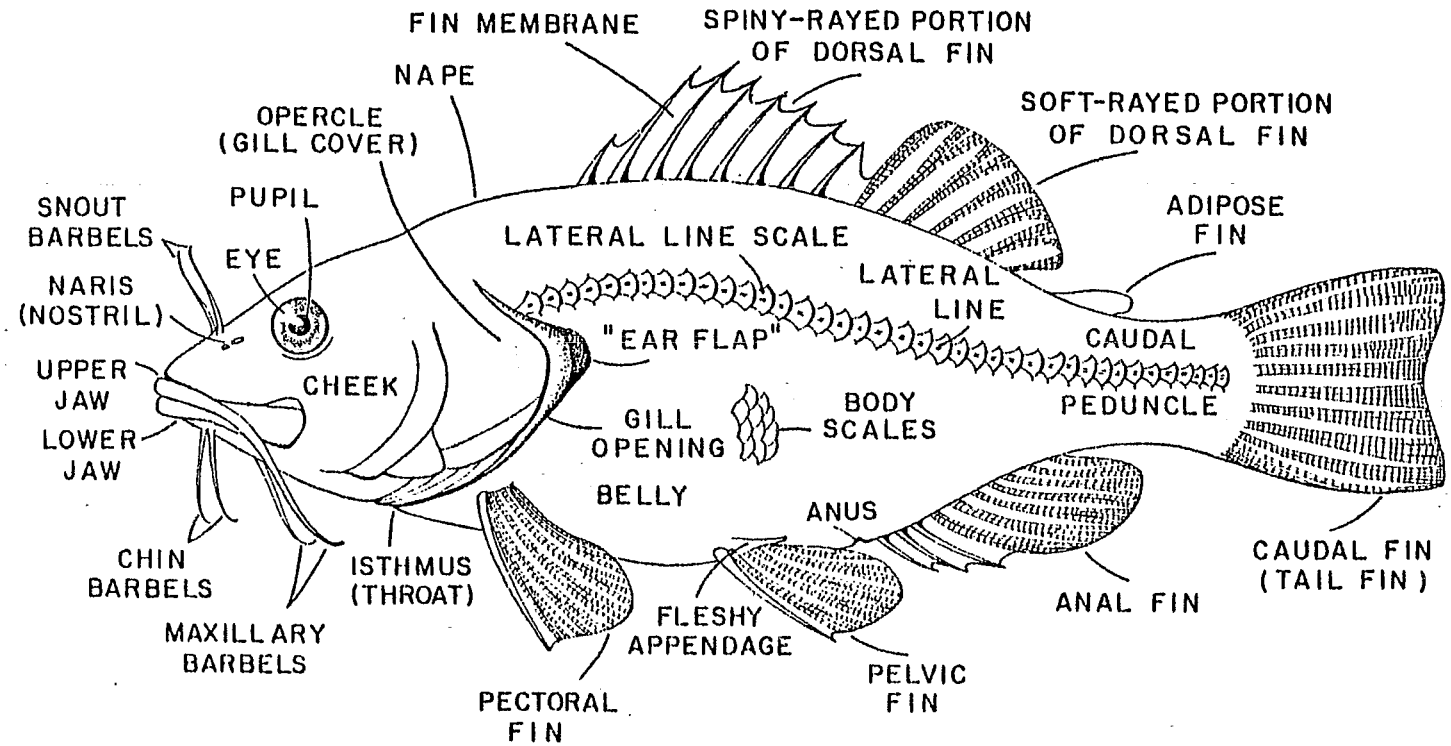

Figure 1. External features of a composite fish. From Lagler (1962), Atlas of Fish Anatomy, Plate 1, Michigan Fisheries No. 5, Department of Fisheries, School of Natural Resources, The University of Michigan, Ann Arbor, MI.

TABLE 2. NECROPSY CLASSIFICATION OUTLINE

Length:	Total length in millimeters
Weight:	Weight in grams
Ktl:	$= \frac{W \times 10^5}{L^3}$ See Subsection 10.9.
Eyes:	Normal (N), Exopthalmia (E1, E2), Hemorrhagic (H1, H2), Blind (B1, B2), Missing (M1, M2), Other (OT)
Gills:	Normal (N), Frayed (F), Clubbed (C), Marginate (M), Pale (P), Other (OT)
Pseudobranch:	Normal (N), Swollen (S), Lithic (L), Swollen and Lithic (S&L), Inflamed (I), Other (OT)
Thymus:	No Hemorrhage (0), Mild Hemorrhage (1), Severe Hemorrhage (2)
Fins:	No active erosion or previous erosion healed over (0), Mild active erosion with no bleeding (1), Severe active erosion with hemorrhage and/or secondary infection (2)
Opercles:	No shortening (0), Mild shortening (1), Severe shortening (2)
Mesentery Fat:	Internal body fat expressed with regard to amount present: 0 - None 1 - Little, where less than 50% of each cecum is covered 2 - 50% of each cecum is covered 3 - More than 50% of each cecum is covered 4 - Ceca are completely covered by large amount of fat
Spleen:	Black (B), Red (R), Granular (G), Nodular (NO), Enlarge (E), Other (OT)
Hind Gut:	No inflammation (0), Mild inflammation (1), Severe inflammation (2)
Kidney:	Normal (N), Swollen (S), Mottled (M), Granular (G), Urolithic (U), Other (OT)
Liver:	Red (A), Light red (B), "Fatty" liver, "Coffee with cream" color (C), Nodules in liver (D), Focal discoloration (E), General discoloration (F), Other (OT)

TABLE 2. NECROPSY CLASSIFICATION OUTLINE (CONTINUED)

Bile:	0 -	Yellow or straw color, bladder empty or partially full
	1 -	Yellow or straw color, bladder full, distended
	2 -	Light green to "grass" green
	3 -	Dark green to dark blue-green
Blood:	Hematocrit -	Volume of red blood cell (erythrocytes) expressed as percent of total blood volume. "Buffy" zone of the packed cell column.
	Leucocrit -	Volume of white blood cells (leucocytes) expressed as percent of total blood volume. "Buffy" zone of the packed cell column.
	Plasma Protein -	Amount of protein plasma, expressed as gram percent (grams per 100 mL).

Fish Necropsies

Wildlife Resources
2/91 FES-25

Date_____ Unit_____ Strain_____ Quality Control #_____
Location_____ Fish Source_____ Age_____ Case History #_____
Mark/Lot_____ Hat. Date_____ Tissue Collection #_____
Investigator(s)_____ Water Temp._____ Water Hardness_____
Reason for Autopsy_____ Remarks_____

Smp no	Lgth mm	Wght gm	Ktl	Eye	Gill	Psbr	Thy	Fat	Spl	Hind Gut	Kid	Liv	Bile	Sex	Hem	Leu	Pl. Pro	Fin	Opl	Remarks
1																				
2																				
3																				
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20																				

GENERAL REMARKS

Fins_____ Gonads_____

Skin_____ Other_____

Figure 2. Fish necropsy worksheet.

10.7.2.2 "Frayed" (F) - This generally refers to erosion of tips of gill lamellae resulting in "ragged" appearing gills. Mere separation of gill lamellae can be construed to be "frayed" but that condition may have been caused by something as simple as the manner in which the gill was exposed by the investigator.

10.7.2.3 "Clubbed" (C) - This refers to swelling of the tips of the gill lamellae. They can often appear bulbous or "club-like". The causes are not pertinent until interpretation is considered.

10.7.2.4 "Marginate" (M) - a graphic description of a gill with a light discolored margin along the distal ends or tips of the lamellae or filaments. Margination can be and often is associated with "clubbing". If both (C) and (M) seem to apply, it is not a problem. It is important that you note that it was not normal. Use the one which seems most appropriate.

10.7.2.5 "Pale" (P) - This refers to gills which are definitely very light in color. Severe anemia can result in gills which are discolored to the point of being white. Severe bleeding induced during sampling of blood can also result in somewhat pale gills. Gills begin to pale somewhat after death also. This is not uncommon in fish taken from nets. All of this should be considered in making the observation.

10.7.2.6 Other (OT) - Any observation which does not fit above. Describe in remarks.

10.7.3 Pseudobranchs (The pseudobranch is located dorsally and anterior to the gills in the branchial cavity and can be easily observed under the opercula.) Some species lack pseudobranchia entirely.

10.7.3.1 Normal (N) - The normal pseudobranch is quite "flat" or even concave in aspect and displays no aberrations.

10.7.3.2 Swollen (S) - The "swollen" pseudobranch is convex in aspect and not difficult to discern upon close examination.

10.7.3.3 Lithic (L) - Mineral deposits in pseudobranchs, manifested by appearance of white, somewhat amorphous spots or foci.

10.7.3.4 Swollen and Lithic (S&L) - Lithic pseudobranchs are often also swollen.

10.7.3.5 Inflamed (I) - This is a generic use of the term, inflamed, and would more appropriately be termed "redness" because it also includes observations of hemorrhage and any other cause of redness. The term, "inflamed" has been traditionally used to describe this condition and is thus contained for that reason.

10.7.3.6 Other (OT) - This term will cover any manifestation observed in the pseudobranch which is not covered in the categories. Be sure to describe in remarks.

10.7.4 Thymus (Assessment of the thymus involves degree of petechial or "pinpoint" hemorrhage).

10.7.4.1 No Hemorrhage (0) - The thymus displaying no hemorrhage is considered to be a normal condition, although this assumption is still under investigation. Caution must be exercised here because when the thymus involutes or ceases to function there is no observable petechial hemorrhage. This happens normally as the fish mature. In salmonids involution of the thymus is thought to happen at two or three years of age but there is considerable disagreement among investigators about this point.

10.7.4.2 Mild Hemorrhage (1) - A few red spots or petechial hemorrhages in evidence. This might be only two or three small spots.

10.7.4.3 Severe Hemorrhage (2) - Many "pin point" hemorrhages in evidence with some of them coalescing. The general area may also have a swollen tumescent appearance but that should be recorded in remarks.

10.7.5 Fins - It must be remembered that this particular assessment procedure is concerned primarily with health and condition. It is not concerned with aesthetic values. Eroded or "ragged" fins are definitely indicative of a departure from normal condition and health. Previously eroded fins which are completely healed over and showing no evidence of the active erosion are, for the purposes of this assessment, considered normal. The evaluation of fins is relative to the degree of active erosion process in evidence. For the purposes of this procedure the number and location of fins involved is not significant. If only one fin is displaying active erosion, the observation must be ranked and recorded. If several fins are displaying erosion with unequal severity, the observation must refer to the most severe in evidence. This unequal nature of the observations, in this case, is less significant in a full 20 fish sample. The classification is as follows:

10.7.5.1 No Active Erosion (0) - Normal appearing fins with no active erosion. This would include previously eroded fins which were completely healed over.

10.7.5.2 Mild active erosion (1) - Active erosion process but no hemorrhage or secondary infection in evidence.

10.7.5.3 Severe Active Erosion (2) - Active erosion with hemorrhage and/or secondary infection in evidence.

Note: Make a general remark relative to which fins were involved and any other observation of special significance. There is a space for this type of entry at the bottom of the data collection worksheet. This is particularly important in the summary.

10.7.6 Opercles (It is necessary only to observe the degree of shortening of the opercles. The classification is as follows:)

10.7.6.1 Normal Opercle (0) - No shortening; gills completely covered.

10.7.6.2 Slight Shortening (1) - Slight shortening of the opercle with a very small portion of the gill exposed

10.7.6.3 Severe Shortening (2) - Severe shortening of the opercles with a considerable portion of the gill exposed.

10.8 **Internal Examination (or Necropsy)**

10.8.1 Figure 3 reveals the key internal anatomical features of a typical soft-rayed fish (brook trout), and Figure 4 displays the anatomical features of a characteristic spiny-rayed fish (largemouth bass).

10.8.1.1 If the fish was not "opened" as suggested above, it should be done now to permit access to the internal systems. Remember to proceed, where possible, in the order listed on the data sheets. This facilitates recording. The order was established beginning posteriorly with the mesenteric fat depot, proceeding anteriorly through the spleen and hindgut, to the kidney, liver, and gall bladder, to the gonads for determination of gender and state of development. At this point, it is wise to observe the mesentery tissue for hemorrhage or inflammation and record in remarks if not normal.

10.8.2 Mesenteric Fat

10.8.2.1 The ranking of mesenteric fat depot has been developed around salmonid fishes with prominent pyloric caeca. It must be noted here that there is great variation among the different fish species in the way that they store this fat. If the system is to be applied to other groups of fishes, alternate ranking criteria will have to be developed. It should be further noted that as long as the ranking is 0 through 4 the computer program, AUSUM, for summarizing data, can still be used. The following ranking system was developed for the rainbow trout but has been applied with minor variations to all major groups of salmonids.

0 - No fat deposited around the pyloric ceca. If there is no fat deposit in evidence anywhere in the visceral cavity it is clearly a "0" fat.

1 - Slight, where less than 50% of each cecum is covered with fat. There are cases where there will be no fat in evidence on the ceca, but there will be a slight fat currently classes as a "1".

2 - 50% of each cecum is covered with fat.

3 - More than 50% of each cecum is covered with fat.

4 - Pyloric ceca are completely covered by a large amount of fat.

10.8.3 Spleen

Black (B) - The "black" is actually a very dark red color of the spleen.

Red (R) - Red coloration of the spleen. There is subjective variation among

investigators as to whether the spleen is black or red, but both conditions are considered normal

Granular (G) - Granular or "rough" appearance of the spleen.

Nodular (NO) - The spleen contains or manifests fistulas or nodules of varying sizes. These are often cysts, such as those encountered with mycobacterial infections.

Enlarged (E) - Spleens can, on occasion, be significantly and noticeably enlarged.

Other (OT) - Occasionally there are observable, gross aberrations which do not fit the above. There may be spleens with a gray mottling and some with very small spleens. These should be classed as "other" and described in remarks.

10.8.4 Hindgut

10.8.4.1 A short distance of the hind gut should be "opened". This should, in fact, have been accomplished as mentioned above when the body cavity is incised. If not, it must be opened to expose the "inner lining" or mucosa. Using the handle of a forceps or some other appropriate blunt instrument, lightly "scrape" out the contents of the hindgut so that you can observe relative reddening or inflammation.

No inflammation (0) - No inflammation or reddening of the hindgut.

Slight inflammation (1) - Mild or slight inflammation or reddening of the hindgut.

Severe inflammation (2) - Considerable, severe inflammation or reddening of the high gut.

10.8.5 Kidney

Normal (N) - Good firm dark red color lying relatively flat dorsally in the visceral cavity along the length of the ventral surface of the vertebral column. It will be necessary to pull the swimbladder and some of the mesentery aside to expose the kidney to view.

Swollen (S) - Enlarged or swollen wholly or in part.

Mottled (M) - Gray discoloration, mottled or "patchy" in appearance ranging from scattered patches of gray to total gray discoloration. This is not to be mistaken with the superficial gray appearance induced by the mesenteric membrane on the surface of the kidney. This should be moved aside before observation is recorded.

Granular (G) - The kidney may have a "granular appearance and texture. This may be induced by granulomatous concretions.

