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FIELD METHODS AND STATISTICAL ANALYSES FOR MONITORING SMALL SALMONID STREAMS

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FIELD METHODS AND STATISTICAL ANALYSES FOR MONITORING SMALL SALMONID STREAMS

by

Carl L. Armour
Kenneth P. Burnham
Western Energy and Land Use Team
U.S. Fish and Wildlife Service
Drake Creekside Building One
2627 Redwing Road
Fort Collins, CO 80526

and

William S. Platts
U.S. Forest Service
Intermountain Forest and Range
Experiment Station
316 East Myrtle
Boise, ID 83702

Western Energy and Land Use Team
Division of Biological Services
Research and Development
Fish and Wildlife Service
U.S. Department of the Interior
Washington, DC 20240

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PREFACE

This document is written primarily for field workers responsible for designing and conducting monitoring programs in small western salmonid streams affected by various land uses, including grazing and timber harvest practices. Variables to measure and types of statistical tests used to evaluate responses of salmonids and habitat to land use practices are presented. Users of this document will need to be familiar with statistical concepts, including sampling variance, confidence intervals, probability distributions, and hypothesis testing. Statistical tests presented in this document can be performed on a hand-held calculator with log, antilog, mean, variance, standard deviation, regression, and correlation functions. A statistician should be consulted prior to designing and conducting any monitoring program. Monitoring programs should be coordinated with the appropriate State fish and game agency prior to their initiation. The authors recommend that users obtain a copy of Methods for Evaluating Stream, Riparian, and Biotic Conditions (Platts et al. 1983, U.S.D.A. Forest Service, Intermountain Forest and Range Experiment Station, 507 25th Street, Ogden, UT 84401) for use in combination with this document.

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ACKNOWLEDGMENTS

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CHAPTER I. INTRODUCTION

The western United States is influenced by many land management practices that can affect fish, including energy development, livestock grazing, timber harvest, reclamation of desert land for agriculture, and use of water for irrigation. This document is intended to aid field personnel in designing monitoring programs to evaluate the effects of land management practices on aquatic resources, especially on small salmonid streams in the West. Sampling techniques and statistical tests for analyzing data are emphasized.

The scope of a monitoring program depends on its purpose and available human resources and funds. Monitoring programs may be initiated for several reasons; e.g., to provide the data for use in court to substantiate an agency's position on management approaches, to justify implementing a management program elsewhere, or to evaluate the general condition of an area following a land use change. If data are to be used in court, Guidelines for Preparing Expert Testimony in Water Management Decisions Related to Instream Flow Issues, by Lamb and Sweetman (1979), should be consulted.

Steps for planning a successful stream monitoring program are outlined in Figure 1. Step 1 (Baseline Evaluation) is critically important. Documentation of baseline conditions and factors affecting aquatic resources is a necessary basis for a sound management program.

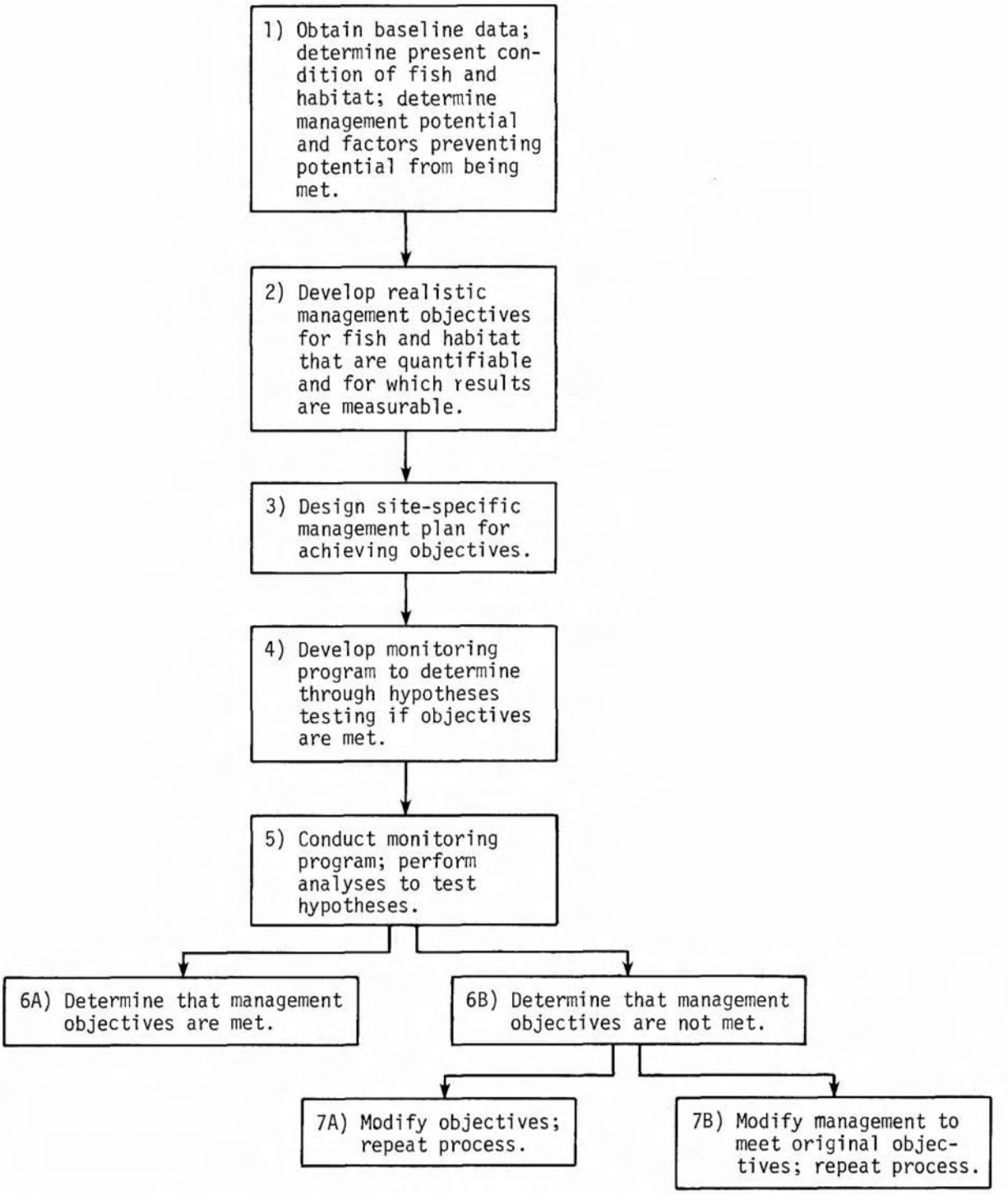

Figure 1. Steps in a stream monitoring program.

When baseline conditions are measured in order to evaluate the status of habitat and fish communities, a preliminary pilot survey is essential in determining if planned sampling approaches and methods are feasible (Green 1979). Advantages and disadvantages of a given method, time and financial constraints, and personnel availability and their expertise should be considered on a site-specific basis in determining the best method. The practicality of the sampling technique also needs to be considered; e.g., sampling equipment must be portable if a study site is not easily accessible. It is advisable to use the same methods in areas where sampling has previously occurred if data comparability is desired. If satisfactory sampling methods have not been developed for a variable, it might be necessary to select another variable for measurement or to develop new sampling methods. Selection of a substitute variable with established sampling methods may be preferable to trying to develop a new, untested sampling method.

Criteria for use in selecting the variables to measure include:

1. Expected responsiveness of variables to habitat management actions and measurability of the responsiveness;

2. Feasibility of precise sampling (Green 1979);

3. Feasibility of sampling at reasonable costs (Green 1979; Hirsch 1980);

4. Legal status of the variables; e.g., endangered species; and

5. Level of the variables in the trophic structure, such as top predators or organisms that can serve as integrators of habitat quality (Hirsch 1980).

Variables chosen must be closely related to the cause and effect relationship to be effective in the evaluation. For example, if the program objectives are to determine the effects of grazing on trout biomass, changes in the

habitat resulting from grazing and changes in the trout biomass should be measured. A more comprehensive process for selecting measurement variables is described by Fritz et al. (1980).

The cost of the monitoring program will affect its design. If the planned cost is not within the financial means of the involved agencies, the monitoring program may not be implemented. Green (1979:180) advises:

> The best rule to follow for both the number of biotic variables and the number of environmental variables is the fewer the better, consistent with adequate description of the impact effects and any natural background variation.

Management objectives (Step 2) should be stated clearly and precisely. For example, the objective might be to narrow the stream width by 50% in a badly degraded area or to establish enough streamside vegetation to lower the water temperature by 3° C during the hottest periods of the summer. A fisheries management objective might be to improve habitat to such a degree that mean length of fish would increase by 25%.

The site-specific management plan (Step 3) for meeting the objective is best developed through an interdisciplinary approach. For example, if the study site is on a rangeland, the plan should be developed with participation of specialists in range conservation, as well as watershed management, soils, hydrology, and aquatic biology. This interdisciplinary approach helps ensure that the management plan will be practical, technically feasible, and compatible with objectives for fish and aquatic habitat. Management plans should be designed to solve and prevent problems affecting the resources, not to provide temporary stop-gap improvements with no lasting impact.

Considerations for designing a successful monitoring program (Step 4) are discussed in Chapters IV and V. Above all, the purpose of the program should be to determine if management objectives for fish and aquatic habitat are met, not merely to collect data. When the program is designed, the appropriate

sampling frequency and dates, the number of replicates, and the stratification of sampling, if necessary, need to be included. Green (1979:70) lists the following prerequisites for optimal program design:

> ... at least one time of sampling before and at least one after the impact [or management program] begins, at least two locations differing in degree of impact [or management], and measurements on an environmental as well as a biological variable set in association with each other.

A control is needed in both time and space whenever circumstances permit this type of design. Also, it is advisable to take a series of photographs at permanent locations before, during, and after management to visually document changes.

The sampling design must be suitable for testing hypotheses related to responses of the site to change. Therefore, the statistical design of the program must be appropriate for the statistical tests to be performed, the sampling strategy, and the properties of the data that will be collected.

After the monitoring program is designed, data are collected (Step 5). It is important to emphasize that even a correctly designed monitoring program will fail if poor data collection occurs in the field. Hunter (1980) emphasized the need for obtaining high quality data with dependable measuring techniques. The use of trained, experienced, and reliable field personnel is necessary to obtain dependable results. Factors other than poor data collection techniques (Chaper IV) can adversely affect monitoring programs if precautionary measures are not taken. Unusual field conditions that could affect the results of a program in progress should be documented. If these conditions are detected early enough, corrective measures to prevent the program from failing may be possible.

The collected data should be analyzed to evaluate the statistical significance of any differences between managed sites and control sites. As pointed out by Green (1979:63-64):

> Having chosen the best statistical method to test your hypothesis, stick with the result. An unexpected or undesired result is not a valid reason for rejecting the method and hunting for a "better" one.

If an unexpected result is obtained, an explanation should be attempted. The lack of a significant difference between pre- and postmanagement values does not necessarily mean that a change has not occurred. Failure to detect a change may be due to several reasons, including poor program design, extreme variability in the data, insufficient sample size, and statistical tests that are not sufficiently sensitive.

Holling (1978) lists four types of environmental assessment information that should be considered in data interpretation: (1) the data base, both actual measurements and assumptions; (2) the technical methods used in the analysis and their assumptions; (3) the results of the analyses; and (4) the conclusions derived from the results. Holling further states that the last two types of information have the highest priority; both of these types have two facets, the literal meaning of the results and the degree of professional confidence in the results. Information obtained from the monitoring program should be assembled into a format that is understandable by resource specialists and decisionmakers (States et al. 1978).

After Step 5 (Fig. 1) is completed, a field specialist can conclude, with an established degree of statistical confidence, whether or not management objectives are met (Step 6A or Step 6B). If objectives are not met, assuming adequate time has lapsed for the site to respond to management, the original objectives can be modified (Step 7A) or different management actions can be taken to meet the original objectives. Management practices can be advanced when unsuccessful practices documented during a monitoring program are avoided at other sites.

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CHAPTER II. LAND USE IMPACTS AND VARIABLES TO MEASURE

ADVERSE IMPACTS OF LAND USES

Management programs can be undertaken to improve stream conditions adversely impacted by various land uses. Therefore, it is necessary to understand how land use practices can impact streams (Fig. 2). Impacts are not always detrimental, and the importance of individual impacts will vary among streams. For instance, an increase in water temperature due to removal of riparian vegetation can be beneficial in areas where the waters are too cold for good salmonid growth. However, only potential adverse impacts are discussed in this document. In the West, overgrazing and improper timber harvesting and mining practices are among the several factors that can damage aquatic habitats and salmonid populations.

Overgrazing by livestock has a variety of potential adverse impacts (Lusby 1970; Armour 1977; Behnke and Raleigh 1978; Bowers et al. 1979; Cope 1979; Platts 1979). Livestock can compact the soil, reduce ground cover, and trample stream banks, which can result in increased erosion and sedimentation in the stream. Salmonid spawning and rearing habitat may be lost, in addition to reductions in macroinvertebrate populations, which are important salmonid food. Overgrazing can affect stream depth, pool and rubble relationships, water temperature, and protective cover to the detriment of salmonids.

Timber harvest and associated activities (e.g., road construction) can impact streams in similar ways to overgrazing, including compacting soil and decreasing ground cover, resulting in increased surface runoff, erosion, and sedimentation in the stream (Brown and Krygier 1970, 1971; Burns 1970; Gibbons and Salo 1973; Brna 1977; Harr et al. 1979; Yee and Roelofs 1980).

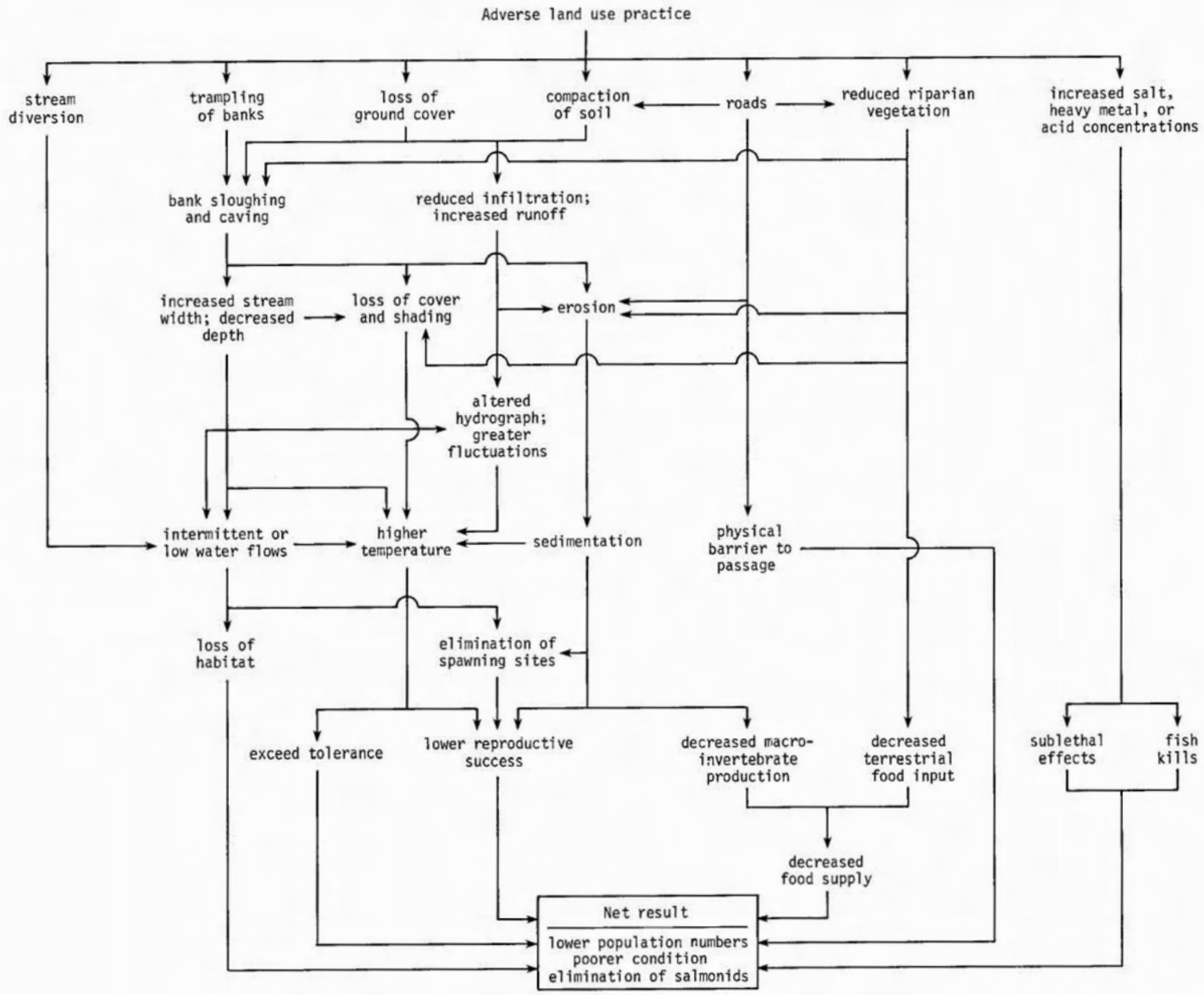

Figure 2. Potential impacts of diverse land uses on salmonids. The impacts can result from several factors, including improperly managed grazing, mining, timber harvesting, and recreation uses.

Impacts due to mining vary depending on the proximity of the mine to the stream, mining methods, and the ore being mined. Surface mining disturbance can increase runoff by decreasing the infiltration rate and reducing the hydraulic resistance of the surface (U.S. Forest Service 1980). A major potential impact of surface mining is the concentration of salts and heavy metals in the runoff water. Overland flow water and seepage from the spoil materials may be contaminated with materials that are toxic to aquatic organisms. Runoff and surface drainage flowing over and through copper spoil tends to contain heavy metals and be slightly acidic, while waters flowing over and through coal, bentonite, oil shale, phosphate, uranium, and gypsum may contain substances that adversely impact salmonids (Moore and Mills 1977). Roads associated with a mine may have a greater impact on the surface water flow and water pollution than impacts directly associated with a disturbed mine site (U.S. Forest Service 1980).

SELECTION OF VARIABLES TO MEASURE

Variables to be monitored (Table 1) should be selected carefully for the most direct cause and effect relationships. For example, symptoms of overgrazing are bank sloughing, increases in stream width, and decreases in stream depth. Improved management should result in the reestablishment of a deeper, narrower stream channel that supports more salmonids. Key variables to measure in this situation would be stream width and depth, streambank stability, amount of riparian vegetation, and salmonid population size.

Key Habitat Variables

Width and depth. The width and depth of streams (Fig. 2) can change with different land uses, due to changes in stream bank stability. The recovery of a degraded stream is accompanied by changes in stream width, depth, substrate, cover for fish, and bank and channel stability. Stream width and depth are especially important because several types of improper land use practices may result in instability and sloughing of stream banks.

Table 1. Key variables for which measurement methods are presented in Chapter III of this manual.

Variables	
Habitat	Fisheries
Stream width	Species composition
Stream depth	Relative abundance
Discharge	Lengths
Water velocity	Weights
Bottom surface substrate	Population numbers
Embeddedness	Biomass
Streambank stability rating	
Cover	
Pools and riffles	
Temperature	

Stream discharge and velocity. Stream discharge can be affected by timber harvesting, overgrazing, and mining when vegetation on lands adjacent to the stream is removed or damaged. Generally, when vegetation is adversely affected, the result is greater fluctuations in discharge on an annual basis with a greater peak runoff and reduced low flows. Intermittent stream conditions also may develop. Streams with unstable discharge regimes are poor habitats for fish (Hynes 1970). Hynes considers the rate of flow and fluctuation in discharge to be two of the most important abiotic factors affecting fish in running waters. Velocity is, by itself, an important attribute, especially as it relates to substrate.

Bottom substrates. Substrate is an important aspect of the fish habitat and is affected by sedimentation. Where sediment influx to the stream exceeds the capacity of the stream to transport the sediment or flush it out, deposition occurs. Sedimentation can be harmful to salmonid reproductive success. Salmonids spawn in gravel relatively free of sediments; otherwise eggs and larval fish may suffocate (Bell 1973; Armour 1977). Suffocation occurs because sediment fills intergravel spaces which reduces percolation, lessening oxygenation and the flushing of embryonic waters. The "smothering" of eggs by sediment also can promote the growth of fungi, which may spread from dead eggs throughout the entire redd. Additionally, hatched fish can be trapped by sediment during emergence from the gravel. Embeddedness pertains to the degree that the larger particles (boulder, rubble, or gravel) are surrounded or covered by fine sediment (Platts et al. 1983). As the percent of substrate embeddedness decreases, the biotic productivity increases.

Bank and channel stability and cover. When the banks and channel are unstable, the resulting erosion can decrease fish cover and increase sedimentation downstream. Cover for salmonids consists of sheltered areas in a stream channel where fish can rest and hide from predators. Thus, cover is a primary requirement of suitable habitat. In small streams, important sources of cover are streambank (riparian) vegetation and overhanging banks, both of which can be adversely affected by several land uses, including overgrazing.

Pools and riffles. Although pools are important to fish as resting areas and cover, food production by benthic macroinvertebrates is often greatest in the riffle areas (Usinger 1974). To sustain good fish populations, there should be a balance between the amount of pools and riffles.

Water temperature. Water temperature elevations can affect salmonid growth, larvae and egg development, feeding, swimming endurance, and reproduction. Temperatures that are too warm also can result in direct mortality and increased disease problems. Hynes (1970) considers water temperature one of

the most important abiotic factors in the habitat of fish in lotic waters. Water temperatures are particularly critical in small streams with limited volumes of water where even small changes in the amount of shading can result in drastic temperature fluctuations.

Key Salmonid Variables

The key variables for salmonids include species composition, relative abundance, length-weight relationships, population numbers, and biomass. Improvements of these variables should be the objective of a salmonid management plan. For example, a management objective may be to produce longer, heavier fish. After management has been implemented long enough to affect fish growth, fish lengths and weights can be monitored to determine if the management objective was met.

OTHER MEASUREMENTS

There are stream features, other than the key variables discussed in this document, that may be of interest from a management standpoint. These variables can be measured if sufficient time and money are available. For example, if the response of the ecosystem as a whole is of concern, units of the aquatic community (including benthic macroinvertebrates) can be studied. Macroinvertebrate variables that might be measured include biomass, species composition, and drift or emergence. Other salmonid variables that might be of interest under some circumstances include net production, age and growth estimates, fecundity, parasitism, and disease incidence.

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CHAPTER III. MEASUREMENT TECHNIQUES

Sampling and measurement techniques for the variables to be monitored are presented in this chapter. Techniques discussed do not include all those currently used. Procedures selected for inclusion are relatively easy to apply, can be analyzed statistically, and are applicable to small western streams. Additional techniques that may be needed are referenced.

The following general sampling procedures should be followed in any monitoring program:

1. Before going into the field:

    a. Compile a checklist of necessary equipment;

    b. Check equipment to make certain it is operating correctly;

    c. Inform personnel of their program responsibilities and train them as needed to perform the necessary field work; and

    d. Document selected sampling procedures.