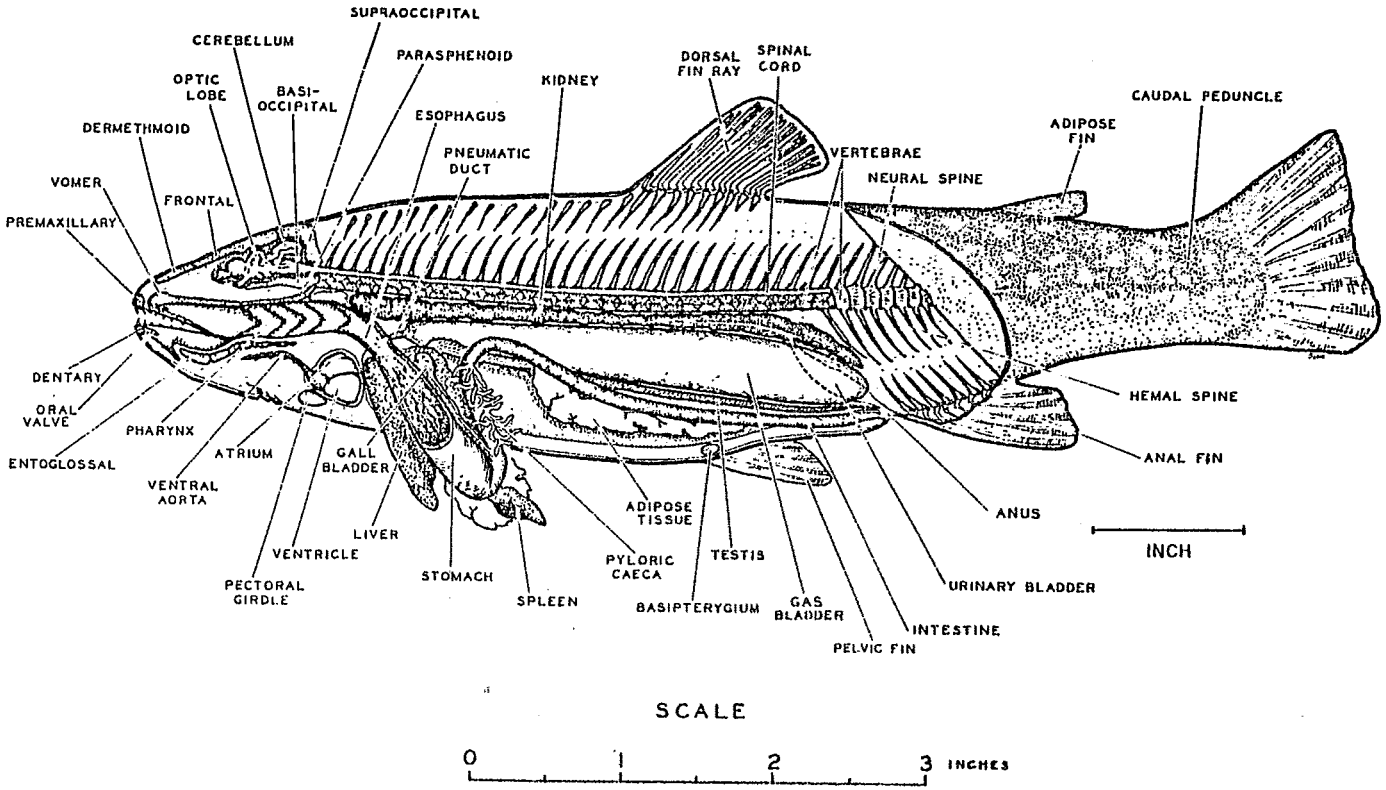

Figure 3. Anatomy of a soft-rayed bony fish, the brook trout, *Salvelinus fontinalis*. From Lagler (1962), Atlas of Fish Anatomy, Plate IV, Michigan Fisheries No. 5, Department of Fisheries, School of Natural Resources, The University of Michigan, Ann Arbor, MI.

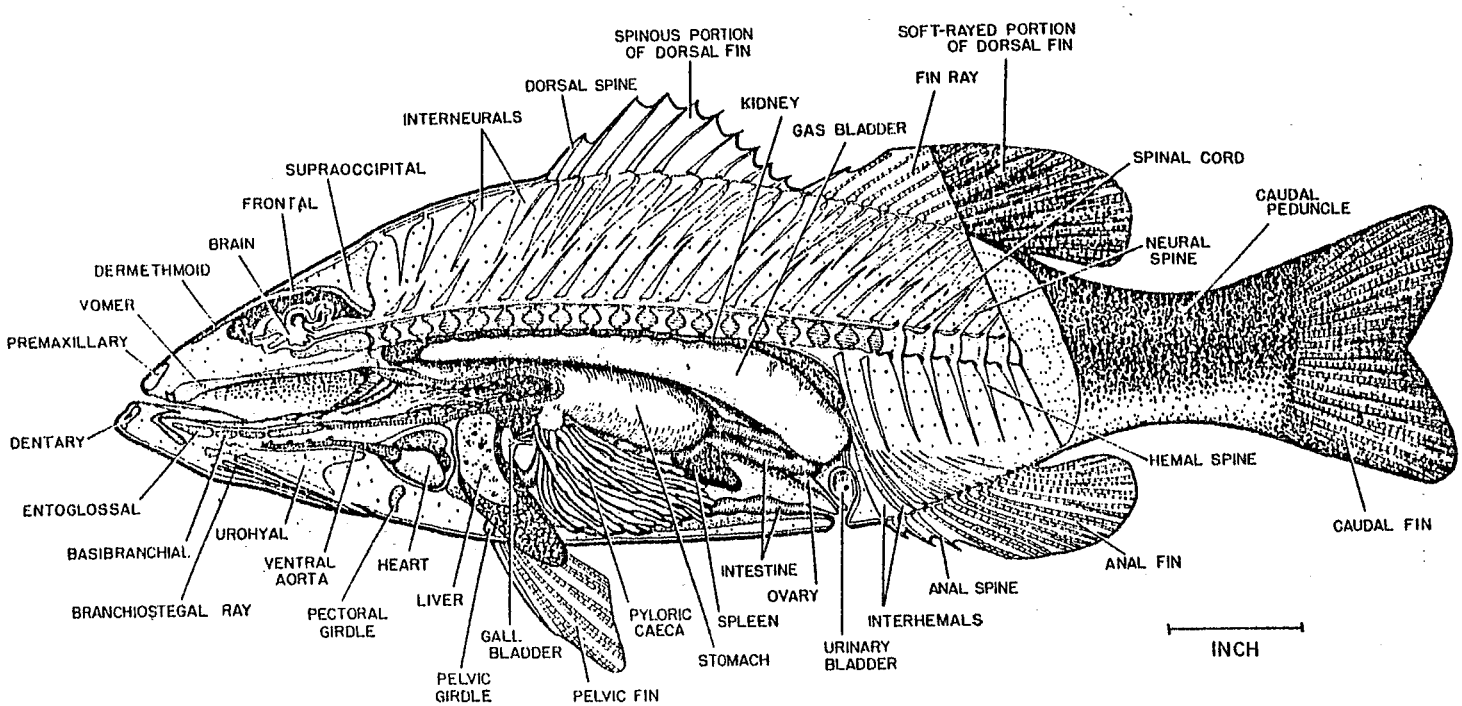

Figure 4. Anatomy of a spiny-rayed bony fish, the largemouth bass, *Micropterus salmoides*. From Lagler (1962), Atlas of Fish Anatomy, Plate V, Michigan Fisheries No. 5, Department of Fisheries, School of Natural Resources, The University of Michigan, Ann Arbor, MI.

Urolithiasis (U) - This condition is known as nephrocalcinosis and involves deposition of a white or "cream-colored" amorphous mineral material in the tubules of the kidney. It can range in appearance from very small white spots to severe involvement with very large "serpentine" deposits. These sites of deposition are not to be confused with the Stannius bodies or corpora of Stannius which are present in salmonid kidneys and have an endocrine function. The Stannius bodies are generally not associated with the tubules and usually occur at the "edges" in an area about midway along the kidney. They appear more globular than do the urolithic deposits.

Other (OT) - This is used to class any aberrations which do not fit into the above scheme. Record it as T and describe it in the remarks.

10.8.6 Liver

10.8.6.1 The appearance of the liver can very well be an artifact of the sampling and the investigator should take that into consideration. Appearance may, for example, vary with the length of time from collection to observation. It also depends to a certain extent on the nature and extent of the loss of blood during sampling. For this reason, categories "A" and "B" are both considered as normal.

A - Normal. Good solid red color.

B - Lighter or less vivid red color than in A. Not so pale as to be classed as general discoloration. Still considered to be normal.

C - "Fatty" liver. Light tan color, such as "coffee with cream".

D - Nodules in the liver, i.e., white mycobacterial cysts and incipient nodules, such as those in hepatoma.

E - Focal discoloration. Color change in the whole liver.

OT - Aberration or deviation in liver which does not fit into above scheme. Class as OT and describe in remarks.

10.8.7 Bile

10.8.7.1 The bile is observed indirectly through observation of the color of the gall bladder. The ranking scheme considers "fullness" of the bladder and degree of "green".

0 - Yellow or straw color; bladder empty or only partially full.

1 - Yellow or straw color; bladder full, distended.

2 - Light green to "grass" green.

3 - Dark green, dark blue-green.

10.8.8 Sex

10.8.8.1 Observation of the gonads when possible should permit determination of gender of the fish. It is also recommended that a remark be entered if the fish are "ripe" or approaching spawning condition.

Male (M) - Observation of testes

Female (F) - Observation of ovaries

10.8.9 General Observations and Remarks

10.8.9.1 Anything which appears to be abnormal should be noted. It is recommended that the mesenteric tissues in the visceral cavity be checked for hemorrhage and inflammation and if these conditions are present, they should be so noted in general remarks.

10.9 Calculation and Summary of Fish Health and Condition Assessment

10.9.1 Now that the fish have been sampled, examined, and as the observations have been made and recorded on the worksheets, all the necessary calculations should be made and summarized.

10.9.2 The format for "Summary of Fish Necropsy" is presented in Table 3. That form will be used for the purpose of this discussion. The section dealing with the heading information will be discussed in a later section, as will the use of the computer. It is more than helpful to use a pocket calculator which is provided with a function for standard deviation.

Ktl - The values of "K" (= coefficient of condition for the metric system) have been used widely by fishery biologists to express the relative robustness of fishes. Also, the values of "K" have been used additionally for age and growth studies to indicate the suitability of an environment for a species by a comparison of the value for a specific habitat with that of other aquatic habitats. The value for Ktl is actually expressed here as Ktl x 10^5. This was done to mitigate the problem of carrying a large number of decimal places in the records. The equation used to obtain the value is as follows:

$$Ktl \times 10^5 = \frac{W \times 10^5}{L^3}$$

Where W = Weight in grams

L = Total length in millimeters

10.9.3 The condition factor used in the English system is Ctl. This value tends to be used by some fish culturists. Ctl x 10^4 is obtained by multiplying (Ktl x 10^5) bu 3.613.

10.9.3.1 The mean, standard deviation and coefficient of variation are to be calculated for the length, weight, Ktl, hematocrit, leucocrit, and plasma protein.

Mean - The mean is determined by totaling all of the values for the observations and dividing by the number of the observations.

Standard deviation - Indepth discussion of the standard deviation is beyond the scope of this presentation. A pocket calculator equipped with a standard deviation function permits very easy determination of that value. To calculate the value without the aid of such a tool would require a prohibitive amount of time.

Coefficient of variation - This value is defined as the ratio of standard deviation to the mean. To obtain this value, divide the standard deviation by he mean and multiply by 100 to convert the answer to percent. This value expresses variation as percent of the mean. Units are not used. Record the results on the necropsy summary sheet.

10.9.4 Values As Percents Of Total Sample

10.9.4.1 This portion expresses the percent of the total sample constituted by each category. As an example, you can consider the eyes. The number of fish with normal eyes divided by the total number of fish in the sample yields the percent normal and should be recorded. The percent of fish with one blind eye (B1) is calculated in the same manner and so on. This is repeated for each category of organ or tissue observation and results are recorded on the necropsy summary sheet.

10.9.5 Summary of Normals

10.9.5.1 This section of the necropsy summary is included to facilitate easier reading with respect to departure from normal. This also facilitates a more accurate summary for those organs and tissues with more than one category considered to be normal, i.e., liver and spleen. It must be further noted that "0" is considered to be normal with respect to degree of hemorrhage in the thymus and degree of inflammation in the hind gut. "N", when present, is understood to be normal and the percent of the sample is indicated in the value distributions. In these instances, merely carry that figure down to the summary of normals. In the following instances the "normal" is not so readily apparent:

Spleen - Black, red, and granular are all considered to be normal manifestations of spleen condition. If the sample demonstrated 70% black, 15% red, and 15% granular, you would combine these and list 100% normal in the summary tables.

Liver - The A and B categories are both considered to be normal. Combine these normals in the summary or normals.

Thymus - The categories included in the observation of the thymus represent degree of petechial or "pin-point" hemorrhage. It is, therefore, understood that "0" hemorrhage is normal. The percent of fish with "0" thymus is carried down to the section dealing with summary of normals.

Hindgut - Degree of inflammation is being measured here so a reading of "0",

TABLE 3. SUMMARY OF FISH NECROPSY

LOCATION:_____ QUALITY CONTROL #_____

Species:_____ Autopsy Date:_____ Sample Size:_____

Strain:_____ Hatching Date:_____ Age:_____

Mark/Lot:_____ Unit:_____ Case History #:_____

Fish Source:_____ Egg Source:_____

Water Temp.:_____ Water Hardness:_____ Investigator(s)_____

Reason for Autopsy:_____

Remarks:_____

MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Length		
Weight		
Ktl*		
Ctl**		
Hematocrit		
Leucocrit		
Plasma Protein		

*Expressed as Ktl times 10 to the fifth power
**Converted from Ktl: expressed as Ctl times 10 to the fourth power

VALUES AS PERCENTS OF TOTAL SAMPLE

EYES	GILLS	PSEUD	THY	MES FAT	SPL	HIND GUT	KID	LIV	BILE	FIN	OPER
N	N	N	0	0	B	0	N	A	0	0	0
B1	F	S	1	1	R	1	S	B	1	1	1
B2	C	L	2	2	G	2	M	C	2	2	2
E1	M	S&L	-	3	NO	-	G	D	3	-	-
E2	P	I	x=	4	E	x=	U	E	-	x=	x=
H1	OT	OT		-	OT		OT	F	x=		
H2				x=				OT			
M1											
M2											
OT											

SUMMARY OF NORMALS

				xxxxx					xxxxx		

SUMMARY OF MEANS

xxxxx	xxxxx	xxxxx			xxxxx		xxxxx	xxxxx			

SEX M: F: U:

INDEX SUMMARY

Fat Index	Gut Index	Normality Index
Bile Index	Opercle Index	Severity Index
Thymus Index	Fin Index	

GENERAL REMARKS

FINS:

SKIN:

GONADS:

OTHER:

indicating no inflammation, would be considered to be the normal. The percent of the sample with "0" is carried down to the summary of normals.

Fins - Degree of active erosion is being measured here so a reading of "0", indicating no active erosion would be considered to be normal. The percent of the sample with "0" is carried down to the summary of normals.

Opercles - The relative degree of shortening of the opercles is being assessed here so a reading of "0", indicating no "shortening", would be considered normal. The percent of the sample with "0" is carried down to the summary of normals. Mesenteric Fat and Bile - There are no normal categories for mesenteric fat deposit and bile.

10.9.6 Summary of Means

10.9.6.1 This Subsection deals only with categories quantifying relative degrees of some manifestation. Those categories involved in this section are thymus, mesenteric fat depot, hind gut, and bile. This appears to be confusing to people but the means are obtained in the usual manner. Total the values in the appropriate columns and divide that total by the number of observations. The "x" listed in the summary section dealing with values as percents of total sample is the mean of the values and should be carried down to the summary of the means. Numerous investigators using these systems have referred to these means as indices, i.e., thymus index, fat index, etc.

10.9.7 Index Summary

10.9.7.1 The fat index and the bile index are the same as the means for those observations as listed in the summary of means. The thymus, gut, fin, and opercle indices are calculated by dividing the mean (listed in the "summary of means") by the highest level possible and multiplying it by 100 to express it as a percent. If, for example, the thymus mean would be .75, one would divide this by 2 (the highest level possible) and multiply by 100 to yield 37.5 percent. This then becomes the thymus index. The severity index is calculated by averaging the thymus, gut, fin, and opercle indexes. The normality index is calculated by averaging the normals as listed in the summary of normals. All of the indices are to be placed in the index summary of the report for clarity.

10.9.8 Miscellaneous Observations

Sex - The relative proportion of gender should be entered if that information is available. Here, as above, merely count the numbers of each category and divide by the number of fish in the sample. If the investigator(s) is unable to determine the gender, be sure to enter "U" for unknown.

General remarks - Any remarks made in the remarks column of the worksheet and any general remarks, the investigator wishes to make should be made in this section. There is a great deal of latitude here. One might, for example, list under "Fins" that 10 fish or 50 percent of fish had badly eroded, bleeding pectoral fins.

10.9.9 Heading Information

10.9.9.1 The information entered into the heading of the worksheet and summary is very important. It is that information which identified the investigation and which ties it into the greater data base which will permit future recall, manipulation, etc. It is very important that standard terminology, abbreviations, ID systems, and cross-referencing be developed and used to facilitate use in a data base. This is particularly true where computers are to be used. It is likely that even more information will be saved in relational data bases to enhance the value of the information. It should be remembered that the worksheet and necropsy summary were developed to be used both in hatcheries and free-ranging populations. This is in evidenced more in the heading information than in any other portion of the investigation. Many of the categories are self-explanatory, but some are confusing enough to require a brief description. The following is a list of categories with brief statements on some of the less obvious:

Location - Site or location of the study, such as Midway Hatchery or Green River.

Quality Control Inspection No. - This is the number assigned to this particular investigation.

Species - Species of fish being investigated. If abbreviations are to be used, they should be standardized, i.e., RBT for rainbow trout.

Strain - Strain of fish under investigation, i.e., Sand Creek.

Necropsy Date - Date the necropsy was performed.

Sample Size - Number of fish in that particular sample.

Age - Age of fish using standard expression, such as months.

Mark/Lot - Identifying mark, such as dye mark or fin clip in free-ranging fish or a production lot number in a hatchery.

Unit - Raceway number in a hatchery or specific station location, such as Little Hole, Green River.

Water Temperature - The temperature of the water at the sampling site.

Fish Source - This generally refers to the original source of fish. The investigation may be on fish in the Green River, but they may have been stocked by a hatchery. The hatchery would be listed as the fish source in this case. If they were natural reproduction, the Green River would be listed as the fish source.

Egg Source - This refers to the original source of the eggs. In the example above, the eggs may have been shipped to the hatchery by a brood station at some other location. That brood station would be listed as the egg source.

Water Hardness - This is expressed as parts per million (ppm).

Investigator(s) - Name of all investigators.

Hatching or Station Date - The date fish samples for collected.

Reason for Necropsy - Indicate reasons; such as research, routine, trouble shooting, etc.

Remarks - Any information which might have an effect on interpretation of results, i.e., fish were electro-shocked and hauled in tub for half an hour or fish were taken in an overnight gill net set.

Tissue Collection No., Disease Survey No., Case History No., and Custody No. - These are all cross-references to other investigations which should be carried in the data base and which might have bearing on interpretation of results.

Purpose Code - Relates somewhat to "reason for Necropsy". It is included because it makes it possible to do better sorts and queries later when working with the assimilated database. It is very important that his be filled in. A single letter coded is used as listed below:

A = Routine quality control inspection

B = Prestocking quality assessment

C = Trouble shooting

D = Research or special investigation

E = Administrative request for quality control

O = Other, make entry in Remarks area

10.9.9.2 Other letters will be included later as we add letters more relevant for fisheries biologists. This is why "O" is used for "other" rather than "F". It is possible in this case to use more than one letter in combination if it seems necessary. It may, for instance, seem appropriate to use AB because the last routine quality control inspection may also be a prestocking quality assessment and may be important in the use of the accumulated database. All of this will be even more useful when viewed along with "Reason for Necropsy" above.

10.9.9.3 The importance of the heading information cannot be overstated. It is not uncommon to find that individuals have not been as diligent as they might have been in achieving this portion of the investigation. It requires only a few minutes more and makes a difference in the preparation of the results. It is also necessary to the retrieval and manipulation of information in data bases. This permits it to move from project significance to program significance.

10.9.9.4 Once completed, the necropsy summary presents a fish health and condition profile of the population of fish sampled (see Tables 4 and 5; Subsection 10.10.2).

10.9.10 Computer

10.9.10.1 This system lends itself very well to spreadsheet analysis and data base management. A computer program has been developed for calculation, summary, and reporting of the fish health and condition assessment necropsy. AUSUM is a template for Lotus 1-2-3^R. It requires a copy of Lotus 1-2-3^R, version 2.0 or later and an IBM compatible PC with at least a 512 K memory. The report is formatted in such a way that the printer must be capable of 12 characters per inch and 8 lines per inch. It is a very user-friendly template. The computer program is not necessary to use this methodology, but it makes the task much easier, facilitates standard reporting, and provides the basis for a data base. Instructions for using the AUSUM template are given in Subsection 10.10, and a separate 30 page user's manual has been prepared for the AUSUM 2.6 program and is available from Ronald W. Goede, Utah Division of Wildlife Resources, Fisheries Experiment Station, 1465 West 200 North, Logan, UT 84321.

10.10 AUSUM 2.6--Computer Program for the Necropsy-Based Fish Health And Condition Assessment System[2]

10.10.1 INTRODUCTION

10.10.1.1 The computer program is written for Lotus 1-2-3^R, version 2.0. It is a large worksheet so a computer with at least 512 K memory is needed. The program calculates and summarizes the information and produces a printed report. The printed report is formatted for 12 pitch and 8 lines per inch. the printer should be capable of this or the report will not fit properly.

10.10.1.2 AUSUM is a computer program that has been specifically designed to supplement the Necropsy-Based Condition Assessment System developed by Ron Goede. The program, which is based on Lotus 1-2-3^R, provides a standard report format and facilitates interpretation of the results. The following features are provided:

* Menus for ease of use
* Defined format for data entry
* Capability to process 60 sample records
* Automatic calculation of the condition factor (Ktl) and all summary information
* Summary information produced in report format
* Hardcopy of sample data produced for reference
* Ability to view Summary information prior to printing the report

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[2]Prepared by Ronald W. Goede and Sybil Houghton (1987), Utah Division of Wildlife Resources, Fisheries Experiment Station, Logan, Utah 84321.

TABLE 4. SAMPLE OF FISH NECROPSY COMPUTER SUMMARY REPORT I

SUMMARY OF AUTOPSY

LOCATION: Midway QUALITY CONTROL NO.: M22Y84

Species: CT
Strain: CTBL
Mark/Lot: 22-Y-8
Unit: 11 & 12
Fish Source: MW
Egg Source: BL
Hatching Date: 07/01/89
Remarks: No unusual variables

Autopsy Date: 07/26/90
Age: 13 mos
Water Temp.: 56 F
Water Hardness: 550 ppm
Investigator: Eric
Reason for Autopsy: Regular autopsy

Sample Size: 20
Tissue Collection No.: NA
Disease Survey No.: NA
Case History No.: NA
Custody No.: NA
Purpose Code: A

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Length	199.000 mm	22.34 mm	11%
Weight	70.400 gr	25.67 gr	36%
Ktl*	0.890	0.09	11%
Ctl**	3.215		
Hematocrit	37.900	3.03	8%
Leucocrit	0.880	0.41	47%
Plasma Protein	4.130	1.05	25%

*Expressed as Ktl times 10 to the fifth power
**Converted from Ktl; expressed as Ctl times 10 to the fourth power

VALUES AS PERCENT OF TOTAL SAMPLE

EYES	GILLS	PSEUDO-BRANCHS	THYMUS	MESEN. FAT	SPLEEN	HIND GUT	KIDNEY	LIVER	BILE	FIN	OPERCLE
N 100%	N 100%	N 45%	0 90%	0 0%	B 20%	0 100%	N 100%	A 80%	0 85%	0 90%	0 85%
B1 0%	F 0%	S 55%	1 10%	1 20%	R 75%	1 0%	S 0%	B 20%	1 15%	1 10%	1 15%
B2 0%	C 0%	L 0%	2 0%	2 20%	G 5%	2 0%	M 0%	C 0%	2 0%	2 0%	2 0%
E1 0%	M 0%	S&L 0%	x 0.1	3 45%	NO 0%	x 0.0	G 0%	D 0%	3 0%	x 0.1	x 0.1
E2 0%	P 0%	I 0%		4 15%	E 0%		U 0%	E 0%	x 0.2		
H1 0%	OT 0%	OT 0%		x 2.6	OT 0%		OT 0%	F 0%			
H2 0%								OT 0%			
M1 0%											
M2 0%											
OT 0%											

Summary of Normals

100%	100%	45%	90%	xxxxxxx	100%	100%	100%	100%	xxxxxxx	90%	85%

Summary of Means

xxxxxxx	xxxxxxx	xxxxxxxx	0.1	2.6	xxxxxxx	0.0	xxxxxxx	xxxxxx	0.2	0.1	0.1

SEX: M: 65% F: 35% U: 0%

GENERAL REMARKS

FINS Some upper caudals nipped

SKIN Clear

GONADS Developing

OTHER #11, 12, 14, 15 twisted intestine

TABLE 4. SAMPLE OF FISH NECROPSY COMPUTER SUMMARY REPORT I (CONTINUED)

Quality Control No. M22Y84

SN	LGH	WGT	Ktl	EYE	GILL	PSBR	THY	FAT	SPL	GUT	KID	LIV	BILE	SEX	HEM	LEU	PLPR	FIN	OPCL
1	242	119	0.84	N	N	S	0	4	B	0	N	A	1	M	36	1	3.8	0	0
2	173	41	0.79	N	N	N	1	2	B	0	N	A	1	M	35	1	4.8	0	0
3	211	72	0.77	N	N	S	0	3	R	0	N	A	0	F	38	1.5	3.8	1	0
4	187	60	0.92	N	N	N	0	3	R	0	N	A	0	F	44	0.5	6.1	0	1
5	183	52	0.85	N	N	S	1	2	R	0	N	A	0	M	37	0.5	3.3	0	0
6	193	65	0.90	N	N	S	0	3	R	0	N	A	0	M	41	1.5	4.6	0	0
7	203	70	0.84	N	N	N	0	3	R	0	N	A	0	F	35	0.5	4.0	0	0
8	222	88	0.80	N	N	N	0	3	B	0	N	A	0	F	44	1.5	5.1	0	1
9	180	53	0.91	N	N	N	0	2	R	0	N	A	0	F	40	0.5	4.0	0	0
10	198	72	0.93	N	N	N	0	3	R	0	N	A	0	M	39	0.5	4.3	0	0
11	178	35	0.62	N	N	S	0	1	R	0	N	A	0	M	39	0.5	2.2	0	0
12	189	50	0.74	N	N	S	0	1	R	0	N	B	0	M	39	0	3.2	1	0
13	210	93	1.00	N	N	N	0	4	R	0	N	A	1	M	37	1	4.8	0	0
14	203	64	0.77	N	N	S	0	1	R	0	N	B	0	M	37	1	3.1	0	0
15	143	22	0.75	N	N	S	0	1	R	0	N	A	0	F	36	1	2.0	0	0
16	185	57	0.90	N	N	S	0	3	B	0	N	B	0	F	31	0.5	3.7	0	1
17	230	122	1.00	N	N	N	0	3	R	0	N	B	0	M	35	1	4.2	0	0
18	215	97	0.98	N	N	S	0	3	G	0	N	A	0	M	36	1.5	6.0	0	0
19	223	96	0.87	N	N	N	0	4	R	0	N	A	0	M	40	1	5.1	0	0
20	212	80	0.84	N	N	S	0	2	R	0	N	A	0	M	39	1	4.5	0	0
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TABLE 5. SAMPLE OF FISH NECROPSY COMPUTER SUMMARY REPORT II

SUMMARY OF AUTOPSY

LOCATION: Green River QUALITY CONTROL NO.: 88-23B

Species: Cutthroat
Strain: Bear Lake
Mark/Lot: 15Z6
Unit: Little Hole
Fish Source: Whiterocks
Egg Source: Egan
Hatching Date: 4-23-87
Remarks: Plasma samples: A403 to 414

Autopsy Date: 7-6-88
Age: 14 mos
Water Temp.: 50 F
Water Hardness: 260 ppm
Investigator: Barton,
Reason for Autopsy: Green River Project

Sample Size: 60
Tissue Collection No.: NA
Disease Survey No.: NA
Case History No.: NA
Custody No.: NA
Purpose Code: D

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Length	222.330 mm	20.69 mm	9%
Weight	117.620 gr	39.81 gr	34%
Ktl*	1.070	0.94	88%
Ctl**	3.866		
Hematocrit	40.710	4.69	12%
Leucocrit	1.690	0.51	30%
Plasma Protein	6.660	0.72	11%

*Expressed as Ktl times 10 to the fifth power
**Converted from Ktl; expressed as Ctl times 10 to the fourth power

VALUES AS PERCENT OF TOTAL SAMPLE

EYES	GILLS	PSEUDO-BRANCHS	THYMUS	MESEN. FAT	SPLEEN	HIND GUT	KIDNEY	LIVER	BILE	FIN	OPERCLE
N 100%	N 100%	N 100%	0 43%	0 20%	B 27%	0 83%	N 100%	A 12%	0 63%	0 47%	0 77%
B1 0%	F 0%	S 0%	1 52%	1 40%	R 73%	1 17%	S 0%	B 88%	1 30%	1 35%	1 13%
B2 0%	C 0%	L 0%	2 5%	2 7%	G 0%	2 0%	M 0%	C 0%	2 7%	2 18%	2 10%
E1 0%	M 0%	S&L 0%	x 0.6	3 25%	NO 0%	x 0.2	G 0%	D 0%	3 0%	x 0.7	x 0.3
E2 0%	P 0%	I 0%		4 8%	E 0%		U 0%	E 0%	x 0.4		
H1 0%	OT 0%	OT 0%		x 1.6	OT 0%		OT 0%	F 0%			
H2 0%								OT 0%			
M1 0%											
M2 0%											
OT 0%											

Summary of Normals

100%	100%	100%	43%	xxxxxxx	100%	83%	100%	100%	xxxxxxx	47%	77%

Summary of Means

xxxxxxx	xxxxxxx	xxxxxxxx	0.6	1.6	xxxxxxx	0.2	xxxxxxx	xxxxxx	0.4	0.7	0.3

SEX: M: 62% F: 38% U: 0%

GENERAL REMARKS

FINS Left pelvic fin clipped; avg. fin index = 0.7

SKIN Red dye marked

GONADS NA

OTHER 3 fish w/mild inflamation of hind gut

TABLE 5. SAMPLE OF FISH NECROPSY COMPUTER SUMMERY REPORT II (CONTINUED)