2. A complete description of the sampling sites should be made during the first sampling trip so that the sites can be easily relocated by new personnel.

3. Photograph the sites before, during, and after treatment from permanent photo points.

4. Take careful field notes on each sampling trip, including information on the sampling site, time of sampling, weather conditions, and any unusual habitat conditions (e.g., especially turbid water).

5. When sampling, do not disturb the site to such a degree that measurements of other attributes are affected.

Both control and sample sites should be at least 100 m in length, if possible, and should be permanently marked with stakes or flags. Control sites should be both physically and biologically similar to the site that will be managed. If only one control site is used, it should be upstream from the treatment site. If the control site must be in another stream, the streams should be similar or the differences should be well documented in advance of any management changes or monitoring activities. The control and treatment sites should be the same size and have the same stream gradient. Walkotten and Bryant (1980) describe a simple instrument that does not require line of sight that can be used to measure stream channel gradient and profiles. Topographic maps produced by the U.S. Geological Survey can be used to estimate gradient.

Sampling should be conducted at similar times for each site and year. High and low water conditions have profound impacts on the physical and biological environment of the stream so these conditions must be considered when sampling programs are designed and conducted.

It is recommended that metric units be used in all sampling measurements. If English units are used, they can later be converted to metric units (see Appendix A for common conversions).

KEY HABITAT VARIABLES

Width

Stream width measurements, at the water surface level, should be made at several equally spaced transects along both the control and managed sites (Fig. 3). The number of transects depends on the variability in width in the sample sites. Minimally, 10 permanently marked transects should be measured. Measurements should be taken perpendicular to the flow of the water with a tape measure stretched across the stream from one bank to the other (Fig. 4). If the stream is divided into two channels, each channel should be measured separately. If the stream is too wide to use a tape measure, a survey instrument should be used to determine width. Stream width can be computed as the average of the "n" measured widths:

$$\bar{W} = \frac{1}{n} (W_1 + W_2 + ... + W_n)$$

where W_i = individual width measurements

n = number of transects in the sample

The channel width can be measured as an alternative to stream width. This type of measurement may be more useful if large fluctuations in discharge are expected. The width of the channel should be measured at maximum bankful water levels.

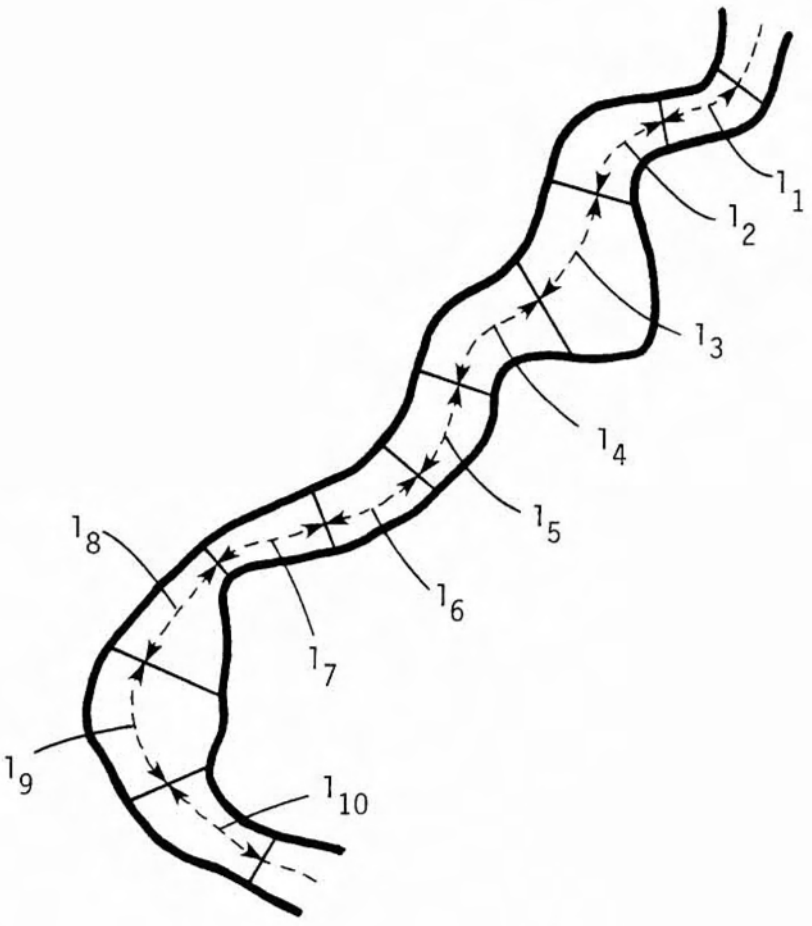

Figure 3. Spacing of transects along the thalweg of a stream should be equidistant; e.g., each length indicated by an $l_{(1-10)}$ is the same throughout.

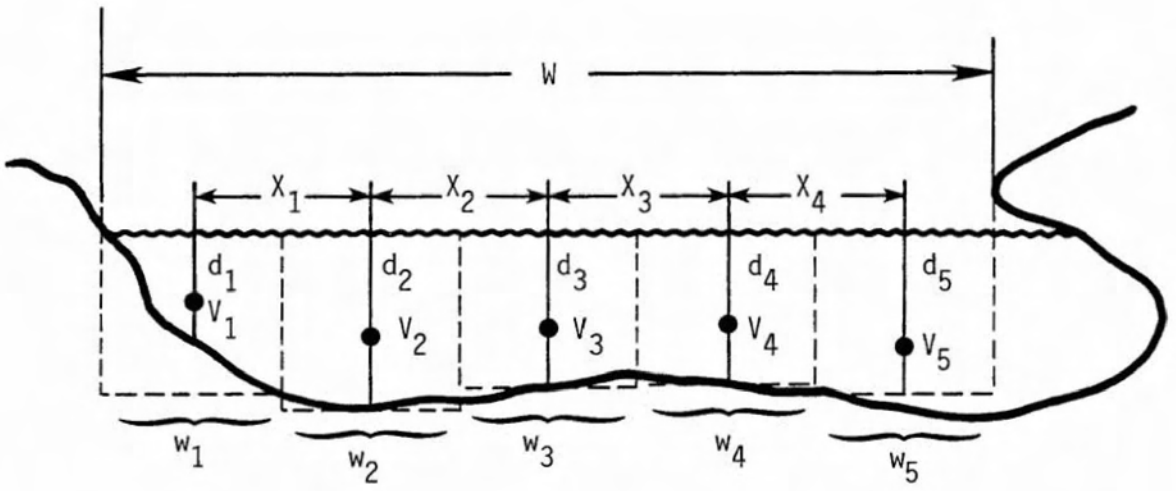

Figure 4. Stream width (W), depth (d), and velocity (V) measurement locations on a transect. Stream width usually is measured as the distance of the observable water surface between banks. Depth is calculated as the average of several values across a transect. Distances between sampling points (e.g., X_1 and X_2) are equal. Widths of sampling cells (e.g., w_1 and w_2) are also equal.

Depth

Stream depth should be measured along the permanent transects established for measuring stream width (Fig. 4). For each transect, the average depth is:

$$\bar{d} = \frac{1}{n}(d_1 + d_2 + \ldots + d_n)$$

where d_i = an individual depth measurement on the transect

n = number of measurements taken on the transect. The average depth of the site is the average of the depths for all the transects if the transects are equally spaced.

Velocity and Discharge

The procedure used to measure velocity and discharge depends on the purpose of the monitoring program and the precision required. Mean channel velocity or discharge are measured along a transect perpendicular to the stream flow. Alternatively, the velocity of salmonid microhabitat (e.g., velocity of water through spawning gravel) may be measured.

Velocity. Current meters are commonly used to determine velocity (m/sec or ft/sec). Some current meters register revolutions per minute, from which the velocity is calculated; other current meters measure velocity directly. The meter must be facing directly into the stream flow and sampling should not be done in turbulent areas because inaccurate readings will result. Current meters need to be carefully used and calibrated.

Velocity varies with stream depth (Fig. 5) and width. The velocity approximates zero at the channel bed and increases toward the water surface. The velocity measured at 0.6 of total depth from the surface of the water is approximately the mean velocity for the vertical section. The average of the velocity taken at 0.2 and 0.8 of total depth is a close approximation of the

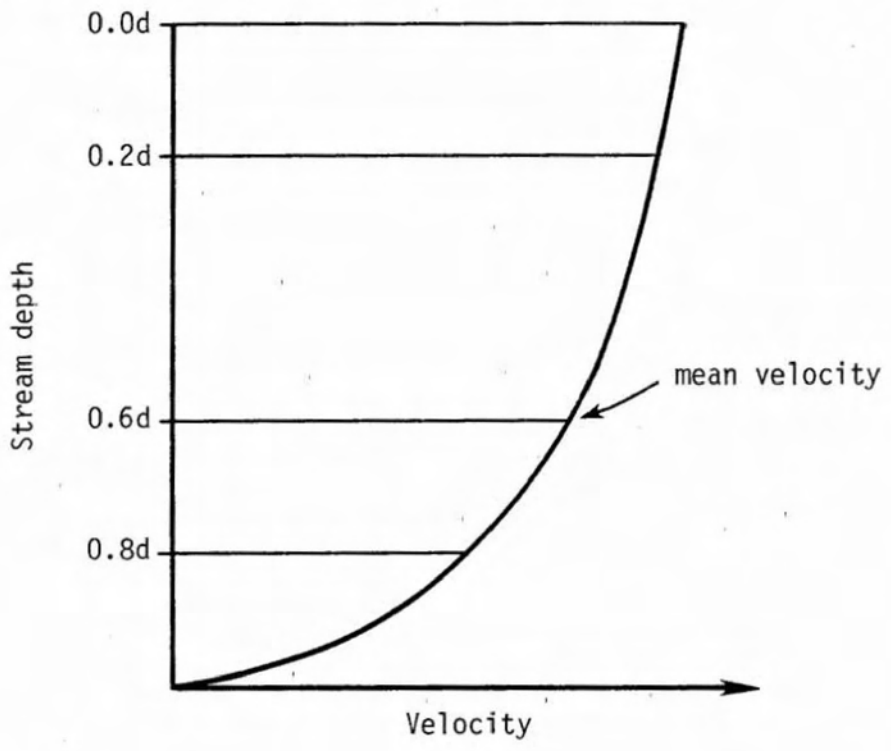

Figure 5. Variation of stream velocity with depth.

mean velocity value (Leopold et al. 1964). The shape of the velocity distribution curve depends on the roughness of the stream bed. For a given depth of flow, the rougher the stream bed, the greater the loss of turbulent energy at the bed, which results in a steeper gradient of velocity toward the bed (Leopold et al. 1964). Velocity measurements should be taken at equally spaced locations along the transect so that an average velocity can be easily calculated. The mean velocity of the channel varies along the stream section, depending on cross sectional area. It is recommended by the authors that the velocity measurements be taken at 0.6 of the total depth from the surface of the water at the same locations that depths are measured (Fig. 4).

It is possible to approximate water velocity by placing an object of neutral buoyancy in the main current and timing how long it takes the object to reach a predetermined place in the stream. Leopold et al. (1964) state that an estimate of mean velocity in a given vertical position can be obtained by timing the rate of travel of an upright float and multiplying this rate by 0.8. Fluorescent dyes and salt solutions can also be used to determine the flow rate (Stalnaker and Arnette 1976a). The advantage of these methods is that they do not require a current meter; however, the estimate of velocity is only for the path the float takes, not the entire channel.

Microhabitat velocities can be monitored with a current meter at specific areas in the stream, depending on the microhabitat of interest (e.g., spawning areas or adult resting areas). Bottom channel velocities are probably of greater significance to fish than average velocities. Bottom channel velocities are a better indication of the velocity the fish are experiencing and are probably more sensitive to velocity changes than are mean channel velocities. Spawning velocity criteria for various species of salmonids are listed in Stalnaker and Arnette (1976b).

Discharge.[1] Stream discharge can be determined at a single transect along the reach because it does not change significantly along the length of the reach (provided water input is constant). The transect where discharge is measured should be where the channel is relatively straight and the channel bottom is as stable and smooth as possible. Sections with backwater areas and turbulence should be avoided.

Basically, the procedure for calculating discharge (Q) requires the measurement of velocity, depth, and width for a number of cells (Fig. 4). The total discharge at the transect is calculated by summing values for all cells as follows:

$$Q = \sum_{i=1}^{n} w_i d_i V_i$$

The number and location of measurements needed to calculate discharge varies. The U.S. Geological Survey (Corbett et al. 1945; U.S. Geological Survey 1977) recommends that velocity be measured at the 0.6 depth for stream depths between 0.5 ft (0.15 m) and 1.5 ft (0.46 m). This sampling approach may need to be modified for other stream depths and conditions.

Stage-discharge curves can be developed if discharge measurements are important in the monitoring program. A discussion of these curves is in U.S. Geological Survey (1977). Other methods for estimating annual and monthly discharge are in Stalnaker and Arnette (1976a). Additional information on the principles involved in these measurements can be found in Corbett et al. (1945), Leopold et al. (1964), U.S. Geological Survey (1977), and standard texts on hydrology. Discharge data may be obtained from the U.S. Geological Survey if they have a gaging station on the stream.

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[1]The discussion in this section relies heavily on information in Corbett et al. (1945) and U.S. Geological Survey (1977).

Substrate and Sedimentation

Substrate composition can vary in a stream reach, especially between slow and fast water areas. Slow velocity areas generally have more small particles than do fast water areas. The location of the samples taken depends on the purpose of the measurement. If a representative composition measurement is desired, several samples should be taken and divided proportionately between slow and fast water areas. If excessive sedimentation of spawning sites is of concern, as is most often the case, substrate samples from potential or documented spawning sites should be collected.

Surface visual analysis.[2] The composition of the channel substrate (Table 2) is determined along the transect line from streamside to streamside. A measuring tape is stretched between the end points of each transect, and each 1 ft (0.3 m) division of the measuring tape is vertically projected by eye to the stream bottom. The predominant sediment class is recorded for each 1-ft division of the bottom. For example, 1 ft of stream bottom that contains 4 inches of small cobble, 6 inches of coarse gravel, and 2 inches of fine sand would be classified as 1 ft of coarse gravel (if a user elects not to use the predominant sediment class approach, information for all sediment classes can be documented). The individual 1-ft classifications across the transect are totaled to obtain the amount of bottom in each of the size classifications. Reference sediment samples for the smaller classes can be embedded in plastic cubes that can be placed on the bottom during analysis. The classification in Table 2 presents the accepted terminology and size classes for stream sediments.

A rating for embeddedness is given in Table 3. The rating is a measurement of how much of the surface area of the larger sized particles is covered by fine sediment.

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[2]This section is based on Platts et al. (1983).

Table 2. Classification of stream substrate channel materials by particle size from Lane (1947), based on sediment terminology of the American Geophysical Union (based on Platts et al. 1983).

Class name (1)	Size range: Millimeters (2)	Millimeters (3)	Microns (4)	Inches (5)	Approximate sieve mesh openings per inch: Tyler screens (6)	United States standard (7)
Very large boulders		4,096-2,048		160-80		
Large boulders		2,048-1,024		80-40		
Medium boulders		1,024-512		40-20		
Small boulders		* 512-256		20-10		
Large cobbles		256-128		10-5		
Small cobbles		* 128-64		5-2.5		
Very coarse gravel		64-32		2.5-1.3		
Coarse gravel		* 32-16		1.3-0.6		
Medium gravel		16-8		8.6-0.3	2-1/2	
Fine gravel		8-4		0.3-0.16	5	5
Very fine gravel		* 4-2		0.16-0.08	9	10
Very coarse sand	2-1	2.000-1.000	2,000-1,000		16	18
Coarse sand	1-1/2	*1.000-0.500	1,000-500		32	35
Medium sand	1/2-1/4	0.500-0.250	500-250		60	60
Fine sand	1/4-1/8	0.250-0.125	250-125		115	120
Very fine sand	1/8-1/16	*0.125-0.062	125-62		250	230
Coarse silt	1/16-1/32	0.062.0.031	62-31		270	
Medium silt	1/32-1/64	0.031-0.016	31-16			
Fine silt	1/64-1/128	0.016-0.008	16-8			
Very fine silt	1/128-1/256	0.008-0.004	8-4			
Coarse clay	1/256-1/512	0.004-0.0020	4-2			
Medium clay	1/512-1/1,024	0.0020-0.0010	2-1			
Fine clay	1/1,024-1/2,048	0.0010-0.0005	1-0.5			
Very fine clay	1/2,048-1/4,096	0.0005-0.00024	0.5-0.24			

Recommended sieve sizes are indicated by an asterisk (*).

Table 3. Embeddedness rating for channel materials (gravel, rubble, and boulder) (based on Platts et al. 1983).

Rating	Rating description
5	Gravel, rubble, and boulder particles have less than 5% of their surface covered by fine sediment.
4	Gravel, rubble, and boulder particles have between 5 to 25% of their surface covered by fine sediment.
3	Gravel, rubble, and boulder particles have between 25 and 50% of their surface covered by fine sediment.
2	Gravel, rubble, and boulder particles have between 50 and 75% of their surface covered by fine sediment.
1	Gravel, rubble, and boulder particles have over 75% of their surface covered by fine sediment.

Subsurface analysis.[3] Methods of sampling and analyzing the particle size distribution of gravels used by spawning salmonids have evolved slowly during the past 20 years. The first quantitative samplers to receive general use were metal tubes, open at both ends, that were forced into the substrate. Sediments encased by the tubes were removed by hand for analysis. A variety of samplers using this principle have been developed, but one described by McNeil (1964) and McNeil and Ahnell (1964) has become widely accepted for sampling streambed sediments.

The McNeil core sampler is usually constructed out of stainless steel and can be modified to fit most sampling situations. The sampler is worked into the channel substrate; the encased sediment core is dug out by hand and deposited in a built-in basin. When all sediments have been removed to the level of the lip of the core tube, a cap is placed over the tube to prevent

[3]This section is based on Platts et al. 1983.

water and the collected sediments from escaping when the tube is lifted out of the water. Suspended sediments in the tube below the cap are lost, but this loss is generally considered a statistically insignificant percentage of the total sample.

The sediments and water collected are strained through a series of sieves to determine the particle size distribution, percent fines, or geometric mean diameter of the sediment size distribution. The sediments collected can be analyzed in the laboratory using the "dry" method or in the field using the "wet" method.

Disadvantages in using the McNeil sampler are that: (1) particle size diameter that can be measured is limited to the size of the coring tube; (2) core materials are mixed and no interpretation of vertical and horizontal differences in particle size distribution can be made; (3) the locations at which sediments can be measured is limited by where the core sampler can enter the channel substrate, a factor controlled by the water depth, length of the collector's arm, and the depth the core sampler can be pushed into the channel; (4) the sample will be biased if the core tube pushes larger particle sizes out of the collecting area; (5) suspended sediments in the core sampler are lost; and (6) the core sampler cannot be used if the particle sizes are so big or the channel substrate so hard that the core sampler cannot be pushed into the required depth.

Even though there are limitations to this method, it is probably the most economical method available in terms of time and money to obtain estimates of channel substrate particle size distributions in channel depths up to 12 inches (305 mm). The diameter of the McNeil tube should be at least 12 inches (305 mm).

More recently, scientists have experimented with cryogenic devices to obtain sediment samples. These devices, generally referred to as "freeze-core" samplers, consist of a hollow probe driven into the streambed and cooled with a cryogenic medium. After a prescribed time of cooling, the probe and a

frozen core of surrounding sediment are extracted. Liquid nitrogen; liquid oxygen; solidified carbon dioxide ("dry ice"); liquid carbon dioxide (CO_2); and a mixture of acetone, dry ice, and alcohol have been used experimentally as freezing media. Several years of development have produced a sampler (Walkotten 1976) that uses liquid CO_2. The freeze-core sampler, like the McNeil core sampler, has become widely accepted for sampling stream substrates.

All of the freeze-core equipment presently available utilize the same principles, although one to many probes may be used. The size of sample collected is directly related to the number of probes and the amount of cryogenic medium used per probe. Walkotten (1976), Everest et al. (1980), Lotspeich and Reid (1980), and Platts and Penton (1980) discuss the construction, parts, and operation of freeze-core samplers and the analysis of samples collected by the freeze-core method. Platts and Penton (1980) and Ringler (1970) believe that the single probe freeze-core sampler may be biased toward the selection of larger sized sediment particles.

The accuracy and precision of sample results with the freeze-core and McNeil samplers have been compared in laboratory experiments. Samples collected by both devices were representative of a known sediment mixture, but results with the freeze-core sampler were more accurate (Walkotten 1976). It is also more versatile and functions under a wider variety of weather and water conditions. However, the freeze-core sampler has several disadvantages. It is difficult to drive probes into substrates that contain many particles over 10 inches (25 cm) in diameter, and the freeze-core technique is equipment-intensive, requiring CO_2 bottles, hoses, manifolds, probes, and sample extractors. It is also necessary to subsample cores by depth for accurate interpretation of gravel quality (Everest et al. 1980). Therefore, it is often necessary to collect larger cores with freeze-core equipment than can be easily obtained by the single-core technique.

A major advantage of the freeze-core sampler is that it allows for vertical stratification of substrate cores. Everest et al. (1980) have developed a subsampler that consists of a series of open-topped boxes made of 26-gage

galvanized sheet metal. The core is laid horizontally across the boxes of the subsampler and thawed with a blowtorch. Sediments freed from the core drop directly into the boxes below.

Sample analysis. Sediment samples can be analyzed either in the field or in the laboratory. The "wet method" can be done onsite and is the least expensive, but also the least accurate, method. The "wet method" usually uses a water-flushing technique with some hand shaking to sort sediments through a series of sieves. The trapped sediment on each sieve is allowed to drain and then poured into a water-filled graduated container. The amount of water displaced determines the volume of the sediment plus the volume of any water retained in pore spaces in the sediment. When the wet method is used, water retained in the sediment must be accounted for, because water retention per unit volume of fine sediments is higher than for coarse sediments. A conversion factor based on particle size and specific gravity can be used to convert wet volume to dry volume.

For more accurate results, sediment samples can be placed in containers and transported to the laboratory for analysis. Laboratory analysis of dry weights is the most accurate way to measure sediments because all of the water in the sample can be evaporated, thus eliminating the need for the conversion factors associated with the wet method. In the laboratory method, the sediment sample is oven-dried [24 hours at 221° F (105° C)] or air-dried, passed through a series of sieves, and the portion caught by each sieve is weighed. The Wentworth sieve series can be adapted for sampling size classes (Table 2) ranging from 0.002 inch to 3.94 inches (0.062 to 100 mm). The upper size limit approximates the largest size particles in which most salmonids will spawn. Consequently, few grains larger than 5 inches (128 mm) are present in preferred spawning areas. The size class [10.1 to 20.2 inches (256 to 512 mm)] is difficult for salmonids to move to deposit and cover their eggs.

Quality indices. The quality of gravels for salmonid reproduction has traditionally been estimated by determining the percentage of fine sediments (less than some specified diameter) in samples collected from spawning areas.

The field data can be compared (Hall and Lantz 1969) to results of several laboratory studies (for example, Phillips et al. 1975) to estimate survival to emergence of various species of salmonids. An inverse relationship between percent fines and survival of salmonid fry has been demonstrated by several researchers, beginning with Harrison (1923). Use of percent fines alone to estimate gravel quality has a major disadvantage; it ignores the textural composition of the remaining particles, which can have a mitigating effect on survival. For example, two samples may each contain 20% by weight of fine sediment less than 1 mm in diameter, while the average diameter of larger particles is 10 mm in one sample and 25 mm in the other. Interstitial voids in the smaller diameter material would be more completely filled by a given quantity of fine sediment than would voids in the larger material, and the subsequent effect on survival of salmonid fry would be very different.

Other gravel quality indexes have been developed recently in an attempt to improve on the percent fines method. Platts et al. (1979) used the geometric mean diameter (d_g) method for evaluating sediment effects on salmonid incubation success. This method has three advantages over the commonly used percent fines method: (1) it is a conventional statistical measure used by several disciplines to represent sediment composition; (2) it relates quality to the permeability and porosity of channel sediments and to embryo survival as well or better than does percent fines; and (3) it is estimated from the total sediment composition. Despite these advantages, d_g was shown by Beschta (1982) to be rather insensitive to changes in stream substrate composition in a Washington watershed. Lotspeich and Everest (1981) have shown that the use of d_g alone can lead to erroneous conclusions concerning gravel quality because d_g alone does not give a true analysis of the particle size distribution. Because of these problems, Beschta (1982) raised serious questions regarding the utility of geometric mean diameter as a quality index.

Tappel (1981) developed a modification of the d_g method that uses a linear curve to depict particle size distribution. The points 0.03 inch (0.8 mm) and 0.37 inch (9.5 mm) are used to determine the line. According to Tappel, the slope of this line gives a truer representation of fine sediment

classes detrimental to incubation. A major drawback of this procedure, as with percent fines, is that it ignores the characteristics of the larger particles in the sample.

A recent spawning substrate quality index that appears to overcome the limitations of percent fines measurements and geometric means has been reported by Lotspeich and Everest (1981). Their procedure uses measures of the central tendency of the distribution (refer to Chapter IV) of sediment particle sizes in a sample and the dispersion of particles in relation to the central value to characterize the suitability of gravels for salmonid incubation and emergence. These two parameters are combined to derive a quality index called the "fredle index", which indicates both sediment permeability and pore size. The measure of central tendency used is the geometric mean (d_g). Pore size is directly proportional to mean grain size, regulates intragravel water velocity and oxygen transport to incubating salmonid embryos, and controls intragravel movement of alevins. These two substrate parameters are the primary determinants of salmonid embryo survival to emergence (Platts et al. 1983).

Bank and Channel Stability

Well vegetated banks are usually stable, even if there is bank undercutting, which provides excellent cover for fish. Valuable fish cover is ultimately lost when bank vegetation decreases, banks erode too much, or banks undercut too quickly and slough off onto the stream bottom.

Streambank soil alteration.[4] Certain land uses, especially livestock grazing, can reduce the stability of a streambank, resulting in the modification of the stream. The streambank alteration rating may well provide an early warning of changes that will eventually affect fish populations in the stream.

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[4]This section is from Platts et al. (1983).

The streambank alteration rating reflects the changes taking place in the bank from any force (Table 4). The rating is separated into five classes. Each class, except the one where no streambank alteration has occurred, has an evaluation spread of 25 percentage points. Once the class is determined, the observer must decide the actual percent of instability within that 25 point spread. Streambanks are evaluated on the basis of how far they have moved away from optimum conditions for the respective stream habitat type being measured. Therefore, the observer must be able to visualize the streambank as it would appear under optimum conditions. This visualization requirement makes uniformity in rating alterations difficult. Any natural or artificial deviation from this optimum condition is included in the evaluation. Natural alteration is any change in the bank resulting from natural events. Artificial alteration is any change not related to natural events, such as trampling by humans or livestock, disturbance by bulldozers, or vegetation removal. Natural and artificial alterations are reported individually, but together cannot exceed 100%. It is often difficult to distinguish artificial from natural alterations; if there is any doubt, the alteration is classified as natural. It is possible to have artificial alterations masking already existing natural alterations and vice versa. Only the major type of alteration on a unit area is entered into the rating system in this case.

Streambank vegetative stability. The ability of vegetation and other materials on the streambank to resist erosion from flowing water is also rated (Table 5). The rating relates primarily to the stability that results from vegetative cover, except in those cases where bedrock, boulder, or rubble stabilizes the streambanks. The rating takes all protective coverings into account. The rated portion of the bank or flood plain includes only that area intercepted by the transect line from the water surface shoreline to 5 ft back from the shoreline or to the top of the bank, whichever is greatest. Precision and accuracy for this rating system are only fair so care has to be taken when ratings are performed.

Table 4. Streambank soil alteration rating based on Platts et al. (1983).

Rating	Description
0	Streambanks are stable and are not being altered by water flows or animals.
1 to 25	Streambanks are stable, but are lightly altered (less than 25%) along the transect line. Less than 25% of the streambank is false, broken down, or eroding.
26 to 50	Streambanks moderately altered along the transect line. At least 50% of the streambank is in a natural, stable condition. Less than 50% of the streambank is false, broken down, or eroding. False banks[a] are rated as altered. Alteration is rated as natural, artificial, or a combination of the two.
51 to 75	Streambanks have major alteration along the transect line. Less than 50% of the streambank is in a stable condition. Over 50% of the streambank is false, broken down, or eroding. A false bank with some stability and cover is still rated as altered. Alteration is rated as natural, artificial, or a combination of the two.
76 to 100	Streambanks along the transect line are severely altered. Less than 25% of the streambank is in a stable condition. Over 75% of the streambank is false, broken down, or eroding. A bank damaged in the past that has gained some stability and cover and is now classified as a false bank is still rated as altered. Alteration is rated as natural, artificial, or a combination of the two.

[a]False stream banks are banks that have been eroded away and have receded back from the edge of the water. They can become stabilized by vegetation, but the edges do not hang over the water to provide cover for fish.

Table 5. Streambank vegetative stability rating based on Platts et al. (1983).

Rating	Description
4 (Excellent)	Over 80% of the streambank surfaces are covered by vegetation in vigorous condition. If the streambank is not covered by vegetation, it is protected by materials that do not allow bank erosion, such as boulders and rubble.
3 (Good)	Fifty to seventy-nine percent of the streambank surfaces are covered by vegetation. Areas not covered by vegetation are protected by materials that allow only minor erosion, such as gravel or larger material.
2 (Fair)	Twenty-five to forty-nine percent of the streambank surfaces are covered by vegetation. Areas not covered by vegetation are covered by materials that give limited protection, including gravel or larger material.
1 (Poor)	Less than 25% of the streambank surfaces are covered by vegetation or by gravel or larger material. Areas not covered by vegetation have little or no protection from erosion, and the banks are usually eroded some each year by high water flows.

Cover

Cover is variously defined and not easily quantified. No completely acceptable method to rate cover was identified. Arnette (1976:10) defines instream cover as "... areas of shelter in a stream channel that provide aquatic organisms protection from predators and/or a place in which to rest and conserve energy due to a reduction in the force of the current" and riparian cover as (page 10) "... areas associated with or adjacent to a stream or cover that provide resting, shelter and protection from predators." Cover can be furnished by water depth, surface turbulence, undercut banks, large rocks and other submerged obstructions, instream vegetation, overhanging vegetation, plant roots, and debris (Binns 1979).

Wesche (1973, 1974) developed a trout cover rating system that can be used to compare cover ratings of the same stream section at different levels of flow or different stream sections at the same level of flow. The equation used is:

$$CR = \frac{L\ obc}{T} (PF\ obc) + \frac{A}{SA} (PF\ a)$$

where CR = cover rating of stream section for trout

L obc = length (ft or m) of overhead bank cover in the stream section having a water depth of at least 0.5 feet (0.1524 m) and a width of at least 0.3 feet (0.0914 m)

T = length (ft or m) of thalweg[5] line through the stream section

A = surface area (ft^2 or m^2) of the stream section having a water depth of at least 0.5 feet (0.1524 m) and a substrate size of at least 3 inches (7.6 cm) in diameter

SA = total surface area (ft^2 or m^2) of the stream section at the average daily flow (equals 0.75 for trout at least 6 inches in length; 0.5 for trout less than 6 inches in length)

PF obc = preference factor of trout for overhead bank cover

PF a = preference factor of trout for instream rubble-boulder areas (0.25 for catachable trout and 0.5 for subcatchables)

When different stream reaches are being sampled and compared and the average daily flow cannot be determined, measurements should be taken when both stream sections are at the same percentage of the average daily flow. Measurements should be taken at the highest flow for which a cover rating is being made when the same stream section is being compared at different flow levels (Wesche 1974). This method does quantify cover to some degree. However, Stalnaker and Arnette (1976b) point out that this technique appears to be valid for cover-oriented salmonids.

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[5]The down-channel course of greatest cross sectional depths (Eiserman et al. 1975).

To evaluate instream cover, Eiserman et al. (1975) recommend counting the number of submerged rocks that are at least 2 feet (0.61 m) in diameter and project at least 1 foot (0.3 m) above the stream bed. Patches of aquatic vegetation or other cover material that are at least 2 feet in diameter and that provide cover are also included in the evaluation.

The rating system for streambank cover described in Platts et al. (1983:24) "... considers all material (organic and inorganic) on or above the streambank that offers streambank protection from erosion and stream shading and provides escape cover or nesting security for fish" (Table 6). The area of streambank to be rated is defined by a transect line covering the exposed stream bottom, bank, and top of bank.

Table 6. Streamside cover rating system (based on Platts et al. 1983).

Rating	Description
4	The dominant vegetation influencing the streamside and/or water environment consists of shrubs.
3	The dominant vegetation consists of trees.
2	The dominant vegetation consists of grass and/or forbs.
1	Over 50% of the streambank transect line intercepts have no vegetation, and the dominant material is soil, rock, bridge materials, road materials, culverts, and mine tailings.

Instream vegetative cover is measured along each 1-ft (0.3 m) division of the measuring tape across the transect (Platts et al. 1983). If more than 50% of the 1-ft distance contains cover, the entire 1-ft division is classified by

the type of cover present; if less than 50% of the 1-ft distance contains cover, the division is not included in the measurement. Cover includes several forms (e.g., algal mats, mosses, rooted aquatic plants, organic debris, downed timber, and brush capable of providing protection for young-of-the-year fish); however, it excludes thin films of algae on the channel substrate.

Pools and Riffles

Pools and riffles are commonly evaluated by determining the percentage of the stream consisting of each category and expressing these percentages as a ratio. The resulting ratio is compared to the assumed optimum ratio of 1:1 (based on surface area). Pools are portions of the stream that are deeper and of lower velocity than the main current (Arnette 1976). Riffles are faster, shallower areas with the water surface broken into waves by wholly or partly submerged obstructions. Glides and runs, sections where the water surface is not broken but is shallow and has a fast velocity (Duff and Cooper 1976), also may be present in a stream.

Pool quality[6] (Table 7) is an estimate of the ability of a pool to promote fish survival and meet fish growth requirements. Platts (1974) found it is a significant relationship between high quality pools and high fish standing crops. Small, shallow pools, needed by young-of-the-year fish for survival, rate low in quality, even though they are essential to fish survival. The rating system in Table 7 was based mainly on the habitat needs of fish of catchable size. In actuality, a combination of pool classes are required to maintain a productive fishery.

The pool quality rating (Table 7) combines direct measurements of the greatest pool diameter and depth with a cover analysis. Pool cover is any material or condition that provides protection to fish, such as logs, other organic debris, overhanging vegetation within 1 ft (0.3 m) of the water surface, rubble, boulders, undercut banks, or water depth.

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[6]This section on pool quality is based on Platts et al. (1983).

Table 7. Rating of pool quality in streams between 20 and 60 feet wide (Platts et al. 1983).[a]

	Description	Pool rating
1A	If the maximum pool diameter is within 10% of the average stream width of the study site ..... Go to 2A, 2B	
1B	If the maximum pool diameter exceeds the average stream width of the study site by at least 10% ..... Go to 3A, 3B	
1C	If the maximum pool diameter is less than the average stream width of the study site by 10% or more ..... Go to 4A, 4B, 4C	
2A	If the pool is less than 2 ft in depth ... Go to 5A, 5B	
2B	If the pool is more than 2 ft in depth ... Go to 3A, 3B	
3A	If the pool is over 3 ft in depth or the pool is over 2 ft in depth and has abundant fish cover[b]	Rate 5
3B	If the pool is less than 2 ft in depth or if the pool is between 2 and 3 ft deep and lacks fish cover	Rate 4
4A	If the pool is over 2 ft deep with intermediate[c] or better cover	Rate 3
4B	If the pool is less than 2 ft in depth but pool cover for fish is intermediate or better	Rate 2
4C	If the pool is less than 2 ft in depth and pool cover is classified as exposed[d]	Rate 1
5A	If the pool has intermediate to abundant cover	Rate 3
5B	If the pool has exposed cover conditions	Rate 2

[a]For streams less than 20 ft wide, deduct 1 ft from all entries with foot values and add 1 ft to the values for streams wider than 60 ft.

[b]If cover is abundant, the pool has excellent instream cover and most of the perimeter of the pool has a fish cover.

[c]If cover is intermediate, the pool has moderate instream cover and one-half of the pool perimeter has fish cover.

[d]If cover is exposed, the pool has poor instream cover and less than one-fourth of the pool perimeter has any fish cover.

As the transect line crosses the water column surface, it can intercept any combination of pools and riffles. If more than one pool is intercepted by the transect line, then the width of each pool is multiplied by its quality rating and the products for all pools intercepted are summed. This total, divided by the total pool width, is the weighted average pool rating.

As an alternative, reaches can be divided into three categories: pools; riffles; and glides or runs. The ratio among these three categories is determined. Eiserman et al. (1975) consider an optimum condition to be 35% pools, 35% riffle, and 30% glides. This method has the advantage of classifying glides, as well as pools and riffles.

The location and size of pools and riffles can change with changes in discharge. Therefore, determinations of pool-riffle relationships need to be made during the same discharge so they can be directly compared.

Temperature

The type of instrument selected to measure water temperature depends on the kind and frequency of data needed. A hand-held mercury thermometer used during routine sampling trips is adequate if only general temperature data is needed. However, if more detailed or exact information is needed, at least a maximum-minimum thermometer should be used and, ideally, a recording thermometer (thermograph).

A maximum-minimum thermometer is a U-shaped liquid-in-glass thermometer that records the maximum and minimum temperatures during the period that it is in water (Stevens et al. 1975). Neither the time of occurrence nor the duration of the maximum or minimum temperature are recorded. The thermometer needs to be quickly replaced in the water when reset to avoid affecting the temperatures recorded by exposing the thermometer to air.

Recording thermometers provide a continuous pen trace of temperature data on a strip or circular chart (Stevens et al. 1975). These thermometers are useful if information about temperature fluctuations is important to the study or if sampling trips are fairly infrequent because of the inaccessibility of the sample site or for other reasons.

Thermometers should be calibrated before their first use and periodically during the field season. Two water baths, 5° C and 20° C, are used to calibrate the thermometer; accuracy should be within 0.5 ° C at both temperatures (Stevens et al. 1975). Maximum-minimum thermometers should be put in a pipe for protection, and the encased thermometer placed where water is flowing but where the thermometer is somewhat protected. The thermometer should be placed where it will not be exposed to the air during low flow periods or exposed to high flows that could damage it.

Temperatures should be taken in the shade in the main flow of the stream because these conditions are usually representative of the entire water mass. To prevent wetbulb cooling, read the temperature without removing the thermometer from the water or while the thermometer is submerged in a container filled with water. If a recording thermometer is used, the water temperature should be checked near the sensor with a calibrated thermometer. Stevens et al. (1975) explain how to correct any instrument error. Mean temperatures can be calculated several ways if the temperature does not vary across the stream channel (e.g., arithmetic mean, area-weighted average, or discharge-weighted average). Temperatures are usually most critical during low flow periods, and temperature measurements should be concentrated at these times.

KEY FISH VARIABLES

A variety of techniques are available to sample fish populations in streams and to analyze the resulting data. Each technique has different assumptions, advantages, and disadvantages. It is important to understand the

characteristics of the technique used so that valid conclusions can be drawn from the data. The most commonly used sampling technique is electrofishing, primarily because it does not result in fish mortality if done properly and it can be very effective in small streams.

Fish distribution is usually "clumped" in response to the nonrandom distribution of many habitat variables (Hendricks et al. 1980), and all sampling gear is selective to some degree (Weber 1973; Lagler 1978; Gulland 1980; Henderson 1980). Selectivity causes the probability of capture to vary in relation to some characteristic of the fish (Backiel 1980), such as species, sex, size, or life stage. Therefore, the sample obtained usually is not totally representative of the population. Selectivity results from extrinisic factors (e.g., construction of the gear), intrinisic factors (e.g., behavioral differences among or within species), or the interaction of both types of factors (Lagler 1978). Bias may also be introduced by the sampling design, particularly sampling time and place (Gulland 1980). Practical considerations often make it easier to sample at certain places or times of the year (e.g., shallow water areas or during low flow). Gulland (1980) advises that the amount of bias introduced by sample design and equipment be examined, if possible, by taking at least a few samples at less convenient times and places. This bias can be more serious than a large variance because a large variance soon becomes apparent in the data from different samples. Samples with a large bias, however, may give consistent results that are incorrect. Procedures to reduce sampling bias through sampling design are discussed in Chapter IV.

Electrofishing.[7] Electrofishing is an efficient capture method that can be used to obtain reliable information on fish population abundance, length-weight relationships, and age and growth for most streams of order 6 or less. Electrofishing devices tend to have higher capture probabilities for larger fish than for smaller fish, although the newer electrical transformers have

[7]The first two paragraphs of this section are based on Platts et al. (1983).

adjustable voltage, pulse, and frequency, which can be used to reduce size selectivity. Electrofishing efficiency is also affected by stream conductivity, temperature, depth, and water clarity. The effects of each condition need to be considered to obtain a reliable population estimate. Electrofishing can be more efficient than other methods to evaluate populations, such as seining and underwater observation, which can be biased by boulder-rubble substrate, turbidity, aquatic vegetation, and undercut banks.

During electrofishing, fish tend to swim or drift downstream, and a downstream blocking net needs to be in place. Sometimes the upstream end of the sample area can be located at a fish passage restriction area. If a restriction area is not available, a blocking net is also needed at the upstream area. Platts et al. (1983) found that salmonids less than 6 inches (152.4 mm) in length seldom tried to leave the electrofished area, while large salmonids attempted to escape. Also, a constant capture probability is difficult to obtain when sampling sculpin populations because of their tendency to remain in the substrate.

Electrofishing is potentially dangerous to operators; therefore, precautions should be taken. Persons involved in electrofishing should have waterproof hip boots or waders and rubber gloves. Hand-held electrodes should be equipped with a "dead-man" automatic shut-off switch. Operators should wear protective gloves if they will be placing their hands in the water. Electrodes should be turned off immediately if anyone falls in the water.

Electrofishing has the following advantages over other fish sampling techniques:

1. Preliminary preparation of the site, with consequent delay and disturbance of the fish, is not needed (Hartley 1980).

2. Sampling can be performed with a limited number of people within a short period of time (Hartley 1980).

3. It is more efficient than most other techniques (e.g., seining) when sampling over irregular substrates and in areas with a strong current (Dauble and Gray 1980).

4. The fish are not killed or damaged when electrofishing is done correctly.

Other fish sampling techniques. Although electrofishing is probably the most commonly used method of sampling fish in small streams, other methods are available that are applicable under certain circumstances. These methods include chemical ichthyocides, traps, seines, gill nets, explosives, and direct observation (see Platts et al. 1983).

Chemical ichthyocides include poisons, such as rotenone, antimycin, copper sulfate, cresol, and sodium cyanide (Weber 1973). The ideal ichythocide is: (1) nonselective; (2) easily, rapidly, and safely used; (3) readily detoxified; and (4) not detected and avoided by fish (Hendricks et al. 1980). Prior to use of an ichthyocide, care must be taken to ensure that it will be used correctly, and approval for use should be obtained from proper authorities.

The most commonly used poison is rotenone, obtained from the derris root. It is effective in a short time period, has low toxicity to birds and mammals (Hendricks et al. 1980), and is quickly dispersed in streams (Weber 1973). Some fish may become trapped under rocks or other obstacles, so the entire treated reach should be carefully examined for any dead fish. Detoxification of rotenone can be achieved with potassium permanganate (Lawrence 1956). Sensitivity to rotenone varies appreciably among species and among life stages within a species (Holden 1980). The toxicity is affected by temperature, pH, oxygen concentration, and light (Weber 1973; Hendricks et al. 1980; Holden 1980). Weber (1973) suggests that a concentration of 0.5 mg/l be applied in acidic or slightly alkaline waters. A concentration of 0.7 mg/l is recommended if bullheads and carp are present. Tracor Jitco, Inc. (1978) recommends a concentration of 0.1 mg/l for sensitive species. Improper application of rotenone can have disastrous effects downstream (Hendricks et al. 1980).

Passive traps, made of wood, metal, netting, or plastic, are static and rely on the movement of fish (Craig 1980). Traps are highly selective for species and size of fish. Swift currents and debris may complicate use of traps (Hendricks et al. 1980). Traps have the advantage of collecting fish alive, although some predation may occur in the trap.

Species Identification

Lowe-McConnell (1978) suggests the following procedure for fish identification:

1. Assemble the best available keys, checklists, and descriptions of the fishes of the region.

2. Key the fish to its proper species identification.

3. Verify identification by comparing fish with:

    a. pictures;

    b. detailed published descriptions;

    c. known geographic range of the species; and

    d. identified materials in museum collections or specimens identified by a specialist.

4. Confirm identifications with a specialist.

It may not be necessary to go through this entire procedure for species that are readily identified; however, identification of difficult species should be confirmed by a specialist. Correct identification of species is especially important if several species are present and one objective of the study is to monitor changes in species composition.

Preservation of Samples

Fish specimens may be preserved during the monitoring study for species identification; taxonomic studies; or studies of parasites, disease, or food habits. Fish should be preserved in 10% formalin. Specimens larger than 7.5 cm that will be used for taxonomic or food habit studies should be slit along the right side (the left side is usually used for measurements) so that the formaldehyde can penetrate the body cavity. Colors will fade when the fish are placed in preservatives, so the various markings and colors of the fish should be documented before preservation if the specimens will be identified later.

Each specimen should be carefully labelled with the following information (Traco Jitco, Inc. 1978):

1. Date;

2. Name of the study area;

3. Site of sampling station;

4. Type of sample (qualitative or quantitative);

5. Name of collector; and

6. Method of sample collection.

Standard Measurements

For some variables, standard measurements, such as length and weight, will be taken. Live fish should be handled with care because they are easily stressed by handling.

Length. Lagler (1978) describes three length measurements that can be taken: standard length; fork length; and total length (Fig. 6). Standard length is the length of a fish from its most anterior extremity (mouth closed) to the hidden base of the median tail fin rays, where these rays articulate on the caudal skeleton. This spot can be located by flexing the tail; a crease will be evident at the point of articulation. Fork length is measured from most anterior extremity of the fish to the tip of the median rays of the tail. In species where the tail fin is not forked, fork length is the same as total length. Total length is the greatest length of a fish from its anteriormost extremity to the end of the tail fin. For fish with forked tail fins, the two lobes are squeezed together to give a maximum length. If the lobes are unequal, the longer lobe is used. Any of these lengths can be used in monitoring studies; however, total length is used most often.