Qual. Control No. 88-23B

SN	LGH	WGT	Ktl	EYE	GILL	PSBR	THY	FAT	SPL	GUT	KID	LIV	BILE	SEX	HEM	LEU	PLPR	FIN	OPCL
1	209	74	0.81	N	N	N	1	0	R	0	N	B	0	F	38	1	6.8	2	1
2	220	90	0.85	N	N	N	0	0	R	0	N	B	0	M	44	1.5	7.1	2	1
3	195	68	0.92	N	N	N	0	1	B	0	N	B	1	F	42	1.5	6.1	1	1
4	207	81	0.91	N	N	N	1	1	B	1	N	B	1	M	46	1	7.3	0	1
5	210	79	0.85	N	N	N	1	0	R	0	N	B	0	M	42	1.5	6.0	2	1
6	214	86	0.88	N	N	N	0	1	R	0	N	B	0	M	40	1.5	6.5	0	2
7	221	89	0.82	N	N	N	1	1	R	0	N	B	0	M	41	2	7.0	1	2
8	210	85	0.92	N	N	N	1	1	R	0	N	B	1	M	38	2	6.8	0	2
9	219	85	0.81	N	N	N	1	1	R	0	N	B	1	F	42	2	6.1	2	2
10	215	82	0.83	N	N	N	0	1	R	0	N	B	0	F	45	1.5	6.4	1	1
11	195	60	0.81	N	N	N	0	0	R	0	N	B	0	M	41.5	2	5.7	1	2
12	195	63	0.85	N	N	N	0	0	R	0	N	B	0	M	37	2	7.1	0	1
13	226	111	0.96	N	N	N	1	1	R	0	N	B	0	M	38	2.5	6.6	1	2
14	230	99	0.81	N	N	N	0	1	R	0	N	B	1	M	41	2	5.8	0	1
15	222	98	0.90	N	N	N	1	1	R	0	N	B	1	F	36	2	6.0	0	0
16	223	102	0.92	N	N	N	1	1	R	1	N	B	1	F	40	1	7.0	2	0
17	205	70	0.81	N	N	N	0	1	B	0	N	B	0	M	52	1.5	6.9	2	0
18	208	69	0.77	N	N	N	1	0	B	1	N	B	0	F	47	1	6.1	0	0
19	230	116	0.95	N	N	N	1	1	R	0	N	B	1	F	36	1.5	6.0	2	0
20	203	75	0.90	N	N	N	0	3	R	1	N	A	1	M	41	2	6.7	2	0
21	218	89	0.86	N	N	N	0	0	R	0	N	B	0	M	37	2.5	6.3	0	0
22	235	114	0.88	N	N	N	0	1	B	0	N	B	0	F	38	2.0	6.6	1	0
23	233	116	0.92	N	N	N	0	1	R	1	N	B	0	M	34.5	2.5	6.2	0	0
24	238	121	0.90	N	N	N	2	1	B	0	N	B	1	M	36	2.0	6.3	1	0
25	232	108	0.86	N	N	N	0	0	B	0	N	B	0	F	33	1.5	6.1	1	0
26	270	186	0.94	N	N	N	1	2	R	0	N	B	0	M	42	2.0	6.0	2	0
27	255	136	0.82	N	N	N	0	0	R	0	N	B	0	F	42.5	2.0	6.5	1	0
28	225	99	0.87	N	N	N	1	1	R	0	N	B	0	F	36.5	2.5	6.4	1	0
29	226	105	0.91	N	N	N	1	1	R	0	N	B	0	F	40	2.5	7.0	0	0
30	251	151	0.95	N	N	N	1	2	R	0	N	B	0	M	35.5	2.0	6.7	1	0
31	232	112	0.90	N	N	N	0	2	B	0	N	B	0	M	38	2.0	6.0	1	0
32	220	93	0.87	N	N	N	1	1	R	1	N	B	1	F	35	2.0	5.9	2	0
33	217	82	0.80	N	N	N	1	1	R	0	N	B	0	M	37	2.0	5.5	1	0
34	227	101	0.86	N	N	N	1	1	R	0	N	B	0	M	37	1.5	6.5	1	0
35	209	81	0.89	N	N	N	0	1	R	1	N	B	1	F	37.5	2.5	7.1	1	0
36	230	115	0.95	N	N	N	0	1	R	0	N	B	0	M	33	1.5	5.0	1	0
37	217	91	0.89	N	N	N	1	0	B	0	N	B	0	F	34	2.0	6.5	2	0
38	207	78	0.88	N	N	N	1	1	R	0	N	B	0	F	34	2.0	7.1	1	0
39	205	75	0.87	N	N	N	0	0	R	0	N	B	0	M	41	1.5	6.7	1	0
40	220	90	0.85	N	N	N	1	0	R	1	N	B	1	F	41	2.0	6.3	1	0
41	236	187	1.42	N	N	N	0	4	R	1	N	B	0	M	46	0.5	7.9	0	0
42	215	128	1.29	N	N	N	1	3	R	0	N	A	0	M	49	2.0	7.1	1	0
43	232	153	1.23	N	N	N	1	3	R	0	N	B	2	F	41	1.0	6.8	0	0
44	247	200	1.33	N	N	N	2	3	B	0	N	B	2	M	41	1.0	7.0	0	0
45	232	169	1.35	N	N	N	0	3	B	0	N	A	0	F	49	1.0	9.4	0	0
46	227	153	1.31	N	N	N	1	3	B	0	N	B	1	M	46	1.0	8.1	0	0
47	236	200	1.52	N	N	N	0	3	R	0	N	B	1	F	40.5	0.5	8.1	0	0
48	218	169	1.63	N	N	N	1	3	R	0	N	B	1	M	44	1.0	7.0	0	0
49	234	153	1.19	N	N	N	1	3	R	0	N	B	2	M	42.5	1.5	7.2	0	0
50	241	172	1.23	N	N	N	1	4	R	0	N	A	0	M	45	2.0	7.1	0	0
51	241	133	0.95	N	N	N	0	4	B	0	N	B	0	M	39	2	6.7	0	0
52	234	164	1.28	N	N	N	1	2	R	0	N	B	0	M	41	2	6.3	0	0
53	250	210	1.34	N	N	N	1	3	R	0	N	B	2	M	30	1.5	5	0	0
54	210	175	1.89	N	N	N	2	3	R	0	N	B	0	M	44	1	7.3	0	0
55	220	162	1.52	N	N	N	0	3	B	0	N	B	0	F	43.5	1	7	0	0
56	215	162	1.63	N	N	N	1	3	R	0	N	A	0	M	46	1.5	7.1	1	0
57	250	107	0.68	N	N	N	1	3	B	0	N	B	0	M	50	2	7.4	0	0
58	223	141	1.27	N	N	N	0	3	R	0	N	B	0	M	49	2.5	7.1	0	0
59	115	123	8.09	N	N	N	0	4	R	0	N	A	1	F				0	0
60	240	171	1.24	N	N	N	0	4	B	1	N	A	1	M	45.5	1.5	6.8	0	0

Prior to use of AUSUM, the following Subsections should be read by all users:

INTRODUCTION
COMPUTER REQUIREMENTS
BEGINNING STEPS
PRINTER SETUP
MENU PRINTER
MACRO PRIMER
AUSUM PROGRAM USE
OTHER PROGRAM SELECTIONS

10.10.1.3 Keyboard Primer (page 279) is provided for those who are not familiar with computers. Lotus Primer (page 280) gives background information for those who are unfamiliar with Lotus 1-2-3^R. Entry Requirement (page 284) lists the data-entry requirements. Sample reports are provided (see pages 262-265, 286-287).

Keyboard Primer is provided for those who are not familiar with computers. **Lotus Primer** gives background information for those who are unfamiliar with Lotus 1-2-3^R. **Entry Requirements** lists the data-entry requirements.

COMPUTER REQUIREMENTS

AUSUM has been designed for the following computer configuration:

2 floppy disks

IBM PC or compatible with at least 512 K memory

Lotus 1-2-3^R, version 2.0

Epson dot matrix printer (see Printer Setup, page 267) for instructions to change the print setup to accommodate other printers.

BEGINNING STEPS

The AUSUM master disk is to be kept for backup purposes only. Before using AUSUM, you need to copy the program onto your own formatted disk. You will also need a formatted disk for a data disk. Use the following instructions to format your disks and copy the program disk:

A. Format a new disk.

1. Place the DOS system disk in drive A.

2. Place the new, unformatted disk in drive B.

3. At the A> prompt, type:
   FORMAT B: (Then press Return key)

B. Copy the AUSUM master disk.

1. Place the AUSUM master disk in drive A.

2. Place a formatted disk in drive B.

3. At the A> prompt, type:
   COPY *.* B: (Then press Return key)

4. Store the original AUSUM master disk in a safe, dry place. This disk should never be used to run the program.

C. Follow Step A directions to format a new disk to be used for your data disk.

PRINTER SETUP

AUSUM has been designed to use an Epson dot matrix printer. The reports are designed to be printed using elite type (12 pitch), 8 lines per inch; thus, the program uses the following command (setup string):

\027\077\0270

Should your printer need a different setup string for elite type, 8 lpi, you may use the Print Set option from the Submenu of the AUSUM program. You will be asked to enter the elite, 8 lpi, setup string for your printer. Simply enter the correct setup for your printer, and the program will automatically setup the printer command for you.

MENU PRIMER

There are two menus for AUSUM:

Main Menu and Submenu

To activate the Main Menu, press Alt-M. To go the Submenu, select the Submenu option from the Main Menu.

Selections may be made from the menus by either of the following methods:

(1) Press the beginning letter of the desired selection, such as H for Heading
(2) Move the Control Panel cursor to highlight the desired selection,then press ENTER.

The following is a brief description of the menu options:

Main Menu

Heading -	Enter heading and general remarks
Data -	Enter sample data

Calculate -	Calculate Ktl and summary data
Report -	Print a report and hardcopy of the data
Xtract -	Extract data and heading for later use
Prepare -	Prepare worksheet for new data entry
Load -	Load previously saved data file
Submenu -	Unlock, Printset, Extract-Edit, End Lotus, List files, Summary, and Main Menu

Submenu

Unlock -	Unlock titles
PrintSet	Set elite command for your printer
Main Menu -	Return to Main Menu
End -	End work with Lotus/return to MS-DOS
Summary -	To view summary information
List	List files on data disk
Xtract-Edit -	Extract edited data using previous or new file name

CAUTION: Prior to using the menus, you must be certain to deactivate any commands that are currently in use; in other words, the status indicator CMD must not be showing at the bottom of the screen. (To deactivate the CMD, press Ctrl-Break and the ESC.)

MACRO PRIMER

In Lotus 1-2-3[R] it is possible to program a set of commands. These programs are called macros. There are four macros which you will be using while entering the processing the Necropsy (Autopsy) System data. Each of these macros is invoked by pressing the Alt key simultaneously with the letter that names the macro. For instance, to bring the D macro, press Alt-D. The following is a list of the AUSUM macros, directions for their use, hints about when you will utilize them, and directions to end them:

M - This macro brings the AUSUM Main Menu Control Panel area (top portion) of the screen. (See the Menu Primer, page 267, for an explanation of the menu options.) Use the menu whenever you need to select the next processing step. Press ESC to deactivate the Main Menu. Press ESC twice or press Ctrl-Break and press ESC to deactivate the Submenu.

D - This macro automatically shifts the cursor down to the next cell whenever ENTER is pressed. You will want to use this when entering the Heading Data and any columns in the Sample Data where the entries vary down the column, such as lengths or hematocrits. To end this macro, press Ctrl-Break (you will hear a beep) and the ESC.

C - This macro permits you to copy a specific cell entry to a specified range. You will want to use this when an entire column is all the same entry, such as all N for Eyes. To use this macro, do the following:

(1) Place the cursor on the cell which contains the data to be copied.

(2) Press Alt-C.

(3) Notice a message on the Control Panel will say:

Enter range to copy FROM:

Following the colon will be the current cell location, repeated twice, such as A23..A23.

(4) Press ENTER.

(5) The message will now say:

Enter the range to copy TO:

After the colon, the current cell location will again be repeated twice. (**CAUTION:** Be sure NUM LOCK is <u>off</u> before using the arrow keys to highlight the copy region.) Press the down arrow key to go down the column as far as you want to copy the data. Notice that the copy range is now highlighted. Also notice that the second cell location on the Control Panel has changed as you have moved the cursor. After the desired range is highlighted, press ENTER. **<u>HINT</u>:** If you desire to have two or more columns next to each other with the same entry, such as two columns of N, then highlight both columns by pressing the appropriate arrow keys.

(6) The macro ends itself with no further entry needed from you.

<u>E</u> - This macro will erase a specific range--or even just one cell. This macro must be used with **extreme caution** because you want to erase <u>only incorrect data</u>. To use this macro, do the following:

(1) Place the cursor on the cell to be erased or on the top left corner cell of the range to be erased.

(2) Press Alt-E.

(3) Notice a message on the Control Panel will say:

Enter range to erase:

Immediately following the colon will be the current cell location.

(a) If one cell is to be erased, press Enter.

(b) If a range is to be erased, use the appropriate arrow keys to highlight the range. Be sure you want to erase all the highlighted area! Press ENTER.

(4) The macro will end itself with no further entry needed from you.

HINT: What to do if you begin a macro and something is wrong? You may have entered a wrong character or the mode indicator says ERROR. To end a macro at any time, press Ctrl-Break (you may hear a beep) and then pres ESC. Of the ERROR message shows, you will probably only need to press the ESC key.

HINT: Lotus 1-2-3^R will not permit you to use more than one macro at any one time. You will need to deactivate the menu or any other macro before activating a new macro.

AUSUM PROGRAM USE

Program Startup

(1) Start the computer and load with MS-DOS 2.0 or later version.

(2) Insert the Lotus 1-2-3^R system disk in drive A.

(3) At the A> prompt, type 123 and then press ENTER.

(4) As soon as the Lotus 1-2-3^R program is loaded (The worksheet format will show on the screen), remove the Lotus 1-2-3 system disk and insert your copy of AUSUM in drive A.

(5) Insert the formatted data disk in drive B.

(6) To being the program, type: /FR (The file name, AUSUM, will be highlighted on the third line of the Control Panel).

(7) Press ENTER. The screen will then appear as Figure 1.

(8) Press **ENTER** as directed, and the screen will then appear as in Figure 2.

Figure 1. Introduction to AUSUM

AUSUM
Version 2.6

Developed December 1986

by

Ron Goede and Sybil Houghton

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Press **ENTER** to continue . . .

Figure 2. Continuation of Introduction

AUSUM is used to summarize data from the most recent version of the necropsy (autopsy) system which includes observations of bile but not mesentery.

NOTE: AUSUM is not be used for data which include observations of mesentery.

Press **ENTER** to continue . . .

(9) Press **ENTER** as directed, and the screen will then appear as in Figure 3.

Figure 3. Data Disk Drive Entry Screen

On the line at the top of the screen, enter the drive in which data disk is to be placed . . .
Then press ENTER to continue . . .

(10) Enter the letter for the drive in which the data disk is to be placed. (for a configuration with two floppy disk drives, you will enter B for the drive letter.)

(11) Then press **ENTER** to continue. The screen will then appear as in Figure 4. You are now ready to begin entering the Heading information. See Program Order (page 273) for steps to follow when using the AUSUM program. The cursor is already located for the first entry.