A measuring board, commonly used to measure length, is efficient and sufficiently precise for most studies. These boards contain a graduated scale and can be made of wood, plastic, stainless steel, or aluminum. Herke (1977) describes a basic measuring board that can be constructed out of acrylic plastic. The boards can be made more useful by constructing them in a V-shape and at an angle so the fish are held in place to measure. Lagler (1978) identifies the following possible contributors to error or inconsistency in measurements:

1. Muscular tension while fish are alive, with muscle relaxation after death;

2. Shrinkage of fish following preservation;

3. Variation in the pressure used to put the jaws into a normal closed position;

4. Inconsistency in squeezing the tail together to get the maximum total length; and

5. Operator skill and consistency.

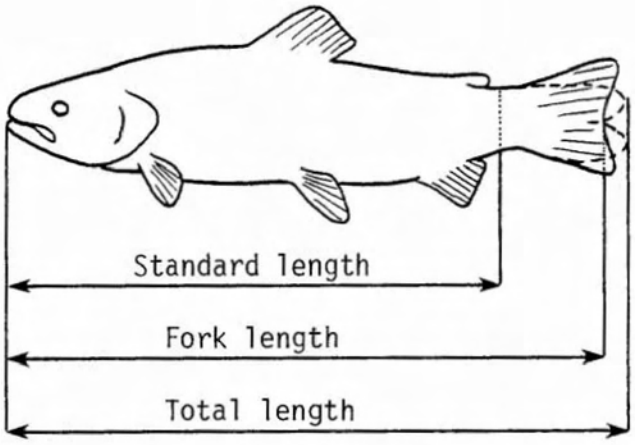

Figure 6. Three common length measurements.

"Numeral bias" may also be introduced; i.e., a tendency to record the "even" divisions of a scale or to prefer scale divisions to interpolated length estimates (Lagler 1978).

Weight. Measurements of weight should be taken with an accurate scale that is sturdy enough to be used in the field. Extreme precision in weight measurements is not possible because of variation in the amount of stomach contents and the amount of water engulfed at capture (Lagler 1978). Because weighing problems can be caused by fish flopping around, anesthetizing the fish with MS222 during weighing is recommended. Weights of live fish and preserved specimens are not comparable unless percentage of shrinkage is known. If the fish being weighed are very small, groups of fish (e.g., five fish per group) can be weighed and an average weight obtained. If too many fish are captured to be weighed separately, weigh 10 in each size class (10 cm intervals), using the first 10 encountered (Keller and Burnham 1982).

Species Composition

Data used to compile a species list can be collected with any technique, or combination of techniques, that does not completely select against one or more species. Sampling should be thorough enough to include species that are in low numbers or that are small in size. Sampling should be conducted several times during the year so that seasonal residents will also be identified.

Relative Abundance

Relative abundance data are used to determine the quantitative composition of the community and can be calculated using fish biomass or population numbers. Data are given as percentages of occurrence. Species must be collected proportionately to their occurrence to obtain accurate composition data. Therefore, sampling techniques that are species selective should not be used. All sampling gear is selective to some degree; consequently, relative abundance data should be analyzed with the selectivity of the gear used in mind.

Length-Weight Relationships

In fish, the length-weight relationship can be expressed by the following equation (Ricker 1975; Bagenal and Tesch 1978):

$$W = aL^b$$

where W = weight

L = length

Generally, the equation is transformed to:

$$\log(W) = \log(a) + b[\log(L)],$$

and the data are then analyzed by simple regression methods.

When the logarithm of the weight is plotted against the logarithm of the length, the antilog of the Y-intercept is equal to "a" and the slope of the fitted line is equal to "b" (b typically is "near" 3.0). These coefficients vary among species and sometimes within the same species. Fish typically pass through several stages of growth between which rather abrupt changes in structure or physiology may occur. Each growth stage may have its own length-weight relationship (Ricker 1975) and, therefore, need to be analyzed separately.

The length-weight relationship varies during different times of the year, primarily because fish typically lose weight during the winter and gain weight during the summer. Weights are also affected by spawning condition and amount of stomach contents. The length-weight relationship may also vary between sexes.

Population Estimation

The only population estimation method recommended for small streams is the removal method based on electrofishing because this method is very efficient. In a 100 m stream section (one study site), two to four removal passes are adequate and can be made in less than one-half day.

Field methods and considerations for electrofishing were discussed previously in this chapter. Obtaining reliable data requires three criteria: (1) fish cannot be lost from the study site while sampling (block-off the site with nets if necessary); (2) all stunned fish must be captured; and (3) equal effort must be used on all removal passes. The equal effort requirement is especially important because estimates of population size can be badly biased with unequal sampling effort.

One removal pass in a study area usually consists of going first upstream and then downstream. At least two passes need to be made for an adequate sample and three or more passes may be needed unless the efficiency of the sampling gear is very high (i.e., a capture probability of 0.8 or more on each pass). The optimal sampling situation is when 100% of the fish are removed in the first pass; then the purpose of the second pass is to verify that all the fish have been counted. In practice, capture probabilities as high as 0.8 are uncommon, although this may be a reflection of the efficiency of the electrofishing gear in use, and significant numbers of fish are usually caught on the second and subsequent passes.

If all of the fish are caught by the last removal pass, the population estimate is the total number of fish captured. This estimate does not rely on any assumptions about capture probabilities. For example, if the removal counts (data) for four passes were 157, 15, 1, and 0, it is reasonable to assume that all of the fish were caught and to use 173 (157 + 15 + 1 + 0) as the population estimate for that site. However, if the capture data for the four passes was 35, 25, 20, and 18, the population size is not obvious. In this case, it is necessary to use the removal data to estimate the population

size for the site. In that case, the estimate may not be very precise because the sampling was inefficient. Statistical analysis can partially solve the problem. However, the real "solution" is to obtain more reliable data through the use of better equipment and field procedures, with an increased capture probability (Capture probability in the first example above is 0.90; in the second example, capture probability is 0.20. The population size is the same in both cases.)

For comparative purposes, abundance data should be expressed as a consistent density measure; for example, fish per linear mile of stream or fish per surface area (see, e.g., Keller and Burnham 1982).

Computations for two removal passes. Let U_1 = the number of fish removed (captured) on the first pass and U_2 = the number removed on the second pass. An estimate of population size is:

$$\hat{N} = \frac{U_1}{1-U_2/U_1}$$

Estimated capture probability is:

$$\hat{p} = 1 - \frac{U_2}{U_1}$$

This quantity is the estimated probability of capture of a fish on one removal pass. If the two capture probability on each pass is at least 0.80, this is a reliable estimate of population size, without requiring exactly equal capture probabilities on each pass.

Computational examples for $\hat{N}$ and $\hat{p}$ are given below for two sets of data:

Example 1 ($U_1 = 157$, $U_2 = 15$)

$$\hat{N} = \frac{157}{1 - \frac{15}{157}} = \frac{157}{0.9045} = 173.6 = 174 \text{ fish}$$

$$\hat{p} = 1 - \frac{15}{157} = 0.9045$$

Example 2 ($U_1 = 35$, $U_2 = 25$)

$$\hat{N} = \frac{35}{1 - \frac{25}{35}} = \frac{35}{0.2857} = 122.5 = 123 \text{ fish}$$

$$\hat{p} = 1 - \frac{25}{35} = 0.2857$$

For the lower estimated p (0.2857) in example 2, the estimate of N is unreliable in two ways: (1) it has a large within-site sampling variance; and (2) $\hat{N}$ may be badly biased if the assumption of equal capture probability on each removal pass is invalid. The solution to the problem is to make more removal passes. With three or more removal passes, the assumption of equal capture probability on every pass can be tested. However, if enough removal passes are made so that all of the fish are caught, no assumptions or sophisticated analyses are needed to estimate the population size.

The formula to determine the sampling variance of $\hat{N}$ when two passes are made is:

$$\text{var}(\hat{N}) = \frac{M\ (1-M/\hat{N})}{A-B}$$

where $M = U_1 + U_2$

$A = (M/\hat{N})^2$

$B = (2\ \hat{p})^2(U_2/U_1) \equiv (2\ \hat{p})^2(1-\hat{p})$

The square root of the variance is the standard error of $\hat{N}$, denoted by $\text{se}(\hat{N})$. It measures how reliable $\hat{N}$ is as an estimate of the fish population size in the sampled site at the time of sampling.

A computational example of $\text{var}(\hat{N})$ and $\text{se}(\hat{N})$ when $U_1 = 157$, $U_2 = 15$, $M = U_1 + U_2 = 172$, $\hat{N} = 174$, and $\hat{p} = 0.90$ follows:

$$A = \left(\frac{172}{174}\right)^2 = 0.9771$$

$$B = [(2)\ (0.9)]^2 \left(\frac{15}{157}\right)$$

$$= (3.24)\ (0.09554)$$

$$= 0.3096$$

and

$$\text{var}(\hat{N}) = \frac{172(1-172/174)}{0.9771-0.3096} = 2.96$$

$$\text{or } \text{se}(\hat{N}) = \sqrt{2.96} = 1.72$$

An approximate 95% confidence interval for N (true population size) is:

$$\hat{N} \pm 2 \times se(\hat{N}) = 174 \pm (2 \times 1.72) \text{ or } 171 \text{ to } 177.$$

Because 172 fish were actually removed, the lower bound of 171 should be changed to 172. The narrow interval (172 to 177) indicates that $\hat{N} = 174$ is a precise estimate of the population size at the time of sampling [see information below for more on the meaning of $se(\hat{N})$].

Computations for the example where $U_1 = 35$, $U_2 = 25$, $M = U_1 + U_2 = 60$, $\hat{N} = 123$, and $\hat{p} = 0.2857$ are:

$$A = 0.23795$$

$$B = 0.23323$$

$$var(\hat{N}) = \frac{60(0.51219)}{0.23795-0.23323}$$

$$= \frac{30.7317}{0.00471}$$

$$= 6519.4$$

or

$$se(\hat{N}) = \sqrt{6519.4} = 80.7$$

Such a large standard error for an estimate of 123 indicates that this $\hat{N}$ is an unreliable estimate. The approximate 95% confidence interval is $123 \pm (2 \times 80.7)$ or -38 to 284. The lower bound of -38 is replaced with 60 because 60 fish were actually known to be in the site, and the range becomes 60 to 284, an unacceptably large interval.

A problem would have been identified in the field when counts of $U_1 = 35$ and $U_2 = 25$ were obtained. The recourse in this situation is to do more sampling. This can be accomplished with more passes under the same conditions as the first pass (although this will not help much when the true capture probability, p, is only 0.2) or with increased efficiency of electrofishing. Additional possibilities that should be looked at include equipment failure, very low stream conductivity, and insufficient sampling effort during the pass.

Computations for more than two removal passes. There are no simple estimation formulas when three or more removal passes are made, except to use the total of all fish removed as $\hat{N}$ when that appears justified (see example 1, above). One possible estimation approach relies on a regression analysis of the data, although this approach is not recommended (see Otis et al. 1978; White et al. 1982).[8] A maximum likelihood estimator of N (there are several slightly different versions available) has good properties, but exact computation requires iterative numerical techniques. A very useful compromise is to use the method developed by Zippin (1958), which relies on his published graphs. Zippin's method was modified slightly and the graphs were replaced with simple polynomial functions, in order to provide a method easily applied by field users. Thus, the method of estimating N, given below, is essentially that developed by Zippin (1958).

Equations for three, four, and five removal passes only are presented. The upper limit of five was selected because more than five passes would not be required with good equipment and technique. First, two calculations are made from the removal data:

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[8]This free publication is available from Dr. Gary C. White, Los Alamos National Laboratory, Section LS-6, Mail Stop 495, P.O. Box 1663, Los Alamos, NM 87545.

M = sum of all removals = $U_1 + U_2 + \ldots + U_t$

where t = the number of removal occasions

U_i = number of fish in i^{th} removal pass

$$C = (1)U_2 + (2)U_2 + (3)U_3 + \ldots + (t)U_t$$

C is just a weighted sum. Now form the ratio

$$R = \frac{C-M}{M}$$

This ratio is the basis for the estimate of capture probability ($\hat{p}$), except that the relationship between R and p is complicated. Excellent approximations (one for each t = 3, 4, and 5) to this relationship were obtained by using a polynomial in R. That is, for known coefficients given in Table 8:

$$\hat{p} = (a_o)1 + (a_1)R + (a_2)R^2 + (a_3)R^3 + (a_4)R^4$$

Table 8. Polynomial coefficients, a_i, for computing the estimate of capture probability from removal data for t = 3, 4, and 5 removal occasions (assuming a constant capture probability on each occassion).

Coefficient of term	t		
	3	4	5
1	0.996784	0.984082	0.987419
R	-0.924031	-0.820445	-0.861918
R^2	0.319563	0.320498	0.507360
R^3	-0.390202	-0.141133	-0.239719
R^4	0.000000	0.000000	0.039395

Select the appropriate coefficient set, compute and insert R into the above formula, and compute $\hat{p}$. The estimated population size is:

$$\hat{N} = \frac{M}{1-(1-\hat{p})^t}$$

The estimated standard error is given by:

$$se(\hat{N}) = \sqrt{\frac{\hat{N}(\hat{N}-M)M}{M^2 - [\hat{N}(\hat{N}-M)(t\hat{p})^2/(1-\hat{p})]}}$$

Use of these formulas is illustrated with several examples. First, with the previously introduced data for t = 4: $U_1 = 35$, $U_2 = 25$, $U_3 = 20$, and $U_4 = 18$. M = 98 (= 35 + 25 + 20 + 18). The quantity C is:

$$C = (1)35 + (2)25 + (3)20 + (4)18$$
$$= 35 + 50 + 60 + 72$$
$$= 217$$

The value of R is:

$$R = \frac{C-M}{M} = \frac{217-98}{98} = 1.21428$$

In the calculation of R, $\hat{p}$, $\hat{N}$, and the standard error of $\hat{N}$, numbers should be carried to at least five significant digits. The value of $\hat{N}$ and $\hat{p}$ should be rounded off to fewer decimal places for reporting.

Having computed R = 1.21428, the coefficients in Table 8 for t =4 removal occasions are used to compute $\hat{p}$:

$$\hat{p} = 0.984082 - 0.820445\ (1.21428) + 0.320498\ (1.21428)^2 - 0.141133\ (1.21428)^3$$

$$= 0.984082 - 0.996249 + 0.472566 - 0.252688$$

$$= 0.207710$$

Using this estimate of capture probability, $\hat{N} = \frac{M}{1-(1-\hat{p})^t}$ can be computed:

$$\hat{N} = \frac{98}{1-(1-0.207710)^4}$$

$$= \frac{98}{1-(0.792289)^4}$$

$$= \frac{98}{0.065964}$$

$$= 161.7$$

Finally, the estimated standard error (the square root of the variance) of $\hat{N}$ is computed. The numerator of the sampling variance is:

$$\hat{N}(\hat{N}-M)M = (161.7)\ (161.7 - 98)\ (98) = 1{,}009{,}428.42$$

The denominator is:

$$M^2 - [\hat{N}(\hat{N}-M)\ (t\hat{p})^2/(1-\hat{p})] =$$

$$98^2 - [161.7(161.7 - 98)\ \ (4(0.20771))^{\ 2}]/(1-0.20771)$$

$$= 9604 - [(161.7)\ (63.7)\ (0.83084)^2]/0.792290$$

$$= 9604 - 8974.28943$$

$$= 629.71057$$

The estimated standard error of $\hat{N}$ in this example is:

$$se(\hat{N}) = \sqrt{\frac{1,009,428.42}{629.71057}}$$

$$= \sqrt{1603.00378}$$

$$= 40.0$$

An approximate 95% confidence interval on the unknown population size in the study site is:

$$\hat{N} \pm 2\ se(\hat{N})$$

For this example, the interval is 161.7 ± 2 (40.0) or 81.7 to 244.7. At this point, it is acceptable to round off $\hat{N}$ and the interval limits to integers: $\hat{N}$ = 162 and the approximately 95% confidence limits are 82 to 245 fish.

This example illustrates that the estimate of $\hat{N}$ is imprecise when the capture probability is low (p of 0.20 is definitely low). The standard error of 40, with $\hat{N}$ = 162, demonstrates that these electrofishing data are very imprecise. So poor, in fact, that the lower confidence bound is less than the 98 fish actually removed. When this kind of discrepancy occurs, the lower bound should be replaced by the number of fish actually removed, 98 in this case.

A more abbreviated example is given below using better data: U_1 = 157, U_2 = 15, U_3 = 1, and U_4 = 0. The values of M and C are M = 173 and C = 190. R = (190-173)/173 = 0.09826; $\hat{p}$ is computed from the polynomial specified by the coefficients for t = 4:

$$\hat{p} = 0.984082 - 0.820445(R) + 0.320498(R^2) - 0.141133(R^3)$$

$$= 0.90642$$

The estimate of population size is:

$$\hat{N} = \frac{173}{1-(0.093578)^4} = 173$$

with a standard error of, essentially, 0.0.

When $\hat{p}$ is at least 0.9, it is unnecessary to compute a standard error because it would be essentially zero. The value of computing the standard error is in representing the precision of the estimate $\hat{N}$ (see the section in Chapter IV on interpreting sampling variation). To some extent, the reliability of $\hat{N}$ can be judged by the value of $\hat{p}$. If $\hat{p} \geq 0.8$, results are reliable. For $0.5 \leq \hat{p} < 0.8$, $\hat{N}$ is probably a good population estimate, although some uncertainty remains about the actual number of fish in the sampled stream segment. If $0.25 \leq \hat{p} < 0.5$, the results may not be very reliable, although the estimate of $\hat{N}$ may be acceptable if three (or four, if $\hat{p}$ is near 0.25) removal passes were done. For $\hat{p} < 0.25$, $\hat{N}$ can be very unreliable; it will not only lack precision, but it can be severely biased by problems of unequal capture probabilities that do not have much effect when p is large. If $\hat{p} < 0.10$, the estimate of N is worthless. Note that, in the example above where $\hat{p} = 0.20$ and $t = 4$, $\hat{N}$ was imprecise; with such poor population estimates, monitoring for management effects on fish abundance is a waste of time and other resources.

Assessing the Fit of the Model

Given three or more removal passes, a chi-square goodness-of-fit test can be used to test the assumption of equal probability (see White et al. 1982: Chapter IV for details). As mentioned above, the assumption of equal probability of capture between passes is only critical when $\hat{p}$ ranges from 0.2 to 0.5 for three or four removal occasions. It is unnecessary to apply the test if most of the fish were caught during sampling.

When capture probabilities are low and variable, $\hat{N}$ will be biased low (see, e.g., Mahon 1980). Stratification by fish size and species helps to overcome the problem of heterogeneous capture probabilities. If the data still do not fit the model, the estimate can be accepted anyway or the generalized removal estimator used (White et al. 1982: Chapter IV), which sometimes helps improve the accuracy of the estimate. This approach is complex, difficult to compute, and probably will not be very useful. Therefore, it is not included here. Use of a computer program, especially CAPTURE (White et al. 1982) or CMLE (Platts et al. 1983), is recommended in this analysis.

Stratifying Data by Fish Size or Species

The estimator of population size previously presented is based on an assumption of equal capture probability for all fish on each removal occasion. This assumption is not critical if all of the fish of interest are caught. However, if substantial numbers of fish are uncaught after the final pass, model assumptions may not be met. Stratifying the removal data by fish size classes or by species (or both) greatly helps to meet the assumptions for a valid population estimate. Stratification based on size is especially important in estimating biomass.

When stratifying data by size, two or three sizes classes are usually enough. Data can be stratified on fish length because of the strong correlation of length with weight and body surface area. Two size classes for rainbow trout, for example, could be fish $\leq$ 12 cm and fish > 12 cm.

If estimates are obtained by fish size class, their sum becomes the estimate of the total number of fish of that species. The sampling variance of that total is the sum of the sampling variances of the individual estimates. For example:

Size class		$\hat{N}$	se($\hat{N}$)	var($\hat{N}$)
1		86	5.1	26.0
2		107	8.7	75.7
3		43	3.2	10.2
	Totals	236		111.9

The standard error of $\hat{N} = 236$ is $\sqrt{111.9} = 10.6$, not the sum of the three standard errors. Therefore, $\hat{N} = 236$ is a reasonably good population estimate for this species. If estimates of fish numbers are by species, simply add the separate $\hat{N}$ values and their variances for the species involved to obtain an estimate of the total population size and its variance.

Other population estimation methods. Capture-mark-recapture methods may be desirable when survival rates and/or fish movements are being measured. This method can also be used to estimate population size. For larger bodies of water, other methods, such as capture-recapture or catch-effort may be needed. However, these procedures are complex (see Seber 1973, 1982; Ricker 1975; Brownie et al. 1978; Otis et al. 1978; White et al. 1982). (Note that the catch-effort method is primarily useful in commercial fisheries.)

The above methods generally require marking or tagging fish. An ideal marking or tagging method would have the following characteristics (Laird and Stott 1978):

1. Fish are permanently and unmistakably recognizable to anyone examining them;

2. The method is inexpensive;

3. The method is easy to apply under field conditions; and

4. The marking or tagging has no effect on fish growth, mortality, behavior, susceptability to predation, or commercial value.

Unfortunately, no currently available technique has all of these criteria. Various marking and tagging techniques are listed in Table 9. For further discussion of these methods, see Laird and Stott (1978).

Table 9. Marking and tagging techniques (compiled from Laird and Stott 1978).

Marking techniques	Tagging techniques
Fin clipping	Subcutaneous tags
Opercular and fin punches	External tags - wired on
Branding	wire and plate tags
Tattooing	hydrostatic tag (Lea tag)
Subcutaneous injection	Petersen tag
dyes	double attachment tag
liquid latex	External tags with an internal
vital stains	anchor
fluorescent dyes	spaghetti tag
	strap tag
	opercular tag
	jaw tag

Biomass

Biomass of fish within a site is estimated as $\hat{N}\bar{W}$, where $\bar{W}$ estimates the average weight of all fish of the species or size class that $\hat{N}$ relates to. Also, let se($\bar{W}$) represent the standard error of $\bar{W}$. In the simplest case, a total of M fish are caught ($= U_1 + U_2 + \ldots + U_t$); $\hat{N}$ is based on the successive removals, and $\bar{W}$ is the average weight of the M fish caught. The standard error of $\bar{W}$ is computed from the M individual values of fish weights, as per the "usually" formula presented in Chapter IV. The standard error of total biomass in the site, $\hat{B}(= N\bar{W})$, is approximately:

$$se(\hat{B}) = \hat{B} \left(\frac{var(\hat{N})}{(\hat{N})^2} + \frac{var(\bar{W})}{(\bar{W})^2} \right)^{1/2}$$

If it is necessary to stratify the data for a species in order to estimate the population, then the total biomass in the site must also be computed on this stratified basis. $\hat{N}$ and $\bar{W}$ are first computed for each strata.

If the removal data are stratified into two size classes, two pairs of values $\hat{N}_1$, $\bar{W}_1$ and $\hat{N}_2$, $\bar{W}_2$ are calculated. Total biomass is:

$$\hat{B} = \hat{N}_1\bar{W}_1 + \hat{N}_2\bar{W}_2 = \hat{B}_1 + \hat{B}_2$$

$$var(\hat{B}) = var(\hat{B}_1) + var(\hat{B}_2)$$

Average fish weight in the site is $\hat{B}$ divided by $\hat{N} = \hat{N}_1 + \hat{N}_2$.

These formulae are valid regardless of the way $\bar{W}$ is computed. If many fish are caught, they do not all have to be weighed. Average weight can be estimated from a random subsample of fish caught. A more complex procedure is to take the length of all fish, but weigh only a small number; e.g., the first 10 in each length class.

Length and weight must be recorded for each fish weighed, in addition to the lengths of all fish caught but not weighed. The log of weight vs. log of length (see Chapter V) is used to establish the relationship between length and weight. The length-weight equation can then be used to predict the weight of the unweighed fish.

A less accurate but simpler approach to analyzing stratified data is possible. Assume there are "r" 1-cm length intervals encountered and the first 10 fish encountered in each length interval are weighed (or all are weighed if less than 10 fish in a length interval are captured). The average

weight in each length interval is calculated, and the total number of fish in successive 1-cm length intervals is tabulated. A table can then be developed from these data:

Length class	Average weight	Number caught by length class
1	$\bar{W}_1$	n_1
2	$\bar{W}_2$	n_2
3	$\bar{W}_3$	n_3
•	•	•
•	•	•
•	•	•
r	$\bar{W}_r$	n_r

The sum of the number of fish caught by length class (M) equals the total number of fish removed. The averages $\bar{W}_i$ are not generally based on all n_i fish in that 1-cm length interval because not all of the fish are weighed. The estimator of the average weight of fish for the site is:

$$\bar{W} = \frac{n_1\bar{W}_1 + n_2\bar{W}_2 + n_3\bar{W}_3 + \ldots + n_r\bar{W}_r}{M}$$

Variance estimates for either the regression or weighed size class methods can be derived. However, the procedure for the deviations is complex and is not included in this manual.

SECONDARY VARIABLES

Variables other than those already discussed may be important in some monitoring programs. These secondary variables may be habitat, fishery, or biotic related.

Other Habitat Variables

Abiotic attributes that may be monitored under certain circumstances include bedload, detritus, suspended solids, dissolved oxygen, pH, conductivity, alkalinity, hardness, nutrients, pesticides, metals, and salinity. Literature is available on measurement techniques for all of these variables. Two general references that may be useful are American Public Health Association et al. (1971) and U.S. Geological Survey (1977).

Other Fishery Variables

Other fishery variables that can be monitored include age and growth, food habits, production, survival or mortality, fucundity, parasitism, disease, and net production. Measurement of many of these variables is discussed in Ricker (1975) and Bagenal (1978).

Other Biotic Variables

If changes in the stream ecosystem are monitored holistically, organisms besides fish (e.g., bacteria, periphyton, macrophytes, and macroinvertebrates) can be sampled. There are various sampling techniques available for a number of attributes that can be measured for each group of organisms. For example, variables that may be of interest for macroinvertebrates include species composition, biomass, relative abundance, emergence, and drift. General sampling techniques for nonfish species are discussed in Cummins (1962), Edmondson and Winberg (1971), Mason et al. (1973), Weber (1973), Benfield et al. (1974), Greeson et al. (1977), Mason (1978), Resh (1979), and Platts et al.

(1983). References discussing other biotic variables that could be measured include Edmondson and Winberg (1971), Langford and Daffern (1975), and Greeson et al. (1977).

Identification of organisms requires someone knowledgeable about the taxa sampled. For general information, see Usinger (1974) or Pennak (1978).

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CHAPTER IV. BASIC STATISTICAL AND STUDY DESIGN CONCEPTS

BASIC TERMS

Statistics refers to the science of organizing and summarizing sample data from a population to develop inferences. A population, in the biological context, is the total number of a species in a specific area; e.g., total number of rainbow trout in a given watershed. For most practical purposes, it is impossible to measure all individuals in a population to calculate descriptive features or parameters. Estimates of the parameters (Table 10) can be derived, however, by sampling the population and applying statistical procedures to the data.

Table 10. Parameters and their statistical estimators.

Parameter	Statistical estimator
mean μ	$\bar{X}$
variance σ^2	s^2
standard deviation σ	s

Measurement variables for a statistical population can be either continuous or discrete. Continuous variables are usually measurements; e.g., stream width or water temperature, which can be any value within a range. Discrete variables have a limited number of possible values; e.g., count data (such as numbers of fish in a gill net) or classification values (such as stable or unstable stream banks).

A statistic computed to estimate a population parameter generally differs from sample to sample because of natural variability. However, statistical methods can be used to make inferences about parameters from sample data with defined levels of statistical confidence. Confidence is discussed later in this chapter.

DESCRIPTIVE FEATURES

Summary statistics are used to describe properties of sample data. The sample mean is one of several statistics used to describe central tendency. The equation for the mean is:

$$\bar{X} = \frac{X_1 + X_2 + \ldots + X_n}{n} = \frac{\Sigma X_i}{n}$$

where ΣX_i = the sum of all the sample values

n = the number of observations or sample size

As an example, let a sample of size 15 (e.g., fish lengths rounded to centimeters), recorded in ascending order, be 6, 8, 9, 10, 11, 11, 11, 12, 12, 13, 13, 14, 16, 20, and 22. The mean of these values is:

$$\bar{X} = \frac{6 + 8 + \ldots + 21}{15} = \frac{186}{15} = 12.4$$

This same sample of 15 values is used below to illustrate other statistical procedures.

The sample median is the value that divides arranged data (values arranged from the lowest to the highest) into two equal parts. That is, half of the values in the array exceed the median, and half are less than the median. When there are an odd number of observations (n) in an array, the median is simply the m^{th} value in the sequence, where $m = (n+1)/2$. When the sample size is even, the median is the average of the two central most values: X_m and X_{m+1}, where $m = n/2$.

In the above example, $n = 15$, $m = 16/2 = 8$, and $X_8 = 12$ is the median. If $n = 14$ because $X_{15} = 22$ was not recorded, the median would be computed as:

$$\frac{X_7 + X_8}{2} = \frac{11 + 12}{2} = 11.5$$

The sample mode is the value represented by the greatest number of individual observations in a sample. On a frequency curve, it is the value of the variable where the peak of the curve occurs. In the above sample, the value 11 occurs most frequently ($X_5 = X_6 = X_7 = 11$) and is the sample mode. In this example, the mean, median, and mode are close to each other, but not identical. This is often the case. The mean, median, and mode of a hypothetical set of data are illustrated in Figure 7.

For some types of data; e.g., lognormal (a skewed distribution) or annual survival rates over a period of years, the geometric mean is more appropriate for describing the central tendency than is the arithmetic mean. The geometric mean of n numbers is defined by:

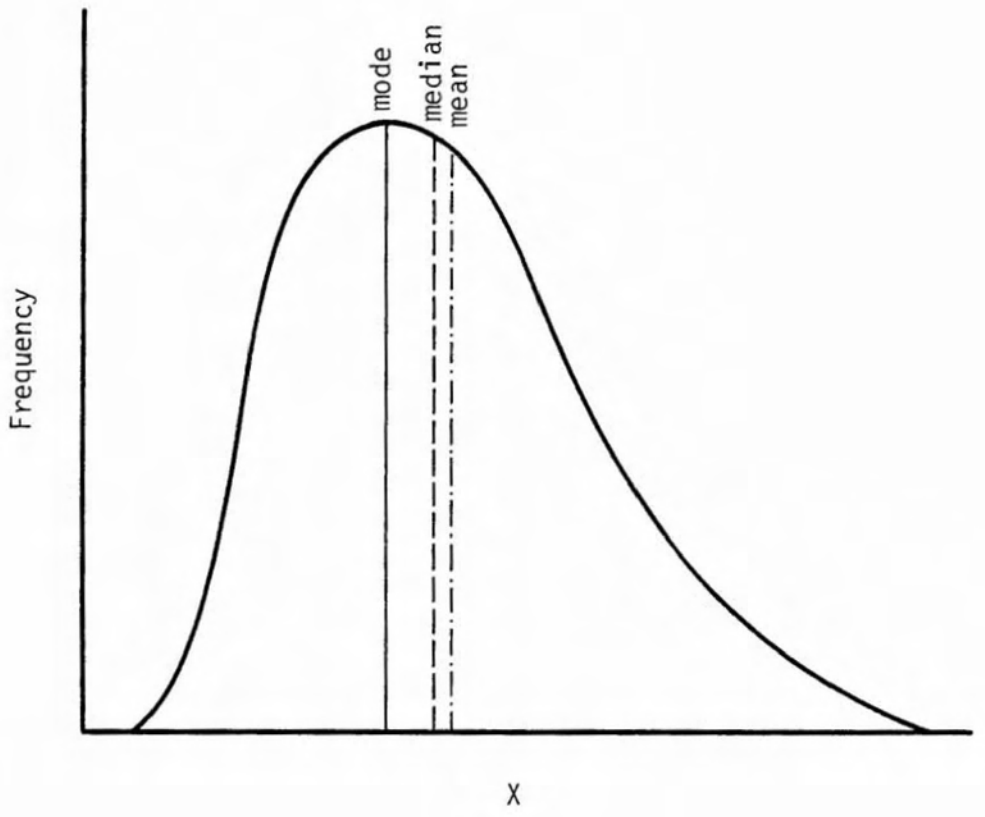


Figure 7. A frequency distribution (skewed to the right) indicating the location of the mean, median, and mode. These values relate to the central tendency for a data set.

$$\bar{X}_g = (X_1 \times X_2 \ldots X_n)^{1/n}$$

which is the product of the numbers raised to the power 1/n. The recommended calculation to obtain a geometric mean is to take the log of each sample value, compute the arithmetic mean of these logs, and then take the antilog of this arithmetic mean:

$$\bar{X}_g = \text{antilog} \left(\frac{\Sigma \log X_i}{n} \right)$$

The logs, to base 10, for the above 15 sample values are 0.7782, 0.9031, 0.9542, 1.0, 1.0414, 1.0414, 1.0414, 1.0792, 1.0792, 1.1139, 1.1139, 1.1461, 1.2041, 1.3010, and 1.3424. The mean of these logs is $\bar{X} = 1.0760$. The geometric mean of the original sample is:

$$\bar{X}_g = \text{antilog } (\bar{X}) = 10^{\bar{X}} = 10^{1.0760} = 11.9$$

(Note: The geometric mean can only be computed if all sample values are greater than zero).

Just as the mean, median, and geometric mean are used to describe the central tendency for a set of data, other statistics can be used to describe the variation or scatter in the sample values. The range, which is simply the difference between the highest and lowest sample values, is an estimate of the variation of values in a sample. (In the above example, the range is 22 - 6 = 16.0.) Because it is based only on the two most extreme values, the range does not indicate the average variation among the sample values.

The sample standard deviation (s) is the statistic typically used to describe the average variation among the sample values:

$$s = \sqrt{\frac{\Sigma (X_i - \bar{X})^2}{n-1}}$$

Except for the divisor being n-1 rather than n, s is the square root of the average squared deviation of each value from the sample mean. Computation of the sample standard deviation by application of the above equation is tedious even for a moderate number of observations. Use of an alternative formula requires computation of only the sum of the sample values (ΣX) and the sum of the squared sample values (ΣX^2):

$$s^2 = \frac{\Sigma X_i^2 - \frac{1}{n} (\Sigma X_i)^2}{n-1}$$

In the example being used here, $\Sigma X_i = n\bar{X} = 186$ and $\Sigma (X_i)^2 = 6^2 + 8^2 + \ldots + 22^2 = 36 + 64 + \ldots + 484 = 2606$. Hence, for this sample:

$$s^2 = \frac{2606 - (186)^2/15}{14} = \frac{299.6}{14} = 21.40$$

$$s = 4.63$$

When the sample mean is used to estimate the population mean (μ), the precision of this estimate depends on both the sample size and the innate sampling variation in the population, as estimated by the standard deviation, s. The sampling variance of $\bar{X}$ is estimated as:

$$\text{var}(\bar{X}) = \frac{s^2}{n}$$

The square root of this variance is an often needed quantity in statistical inference. It is called the standard error of the mean to distinguish it from the standard deviation; i.e., $se(\bar{X}) = s/\sqrt{n}$ (see, e.g., Tacha et al. 1982). For the current example, $se(\bar{X}) = 4.63/\sqrt{15} = 1.20$.

The relative variation among the sample values is often described by the sample coefficient of variation, cv, which is the sample standard deviation expressed as a percentage of the sample mean:

$$cv = \frac{s}{\bar{X}}$$

The coefficient of variation is usually reported on a percent basis; i.e., percent cv = $100s/\bar{X}$. In the example, cv = 4.63/12.4 = 0.3734 or, as a percent, 37.3%.

The sample mean and standard deviation provide "point" estimates of the corresponding population parameters. In addition to such point estimates, it is useful to have "interval" estimates; i.e., an interval such that the true parameter falls inside the interval with a known probability. One easily computed type of interval is confidence intervals. A confidence interval can be calculated for most population parameters estimated by a statistic. For example, the interval for a population mean (μ) for normally distributed data is expressed as:

$$\bar{X} - t_{\alpha,n-1}se(\bar{X}) < \mu < \bar{X} + t_{\alpha,n-1}se(\bar{X})$$

where $t_{\alpha,n-1}$ = the tabular value for the t statistic

α = 1 - the confidence level; e.g., 1 - 0.95 = 0.05

n = number of observations in the sample (the sample size)

n-1 = degrees of freedom for the t statistic

$se(\bar{X})$ = standard error of the mean, $\bar{X}$

By selecting a 95% confidence level, a user can conclude, with 95% confidence, that the unknown value of μ is between the lower $[\bar{X} - t_{\alpha,n-1}se(\bar{X})]$ and the upper $[\bar{X} + t_{\alpha,n-1}se(\bar{X})]$ computed confidence limits.

Methods for computing confidence intervals are included in most statistical texts, including Snedecor and Cochran (1967).

Computational methods for descriptive statistics discussed in this section are demonstrated in Example 1 later in this chapter.

FREQUENCY DISTRIBUTIONS

The basic paradigm of statistics is that sample data can be described (modeled) by probability (frequency) distributions. Most data analysis methods make some assumptions about the type, or properties, of the probability model that describes (fits) the data. If these assumptions are wrong, the results of the analysis may be misleading. Consequently, it is important to know what distribution describes the data. The distribution can be determined on three types of information: (1) theoretical considerations (not usually very applicable in environmental work); (2) past experience; and (3) empirical examination of the present data, especially plotting it.

When samples are obtained from a population, the data should be summarized graphically to determine the applicable type of probability distribution (Fig. 8). Commonly used models for discrete or count data are the positive

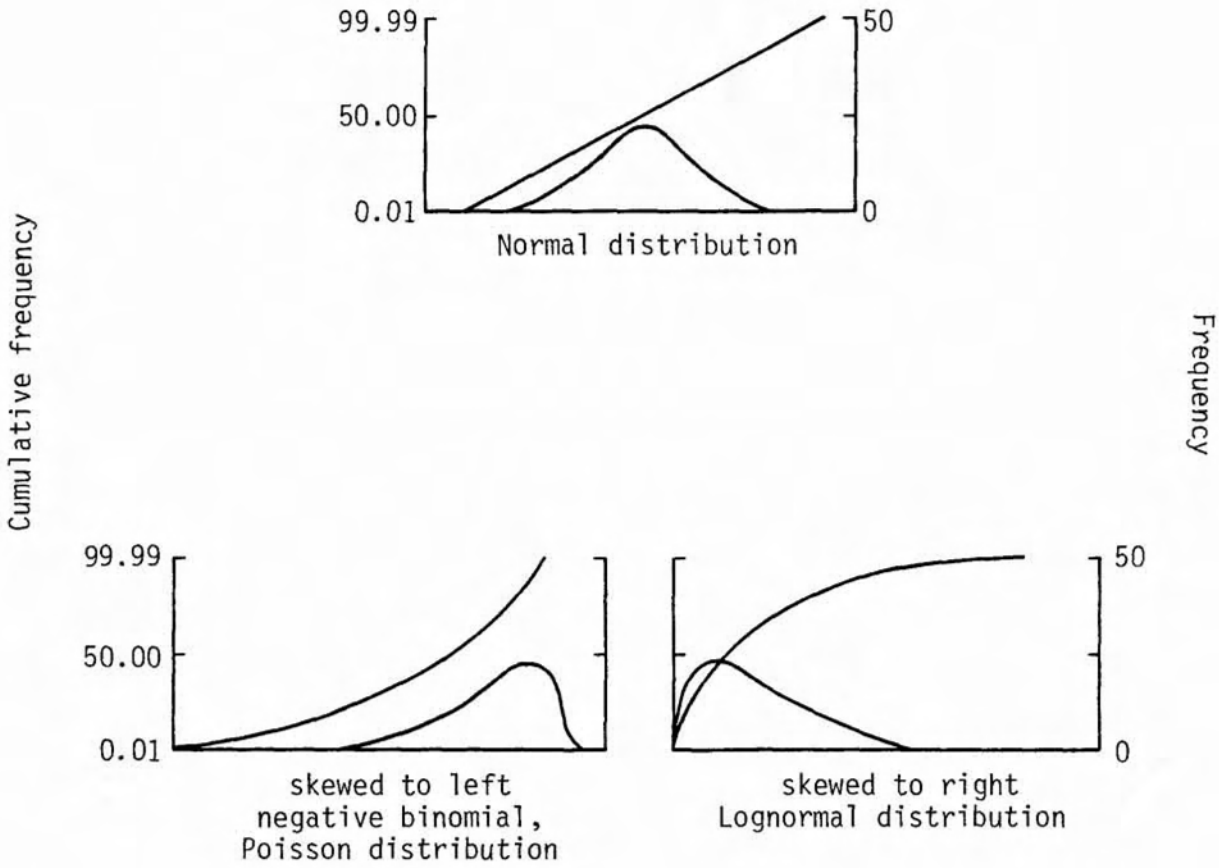


Figure 8. Types of frequency distributions and their plots on normal probability paper. The continuous curves under the upper plots for each example represent a distribution before plotting on normal probability paper (after Sokal and Rohlf 1969). A positive binomial distribution with a large sample size (n) would resemble a normal distribution.

binomial, the negative binomial, and the Poisson distributions. Explanations of these distributions and statistical applications are contained in many basic statistics texts; e.g., Snedecor and Cochran (1967) and Elliot (1977).

The normal distribution is probably the most widely used (and, unfortunately, the most widely abused) model for continuous measurement variables. The normal distribution, colloquially described as the bell-curve, is completely determined by the mean (μ) and standard deviation (σ) of the population. Figure 9 illustrates a normal frequency curve. As indicated in Figure 9, on the average, 68.3% of the sample values will be within $\pm 1\sigma$ of the mean, and 99.7% will be within $\pm 3\sigma$ of the mean. For sample data from a normally distributed population, $\bar{X}$ is substituted for μ, and s is substituted for σ. Several nonnormal frequency distributions have been postulated for application to continuous data (Johnson and Kotz 1970a,b). The lognormal distribution (Fig. 10) has applicability to parametric tests because, when the data are transformed by logarithms, they have a normal distribution. For many variables, such as fish weight or length, the lognormal distribution may be a more reasonable model than the normal distribution. The lognormal distribution has also been used to model discrete variables, such as counts of fish or species abundance (Pielou 1975). Some examples of statistical computations are as follows.

Example 1

Problem: In a stream monitoring study, the following 10 temperatures (°C) were taken in the managed site.

8.0	10.0
8.0	10.5
8.5	11.0
10.0	11.5
10.0	12.0

Give the descriptive statistics for these data, assuming no data transformation is necessary or desired.

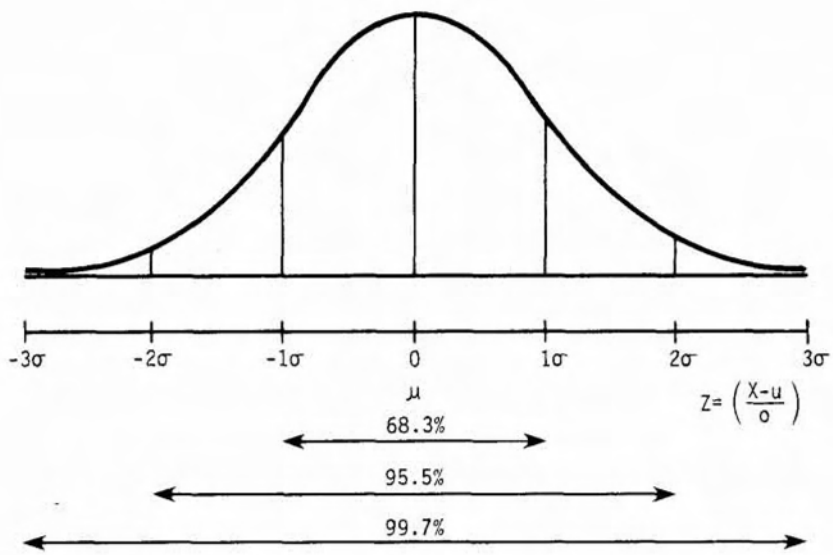


Figure 9. A normal distribution.

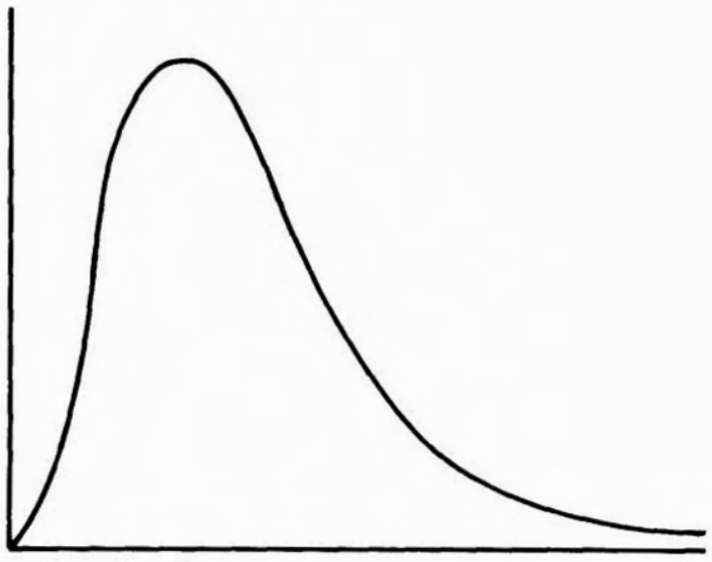


Figure 10. A lognormal distribution.