Figure 4. Beginning Screen

```
D67:  [W10]                                                    READY

 A      B       C       D      E
 64 Enter the heading data in column D
 65    using the specified field lengths {  }:
 66
 67 Location:                    {30}
 68 Species:                     {13}
 69 Strain:                      {13}
 70 Mark/Lot:                    {'13}
 71 Unit:                        {17}
 72 Fish Source:                 {8}
 73 Egg Source:                  {8}
 74 Date of Hatching"            {'MM-DD-YY}
 75 Remarks                      {68}
 76 Necropsy Date:               {'MM-DD-YY}
 77 Age:                         {10}
 78 Water Temp.:                 {2}
 79 Temp. Scale (C or F):        {1}
 80 Water Hardness:              {4}
 81 Investigator:                {15}
 82 Reason for Necropsy          {30}
 83 Qual. Control No.:           {'7}
 84 Sample size                  {2}
 85 Tissue Collection No.:       {'7}
 86 Disease Survey No.:          {'7}
 87 Case History No.:            {'7}
 88 Custody No.:                 {'7}
 89 Fins:                        {65}
 90 Sins:                        {65}
 91 Gonads:                      {65}
 92 Other:                       {65}
 93 Purpose Code:                {2}
```

Program Notes

Before you begin to use the program, read the following notes:

(1) When you begin the program , the cursor is already in position for you to enter the heading data.

(2) You are instructed to enter the data column D according to the specific directions given. There are three types of directions:

(a)	{'MM-DD-YY}	Enter dates, such as '12-06-86. You <u>must</u> use the apostrophe (') in front of the date. (For explanation, see Label/Value section of the Lotus Primer, page 280.)
(b)	{13}	The number (13) indicates the maximum number of characters allowed.
(c)	{'7}	A number used as a label. You must use the '. The number (7) shown indicates the maximum number of characters allowed in addition to the apostrophe. Example: '86-02-1

(3) When the Main Menu us activated, the selections will be displayed on the Control Panel (top portion of the screen).

Program Order

The usual order of menu selections when entering a set of data for the first time is:

(1) Heading
(2) Data
(3) Calculate
(4) Report
(5) Xtract
(6) Prepare

Heading

To enter the Heading information, use the following directions:

(1) Invoke the Down macro by pressing Alt-D.

(2) Enter the information in the appropriate cells.

(3) If there is no information for a particular cell.

To correct entries, do one of the following:

(1) Use the down or up arrow keys to move to the appropriate cell. They type the correct entry.

(2) If you desire to EDIT the entry, do the following:

(a) Move to the appropriate cell.

(b) Deactivate the Down macro by pressing Ctrl-Break and then ESC.

(c) Press the F2 key to EDIT.

(d) Edit the entry line.

(e) Press ENTER.

After all the Heading information has been entered, do the following:

(1) Deactivate the Down macro by pressing Ctrl-Break and then ESC.
(2) Activate the Main Menu by pressing Alt-M.

Data

After you select Data from the Main Menu, the cursor is located in the first cell of the length column. In this area of the worksheet, you may want to enter data in either of the two following ways:

(1) Use the Down macro (see page 268) and enter data in the individual cells as you go down the column.

(2) Use the Copy macro (see page 268) if the column entries are all the same.

NOTE: The first cell of the Ktl column says ERR. This is not a mistake or error! The cell contains the formula to calculate the Ktl. During the calculation process, the formula will be copied down the column and the Ktl will be calculated for each item in the sample. Thus, no entry is required for the Ktl column. (The Ktl column is **not** protected; thus, be careful that you **do not** enter the data in that column.)

To help you with the data entry, the column titles and sample numbers have ben "locked" in place. Thus, as you work you way down and across the worksheet, you will always know the title of the column and number of row for your current cell location.

Enter all the sample data before doing any calculations. After all the data is entered, deactivate the Down macro, if necessary.

REMEMBER: The program is designed for a maximum sample size of 60.

Calculate

(1) Activate the Main Menu (Alt-M).

(2) Select Calculate--the calculations will take a minute or so to complete; thus, the screen will say: Please wait . . .

(3) At the end of the calculation process, the Main Menu will again be displayed and you will be asked to make your next selection.

Report

(1) Be sure the printer is on!

(2) Select Report from the Main Menu.

(3) On the Control Panel will be a question:

Has the printer been turned on? (0 or 1)

After checking to see that the printer is turned on, press 1 and the program will continue. If you decide not to print the report, press 0 (zero) and you will be returned to the Main Menu for your next selection.

(4) A second question will then be shown:

Has all the data been entered? (0 or 1)

If you press 0 (zero), the Main Menu will be displayed so you may make the appropriate data entry selection. If you press 1, the program will continue to execute the print commands.

(5) The screen will say: Please wait . . .

The standard formatted report and a hardcopy of the data will then be printed.

(6) At the end of the printing process, the Main Menu will again be displayed and you will be asked to make your next selection.

NOTE: If you want to save the data on the data disk, you must continue with the next step (Xtract); if not, the data will be permanently lost.

Xtract

By selecting this option, you will be saving (Xtracting) only the heading information and the sample data rather than the entire worksheet. (The program has been designed in this manner to conserve space on your data disk). This selection is only for the first time you save (Xtract) the specific set of data. (See **Xtract-Edit** for edited data, page 278).

To save your data entry on your data disk, do the following:

(1) Select Xtract from the Main Menu.

(2) The screen will show:

ENTER THE NAME OF THE FILE TO BE EXTRACTED . . .

(3) Enter the file name (limited to 8 characters) you wish to use for this set of data. As you enter the file name the *.wkl will disappear from the Control Panel and the file name will appear.

HINT: For easier file name recognition, we suggest you use the specific Quality Control No. (i.e., 87-01) as part of the file name, such as 87AU01. (You cannot use hyphens in a file name.)

(4) At the end of the extraction process, the Main menu will again be displayed and you will be asked to make your next selection.

Prepare

This process will clear the worksheet and prepare it for a new set of data. [**CAUTION:** Be sure you have saved (using the Xtract option) your data before selecting the prepare option!]

(1) Select Prepare from the Main Menu.

(2) No questions to answer--just wait until it is complete.

(3) At the end of the preparation process, the Main Menu will again be displayed and you will be asked to make your next selection. You are now ready to enter a new set of data or load in a previously saved set of data.

HINT: If you are running short on time and do not want to wait for the printer to print the report, or if a printer is unavailable, you may want to skip the Report option and just Xtract (save) the data for now. Then at a later time you may load the data and select the Report option.

OTHER PROGRAM SELECTIONS

List

An additional feature which AUSUM offers is the ability to list the files on your data disk. This List option is helpful for several reasons. First, you may need to know whether the data disk is full before trying to save a new set of data. (A diskette will hold approximately 25 extracted necropsy (autopsy) data files.) Second, it will help you remember the name of the data file that you want to load. To use the List option, do the following:

(1) Select List from the Main Menu.

(2) A list of the files on the data disk will be displayed on the screen.

(3) To end viewing of the file list, press ENTER.

Load

To process and/or edit data that you previously saved, you will need to load that data into the worksheet. [**CAUTION:** Be sure that the worksheet is prepared for new data prior to using the Load option.] **REMEMBER:** You may select the List option to review the names of your data files prior to selecting the Load option.

Place the specific data disk in the drive you selected for the data disk at the beginning of the AUSUM program, and then do the following:

(1) Select Load from Main Menu.

(2) On the screen will be:

ENTER THE NAME OF THE FILE TO LOADED . . .

(3) Type in the appropriate file name.

(4) Press ENTER.

(5) After the data is loaded, the Main Menu will again be displayed and you will be asked to make your next selection.

You may now do any necessary editing using the methods to correct entries described in **Heading** (page 273) and **Data** (page 274). The program may then be continued as if it were the original data entry. [**CAUTION:** Be sure to select Calculate **after** editing and **before** a report is printed. Calculation must be performed each time you re-enter a file and make any changes.]

Unlock

While you are entering the Sample data, the column and row titles are locked into place. To deactivate the locking process, simply select the Unlock option of the Submenu and press ENTER.

End

When you have completed your data entry for AUSUM and are finished with your use of Lotus 1-2-3, select the End option from the Submenu. This will return you to A> prompt of MS-DOS at the system level. [**CAUTION**: Be sure you have saved all your data before you use the End option.]

PrintSet

See **Printer Setup** for an explanation.

Xtract-Edit

When saving (Xtracting) data that has been previously saved, you must use the **Extract-Edit** option--not the Xtract option. To help your memory, you will be reminded of the name of the file which you have been editing. To use this option, do the following:

(1) Select Xtract-Edit from the Submenu.

(2) On the screen will be:

THE NAME OF THE FILE YOU HAVE LOADED IS:

PLEASE ENTER THAN FILE NAME . . . OR YOU MAY CHANGE
TO A NEW FILE NAME . . .

(3) Type in the appropriate file name.

(4) Press ENTER.

(5) At the end of the process, the Submenu will be displayed and you will be asked to make your next selection.

Summary

This option allows you to view the Summary information. You may want to use this option to check the information prior to printing the report. To use this option, do the following:

(1) Select Summary from the Submenu

(2) On the screen will be some of the Summary information. Use the arrow keys to view all of the information.

KEYBOARD PRIMER

You will notice that the keyboard is very similar to that of a typewriter. However, there are some additional keys. A brief description of these additional keys follows:

Functional keys

On the left side (or across the top) of the keyboard are at least 10 keys which are labeled as F1, F2, etc. These keys are pre-programmed by each computer program to have specific capabilities. The only Function key you need to use for this program is the F2 key, which is the Edit key.

Ctrl (Control) Key

This key is used in conjunction with other keys to enact specific directions. An instruction such as Ctrl-Break means to press the Control and Break keys simultaneously.

Scroll Lock/Break Key

This key is used when the instructions call for the Break key. It is used in conjunction with the Control key to abort certain operations in Lotus. The key has many other uses, but that is the only one you will be using for this program. **CAUTION:** If you do not hold the Ctrl and Break keys down simultaneously, the indicator SCROLL may appear at the bottom of the screen. If this happens, press only the Scroll Lock/Break key to erase the SCROLL indictor and then press Ctrl-Break simultaneously.

Alt Key

This key is used in conjunction with any letter key to invoke Lotus macros (programs). For example, Alt-D means to simultaneously press the Alt Key and the letter D. By doing so you would invoke a macro identified by the letter D. Refer to the **Macro Primer** (page 268) for a further explanation.

Number Pad

These keys permit you to efficiently enter numeric data. To invoke the number pad, press the NUM LOCK key. [**CAUTION:** If the number keys have arrows on them, they can be used only as numbers when the NUM LOCK key has been pressed.

The NUM LOCK key is a toggle key; thus, to return to arrow or direction use, press the NUM LOCK key again.

Arrow Keys

Your keyboard may have separate keys with arrows on them, or the arrows may be on the number pad keys. (Be sure to read the caution included in the Number Pad description above.) Use the arrow keys to move the cursor up, down, right or left.

Home Key

This key is located with the arrow keys. While editing a cell entry, you may use this key to go the beginning of the line being edited. [**CAUTION:** Any other time the Home key is used, the cursor will be taken out of the current position to the beginning of the screen. In that case you must return to the menu (press Alt-M), then make you original selection and return to your original position using the arrow keys.]

Del (Delete) Key

While editing a cell entry, you may use this key to delete the character at the same location as the edit-line cursor.

Backspace Key

You may use this key while entering data or when editing. Pressing this key will delete the character just to the left of the cursor location.

End Key

This key is located with the arrow keys. While editing a cell entry, you may use this key to go to the right end of the line being edited.

ESC (Escape) Key

Use this key when you want to end an operation prior to its normal completion. At times you will need to first press Ctrl-Break and then the ESC key to end an operation.

LOTUS PRIMER

Introduction

Lotus 1-2-3^{R} is a spreadsheet-type of computer program. Such a program is based on "cell entries." Picture the worksheet (working area of the program) as a grid with columns named by letters and rows named by numbers. Thus, each "cell" has a specific location such as A1 or X36. (Perhaps you have played the game "Battleship" that is based on this same type of grid identification.) As you enter data in this worksheet, you will be filling a cell with each "piece" of data.

Screen Format

An understanding of Lotus's screen format will be helpful. The Control Panel comprises the top three lines of the screen. When you begin the program, the Control Panel will appear as in Figure 4. The following example is an explanation of the information on the first line:

Information	Explanation
D67	Location of cursor

{W10}	Width of column
READY	Mode of indicator

When using the menus, the selections will be displayed on the second line. The third line will give the explanation for the highlighted menu selection. Use the arrow keys to move the cursor across the second line, and you will see that the third line changes to give the explanation of each menu selection as it is highlighted.

Sometimes the second and third line of the Control Panel will be blank, or there may be a question on the second line of the Control Panel that you will need to answer. At other times you will need to enter the name of a file. Further directives are given in the Menu Primer (page 267).

The lower left-hand corner of the screen, as shown in Figure 4, gives the date and time. The remaining portion of the bottom line is used to tell which "status indicators" are currently in use. This example shows CALC as the current status indicator. While you are running the program, other status indicators may appear, such as NUM, CMD, and CAPS.

The remaining portion of the screen is the actual worksheet area with its column letters and row numbers for reference. All data entry will be made by you in the worksheet area. This is more fully explained within the program directions.

Label/Value

Typical of all computer programs, Lotus 1-2-3 has its own idiosyncrasies. For data entry you must be aware of one particular Lotus requirement. When you type the first character of an entry, Lotus immediately determines whether the entry is going to be a VALUE or a LABEL. (The mode indicator in the top right corner of the screen will change from READY to VALUE or LABEL.) Sometimes this idiosyncrasy can present a problem. For instance, you may want to enter a date as 12-06-85. Lotus assumes this to be a value because the first character is a number. Thus, rather than displaying your entry, Lotus would display -79, the result of 12 minus 6 minus 85! Likewise, if you typed the date as 12/06/85, Lotus would display .02 which is the result of 12 divided by 6 divided by 85!

Fortunately, there is a way to circumvent this "problem." You simply need to begin this entry with an apostrophe, so you will enter '12-06-85. The apostrophe tells Lotus that you want to treat these numbers as a LABEL rather than as a VALUE. Note that as soon as you enter the apostrophe, the READY mode indicator changes to LABEL.

Lotus considers all of the following as indicative of a VALUE entry:

0 1 2 3 4 5 6 7 8 9 + - . $ (

If you desire an entry that begins with one of these characters to be a LABEL instead, you must begin the entry with an apostrophe.

The slash (/) key is reserved for Lotus commands. You will not need to use this key. In fact, it is recommended that you not used this key unless you are familiar with the use of LOTUS. Should you accidentally press this key, you may press the ESC key to negate its effect.

If the first character of an entry is other than those VALUE entries shown above or a slash (/), Lotus assumes the entry us a LABEL. In this case, you do not need to use the apostrophe--Lotus will automatically place it there for you.

Now, what happens if you forget to use the apostrophe? One of two things will happen:

(1) As in the date example above, Lotus will do the calculation instead of accepting your entry as a LABEL. In such a case, you may change the cell entry using either of the following methods:

(a) Edit the cell entry.

(1) Press the F2 key.

(2) Press HOME to go the beginning of the entry line.

(3) Press the apostrophe key.

(4) Press ENTER.

(b) Re-enter the entire cell entry using an apostrophe as the first character.

(2) If you have combined number characters with label characters, such as 80-6C, Lotus will beep and automatically change to the EDIT mode. You may then simply press the HOME key to go to the beginning of the entry line, press ', and then ENTER.

<u>Data Entry Methods</u>

During the program you will be using two different methods for data entry:

(1) To enter data in a single cell, do the following:

(a) Place cursor on cell where data in to be entered.

(b) Type the entry using an apostrophe where appropriate.

(c) Press ENTER.

This is the most efficient methods to use when

entering the Heading information, length and wright columns data, and all other columns where data varies for each sample.

(2) If the entire column is all the same entry, such as all the Eye entries are N or all the Thymus entries are 0 (zero), then it is more efficient to enter the desired character in the first cell of the column and then copy this entry down the column. To do this, use the Copy macro as explained in the **Macro Primer** (page 268).