Solution:

1. The mean, $\bar{X} = \frac{\Sigma X_i}{n}$

$$\bar{X} = \frac{99.5}{10.5} = 9.95$$

2. The median is the average of the $\left(\frac{n}{2}\right)^{th}$ and $\left(\frac{n}{2} + 1\right)^{th}$ (fifth and sixth, in this example) ordered values because there is an even number of temperature values:

$$\text{Median} = \frac{10 + 10}{2} = 10$$

3. The mode is the most common value:

$$\text{Mode} = 10$$

4. The range is 12.0 - 8.0 = 4.0°C.

5. The sample standard deviation s is computed as:

$$s = \frac{\Sigma X^2 - \frac{1}{n}(\Sigma X)^2}{n-1}$$

$$\Sigma X^2 = 64 + 64 + \ldots + 144 = 1007.75$$

$$s = \frac{1007.75 - \frac{1}{10}(99.5)^2}{n - 1} = 1.403$$

6. Percent coefficient of variation, $cv = \frac{s}{\bar{X}} \times 100$

$$cv = \frac{1.40}{9.95} \times 100 = 14.1\%$$

7. The standard error of the mean is:

$$se(\bar{X}) = \frac{s}{\sqrt{n}} = \frac{1.403}{\sqrt{10}} = 0.444$$

8. Confidence limits for the true population mean, μ (L = lower limit, U = upper limit):

$$L = \bar{X} - t_{\alpha,n-1}se(\bar{X})$$

Let $\alpha = 0.05$, which corresponds to a 95% confidence level. Thus, $t_{0.05,9} = 2.262$, and

$$L = 9.95 - (2.262)(0.444) = 8.95$$

$$U = \bar{X} + t_{\alpha,n-1}se(\bar{X})$$

$$U = 9.95 + (2.262)(0.444) = 10.95$$

Therefore, $8.95 < \mu < 10.95$ is the 95% confidence interval.

Example 2

Problem: The same data are used as in example 1, but a lognormal distribution is assumed. The appropriate analysis in this case is to transform each datum X to log(X) (base 10 will suffice), do the same statistical analyses, and back-transform appropriate estimates (it is not appropriate to back-transform variances, standard deviations, or standard errors).

The log(X) data are

0.9031	1.0
0.9031	1.0212
0.9294	1.0414
1.0	1.0607
1.0	1.0792

Solution:

1. The mean of these logs is:

$$\overline{\log(X)} = \frac{9.9381}{10} = 0.9938$$

The antilog of this value is the geometric mean $\bar{X}_g$:

$$\bar{X}_g = \text{antilog } (0.9938) = 9.86$$

Compare this value to the arithmetic mean of 9.95.

Note that the geometric mean is less than the arithmetic mean. This will always be true.

2. The median is:

$$\frac{\log(X_5) + \log(X_6)}{2} = 1$$

Back-transforming, 10 = antilog (1). In general, the median computed this way does not necessarily equal the median of the untransformed data.

3. The mode is:

10 = antilog(1)

Transformations do not change the estimate of the mode.

4. The range of the transformed data (1.0792 - 0.9031 = 0.1761) can be computed, but should not be back-transformed because it does not produce a valid estimate of range for the untransformed data.

5. The standard deviation of the log(X) data is needed to compute a confidence interval on μ:

$$s^2 = \frac{9.912 - \frac{1}{10}(9.9381)^2}{9}$$

$s^2 = 0.003944$ or

$s = 0.06280$

6. The standard error of the mean of the log(X) values is:

$$se(\overline{\log(X)}) = \frac{0.06280}{\sqrt{10}} = 0.01986$$

7. To obtain a 95% confidence limit on the true population mean, μ, first compute the mean from the transformed data, then back transform the resultant lower and upper limits. Using $\alpha = 0.05$, hence $t_{0.05,9} = 2.262$, compute upper and lower limits with the transformed data:

$$L = \overline{\log(X)} - 2.262\ se(\overline{\log(X)})$$

$$L = 0.9938 - 2.262(0.01986) = 0.9489$$

Similarly,

$$U = 0.9938 + 2.262(0.01986) = 1.0387$$

Now back transform both limits by the antilog:

$$L_g = \text{antilog}(L) = 10^{.9489} = 8.89$$

$$U_g = \text{antilog}(U) = 10^{1.0387} = 10.93$$

Therefore, $8.89 < \mu < 10.93$ is the 95% confidence interval when proper analysis requires a log transformation.

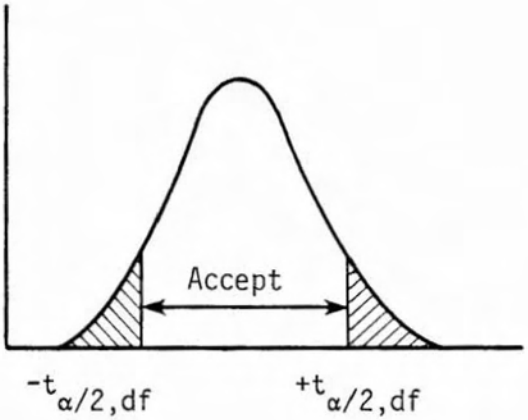
STATISTICAL TESTING

Hypothesis testing is an important facet of statistical analysis. A hypothesis is generally a statement about one or more parameters that needs to be tested. For example, a field biologist might hypothesize that fish under

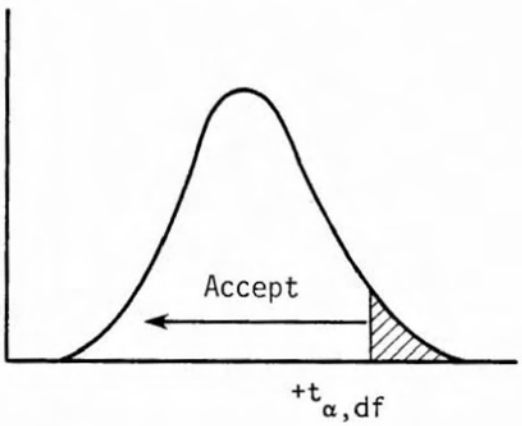
particular environmental conditions are not affected by a new management practice. Statistical tests could be based on mean weights ($\bar{X}$) of samples from the population. The hypothesis could be that the mean weight of fish in a managed area is equal to the mean weight in a control area; symbolically, the null hypothesis is $H_o: \mu_1 = \mu_2$. The symbol μ_1 represents the true population mean for the managed area; μ_2 corresponds to the true mean for the control area. These means are estimated by $\bar{X}_1$ and $\bar{X}_2$, respectively. The null hypothesis is either rejected or fails to be rejected (in which case it is tentatively accepted), depending on the results of the appropriate statistical test.

The alternative hypothesis, denoted by H_a, should be either $\mu_1 \neq \mu_2$, $\mu_1 > \mu_2$, or $\mu_1 < \mu_2$. The three alternative hypotheses represent situations where the mean weights for the two zones are different, the mean weight is greater in the managed zone, and the mean weight is less in the managed zone, respectively. To test the null hypothesis, a significance level is designated; e.g., 0.05. Significance refers to the probability of rejecting the null hypothesis, H_o, when it is true. A significance level of 0.05 means that, if H_o is rejected, there is a 95% confidence that the rejection is correct. An appropriate statistical analysis for testing the null hypothesis against the alternative hypothesis must be selected, along with the significance level. Acceptance or rejection of the null hypothesis is determined by comparing the computed test value, e.g., a t-value, against a critical value (Fig. 11) determined by the theoretical sampling distribution of the test statistics (see White et al. 1982:Chapter 2).

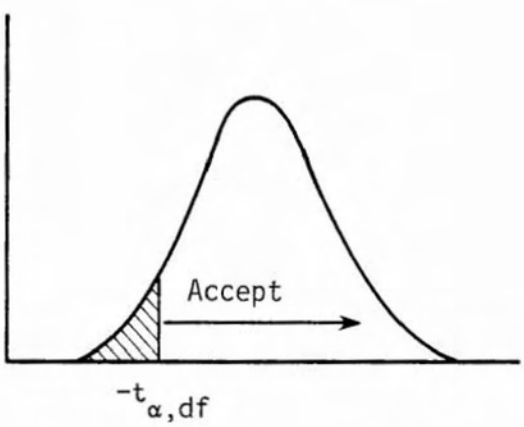
For example, suppose the null hypothesis $H_o: \mu_1 = \mu_2$ versus $H_a: \mu_1 \neq \mu_2$ is to be tested using a t-test, and the designated significance level is 0.05 (denoted as α). This would be a "two-tailed" test and a statistical table would be used to find the critical (i.e., rejection-level) t-value for the appropriate degrees of freedom (df). Suppose this tabular value is $\pm$ 2.07 for $t_{\alpha/2,df}$ and the computed test statistic value is 2.78. Because the test statistic is greater than 2.07, the null hypothesis is rejected with 95% confidence that the true population means are unequal.



A. Acceptance and rejection regions for a computed t-value for H_o: $\mu_1 = \mu_2$ versus H_a: $\mu_1 \neq \mu_2$, a "two-tailed" test.



B. Acceptance and rejection regions for a t-test for H_o: $\mu_1 = \mu_2$ versus H_a: $\mu_1 > \mu_2$, a "one-tailed" test.



C. Acceptance and rejection regions for a t-test for H_o: $\mu_1 = \mu_2$ versus H_a: $\mu_1 < \mu_2$, a "one-tailed" test.

Figure 11. Rejection and acceptance regions for comparing a null versus an alternative hypothesis. Critical rejection regions (the "tails" of the distribution curve) contain slash marks. Computed values for data are compared to table values.

Two types of errors are possible when a hypothesis is tested. The first type (Type I) is rejecting the null hypothesis when it is true; the second type (Type II) is failing to reject the null hypothesis when it is false.

When a 0.05 α-level is stipulated and the null hypothesis is rejected, an asterisk (*) is often used to denote this significance level. The computed statistic can usually be compared against tabular values for $\alpha = 0.01$(**) and $\alpha = 0.001$(***), as well as for $\alpha = 0.05$. The probability of a Type I error is always α, the level of significance. An α of 0.05 represents one chance in 20 that failure to reject the null hypothesis is wrong. The chances of making a Type I error increases as the α value increases.

The probability of a Type II error, often denoted by β, is a function of: (1) the choice of α; (2) the statistical test used (given the choice of α); (3) the difference between the true parameter value and the hypothesized parameter value; and (4) the number of observations (sample size). The power (or sensitivity) of a statistical test is the probability of rejecting the null hypothesis when it is, in fact, false; thus, power is $1-\beta$; i.e., unity minus the probability of a Type II error. When the true parameter value is greatly different than the hypothesized value, the test chosen should have a very high probability of detecting this difference; i.e., have a high power. The "standard" statistical tests (e.g., t-test and F-test) have this property when certain assumptions, such as normality, are met. The power of a statistical test decreases drastically when parameter values for the null and the alternative hypothesis are close together because of the difficulty in differentiating between the hypotheses with a statistical test (Sokal and Rohlf 1969). The sample size must be increased to increase the power of a given test (or decrease β) while keeping α constant for a stated null hypothesis. However, with respect to sample size, a bigger sample does not necessarily mean a substantially "better" test because the power of most statistical tests is a complex function of several factors, including sample size. Power can also be increased by changing the nature of the test, usually through better study design. In fact, use of a good study design is the most efficient way to increase the power of these statistical tests.

In summary, the ideal statistical test has a small probability of rejecting the null hypothesis when it is true and a large probability of rejecting it when it is false (Elliot 1977). Hypotheses are tested to determine if the values obtained from two or more sites (control and managed) are from the same statistical population or from different statistical populations. Two types of errors can be made, Type I and Type II. Because it is always possible (though highly improbable) that a highly deviant test value could be obtained by chance even when H_o is true, a statistical test never proves that a particular null hypothesis is false (Elliot 1977). Similarly, rejection of the null hypothesis does not prove that the alternative hypothesis is true; it only provides good evidence that it is true. Finally, failure to reject H_o does not prove that H_o is true.

The process of hypothesis testing is basic to all areas of science and can be summarized as follows:

1. Formulate the null and alternative hypotheses, H_o and H_a.

2. Specify the significance level α[9].

3. Determine the statistical test to be used.

4. Determine the "rejection region" for the test.

5. Calculate the test statistic.

6. Reject or accept the null hypothesis depending on the numerical value of the computed test statistic relative to the theoretical rejection region.

---

[9]Most α values used for computations in this manual are $\alpha = 0.05$. However, other α values can be selected for these tests.

This general procedure is followed in the examples given in Chapter V. (For more explanation of parametric testing in a biological context, see White et al. 1982:Chapter 2.)

PARAMETRIC AND NONPARAMETRIC TESTS

Two types of statistical tests are discussed in this manual: parametric and nonparametric (discussed briefly). Parametric tests, as the name implies, require certain assumptions about population parameters. Conversely, nonparametric tests are not dependent on a given parametric distribution and, thus, are distribution-free tests (Sokal and Rohlf 1969). Nonparametric tests are often easier to compute than parametric tests but generally have less power. Parametric tests make maximum use of all the information that is inherent in the data when the necessary assumptions are met.

Nonparametric procedures are appropriate in the following situations:

1. The hypothesis to be tested does not involve a population parameter.

2. The data have been measured in some way other than that required for the parametric procedure that would otherwise be appropriate. For example, count or rank data may be available, precluding the use of an otherwise appropriate parametric procedure that requires continuous data.

3. The assumptions necessary for the valid use of a parametric procedure are not met. In many instances, the design of a research project may suggest a certain parametric procedure. Examination of the data, however, may reveal that one or more assumptions underlying the test are not met. In this situation, a nonparametric procedure is frequently the best alternative.

4. Results are needed in a hurry and calculations must be done by hand, so tests that are easily calculated are necessary.

The assumptions that need to be met for classical parametric tests (such as the t-test and various analyses of variance; i.e., the F-test) are (Siegel 1956):

1. The observations must be independent; i.e., randomly obtained;

2. The observations must be drawn from normally distributed populations; and

3. These populations must have the same variances: homogeneity of variances (see Fig. 12) or homoscedasticity (or, in special cases, they must have a known ratio of variances).

The basic assumption of all parametric tests is that sampling of individuals is random (this does not mean haphazard). Nonrandomness of sample selection may be reflected in lack of independence of the sample items, in heterogeneity of variances (i.e., different variances for control vs. treatment sites), or nonnormal distribution of the data.

Before proceeding with a parametric test, it should be determined if the assumptions are reasonable, and verification tests should be conducted (Sokal and Rohlf 1969). Several methods are available to test these assumptions; the less complex tests are presented in this manual. Although many parametric statistical methods are not greatly affected by small departures from normality, a major violation of the required assumption of normality may render any statistical inference based on the sample data almost meaningless.

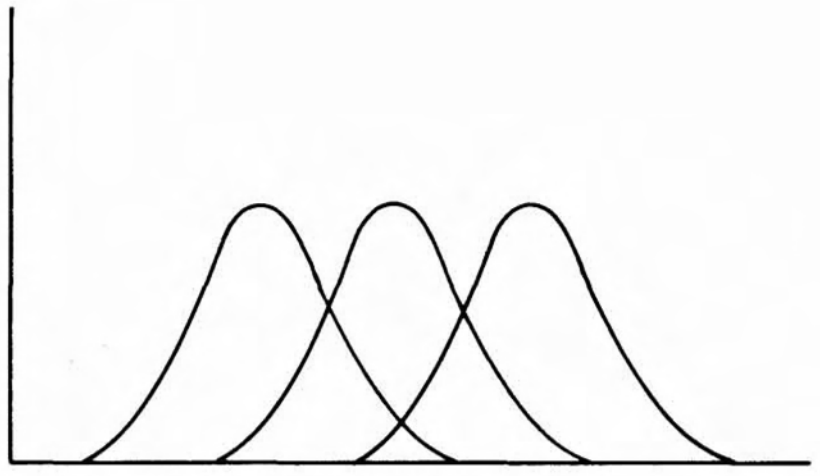


Figure 12. Graphic demonstration of homogeneity of variance. Means are different but shapes of distribution are similar (Huntsberger 1967).

Four common methods for testing the assumption of normality are:

1. The graphic method;

2. The chi-square goodness-of-fit test;

3. The Wilk-Shapiro test (sample size $n < 50$); and

4. The Kolmogorv-Smirnov test ($n > 50$).

The graphic method, which involves plotting the data on normal probability paper, is used for demonstration purposes in this text. When there are indications that the data are not normally distributed, e.g., a straight line is not appropriate for the data points, a transformation of the data should be attempted (Table 11). For example, if the data are plotted in a histogram and the distribution appears to be lognormal (Fig. 10), then the individual values in the data set should be converted to logarithms and replotted on normal probability paper. This transformation usually results in normality, which permits application of parametric tests.

Another approach to testing the appropriateness of a log transformation is to plot the data on lognormal probability paper. If a straight line can be plotted through the data points, the log-transformation is appropriate, and the normal probability plot test is unnecessary. Methods of testing for normality that are more quantitative are described in standard statistical references, including Snedecor and Cochran (1967) and Sokal and Rohlf (1969).

The assumption for homogeneity of variance (Fig. 12), often necessary when multiple data sets are being compared, can be preliminarily tested by the normal probability plot approach. If the lines for the different data sets are parallel, the variances are homogeneous. If the lognormal probability plot approach is used and the lines are parallel, it is a positive test for homogeneity of variance for lognormal data.

Table 11. Data transformations used for various probability distributions or when the population mean μ and standard deviation σ have a given relationship.

Population distribution	Relationship of σ to μ[a]	Transformation
Poisson	$a\sqrt{\mu}$	$\sqrt{x}$ or $\sqrt{x} + 0.5$
Binomial	$c\sqrt{\mu(1-\mu)}$	$\sin^{-1}(\sqrt{x})$
Negative binomial[b]	$f\sqrt{\mu(1 + g\mu)}$	$\sinh(\sqrt{x})$ or $\sinh(\sqrt{x + 1})$
Lognormal or Empirical	$b\mu$	$\log(x)$ or $\log(x + 1)$
Empirical	$d\mu(1-\mu)$	$\log\left(\frac{x}{1-x}\right)$
Empirical	$e(1-\mu)$	$\log\left(\frac{1 + x}{1 - x}\right)$

[a] a, b, c, d, e, f, and g are constants that may be known or unknown.

[b] The transformation is the hyperbolic sine function, $\sinh(y) = (e^{y}-e^{-y})/2$.

The F-test can be used to quantitatively test for homogeneity of variance for two sample sets (e.g., control vs. treatment data) for the hypothesis H_o: $\sigma_1^2 = \sigma_2^2$ versus H_a: $\sigma_1^2 \neq \sigma_2^2$. Homogeneity for more than two sets of data can be tested with Bartlett's test (Sokal and Rohlf 1969).

If the assumptions for parametric tests are not reasonably met, then two basic choices remain: transform the data as previously discussed or use a nonparametric test. Fortunately, a single transformation will often simultaneously solve several departures from the assumptions (Table 11 and see Sokal and Rohlf 1969). For the logarithmic transformation, if the data set contains zeros, use $\log(x + 1)$. When a transformation is done, tests of significance are performed on the transformed data, although estimates of means (and confidence intervals) are usually back-transformed in order to be presented in the untransformed scale (Sokal and Rohlf 1969).

The statistical tests selected for use in a monitoring program depend on the experimental design and the characteristics of the data. The first consideration in choosing the statistical test to be used is the type of data obtained for the variable. If the data are continuous (Table 12), i.e., when values can assume any value within a given range, the choice of the test depends on the study design, including the number of factors and the number of replicates. If the data are discrete, but can be considered continuous because of the wide range of values that can be assumed, the data are treated as if they were continous (Table 12).

In situations where a percentage is used that can range from 0 to 100%, the data can be treated as if they are continuous measurement data. Discrete data that cannot be considered continuous, such as ranks on a small scale (e.g., 0, 1, 2, or 3) or count data (e.g., fish relative abundance), are analyzed using a contingency table. When the objective of the study is to find the relationship between variables, regression or correlation analysis is needed. Guidance for determining whether to use a parametric or a nonparametric test is presented in Figure 13. Parametric and nonparametric test counterparts are listed in Table 13.

Table 12. Types of distributions appropriate for sample data in monitoring studies.

Continuous	Distribution discrete	Summary variables
Stream width	Stream bank and channel stability[a]	Substrate composition[c]
Stream depth	Fish population estimates[b]	
Water velocity	Percent cover	
Discharge	Percent pools and riffles[c]	
Water temperature	Relative abundance	
Length/weight relationships	Relative ranks	
Fish biomass		

[a]If there is a wide range of values, the data can be considered continuous (Pfankuch's method).

[b]If the values can take on any percentage from 0 to 100, the data can be treated as continuous measurement data.

[c]Treat the same as relative abundance.

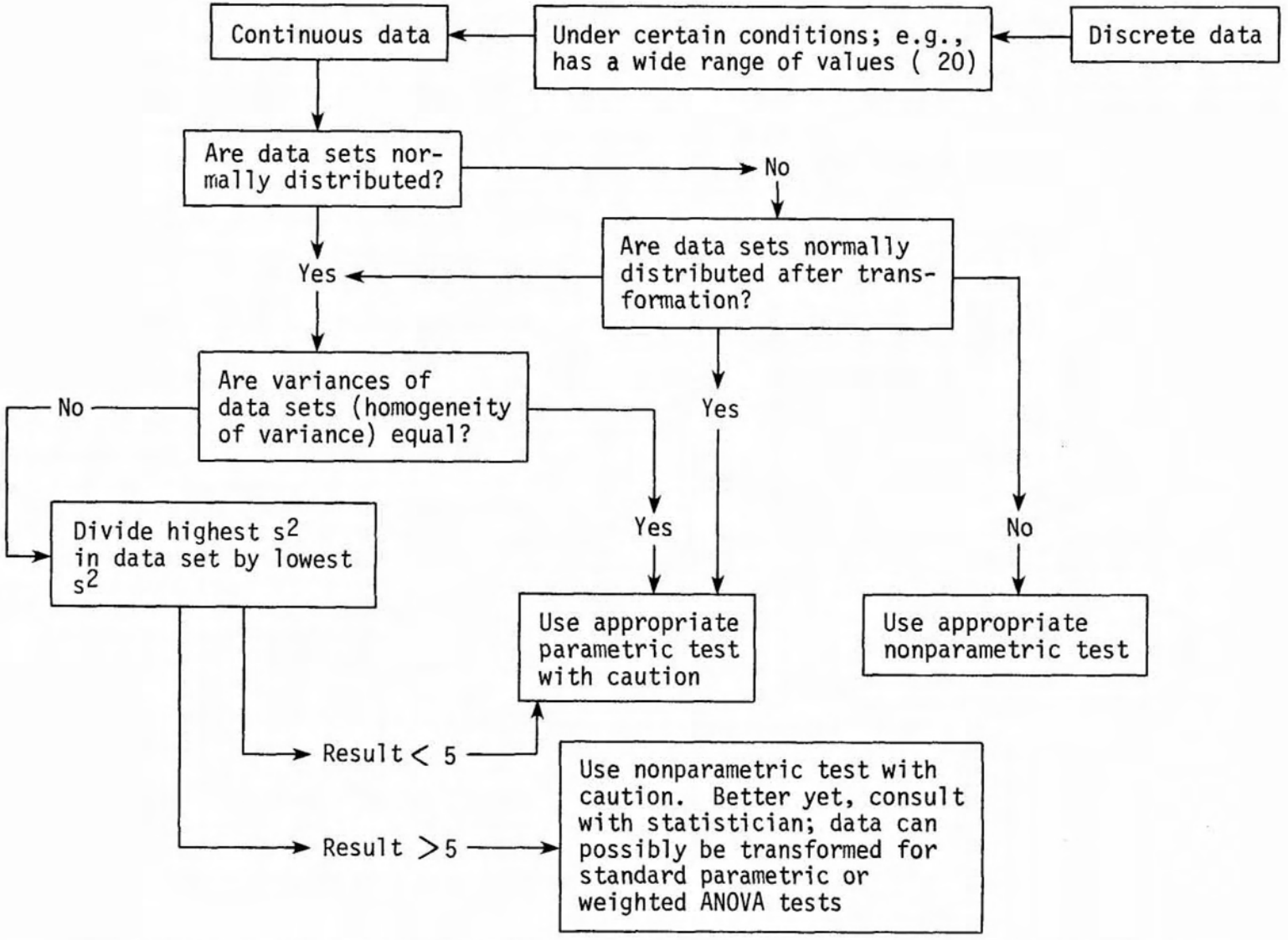


Figure 13. General screening process to choose appropriate statistical tests for comparing single variables, such as means for different data sets.

Table 13. Counterparts for parametric and nonparametric statistical tests.

Parametric	Nonparametric
Two-sample t-test	Mann-Whitney U-test, t'-test
Paired t-test	Wilcoxon Signed-Rank
One-way ANOVA	Kruskall-Wallis
Two-way ANOVA without replicates	Friedman's Test
Two-way ANOVA with replicates	None
(None)	Chi-square contingency table
Regression	(None)

If sample sizes differ among samples, two analysis options are available: (1) decrease the sample size by random elimination of data (results in data loss); or (2) use a weighted analysis of variance. Sometimes data may be missing because samples are lost or were not taken. Sokal and Rohlf (1969) discuss methods for coping with these problems.

STUDY DESIGN

Introduction

No amount of sophisticated statistical analysis can compensate for a poor study design. Conversely, if study design was good and the data were carefully collected, it is always possible to do a good analysis of the results (i.e., an improper, or poor, analysis of the data can be validly replaced by a better analysis). There is a large literature on study design, and yet, designing a

good study remains at least partially an art, based on professional judgement and experience. Some basic principles and guidelines for environmental studies are presented below. However, it is impossible to develop a set formula for designing a study; whereas, it is possible to present specific formulae for data analysis. Because of this difficulty, many books (including this manual) may seem to underemphasize the importance of the design phase of a study.

Designing a good study requires knowledge of statistical design principles, as well as appropriate subject-matter knowledge (e.g., fisheries management, range science, wildlife management, ecology, and related fields). If possible, obtain help from a statistician with the study design before any data collection occurs. For small-scale studies with limited funding, accessing a statistician may be difficult or impossible. Fortunately, when the study involves one simple objective, a short time frame, and measurement of only a few variables, the biologist in charge can often develop a good design without statistical help.

Large scale, long term studies are a different matter, and statistical assistance at the beginning of such studies is recommended. Because there is no after-the-fact remedy for a poorly planned study, it is cost-effective to spend the necessary time and money in planning all phases of the study. It is suggested that at least 5 to 10% of the total study costs be applied to planning. If necessary, statistical help can be contracted. (A good quantitative biologist, especially one that is interested and experienced in field applications, can also be very helpful in designing monitoring studies.) Work closely with the statistician and get them into the field with you. Do not expect immediate answers to design problems. A good study design requires, and is well worth, the effort and expense.

Most books on study design assume a laboratory or agricultural setting, where a high degree of control can be exerted over the system. To a large extent, a high degree of control over relevant variables is not possible in environmental studies. In particular, changes that occur over time periods of months or years (due, for example, to weather) cannot be controlled. Because

of this lack of control, the optimal design for environmental studies differs from that in laboratory and other similar settings, and the analysis of data to test for treatment effects differs from that used in classical analyses of variance. A useful reference on the principles of study design in environmental work is the book by Green (1979) Sampling Design and Statistical Methods for Environmental Biologists. This book begins with the statement (1): "The purpose of this book is to provide biologists with a compact guide to the principles and options for sampling and statistical analysis methods in environmental studies." Ward (1978) is another useful reference in this field.

Considerable evaluation of environmental impact and monitoring methodologies has been done by the U.S. Department of Energy. Their literature is a good source of information on the design and analysis of environmental studies. See, for example, Eberhardt (1976), Thomas (1977), and Eberhardt (1978).

Validity in Study Design

Valid methods are necessary in any monitoring study in order to answer the pertinent question or questions. The question that prompted the study is often general in nature, such as "What are the effects of grazing practices on trout?" In practice, more specific versions of this question need to be formulated in order to provide the basis for the study. For the general question above, there is no reference to a particular time period or to a particular place. The answer should pertain to the entire area for previous years, the year or years of the study and, especially, for future years. If the results apply only to the time period and place of the study, they are of limited use in a monitoring study. However, data cannot be collected for every square foot of ground or from an entire stream. The study must rely on sampling over space; therefore, the answer to the general question requires an extension of the study results (an inference) beyond the spatial-temporal scope of the study. The study design must allow such an inference to be made.

Conclusions (inferences) are valid only if the study design and analysis methodology are valid. Valid methods are those which will, on the average, produce the correct answer as more and more data are collected. Whether or

not the given design and/or analysis methods produce the correct answers is determined by the scientific characteristics of the methods. Much of the construction of study designs and analysis methods falls in the area of statistics. Because of its mathematical and abstract nature, statistics often tend to be confusing. This is unfortunate because statistics need to be used by persons conducting field studies to define valid methods for the design and analysis of inferential studies.

Designing a study involves the allocation of sampling effort over space and time. This allocation is necessary because there is natural variation in biological populations over both space and time. It is the existence of sampling variation that causes the difficulties in design of studies and analysis of the data. Data collected, even by standardized methods, can vary as the result of several factors, including sampling site, year, season, time of day, and impacts on the area sampled. Data can also vary significantly due to the sampling method, plot size, equipment used, the persons taking the sample, and other similar factors. The reality of sampling variation and the need to draw conclusions broader than the specific circumstances of the study motivate most of the principles of valid study design.

Two General Design Principles

Two types of variation in a sampled variable can be recognized: explained and unexplained. Often the source of variation (such as habitat type, elevation, or sampling method) can be identified and the variation in a sampled variable at least partially explained. This type of variable needs to be recognized and incorporated into the study design; e.g., by standardizing the sampling methods and stratifying the sampling by habitat type. Unexplained variation is referred to as sampling variation. For example, replicate samples may vary even when sampling occurs within an apparently uniform habitat, at virtually the same time, using the same sampling methods. This unexplained variation necessitates within treatment replicate sampling. If variability were not a fact of life, there would be little need for statistics or designed studies.

Any deliberate treatment, or management action, is only one possible source of variation in the environment. Studies must be designed so that the effects of the treatment, if any, can be separated, in the statistical analysis, from the effects of all other possible sources of variation affecting the response variable(s). Failure to do so violates the most important principle of valid study design:

1. The study design must allow treatment effects (an "explained" source of variation) to be distinguished from all other sources of variation.

In order to achieve this avoidance of confounding the treatment effect with other sources of variation, all important sources of variation need to be identified and allowed for through design concepts such as fixed plots over time, stratification by habitat type, matched treatment control areas, standardized methodology, and pre- and postimpact sampling.

The second principle of valid study design is:

2. Replicate samples should be taken over space and time.

Replicate sampling must be used to validly judge the significance of differences between "treatment" and "control" conditions because of natural sampling variation over space and time. The determination of how large a sample to take relates, in large part, to how many replicate samples are needed to compensate for this natural within-site sampling variation.

Study Design Guidelines

Green (1979) lists four prerequisites for optimal study design:

1. The impact (management action) must not have occurred yet, so that baseline data can serve as a temporal control.

2. The type of impact and place of occurrence must be known, so that a sampling design appropriate to tests of the hypotheses can be formulated.

3. It must be possible to measure all of the relevant biological and environmental variables for which statistical tests will be conducted.

4. A comparable area that will not be impacted must be available to serve as a control.

Stream monitoring studies should include at least one preimpact (baseline) data set for both the control site(s) and the treatment site(s). The management effect is estimated by comparing the two differences: the difference in the control sites before and after management and the before and after difference in the treatment sites. It is the comparison of these two differences that is the basis for determining the effect of any management action.

Control sites can be either upstream or downstream from the area of the stream where the management action occurs, depending on the type of management and the area of its impact. In some cases, a downstream control area could be considered a "lesser-affected" study site. In other instances, the control sites may need to be in a different, but similar stream. Similarity (at least with respect to the variables of interest) of control and affected sites prior to the impact is essential to the valid interpretation of postimpact sampling. Therefore, control sites should be very carefully selected, including a statistical review of any available historical data and on-site visits to the affected area and potential control sites.

Even when the baseline sample values are very similar for each affected site and its corresponding control site, there is no way to be certain that differences observed between treatment and control sites at postimpact sampling times are due only to management activities because confounding factors may also be affecting the changes.

It is not always possible to include control sites, and appropriate statistical tests for application in this situation are presented in Chapter V. Preimpact sampling is extremely important in the absence of control sites because baseline data becomes the only means to evaluate the effects of management activities.

Green (1979) developed the following criteria for sampling design and selection of statistical methods for data analysis (adapted for management programs):

1. It must be possible to test the null hypothesis that any change in the managed area, over a time period that includes the management action, does not differ significantly from the change in the control area over the same time period.

2. It must be possible to relate a demonstrated change to the management action and to identify any effects resulting from natural environmental variation rather than from the management program.

3. The analysis method must lead to an effective visual display of: (1) change due to management, as opposed to other sources of variation; and (2) the relationship between changes due to management in biological variables and in environmental variables.

4. It must be possible to use the study results to design subsequent monitoring studies in order to detect future impacts of management activities of the same type.

5. The test of the null hypothesis of no change due to management must be as conservative, powerful, and robust as possible.

The basic questions that need to be answered are:

- What do I sample?
- How do I sample?
- When do I sample?
- Where do I sample?
- How many samples do I need?
- Which statistical tests do I use?

What is sampled and how it is sampled depend on the objectives of the study and are discussed in the second and third chapters of this manual. When to sample depends on the natural variation in the variable(s) and on the presence of confounding factors (discussed in a subsequent section). For example, there may be practical limitations to the time when sampling can occur, such as ice cover, fishing pressure, or level of stream flow.

Sample sites are selected on the basis of a variety of criteria. The site to be managed is often chosen because it has a high potential of being managed successfully. If the managed site(s) [and the control site(s)] is not selected at random, the statistical inferences that can be developed from the data are quite restricted. The success of the management program at future sites cannot be inferred when the managed site is deliberately chosen and, therefore, not necessarily representative of other sites subjected to the same management action in the future.

Sampling is discussed by Greeson et al. (1977) and in other available statistical references. The four basic types of sampling are:

1. Simple random sampling;

2. Stratified random sampling;

3. Systematic sampling; and

4. Two-stage sampling (often called double sampling).

Simple random sampling occurs when every potential sampling unit in the population has an equal chance of selection, and each sample unit is representative of the entire population (Elliot 1977). Random sampling is most reliably designed when a random numbers table is used.

Stratified random sampling increases sampling efficiency because the population is divided into several subpopulations or strata (Elliot 1977). These strata should be internally more homogeneous than the population as a whole and should be well defined. Stratified sampling is most useful when the study area contains a variety of different environments; e.g., pools and riffles. The data from the various strata can be analyzed using a one-way analysis of variance (see Chapter V).

Systematic sampling occurs when the first sample site is selected at random, and the other sample sites are spaced at some fixed interval; e.g., every 10 m. Although this technique is easy, Elliot (1977) gives two disadvantages of systematic sampling: (1) the sample may be very biased when the interval between units in the sample coincides with a periodic variation in the population; and (2) there is no valid way to estimate the standard error of the sample mean.

Two-stage sampling is useful when there is a variable that is very difficult or expensive to measure precisely, but there exists an imprecise, quick nondestructive way to measure that variable. The quick method is applied to a large sample of sites and then a more precise method applied to a subset of these sites (second stage sample). Based on the second stage sample, the

imprecise measurement method is calibrated by a ratio or regression method. This method has been used to estimate biomass in terrestrial applications. The expensive, precise method is vegetation clipping; ocular estimation is the quick, imprecise method (see, e.g., Ahmed et al. 1983). A potential application area in stream sampling is the estimation of macroinvertebrate abundance and relative abundance by taxonomic groups, where the weight of samples can be calibrated to the total sample count.

Agricultural and laboratory studies can often start, in essence, from time "zero" (e.g., plowed fields in agriculture). However, this is not the case in environmental studies; where control and treatment plots may differ from each other prior to the treatment (i.e., management activities). Because of this potential difference, optimal study design includes both control and treatment plots, which are sampled both before and after treatment. There should be sampling replicates for these plots; e.g., over habitat types on a given stream, over different streams, or both. Optimal study design goes a step further and "pairs" the control and treatment plots, then replicates these pairs (study designs are illustrated in Chapter V, along with actual analyses).

For example, the effect of grazing in a specific area could be evaluated by randomly selecting a sample of 20 streams in that area. Possible control-treatment sample site pairs are identified on each stream. Then one pair of sites is randomly selected on each stream, and one member of each pair is randomly selected as the treatment plot. Grazing is assumed to have occurred on all plots, hence the "treatment" is the elimination of grazing by fencing (see, e.g., Keller and Burnham 1982). The primary plots should be large, up to 0.5 linear mile or more of stream plus the adjacent habitat. Subsampling is required to measure the response variables on each plot. This combination of primary and secondary levels of sampling is common in environmental work (see, e.g., Eberhardt 1978). The within primary-plot sampling should be based on fixed sampling locations (fixed subplots or transects); these fixed locations are sampled over time.

Selection of sampling sites within larger plots is also subject to the principles of good study design, and random selection of sampling sites is still necessary. If the main plots are large, they can be stratified by habitat type before sample sites are selected. When possible, the response variable(s) should be measured over the entire main plot; subsampling is only done as a matter of necessity.

Interpreting Sampling Variation

There are two components of sampling variation when main plots and subplots are used. The most important variation is between main plots, and this source of variation is the basis of tests of treatment effects. Within-plot sampling effort is sufficient if the response variable(s) in each main plot is precisely measured (see White et al. 1982, Chapter 2, for additional discussion of the concept of levels of sampling variation).

The variance computed for estimates of N from within-plot sampling only estimates the precision of $\hat{N}$ at a given sample plot. This within-plot sampling variance has nothing to do with the natural variation among different main plots or different periods of time. Within-plot sampling variances, therefore, are inappropriate for most statistical tests in monitoring studies.

The most important source of variation is between plots. For example, consider a situation where there are two streams, one a managed stream and one a control stream. Fish numbers will be the response variable and electrofishing will be the within-plot sampling method. To test the hypothesis that fish abundance differs between specified reaches in the two streams, replicate sampling plots are selected at random from the stretches. For this example, sample plots are set at 100 m long, with five plots on each stream. The true population (N) of fish in each of the five plots in the control and the managed stream after management are as follows:

Site	Control stream N	Managed stream N
1	90	175
2	155	211
3	110	160
4	120	190
5	165	258

The correct test to use in this case is an unpaired t-test with 8 df. The t-value is 3.21, which is significant at the $\alpha = 0.01$ level, meaning that the null hypothesis of no difference in fish abundance for the two streams can be rejected at the 99% confidence level. (The reader is encouraged to compute this test as an exercise.) The variation between plots within a stream is natural variation; this between-plots variation is the basis for determining differences between streams.

Within-plot sampling is necessary in order to estimate the unknown fish abundance in each plot. As a result, there is uncertainty associated with the subsequent estimates of fish abundance at each plot. Assume that electrofishing is done and that good point estimates of N are produced and standard errors of $\hat{N}$ are calculated:

Site	Control stream N	Control stream $\hat{N}[se(\hat{N})]$	Managed stream N	Managed stream $\hat{N}[se(\hat{N})]$
1	90	87(1.5)	175	168(6.2)
2	155	160(4.0)	211	222(8.1)
3	110	108(2.2)	160	158(4.0)
4	120	126(4.8)	190	197(5.3)
5	165	155(7.0)	258	245(11.7)

The difference between the true N and the estimate $\hat{N}$ within each plot is due to within-plot sampling variation; it is the average value of this squared difference that is estimated by the formula for var($\hat{N}$). For the above example, based on the values of $\hat{N}$, t = 3.45. There are still 8 df, and there is still a significant difference at the $\alpha = 0.01$ level.

The reason for computing $se(\hat{N}) = \sqrt{var(N)}$ is to determine the reliability of the individual estimates. When there are small standard errors, the estimates are reliable, and the t-test comparing fish abundance for the control vs. the managed stream, based on the values of $\hat{N}$, can be computed with confidence that the results are essentially the same as if the true N were known; i.e., the electrofishing part of the study has been successful. (The values of $se(\hat{N})$ play no role in computing that t-test.)

For larger values of $se(\hat{N})$, the t-test is less reliable. If the estimates are very inaccurate, it may be impossible to tell if there is a difference in control and managed streams. For example, suppose that the point estimates and standard errors for each plot are:

	Control stream		Managed stream	
Site	N	$\hat{N}[se(\hat{N})]$	N	$\hat{N}[se(\hat{N})]$
1	90	40(23.1)	175	250(107.9)
2	155	230(70.5)	211	130(61.1)
3	110	180(57.0)	160	80(37.1)
4	120	60(28.7)	190	201(74.0)
5	165	185(68.8)	258	150(43.4)

By looking at the sampling standard errors of $\hat{N}$, it is obvious that the study has failed because these values are too large. The estimates of N are, therefore, too inaccurate to reliably detect any difference between streams. The computed t-test from the above values of $\hat{N}$ is 0.49 (8 df). The result is

not significant, but it would be erroneous to conclude that the populations of the two streams are not different, meaning that management had no effect, because the within-plot estimates of N are poor.

The important two points here are that replicate main plots are generally needed in both control and managed areas to test for impacts and any subsampling of main plots must produce reasonably precise results for each main plot. It is not valid to select one plot in each stream and base the test on the within-plot sampling variance of N. For example, if control plot 5 (true N = 165; $\hat{N}$ = 155) and managed plot 3 (true N = 160; $\hat{N}$ = 158) in the first case above were selected as the only study plots, an apparent test statistic would be:

$$\frac{158 - 155}{\sqrt{4.0^2 + 7.0^2}} = \frac{3}{8.06} = 0.372$$

This would approximate a standard normal variable (a t-test with many degrees of freedom), and the results would not be significant. The test is also invalid because the standard error of the difference in the estimates is based, incorrectly, on within-plot variances (= $4.0^2 + 7.0^2$).

Sample Size Guidelines

Sample size (i.e., sampling effort) needs to be considered at both the main plot and within-plot levels. Unfortunately, standard formulae to determine sample size are often not useful in environmental studies, especially when the main plots are large. When plots are very large, it is difficult to sample enough plots, and the rule of thumb becomes to sample as many as possible. There is a trade-off between the number of main plots and the amount of within-plot sampling that is done, unless the study is such that the response variables can be measured directly for the entire main plot. It is generally better to have more main plots at the expense of less within-plot sampling, at least up to the limit of getting reliable within-plot estimates.

In order to make an inference about some management action for a large area, there should be at least 10 pairs of control-treatment main plots and at least two within-plot sampling sites in each main plot. No inference to a larger area is possible with only one control-treatment pair, even if the pair is randomly selected. No amount of within-plot sampling can compensate for having too few main plots.

Eberhardt (1978) and Green (1979) provide useful guidelines on sample size. The following formula (modified after Calhoun 1966) is sometimes useful; e.g., in determining the sample size needed to estimate the average macroinvertebrate density in a stream section:

$$n = 4\left(\frac{\sigma}{\delta\mu}\right)^2 = 4\left(\frac{cv}{\delta}\right)^2$$

n = the desired sample size to achieve a 95% confidence interval on the true mean μ with a relative confidence interval width of 2δ. The unknown average value of the response variable is μ; the sample-to-sample standard deviation is σ. The ratio $\sigma/\mu = cv$ is the per sample coefficient of variation, which must be known or estimated (e.g., from a pilot study or from existing data). It is often possible, for planning purposes, to let $cv = 1.0$ (100%). With this value, $n = 4/\delta^2$. Thus, to estimate μ with "good" precision, i.e., to obtain a 95% confidence interval with a relative half-width of $\delta = 0.1$, may sometimes require a sample size of:

$$n = 4/(0.1)^2 = 400$$

If $\delta = 0.25$, $n = 4/(0.25)^2 = 64$, which is still very large. Useful values of δ are ≤ 0.25, with $\delta = 0.1$ representing good precision.

The above example illustrates the fact that when optimal target sample sizes are computed, the result often is larger samples than can be taken because of study constraints. Consequently, a common approach is to determine the sample size that can be taken, given time, personnel, and budget resources, and then find out what level of precision can be obtained with this level of sampling. The level of precision that can be obtained will determine whether or not the study can be expected to detect a treatment effect of practical significance. Procedures for determining expected precision given a level of sampling effort are beyond the scope of this document, and statistical assistance may be needed to answer such questions.

There is a complex interplay between sample size and study design. The role of study design is two fold: (1) to produce valid results; and (2) to reduce the level of sampling effort needed through practices such as control-treatment pairing, stratification, use of prior information, before/after measurements, fixed plots, two-stage sampling, and other techniques. Consequently, the question of sample size can only be answered with respect to a given study design.

CONFOUNDING FACTORS

Confounding factors are factors that, if not adequately considered, confuse conclusions regarding the success of a management program. Many confounding factors that may be encountered in a monitoring study are listed below under five basic categories: institutional; equipment; personnel; biological; and statistical.

Institutional Factors

1. There must be a commitment (and, if possible, a guarantee) that the study will be continued until it is finished.

2. Commitments of time, personnel, and money should be enough for the entire study.

3. Communication lines should be kept open between the people responsible for the study and land use managers. If unplanned activities begin at the study site that may interfere with the success of the study (e.g., construction activity), the involved personnel need to be notified and attempts made to halt or modify the activity until the study is completed. There also needs to be continued communication and cooperation with State agencies that have species management responsibilites in the area.

4. Management programs should not be changed during the study.

5. Institutional constraints that may restrict sampling to certain times should be considered when the study is designed.

Equipment

1. Biases in the results due to the sampling procedure used need to be considered so that they do not have an undue affect on the study conclusions. Fish sampling results, in particular, can be differentially biased by the choice of sampling gear.

2. The effect of different water conditions (e.g., turbidity, hardness, and discharge) on the precision and efficiency of the equipment used in the study needs to be understood and accounted for in study results.

3. Equipment should be calibrated, as appropriate and needed.

4. Methods should remain the same throughout the study because results are generally not comparable between methods.

5. Values obtained may be affected if equipment is replaced or modified during the study. For example, the efficiency of electrofishing units may vary with time as the battery loses its charge or if one brand of equipment is replaced with another brand.

Personnel Factors

1. Trial runs should be conducted before study sampling begins to familiarize personnel with equipment and to standardize methods.

2. The number of persons available must meet the requirements for the method chosen. The same number of people should be available each time a method is used that is affected by the number of participants (e.g., electrofishing).

3. The amount of previous training and experience may vary among personnel and can affect the precision of sampling. If differences in sampling efficiency are suspected, personnel should be rotated systematically among sites in order to avoid confounding differences resulting from personnel involved in the sampling with treatment effects.