HINT: If only one or two of the column entries are different, you may still prefer to use the Copy macro. After copying, go to the one or two cells which should be different and enter the particular data using the single cell entry method.

What do you do if you enter incorrect data? You may do either of the following:

(1) For a single cell correction, move the cursor to the appropriate cell and do one of the following:

(a) Type the entire entry again.

(b) Use the EDIT mode (F2 key) to correct the entry.

(2) For a block or range of cells (see definition of range below), you may find it easier to erase the entire range and then re-enter the data. Use the **Erase Macro** to do the erasure (see page 269).

Terminology

To help you understand and use AUSUM, the following Lotus 1-2-3 terms are defined:

Cursor - There ar two types of cursors in Lotus 1-2-3:

(1) In the worksheet area, the cursor is a highlighted area that designates the current cell location. You will move this cursor with the arrow keys when entering data.

(2) In the Control Panel, a blinking line underlines the current location of the cursor.

Macro - A set of special commands that can be executed with one key stroke combination: Pressing and holding down the Alt key while at the same time pressing the key representing the macro's name.

Mode - Displayed in the top right corner of the screen.

Examples are READY, VALUE, LABEL, EDIT, AND MENU. Hopefully, you will not have the ERROR mode! (If you do, press ESC.)

Range - Specific area of the worksheet--one or more cells. It must be a rectangle or square.

Worksheet - The screen area, except of the Control Panel (top three lines) and the status indicator line (bottom line). This is the work area for a Lotus program.

ENTRY REQUIREMENTS

Below is a list of the correct entries to be used for the AUSUM program:

ENTRY	EXPLANATION
	Eyes
N	Normal
B1	One blind
B2	Two blind
E1	One exophthalmic
E2	Two exophthalmic
H1	One hemorrhagic
H2	Two hemorrhagic
M1	One missing
M2	Two missing
OT	Other
	Gills
N	Normal
F	Frayed
C	Clubbed
M	Marginate
P	Pale
OT	Other
	Pseudobranchs
N	Normal
S	Swollen
L	Lithic
S&L	Swollen & lithic
I	Inflamed
OT	Other

ENTRY	EXPLANATION
	Spleen
B	Black
R	Red
G	Granular
NO	Modular
E	Enlarged
OT	Other
	Hind Gut
0	No inflammation
1	Mild inflammation
2	Severe inflammation
	Kidneys
N	Normal
S	Swollen
M	Mottled
G	Granular
U	Urolithiasis
OT	Other
	Liver
A	Normal, red
B	Pale red
C	Fatty
D	Nodules
E	Focal discoloration
F	General discoloration
OT	Other

ENTRY REQUIREMENTS (CONTINUED)

ENTRY	EXPLANATION
	Thymus
0	No hemorrhage
1	Mild hemorrhage
2	Severe hemorrhage
	Mesenteric Fat
0	None
1	Little; <50% coverage
2	50% coverage
3	>50% coverage
4	100%
	Fins
0	Normal
1	Mild active erosion
2	Severe actiave erosion

ENTRY	EXPLANATION
	Bile
0	Yellow bile; <full bladder
1	Yellow bile; full bladder
2	Green bile
3	Dark blue-green bile
	Sex
M	Male
F	Female
U	Unknown
	Opercles
0	Normal
1	Slight shortening
2	severe shortening

10.10.2 Sample Report (Summary of Necropsy).

LOCATION: Green River QUALITY CONTROL NO.: 88-23B

Species: CUT
Strain: Bear Lake
Mark/Lot: 15Z6
Unit: Little Hole
Fish Source: Whiterocks
Egg Source: Egan
Hatching Date: 4-23-87
Remarks: Plasma samples: A403 to 414

Autopsy Date: 7-6-88
Age: 14 mos
Water Temp.: 50 F
Water Hardness: 260 ppm
Investigator: Barton,
Reason for Autopsy: Green River Project

Sample Size: 60
Tissue Collection No.: NA
Disease Survey No.: NA
Case History No.: NA
Custody No.: NA
Purpose Code: D

	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Length	222.330 mm	20.69 mm	9%
Weight	117.620 gr	39.81 gr	34%
Ktl*	1.070	0.94	88%
Ctl**	3.866		
Hematocrit	40.710	4.69	12%
Leucocrit	1.690	0.51	30%
Plasma Protein	6.660	0.72	11%

*Expressed as Ktl times 10 to the fifth power
**Converted from Ktl; expressed as Ctl times 10 to the fourth power

VALUES AS PERCENT OF TOTAL SAMPLE

EYES	GILLS	PSEUDO-BRANCHS	THYMUS	MESEN. FAT	SPLEEN	HIND GUT	KIDNEY	LIVER	BILE	FIN	OPERCLE
N 100%	N 100%	N 100%	0 43%	0 20%	B 27%	0 83%	N 100%	A 12%	0 63%	0 47%	0 77%
B1 0%	F 0%	S 0%	1 52%	1 40%	R 73%	1 17%	S 0%	B 88%	1 30%	1 35%	1 13%
B2 0%	C 0%	L 0%	2 5%	2 7%	G 0%	2 0%	M 0%	C 0%	2 7%	2 18%	2 10%
E1 0%	M 0%	S&L 0%	x 0.6	3 25%	NO 0%	x 0.2	G 0%	D 0%	3 0%	x 0.7	x 0.3
E2 0%	P 0%	I 0%		4 8%	E 0%		U 0%	E 0%	x 0.4		
H1 0%	OT 0%	OT 0%		x 1.6	OT 0%		OT 0%	F 0%			
H2 0%								OT 0%			
M1 0%											
M2 0%											
OT 0%											
Summary of Normals											
100%	100%	100%	43%	xxxxxxx	100%	83%	100%	100%	xxxxxxx	47%	77%
Summary of Means											
xxxxxxx	xxxxxxx	xxxxxxxx	0.6	1.6	xxxxxxx	0.2	xxxxxxx	xxxxxx	0.4	0.7	0.3

SEX: M: 62% F: 38% U: 0%

Index Summary

Fat Index:	1.62	Gut Index	8.3	Normality Index	85.0
Bile Index:	0.43	Opercle Index	16.7	Severity Index	22.9
Thymus index	30.8	Fin Index	35.8		

GENERAL REMARKS

FINS Left pelvic fin clipped;

SKIN Red dye marked

GONADS NA

OTHER 3 fish w/mild inflamation of hind gut

10.10.2 Sample Report (Summary of Necropsy) Continued.

Qual. Control No. 88-23B

SN	LGH	WGT	Ktl	EYE	GILL	PSBR	THY	FAT	SPL	GUT	KID	LIV	BILE	SEX	HEM	LEU	PLPR	FIN	OPCL
1	209	74	0.81	N	N	N	1	0	R	0	N	B	0	F	38	1	6.8	2	1
2	220	90	0.85	N	N	N	0	0	R	0	N	B	0	M	44	1.5	7.1	2	1
3	195	68	0.92	N	N	N	0	1	B	0	N	B	1	F	42	1.5	6.1	1	1
4	207	81	0.91	N	N	N	1	1	B	1	N	B	1	M	46	1	7.3	0	1
5	210	79	0.85	N	N	N	1	0	R	0	N	B	0	M	42	1.5	6.0	2	1
6	214	86	0.88	N	N	N	0	1	R	0	N	B	0	M	40	1.5	6.5	0	2
7	221	89	0.82	N	N	N	1	1	R	0	N	B	0	M	41	2	7.0	1	2
8	210	85	0.92	N	N	N	1	1	R	0	N	B	1	M	38	2	6.8	0	2
9	219	85	0.81	N	N	N	1	1	R	0	N	B	1	F	42	2	6.1	2	2
10	215	82	0.83	N	N	N	0	1	R	0	N	B	0	F	45	1.5	6.4	1	1
11	195	60	0.81	N	N	N	0	0	R	0	N	B	0	M	41.5	2	5.7	1	2
12	195	63	0.85	N	N	N	0	0	R	0	N	B	0	M	37	2	7.1	0	1
13	226	111	0.96	N	N	N	1	1	R	0	N	B	0	M	38	2.5	6.6	1	2
14	230	99	0.81	N	N	N	0	1	R	0	N	B	1	M	41	2	5.8	0	1
15	222	98	0.90	N	N	N	1	1	R	0	N	B	1	F	36	2	6.0	0	0
16	223	102	0.92	N	N	N	1	1	R	1	N	B	1	F	40	1	7.0	2	0
17	205	70	0.81	N	N	N	0	1	B	0	N	B	0	M	52	1.5	6.9	2	0
18	208	69	0.77	N	N	N	1	0	B	1	N	B	0	F	47	1	6.1	0	0
19	230	116	0.95	N	N	N	1	1	R	0	N	B	1	F	36	1.5	6.0	2	0
20	203	75	0.90	N	N	N	0	3	R	1	N	A	1	M	41	2	6.7	2	0
21	218	89	0.86	N	N	N	0	0	R	0	N	B	0	M	37	2.5	6.3	0	0
22	235	114	0.88	N	N	N	0	1	B	0	N	B	0	F	38	2.0	6.6	1	0
23	233	116	0.92	N	N	N	0	1	R	1	N	B	0	M	34.5	2.5	6.2	0	0
24	238	121	0.90	N	N	N	2	1	B	0	N	B	1	M	36	2.0	6.3	1	0
25	232	108	0.86	N	N	N	0	0	B	0	N	B	0	F	33	1.5	6.1	1	0
26	270	186	0.94	N	N	N	1	2	R	0	N	B	0	M	42	2.0	6.0	2	0
27	255	136	0.82	N	N	N	0	0	R	0	N	B	0	F	42.5	2.0	6.5	1	0
28	225	99	0.87	N	N	N	1	1	R	0	N	B	0	F	36.5	2.5	6.4	1	0
29	226	105	0.91	N	N	N	1	1	R	0	N	B	0	F	40	2.5	7.0	0	0
30	251	151	0.95	N	N	N	1	2	R	0	N	B	0	M	35.5	2.0	6.7	1	0
31	232	112	0.90	N	N	N	0	2	B	0	N	B	0	M	38	2.0	6.0	1	0
32	220	93	0.87	N	N	N	1	1	R	1	N	B	1	F	35	2.0	5.9	2	0
33	217	82	0.80	N	N	N	1	1	R	0	N	B	0	M	37	2.0	5.5	1	0
34	227	101	0.86	N	N	N	1	1	R	0	N	B	0	M	37	1.5	6.5	1	0
35	209	81	0.89	N	N	N	0	1	R	1	N	B	1	F	37.5	2.5	7.1	1	0
36	230	115	0.95	N	N	N	0	1	R	0	N	B	0	M	33	1.5	5.0	1	0
37	217	91	0.89	N	N	N	1	0	B	0	N	B	0	F	34	2.0	6.5	2	0
38	207	78	0.88	N	N	N	1	1	R	0	N	B	0	F	34	2.0	7.1	1	0
39	205	75	0.87	N	N	N	0	0	R	0	N	B	0	M	41	1.5	6.7	1	0
40	220	90	0.85	N	N	N	1	0	R	1	N	B	1	F	41	2.0	6.3	1	0
41	236	187	1.42	N	N	N	0	4	R	1	N	B	0	M	46	0.5	7.9	0	0
42	215	128	1.29	N	N	N	1	3	R	0	N	A	0	M	49	2.0	7.1	1	0
43	232	153	1.23	N	N	N	1	3	R	0	N	B	2	F	41	1.0	6.8	0	0
44	247	200	1.33	N	N	N	2	3	B	0	N	B	2	M	41	1.0	7.0	0	0
45	232	169	1.35	N	N	N	0	3	B	0	N	A	0	F	49	1.0	9.4	0	0
46	227	153	1.31	N	N	N	1	3	B	0	N	B	1	M	46	1.0	8.1	0	0
47	236	200	1.52	N	N	N	0	3	R	0	N	B	1	F	40.5	0.5	8.1	0	0
48	218	169	1.63	N	N	N	1	3	R	0	N	B	1	M	44	1.0	7.0	0	0
49	234	153	1.19	N	N	N	1	3	R	0	N	B	2	M	42.5	1.5	7.2	0	0
50	241	172	1.23	N	N	N	1	4	R	0	N	A	0	M	45	2.0	7.1	0	0
51	241	133	0.95	N	N	N	0	4	B	0	N	B	0	M	39	2	6.7	0	0
52	234	164	1.28	N	N	N	1	2	R	0	N	B	0	M	41	2	6.3	0	0
53	250	210	1.34	N	N	N	1	3	R	0	N	B	2	M	30	1.5	5	0	0
54	210	175	1.89	N	N	N	2	3	R	0	N	B	0	M	44	1	7.3	0	0
55	220	162	1.52	N	N	N	0	3	B	0	N	B	0	F	43.5	1	7	0	0
56	215	162	1.63	N	N	N	1	3	R	0	N	A	0	M	46	1.5	7.1	1	0
57	250	107	0.68	N	N	N	1	3	B	0	N	B	0	M	50	2	7.4	0	0
58	223	141	1.27	N	N	N	0	3	R	0	N	B	0	M	49	2.5	7.1	0	0
59	115	123	8.09	N	N	N	0	4	R	0	N	A	1	F				0	0
60	240	171	1.24	N	N	N	0	4	B	1	N	A	1	M	45.5	1.5	6.8	0	0

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SECTION 11

GUIDELINES FOR FISH SAMPLING AND TISSUE PREPARATION FOR BIOACCUMULATIVE CONTAMINANTS

11.1 Introduction

11.1.1 Sampling of fish and shellfish for bioaccumulative contaminants has been conducted for over 35 years. Most fish sampling for contaminants has focused on contaminants of local concern, so data results and program conclusions have not always been comparable. The issues surrounding management of chemical contaminants in fish are of increasing concern for fishery management, environmental and public health agencies. The interdisciplinary multiagency problems caused by chemical contaminants suggests the need for standard sampling protocols. There have been inconsistent warnings given to the public by local, state, and federal regulatory agencies regarding the consumption of sport fish. This has been particularly evident on bodies of water shared by two or more states and on international waters. The Great Lakes States (Great Lakes Fish Consumption Advisory Task Force) and those States and EPA Regions bordering the Mississippi (Mid-America Fish Contaminants Group) and Ohio Rivers (Ohio River Valley Water Sanitation Commission) have endeavored to provide consistent sampling and advisory information but a standard protocol has yet to be agreed upon.

11.1.2 The application of quantitative risk assessment including hazard assessment, dose response assessment, exposure assessment and risk characterization functions best with a standardized protocol. The development of human health fish consumption advisories, whether based on quantitative risk assessment or some other methodology, is fundamentally affected by the procedures used in sampling. This section presents guidance for the sampling and preparation of fish for contaminant analysis, which is a key component of exposure assessment in quantitative risk assessment.

11.1.3 The purpose and goals of each study should be clearly stated prior to the initiation of fish collection for contaminant analysis. One should consider the overall long-term development of a fish contaminant database in each jurisdiction. Frequently short term goals have been the only consideration, where as long term trend assessments may provide a better understanding of the problem because the long view is the only way of gauging important changes occurring in water quality.

11.1.4 Various federal, state, and local agencies have responsibilities for the collection and preparation of fish samples. Thus, numerous collection protocols are available. Fish sampling for contaminant analysis will often be included in other biological surveys to maximize use of the resource and to minimize costs. It must be recognized that any sample collected represents the future expenditure of significant dollar amounts by the time a decision is reached, and can have significant effects on major sectors of our society.

11.1.5 These guidelines present a basic fish sampling protocol designed to give comparable results between studies. Some additional requirements are pointed out which may be needed in special studies where different sizes or species of fish might be targeted or where special collections for spike samples might be needed. A partial discussion of sampling strategy including statistical concerns can be found in USEPA (1989), which should be reviewed during any planning effort.

11.2 Site Selection

11.2.1 Collecting sites should be established according to the specific requirements of each study. Sites may be designed as short- or long-term depending on the frequency with which they are sampled. Most sampling designs for short-term (synoptic) studies will be structured to determine the extent of contamination in a water body or a section of a water body. The determination of contamination gradients extending away from point sources or industrial/urban areas with point and non-point sources provides important information needed to manage contaminant burdens in fish. Some sites will be selected by individual states to address intrastate needs while other sites will be selected to address interstate needs through cooperative programs. Regardless of the various reasons for site selection, long-term comparability is of utmost importance to provide trend information needed to place bioaccumualtive contaminants in perspective.

11.2.2 Sites should be described as sport, commercial, or having both types of fisheries, and additional sites may be identified for ecological risk assessment. Special watershed information should be indicated, including urban areas, mining, manufacturing, agriculture, etc., and any known point or non-point sources of pollution at or near the site in the watershed. Additional information should include average width, depth, and velocity at the sampling station, description of the substrate, duration of the sampling effort, and habitat area sampled (e.g., length of stream or area of lake). Selected water quality measurements (e.g., conductivity, pH, dissolved oxygen, temperature, etc.) may also be useful. It is becoming routine to collect and analyze water, sediment and fish at common stations to gain a more complete understanding of contaminants in aquatic environments.

11.3 Sample Collection

11.3.1 The following three objectives should guide sample collection:

1. Provide comparable data

2. Utilize sizes and ages of species generally available to the fishery and,

3. Yield data which will screen for problems that might indicate that more intensive studies are needed.

11.3.2 Samples should be obtained at each station from the principal fish categories. Fish species are grouped by feeding strategy into predators, omnivores and bottom feeders. To reduce the number of categories, the

omnivores may be placed with the bottom feeders. USEPA (1990a) sampled 388 sites nationwide at which 119 different species of fish representing 33 taxonomic families of fish were collected. The most frequently sampled freshwater and marine species in that study are listed in Table 1.

11.3.3 This national study indicates that of the freshwater species, carp and largemouth bass were the most frequently sampled and are the most likely to provide interstate comparability. The other freshwater species listed may be selected in a declining order of priority; however, additional less common species may not be added except in special situations. The diversity of marine species is much greater resulting in a lack of focus on a limited number. Additional effort will be needed to determine which marine species should receive priority on the Atlantic, Pacific and Gulf Coasts in order to provide long term comparative data.

11.3.4 Cunningham et al. (1990) in a census of state fish/shellfish consumption advisory programs found that approximately 60 species of fish and shellfish are used as the basis for consumption advisories nationwide. The leading fish families are the Ictaluridae (catfish), Centrarchidae (sunfish, largemouth and smallmouth bass), Cyprinidae (carp), and Salmonidae (salmon and trout). Among shellfish, crustaceans (e.g., blue crab) and molluscs (e.g., American oyster, soft-shelled clam, and blue mussel) are the most widely used. The criteria most frequently used for collecting fish/shellfish species were: 1) the dominant species harvested for consumption, 2) the most abundant species and 3) the species representing a specific trophic order.

11.3.5 Consistent sampling of common species over long time periods (several years) and large geographic areas will greatly facilitate future trend analyses. Many species are similar in appearance, and taxonomic identification must be reliable to prevent mixing species. Under no circumstance should two or more species be mixed to create a composite sample. Fish for contaminant analyses may be obtained during studies to determine fish community structure. The measurement of multiple parameters (e.g., fish health condition assessment, histopathological examination, bioindicators of stress, etc.) are encouraged on common samples to provide the information needed in ecological risk assessment.

11.3.6 Screening studies should endeavor to collect the largest individuals available. However, more detailed studies should sample the predominant two or three age classes of the same species in a water body to determine the relationship between contaminant burden and fish size (age) to provide information needed for greater risk management flexibility. This information could allow the lifting of an advisory on smaller, more abundant sizes of a contaminated species with lower body burdens if these were important to a sport fishery.

11.3.7 The frequency of sampling should be considered in each study design. Most long-term monitoring programs will be based on an annual frequency due to the costs of analysis. However, special studies may require seasonal sampling. Fish sampled in the fall may tend to have a higher lipid content than those sampled during the spring. Sampling freshwater in the spring may

TABLE 1. FREQUENCY OF OCCURRENCE FOR FRESHWATER AND MARINE SPECIES IN THE NATIONAL FISH BIOACCUMULATION STUDY (USEPA, 1990a)

FRESHWATER

Bottom Feeder Species	Site Occurrence
Carp	135
White sucker	32
Channel Catfish	30
Redhorse sucker	16
Spotted sucker	10

Game (Predator) Species	Site Occurrence
Largemouth Bass	83
Smallmouth Bass	26
Walleye	22
Brown trout	10
White Bass	10
Northern Pike	8
Flathead Catfish	8
White Crappie	7
Rainbow trout	7

MARINE

Species	Site Occurence
Hardhead catfish	7
Starry flounder	5
Blue fish	5
White perch	4
Winter flounder	4
White sturgeon	4
Red drum	3
Black drum	3
Striped mullet	3
Atlantic croaker	3
Spot	3
Spotted seatrout	3
Weakfish	3
Sheepshead	2
Southern flounder	2
Flathead sole	2
Atlantic salmon	2
Red snapper	2
Gizzard shad	1
Atlantic cod	1
Yellow jack	1
Striped bass	1
American shad	1
Surf smelt	1
Spotted drum	1
Crevalle jack	1
Redstripe rockfish	1
Summer flounder	1
Diamond turbot	1
Hornyhead turbot	1
Bocaccio	1
White surfperch	1
Quillback rockfish	1
Brown rockfish	1
Copper rockfish	1
American eel	1

find fish more available due to spawning movements exhibited by spring spawning species; however, extensive movement may temporarily dislocate fish from the usual area where they have been exposed to contaminants. The various methods of collecting fillets (skin-on versus skin-off, belly flap included or excluded) must be standardized. A skin-on fillet with belly flap included is recommended. A lipid analysis of each sample is required for trend analysis and model validation, however, lipid content is not recommended for use in normalizing the differences among fillet types because it frequently increases the variance in the data (NOAA, 1989). Even when considering the bioaccumulation of lipophilic compounds all of the compound is typically not stored in the lipid. At any given time additional amounts of the compound will be found in the cell moisture and the non-lipid tissue. Lipid content may also provide insight into seasonal changes within species, as well as identify differences between species used in contaminants monitoring.

11.3.8 Active sampling techniques (electrofishing, trawling, seining, etc.) are preferred over passive capture techniques (gill nets, trammel nets, etc.) however, the latter can be used as long as the gear is checked on a frequent basis to avoid sample deterioration. Species that are difficult to collect may be obtained from a commercial fisherman, but only when the collector accompanies the fisherman to verify the time and place of capture. Following collection, fish should be placed on wet ice in clean coolers prior to processing. Fish should be either processed within 24 hours or frozen within 24 hours for later processing if immediate processing is not possible. If analyses of fish eggs or internal organs are required, a sample size of at least 20 grams is required.

11.3.9 Composite samples of three to ten fish (same species) are recommended for each of the predator and bottom feeder categories based on the variability of contaminant concentration in fish at the site. The number of fish/composite selected should remain constant over time and space for each species monitored. Composites are used to reduce the cost of analysis per fish; however, it must be recognized that statistical manipulation of the data is compromised when individual values are not determined. The smallest size fish in a composite should equal 75% of the total length of the largest fish in a composite, e.g., if the largest is 400 mm, the smallest should not be less than 300 mm. Replicate composite samples may be added as needed to meet statistical requirements; (USEPA, 1989) however, the cost of additional samples will quickly become a factor. The most important sport and/or commercial species in each feeding strategy group should be used for analysis. Composite samples can be collected for either fillet analysis (human health risk assessments) or for whole body analysis (ecological risk assessments and worst case monitoring).

11.3.10 When a study is planned, it is not certain that the quantity of each species indicated for analysis can be obtained especially if the water body has had little or no prior sampling activity. In order to meet both the human health and ecological requirements a sample of a sport fish species and a bottom feeder species is needed. The sport fish species is usually filleted and the data used for human health risk assessment. The whole body analysis of bottom feeder species is used both for initial "worst case" monitoring and for ecological risk assessment.

11.3.11 If fish are not abundant or detailed comparisons with other parameters are desired, it may be possible to do a reconstructed analysis (Figure 1) on a single species either sport fish or bottom feeder. To do a reconstructed analysis, the fish are filleted and the remainder of the carcass is saved for analysis. The contaminant concentrations in both the fillet and remaining carcass portions can then be added together to estimate the whole body concentration. A lipid analysis must be performed on both the fillet and remaining carcass to allow normalization of the contaminant concentrations in both samples. A reconstructed analysis may be performed on either single fish or composite fish samples, however, the data may be more reliable if single fish are analyzed.

11.3.12 Sediment samples can sometimes indicate a "hot spot" and can be helpful in determining the source(s) of contamination or the zones of deposition. However, sediment samples cannot be used as a substitute for fish collections, but both can provide complimentary data.

11.4. Sample Preparation For Organic Contaminants in Tissue

11.4.1 Collection Precautions

11.4.1.1 In the field, sources of tissue contamination include sampling gear, boats and motors, grease from ship winches or cables, engine exhaust, dust, and ice used for cooling. Efforts should be made to minimize handling and to avoid sources of contamination. For example, to avoid contamination from ice, the whole samples (e.g., molluscs in shell, whole fish) should be wrapped in aluminum foil, placed in watertight plastic bags, and immediately cooled in a covered ice chest. Many sources of contamination can be avoided by resecting (i.e., surgically removing) tissue in a controlled environment (e.g., a laboratory). Organisms should not be frozen prior to resection if analyses will be conducted on only selected tissues (e.g., internal organs) because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated in the field, the remaining tissue may be wrapped as described above and frozen. Tissue sample collection and preparation requirements are summarized in Table 2 (Puget Sound Estuary Program, 1989).

11.4.2 Processing

11.4.2.1 To avoid cross-contamination, all equipment used in sample handling should be thoroughly cleaned before each sample is processed. All instruments must be of a material that can be easily cleaned (e.g., stainless steel, anodized aluminum, or borosilicate glass). Before the next sample is processed, instruments should be washed with a detergent solution, rinsed with tap water, rinsed in isopropanol, and finally rinsed with organic free distilled water. Work surfaces should be cleaned with isopropanol, washed with distilled water and allowed to dry completely.

11.4.2.2 The removal of biological tissues should be carried out by or under the supervision of an experienced biologist. Tissue should be removed with clean stainless steel or quartz instruments (except for external surfaces). The specimens should come into contact with precleaned glass surfaces only. Polypropylene and polyethylene (plastic) surfaces and implements are a

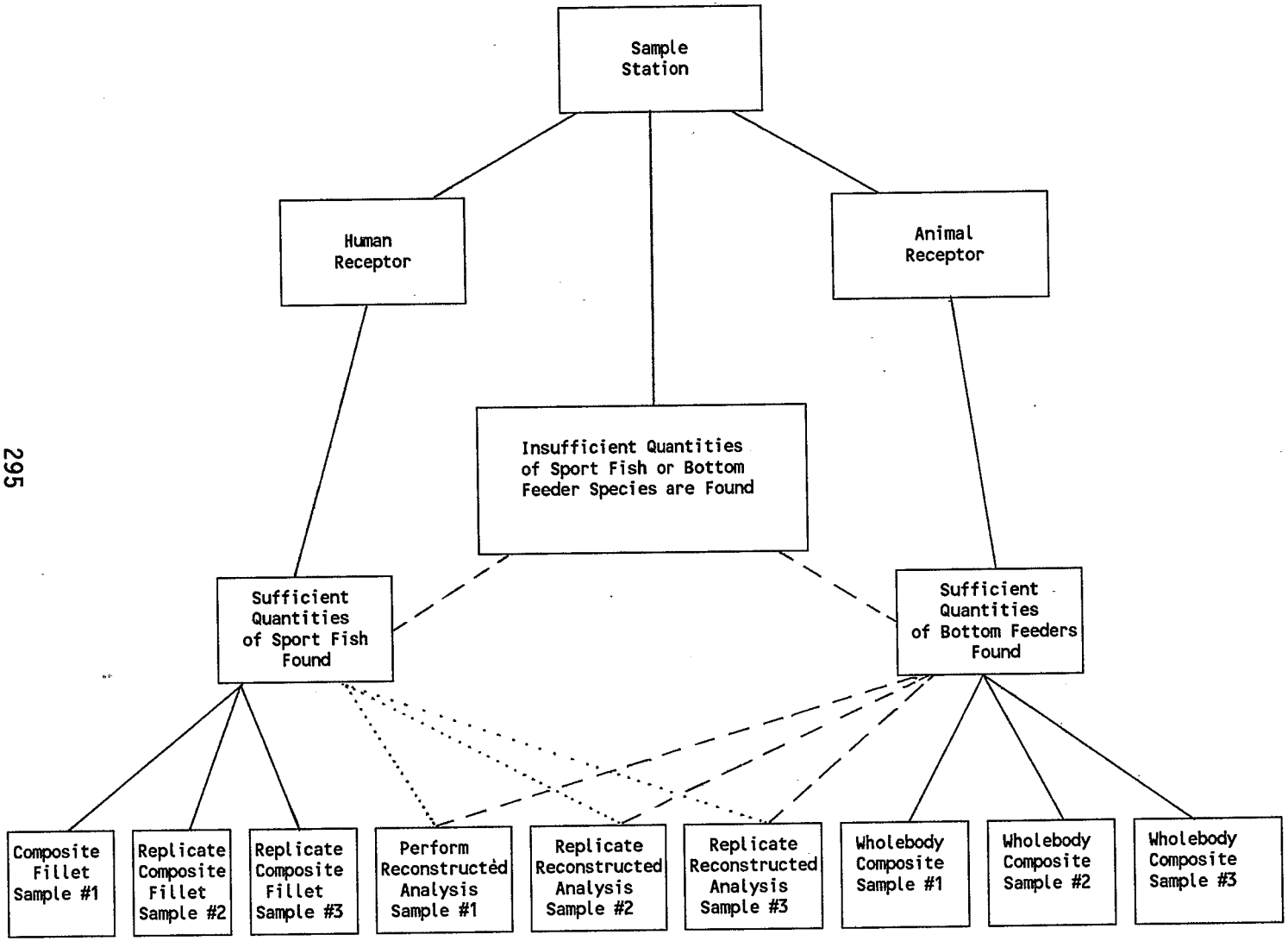

Figure 1. General sampling scheme for bioaccumulative contaminants in fish, multiple age groups will require additional samples.

TABLE 2. SUMMARY OF SAMPLE COLLECTION AND PREPARATION QA/QC REQUIREMENTS FOR FISH TISSUE (MODIFIED FROM PUGET SOUND ESTUARY PROGRAM, 1986, 1989)

Variable	Sample Size (a)	Container (b)	Preservation	Maximum Holding Time (c)	Maximum Extract Holding Time
Organic Compounds					
Wholebody Tissues (after resection)	--	A	Freeze (-18°C)	1 yr	40 days
Semivolatiles	25 g	G,T,A	Freeze (d) (-18°C)	1 yr	40 days
Volatiles	5 g	G,T	Freeze (d) (-18°C)	14 days	--
Trace Metals					
Wholebody Tissues (after resection)	--	W,P,B	Freeze	6 mo	
All Metals (except Hg)	5 g	P,B	Freeze (d)	6 mo	
	0.2 g	P,B	Freeze (d)	28 days	

a. Recommended wet weight sample sizes for one laboratory analysis. If additional laboratory analyses are required (i.e., replicates) the field sample size should be adjusted accordingly. If specific organs are to be analyzed, more tissue may be required.

b. G = glass, A = wrapped in aluminum foil, placed in watertight plastic bags, T = PTFE (Teflon), P = linear polyethylene, B = borosilicate glass, W = watertight plastic bags.

c. This is a suggested holding time. No USEPA criteria exist for the preservation of this variable.

d. Post-dissection

potential source of contamination and should not be used. To control contamination when resecting tissue, technicians should use separate sets of utensils for removing outer tissue and for resecting tissue for analysis.

11.4.3 Preparation of Composite Fillet Samples

11.4.3.1 For fish samples, special care must be taken to avoid contaminating targeted tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The proper handling in the preparation of fish tissue samples to decrease the likelihood of contamination cannot be over emphasized. To reduce variation in sample preparation and handling, samples should be prepared in the laboratory rather than in the field. However, if no laboratory is available, field preparation is acceptable if portable tables are used, dust and exhausts are avoided and proper decontamination procedures are followed. Regardless of where preparation occurs, the following subsections should be followed to insure quality fillet samples:

11.4.3.2 To initiate processing, each fish is measured (total or fork length) to the nearest tenth of a centimeter, weighed (nearest gram) and external condition noted. A few scales should be removed from each fish for age and growth analysis. This presents an excellent opportunity to systematically evaluate each fish using the Fish Health and Condition Assessment Methods (Section 10). Fish are scaled (or skinned: catfish) and filleted carefully, removing bones, to get all of the edible portion flesh.

11.4.3.3 A fillet includes the flesh tissue and skin from head to tail beginning at the mid-dorsal line from the left side of each fish and including the belly flap. The fillet should not be trimmed to remove fatty tissue along the lateral line or belly flap. A comparable fillet can be obtained from the right side of the fish and can be composited with the left fillet, kept separate for duplicate quality assurance analysis, analyzed for different compounds or archived. Each right and left fillet should be weighed individually, recorded and individually wrapped in clean aluminum foil.

11.4.3.4 Care must be exercised not to puncture any of the internal organs. If the body cavity is entered, rinse the fillet with distilled water. Fish sex and condition of internal organs are determined during or after filleting. This skin-on fillet deviates from the skin-off fillets analyzed in the National Fish Bioaccumulation Study (USEPA 1990a), however, skin-on is recommended because it is believed that this is the way most sport anglers prepare their fillets. The issue of skin-on versus skin-off fillets differs greatly among jurisdictions (Hesse, 1990) and is far from settled, however, the above recommendations appear to be the preferred method unless the species specificity is increased in future guidelines.

11.4.3.5 Filleting should be conducted on cutting boards covered with heavy duty aluminum foil, which is changed between composite samples. Knives, fish scalers, measurement boards, scales, etc. should be cleaned with reagent grade isopropanol, followed by a rinse with distilled water between each composite sample.

11.4.3.6 Because of the low limits of detection for many environmental analyses, clean field and laboratory procedures are especially important. Sample contamination can occur during any stage of collection, handling, storage or analyses. Potential contaminant sources must be known and steps taken to minimize or eliminate them.

11.4.3.7 Large sheets of heavy duty aluminum foil should be used to carefully fold and completely wrap the fillet samples. When filling out I.D. labels use pencil or waterproof marker and place the foil wrapped sample in a secured plastic bag.

11.4.4 Storage

11.4.4.1 Recommended holding times for frozen tissue samples have not been established by USEPA, but a maximum 1 year holding time is suggested. For extended sample storage, precautions should be taken to prevent desiccation. National Institute For Standards and Technology is testing the effects of long-term storage of tissues at temperatures of liquid nitrogen(-120° to -190°C). At a minimum, the samples should be kept frozen at -20°C until extraction. This will slow biological decomposition of the sample and decrease loss of moisture. Liquid associated with the sample when thawed must be maintained as part of the sample because the lipid tends to separate from the tissue. Storage of samples should remain under the control of the sample collector until relinquished to the analytical laboratory.

11.4.4.2 Whole fish may be frozen and stored if no resection of internal organs or fillets will be conducted and the ultimate analysis is whole body. However, if resection of fillets or organs is required, these tissues should be removed prior to freezing and can be stored frozen in appropriate individual containers. The tissues may then be ground and homogenized at a later date and refrozen in sample packets for shipment on dry ice to the analytical laboratory(s).

11.4.4.3 It is frequently necessary to ship whole fish, fillets or homogenized tissue samples over long distances to an analytical laboratory. To avoid sample deterioration, it is recommended that all samples be frozen solid prior to shipment. The frozen and logged samples should be wrapped in newspaper to provide additional insulation for the samples which are shipped in well sealed insulated containers with an appropriate quantity of dry ice. The quantity of dry ice should be sufficient to eliminate any defrosting of the samples during the time of priority transport. However, in the event that a delay occurs in transit, these recommendations will provide some assurance that the samples will arrive in usable condition. Under no circumstances should unfrozen tissue be shipped either with or without dry ice because the quality of the sample cannot be assured.

11.4.5 Tissue Preparation

11.4.5.1 Organic contaminants are not evenly distributed throughout biological tissue, especially in fish. This is also true for fish fillets. Therefore, to obtain a homogenous sample, the whole fish or the whole fillet

must be ground to a homogeneous consistency. This procedure should be carried out by the sample collector on partially thawed samples.

11.4.5.2 Chop the sample into 2.5 cm cubes unless the sample is small enough to fit in a hand crank meat grinder (300 gm or less) or a food processor (Hobart Model 8181D or equivalent for large fish) (USEPA, 1990b). Then pass the whole sample through a meat grinder. Grinding of biological tissue is easier when the tissue is partially frozen. This is especially true when attempting to grind the skin. Chilling the grinder with a few chips of dry ice will reduce the tendency of the tissue to stick to the grinder. Do not freeze the grinder since hard frozen tissue is difficult to force through the chopper plate.

11.4.5.3 The ground sample is divided into quarters, opposite quarters are mixed by hand with a clean stainless steel spatula and then the two halves are mixed back together. Repeat the mechanical grinding, quartering and hand mixing two more times. No chunks of tissue should be present at this point as they will not be efficiently extracted. Very small fish or small fillets may be homogenized in a high speed blender.

11.4.5.4 When compositing fillets or whole fish each individual fillet or fish should be ground separately following the above described procedure. Then take equal amounts from each fillet or fish sample to be composited to provide a total equal to that required for extraction or the total number of split and archived samples required by the study plan.

11.4.5.5 If the ground fish is to be re-frozen prior to extraction and analysis, weigh out the exact amount for extraction into a small container. Using a top loading balance, tare a 2 oz. glass jar (or a small sheet of aluminum foil that can be formed into a sealed packet) to 0.0 gm and carefully dispense a 20.0 gm portion of homogenized tissue into the container. Tightly seal the container or foil packet. Repeat with additional containers for duplicates, splits, or archived samples. Lipid material tends to migrate during freezing; therefore, storing a weighed portion ensures extraction of a representative portion of the tissue if the foil or container is completely rinsed with solvent by the analytical chemist.

11.4.5.6 Whenever a ground sample is to be split between two or more labs, the ground sample must also be mixed with reagent grade anhydrous sodium sulfate (previously heated to 400°C to drive off any phthalate esters acquired during storage). To ensure the homogeneity of the sample prior to splitting, transfer 100 gm of ground tissue to a 600 mL beaker. Add 250 gm of anhydrous sodium sulfate and mix thoroughly with a stainless steel spoon or a spatula. There should not be any lumps and the mixture should appear homogeneous. Dispense exactly 70.0 gm of mixture to each lab and note on the package that it contains 20 gm of tissue.

11.4.5.7 When preparing the tissue for volatile analysis, grind it in an area free of volatile organic compounds. The meat grinder or food processor must be heated in an oven for 30 minutes at 105°C after solvent rinsing and then allowed to cool at room temperature. Immediately after grinding the tissue,

weigh duplicate 1 gm portions into culture tubes with screw caps. Analyze immediately or store in a freezer.

11.5 Sample Preparation For Metal Contaminants In Tissue

11.5.1 Collection Precautions

11.5.1.1 The major difficulty in trace metal analyses of tissue samples is controlling contamination of the sample after collection. In the field, sources of contamination include sampling gear, grease from winches or cables, engine exhaust, dust, or ice used for cooling. Care must be taken during handling to avoid these and any other possible sources of contamination. For example, during sampling the ship should be positioned such that the engine exhausts do not fall on deck. To avoid contamination from melting ice, the samples should be placed in watertight plastic bags.

11.5.1.2 Sample resection and any subsampling of the organisms should be carried out in a controlled environment (e.g., dust-free room). In most cases, this requires that the organisms be transported on ice to a laboratory rather than being resected in the field. It is recommended that whole organisms not be frozen prior to resection if analyses will be conducted only on selected tissues, because freezing may cause internal organs to rupture and contaminate other tissue. If organisms are eviscerated in the field, the remaining tissue (e.g., muscle) may be wrapped as described above and frozen (Puget Sound Estuary Program, 1986).

11.5.1.3 Resection is best performed under "clean room" conditions. The "clean room" should have positive pressure and filtered air and also be entirely metal-free and isolated from all samples high in contaminants (e.g., hazardous waste). At a minimum, care should be taken to avoid contamination from dust, instruments, and all materials that may contact the samples. The best equipment to use for trace metal analyses is made of quartz, TFE (tetrafluoroethylene), polypropylene, or polyethylene. Stainless steel that is resistant to corrosion may be used if necessary. Corrosion-resistant stainless steel is not magnetic, and thus can be distinguished from other stainless steels with a magnet. Stainless steel scalpels have been found not to contaminate mussel samples (Stephenson et al., 1979). However, low concentrations of heavy metals in other biological tissues (e.g., fish muscle) may be contaminated significantly by any exposure to stainless steel. Quartz utensils are ideal but expensive. To control contamination when resecting tissue, separate sets of utensils should be used for removing outer tissue and for removing tissue for analysis. For bench liners and bottles, borosilicate glass would be preferred over plastic if trace organic analyses are to be performed on the same sample.

11.5.1.4 Resection should be conducted by or under the supervision of a competent biologist. Special care must be taken to avoid contaminating target tissues (especially muscle) with slime and/or adhering sediment from the fish exterior (skin) during resection. The procedure previously outlined for the preparation of fillet samples should generally be followed. Unless specifically sought as a sample, the dark muscle tissue that may exist in the

vicinity of the lateral line should not be separated from the light muscle tissue that constitutes the rest of the muscle tissue mass.

11.5.1.5 Prior to use, utensils and bottles should be thoroughly cleaned with a detergent solution, rinsed with tap water, soaked in acid, and then rinsed with metal-free water. For quartz, TFE, or glass containers, use 1+1 HNO_3, 1+1 HCl, or aqua regia (3 parts conc. HCl + 1 part conc HNO_3) for soaking. For plastic material, use 1+1 HNO_3 or 1+1 HCl. Reliable soaking conditions are 24 h at 70°C (APHA, 1989; 1992). Do not use chromic acid for cleaning any materials. Acids used should be at least reagent grade. For metal parts, clean as stated for glass or plastic, except omit the acid soak step. If trace organic analyses are to be performed on the same samples, final rinsing with methylene chloride is acceptable.

11.5.1.6 Sample size requirements can vary with tissue type (e.g., liver or muscle) and detection limit requirements. In general, a minimum sample size of 6 g (wet weight) is required for the analysis of all priority pollutant metals. To allow for duplicates, spikes, and required reanalysis, a sample size of 50 g (wet weight) is recommended. Samples can be stored in glass, TFE, or high-strength polyethylene jars.

11.5.2 Processing

11.5.2.1 Samples should be frozen after resection and kept at -20°C. Although specific holding times have not been recommended by USEPA, a maximum holding time of 6 months (except for mercury samples, which should be held a maximum of 28 days) would be consistent with that for water samples.

11.5.2.2 When a sample is thawed, the associated liquid should be maintained as a part of the sample. This liquid will contain lipid material. To avoid loss of moisture from the sample, partially thawed samples should be homogenized. Homogenizers used to grind the tissue should have tantalum or titanium parts rather than stainless steel parts. Stainless steel blades used during homogenization have been found to be a source of nickel and chromium contamination. Some trace metal contamination during processing cannot be avoided and it is therefore necessary to determine and control the amount of contamination introduced during processing. Contamination can be monitored by introducing a dry ice blank into the blender and analyzing the chips.

11.5.2.3 To avoid trace metal contamination during processing the preferred method is to proceed to a chemical digestion process which minimizes or eliminates resection, homogenization, or grinding. Chemical digestion is best limited to specific organ tissues from large fish or to smaller sized whole fish.

11.6 Identification of Composite Whole Fish or Fillet Samples

11.6.1 Composite whole fish samples will be made up of three to ten fish with any deviation in number clearly identified. The limitation on the variance between individual fish in each composite will be as previously described. The length and weight of each fish must be recorded. The same field

information should be provided as described above for both fillet and/or whole body composite samples. The same handling precautions as described above should be followed for either organic or trace metal contaminants. Spines on whole fish should be sheared to minimize puncturing the sample packaging.

11.6.2 The following information should be included on the field/lab form for each sample collected:

11.6.2.1 Project Name

11.6.2.2 Station Code (if applicable)

11.6.2.3 Date

11.6.2.4 Collector's Name

11.6.2.5 Sampling location (river mile and/or other specific information relating to local landmarks)

11.6.2.6 Latitude and Longitude

11.6.2.7 Water body name

11.6.2.8 Sampling technique(s), i.e. 230 vac electrofishing apparatus, hoop nets, etc.

11.6.2.9 Fish species

11.6.2.10 Individual lengths and weights of fish in sample

11.6.2.11 Sample type (Whole or Fillet)

11.6.2.12 Individual fillet weights (whether left or right)

11.6.2.13 Comments or Unusual Conditions, i.e., tumors, sores, fin rot, blind, etc.

11.7 Chain-of-Custody Procedures (USEPA, 1990c; USEPA, 1991) Also See Section 2, Quality Assurance and Quality Control.

11.7.1 All samples should be kept in a secure (locked) area to avoid legal complications in administrative proceedings. Transportation of the samples must be coordinated between the agency responsible for the field collection and the agency responsible for analytical work. When custody of the samples is transferred, the following checks should be implemented:

11.7.1.1 All transfers should be properly relinquished to ensure chain-of-custody. Transfers should be recorded on a form separate from the field data sheet. The chain-of-custody form should include the sample identification number(s). Custody tags must be used and numbered in sequence (if possible).

11.7.1.2 The field data sheet should stay with the sample until it is logged in by the analytical laboratory.

11.7.1.3 Samples can be shipped and chain-of-custody maintained as long as shipping containers are sealed with custody tape.

11.7.1.4 Samples should remain frozen until they are prepared for analysis. Shipping with dry ice is recommended.

11.7.1.5 The laboratory's receiving agent should initial the field data sheet and affix the date of sample receipt. Depending on administrative need, a copy of this form (with initials and date of sample receipt plainly visible) may be required by the lab agency's central office.

11.8 Conclusion

11.8.1 This protocol only addresses the steps to be considered in field sampling fish and sample preparation for human health fish consumption advisories and ecological risk assessment. Additional protocols must be followed to carry out the appropriate analytical chemistry and the risk assessment/management requirements leading to an action. These additional protocols were beyond the scope of this assignment.

11.9 Literature Cited

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SECTION 12

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