4. Personnel changes during the study may introduce error if sampling precision or bias varies among the persons involved in the sampling.

5. Sampling by personnel may vary over time; e.g., they may become more efficient with added experience or be affected by certain times of the day or year.

Biological Factors

1. Biological variables may not be independent of one another.

2. Fishing pressure affects fish population estimates and size distribution and, therefore, should be considered when selecting sampling times.

3. There is considerable natural variation in population numbers in both time and space that can mask management effects (see Hall and Knight 1981).

4. Biological populations may not respond immediately to changes in their environment; i.e., there may be a lag time between the management action and the population response. Studies may have to extend for a number of years after treatment initiation in order to accurately determine responses.

5. Biological populations may adapt or acclimate to conditions and, therefore, not change. However, this phenomenon is rare.

6. Biological populations often have response thresholds, rather than reacting linearly.

7. Factors other than those being monitored may affect populations, and population changes may occur for reasons that are unconnected with the management program.

8. Habitat changes unrelated to management actions may result in a reallocation of fish in the study area, thereby increasing the difference in population numbers between the control and managed areas. In this case, there are the same number of fish but in different places.

Statistical Factors

1. If the assumptions for the parametric tests used are only approximated rather than fully meet, these assumption violations may have serious affects on the study results.

2. Controls in time and space are necessary for valid comparisons; however, they are far from foolproof (Eberhardt 1978).

3. The time of sampling can bias results when changes in the values of the variable being monitored are related to time of day or year.

4. When an insufficient sample size is used, a significant difference may exist but not be apparent. Conclusions drawn from an analysis with an insufficient sample size may, therefore, be invalid. Green (1979:40) advises "If it was not possible to conduct preliminary sampling and a number must be pulled out of a hat, three replicates per treatment combination is a good round number. [However], it is the overall error degrees of freedom that are important."

5. Lack of enough replication makes estimation of natural variability impossible. Replicate samples should be taken (Green 1979:27) "... within each combination of time, location and any other controlled variable. Differences among can only be demonstrated by comparisons within".

6. Considerable error can be introduced when the assumptions of population estimates are not met completely.

7. Unforeseen events (e.g., a 100-year flood) can affect the study site(s) to the extent that comparisons of differences are invalid.

8. A statistically significant relationship is not always proof of causality because many variables are interrelated (Green 1979).

9. Rounding of numbers with several decimal places can cause considerable variation in calculations. It is advisable to retain four digits to the right of the decimal point for computational steps. An example of the error that can result from rounding is demonstrated in the following example of computing a variance estimate:

$$s^2 = \frac{\Sigma(X)^2 - n(\bar{X})^2}{n-1}$$

If $n = 20$, $\Sigma(X)^2 = 478.0499$, and $\bar{X} = 4.8555$, then $s^2 = 0.3438$. But if $\bar{X}$ is rounded to 4.9 and $\Sigma(X)^2$ is rounded to 478.0, the result is $s^2 = -0.1158$, which is impossible for a variance. This illustrates that, in general, if intermediate quantities in a series of calculations are rounded off, the end result of a calculation can be seriously in error.

10. Tabular values can be selected or recorded incorrectly, which can result in incorrect calculations or conclusions.

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CHAPTER V. STATISTICAL TESTS FOR EVALUATING RESPONSES TO MANAGEMENT ACTIVITIES

The following stepwise examples are for the statistical procedures mentioned in Chapter IV. For demonstration purposes, the assumptions necessary for parametric tests are tested for one example. The necessary assumptions are given for the remaining examples. A statistics text by Sokal and Rohlf (1969) and their statistical tables (Rohlf and Sokal 1969) are the primary reference sources for the tests.

DETERMINATION OF THE DATA DISTRIBUTION PATTERN

The following total lengths (mm) of 64 adult trout are used to determine the data distribution pattern:

162	166	148	110	109	164	148	162
219	175	87	135	121	114	115	150
94	140	199	215	150	160	142	202
214	95	282	123	146	313	264	208
127	114	161	81	163	115	155	199
172	175	97	136	173	174	113	138
111	207	136	125	160	79	171	122
93	195	121	122	102	138	110	161

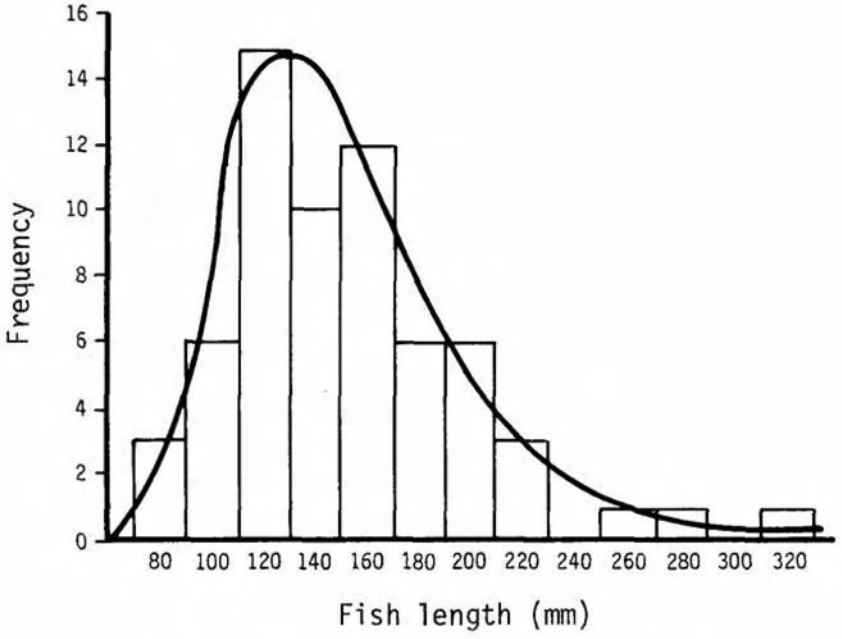
Step 1

Prepare a frequency distribution table.

Fish length (mm)	No. of observations	% of total	Cumulative % of total
70 - 89	3	4.7	4.7
90 - 109	6	9.4	14.1
110 - 129	15	23.4	37.5
130 - 149	10	15.5	53.0
150 - 169	12	18.7	71.7
170 - 189	6	9.4	81.1
190 - 209	6	9.4	90.5
210 - 229	3	4.7	95.2
230 - 249	0	0.0	95.2
250 - 269	1	1.6	96.8
270 - 289	1	1.6	98.4
290 - 309	0	0.0	98.4
310 - 329	1	1.6	100.0
	64		

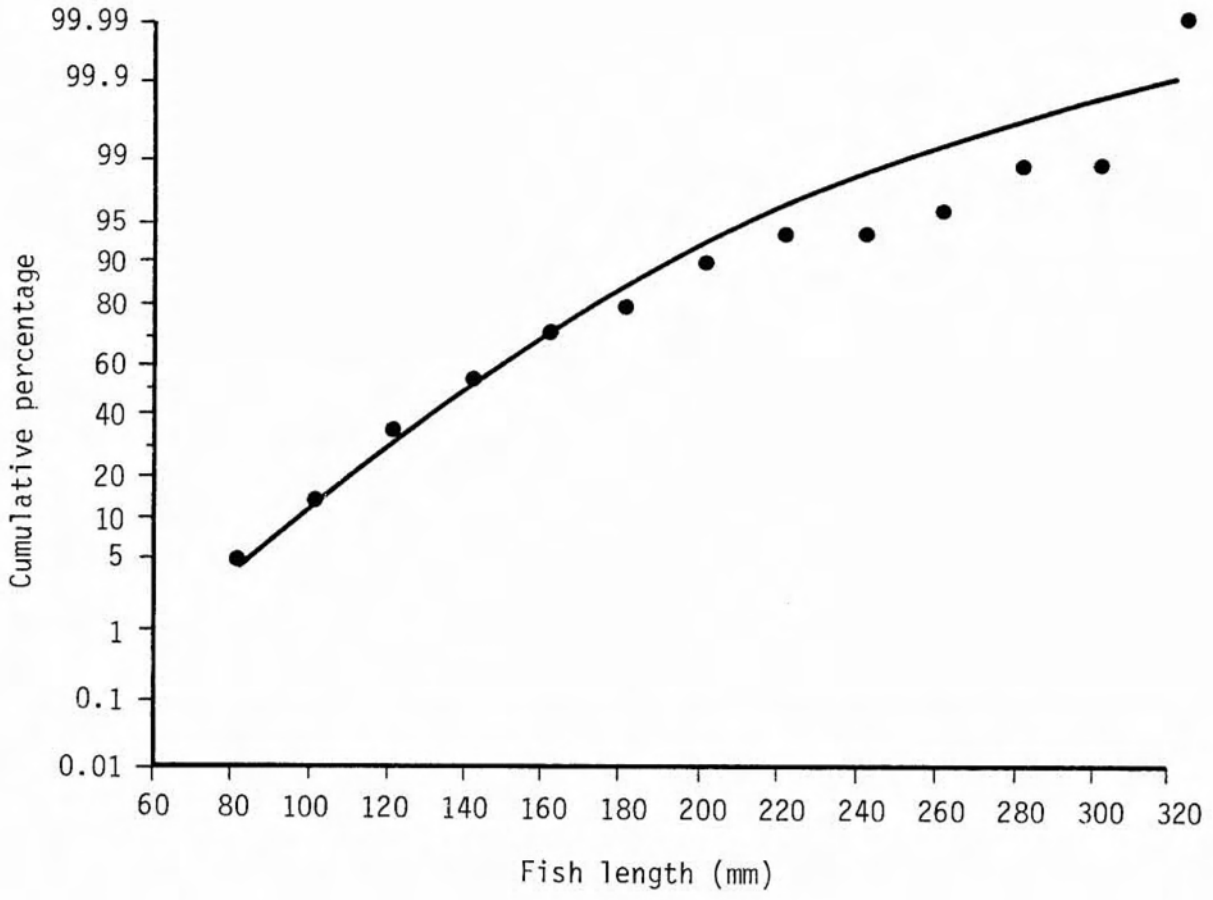
Step 2

Plot the data in a histogram, and draw a curve to approximate the distribution pattern.



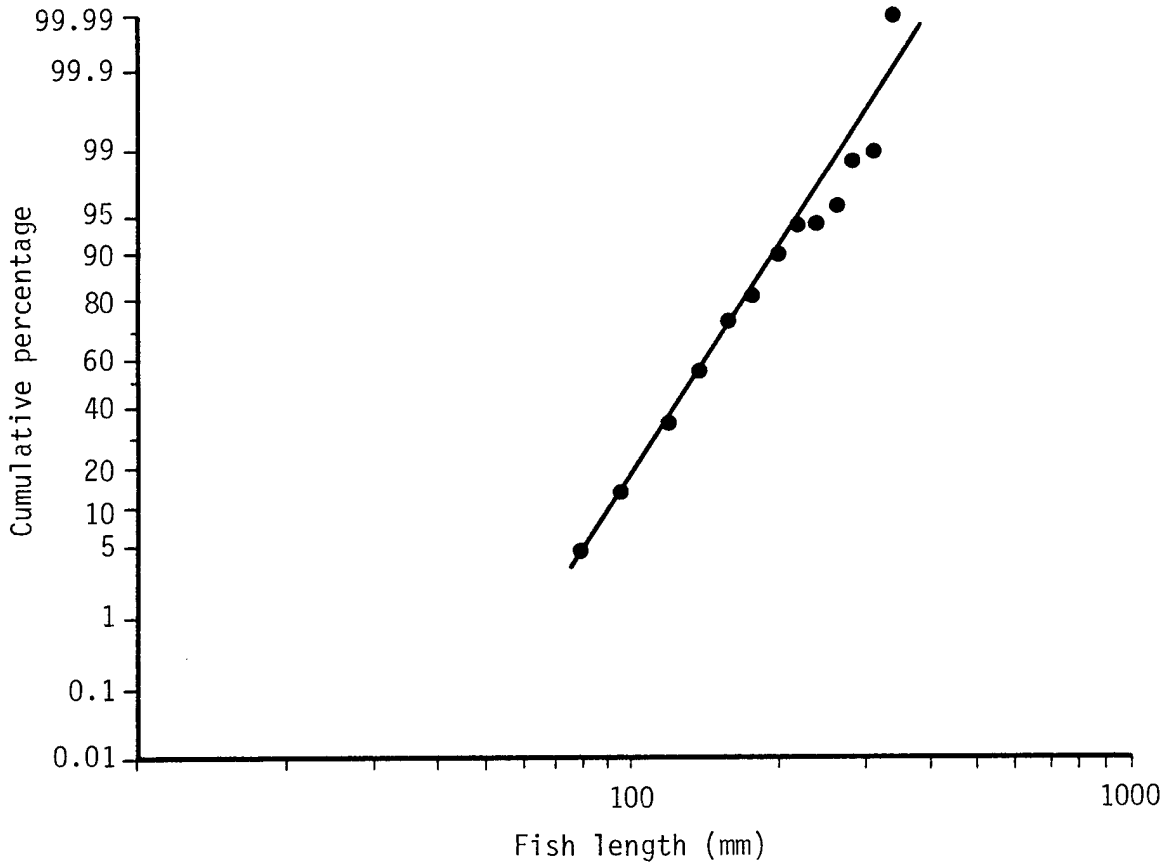
Step 3

Plot the data on normal probability paper and fit a line to the data points by visual observation. Data points are midpoints for each fish length class.



Step 4

The pattern appears to be lognormal. To confirm this assumption, plot the data points on lognormal probability paper and visually fit a curve to the points.



A straight line pattern of the data points strongly supports lognormality.

Step 5

Transform the data by logarithms. If parametric tests will be used, distributions other than lognormal can often be normalized by the appropriate transformations (Sokal and Rohlf 1969). Nonparametric tests should be used when normalization is unsuccessful.

TEST FOR HOMOGENEITY OF VARIANCE

The following water depth data will be used to test for homogeneity of variance:

	Site 1		Site 2		Site 3	
	X_i	X_i^2	X_i	X_i^2	X_i	X_i^2
	1.5	2.25	3.5	12.25	4.1	16.81
	3.0	9.00	4.6	21.16	3.6	12.96
	4.5	20.25	5.2	27.04	1.5	3.25
	6.0	36.00	3.2	10.24	3.2	10.24
	1.6	2.56	4.1	16.81	1.7	2.89
	5.0	25.00	2.0	4.00	6.2	38.44
	3.2	10.24	1.6	2.56	2.8	7.84
	4.5	20.25	5.0	25.00	1.9	3.61
	2.3	5.29	2.3	5.29	3.1	9.61
	4.1	16.81	2.5	6.25	2.7	7.29
ΣX_i =	35.7		34.0		30.8	
$\bar{X}$ =	3.57		3.40		3.08	
ΣX_i^2 =		147.65		130.6		111.94

$$s_1^2 = \frac{147.65 - \frac{(35.7)^2}{10}}{9} = 2.24$$

$$s_2^2 = \frac{130.6 - \frac{(34)^2}{10}}{9} = 1.67$$

$$s_3^2 = \frac{111.94 - \frac{(30.8)^2}{10}}{9} = 1.90$$

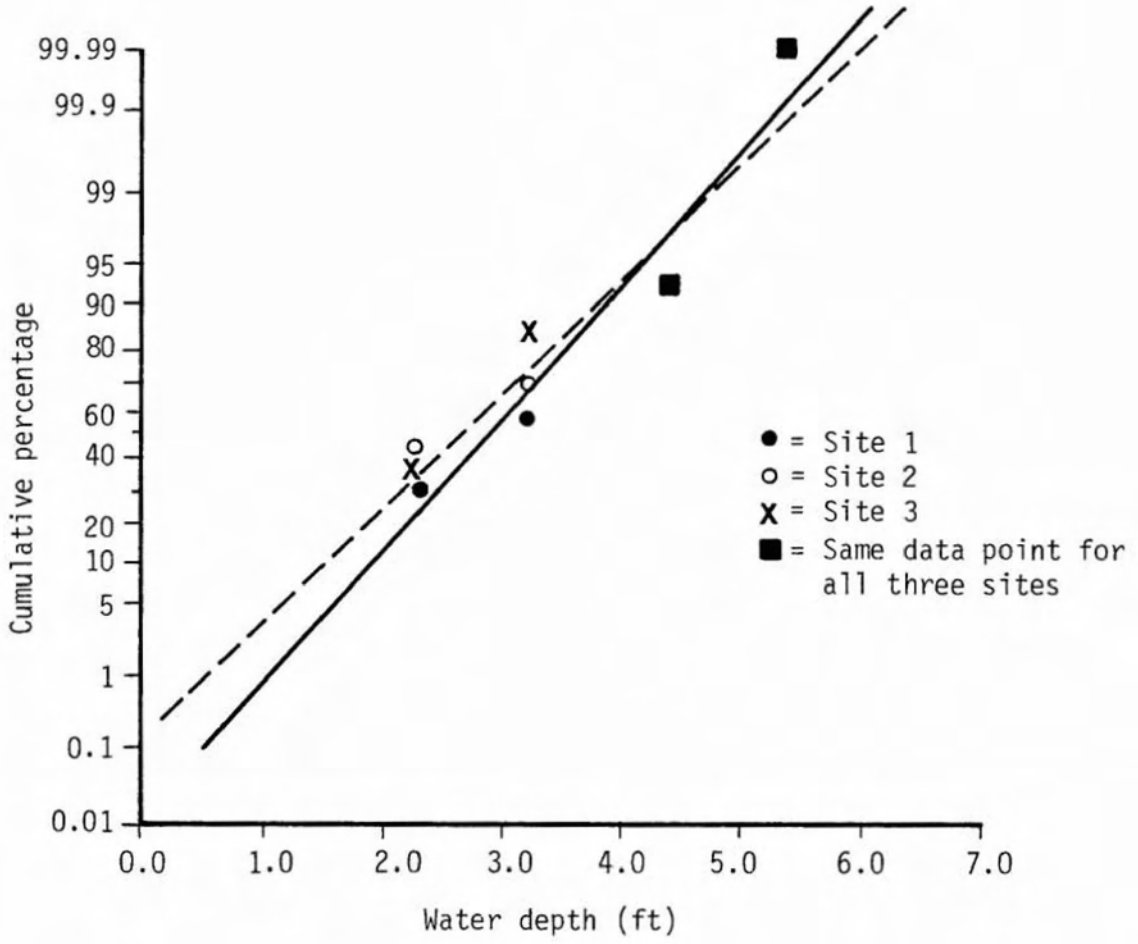
Step 1

Determine the frequency distributions. The frequency distribution of the data for Site 1 is:

	Site 1			
Class	Frequency	% Frequency	Percent cumulative frequency	Class midpoint
1.5-2.6	3	30.00	30.00	2.02
2.7-3.8	2	20.00	50.00	3.22
3.9-5.0	4	40.00	90.00	4.42
5.1-6.2	1	10.00	100.00	5.62
	10	100.00		

Step 2

Graph data on normal probability paper (Sokal and Rohlf 1969).



The slopes of the three lines are very similar, indicating that the variances are probably homogeneous. Additional tests can be used for confirmation. The lines for sites 2 and 3 overlap too much to distinguish them.

Step 3a

The F-test is used to test for homogeneity of variance when there are only two data sets:

H_o: $\sigma_1^2 = \sigma_2^2$

H_a: $\sigma_1^2 \neq \sigma_2^2$

Select a level of confidence; e.g., $\alpha = 0.05$.

Calculate the F-value = F_s:

$$F_s = \frac{s_1^2}{s_2^2} = \frac{2.24}{1.67} = 1.3413$$

Look up the F-value for $F_{\alpha,(n-1),(n-1)}$ in the appropriate statistical table where n = number of observations in each sample (10 in this example).

The calculated F value of 1.3413 is less than the table F-value of 3.18. Therefore, the null hypothesis cannot be rejected, and the conclusion (with a 95% confidence level) is that the variances are equal (homogeneity of variance).

Step 3b

Bartlett's test (Sokal and Rohlf 1969) is used to test for homogeneity of variance when there are more than two data sets:

Sample	df = n-1	s^2	$\log(s^2)$
1	9	2.24	0.35024
2	9	1.67	0.22271
3	9	1.90	0.27875

Compute the weighted average variance:[10]

$$s^2 = \frac{\text{sum of [(variance values) times (their respective degrees of freedom)]}}{\text{sum of df}}$$

$$= \frac{(2.24)\ (9) + (1.67)\ (9) + (1.90)\ (9)}{27} = \frac{20.16 + 15.03 + 17.10}{27}$$

$$= \frac{52.29}{27} = 1.9367$$

Find the logarithm of 1.9367, which is 0.28706.

Sum the logs of each variance multiplied by its respective degrees of freedom:

$$= (0.35024)\ (9) + (0.22271)\ (9) + (0.2875)\ (9)$$

$$= 3.1522 + 2.0044 + 2.5875$$

$$= 7.7441$$

Compute x^2 = 2.3026 (sum of the degrees of freedom multiplied by the log of the weighted average variance) - (sum of the logs of each variance multiplied by its respective degrees of freedom):

$$= (2.3026)\ [(27)\ (0.28706) - 7.7441]$$

$$= 2.3026\ [7.75062 - 7.7441]$$

$$= (2.3026)\ (0.00652) = 0.015$$

Compute correction factor C:

$$= 1 + \frac{1}{3(a-1)} \left[\text{sum of reciprocal of individual df} - \frac{1}{\text{sum of df}}\right]$$

a = number of sample sets (a = 3 in this example)

$$= 1 + \frac{1}{3(2)}\left[\left(\frac{1}{9} + \frac{1}{9} + \frac{1}{9}\right) - \frac{1}{27}\right]$$

$$= 1 + (0.1667)\ (0.3333 - 0.037)$$

$$= 1 + (0.1667)\ (0.2963) = 1.0494$$

---

[10]If any of the s^2 values are less than 1, all of the s^2 values are multiplied by the same multiple of 10 so that there is at least one number to the left of the decimal in each s^2 value. For example, if the smallest s^2 value is 0.224, all s^2 values would be multiplied by 10. This multiplication is necessary to prevent negative logs.

Compute the adjusted x^2:

$$= \frac{x^2}{C} = \frac{0.015}{1.0494} = 0.014$$

H_o: $\sigma_1^2 = \sigma_2^2 = \sigma_3^2$

H_a: $\sigma_1^2 \neq \sigma_2^2 \neq \sigma_3^2$

a-1 = 2 df

Because $x^2_{.05,(2)} = 5.991$ and the adjusted test statistic x^2 of 0.014 is lower, the null hypothesis is not rejected and we are reasonably safe to assume that the variances are equal.

Step 4

F_{max} - test (Sokal and Rohlf 1969)

When Bartlett's test indicates that there is no homogeneity of variance, the F_{max} - test can be used to determine if parametric methods are still acceptable; e.g.:

Compute the $\frac{s^2 \text{ maximum}}{s^2 \text{ minimum}}$ ratio:

$$= \frac{2.24}{1.67} = 1.34$$

Select the tabulated F_{max} statistic:

$F_{max\ \alpha,(a),(n-1)} = F_{max0.05,3,9} = 5.34$

where $\alpha = 0.05$

a = number of data sets = 3

n = samples per set = 10

The calculated value does not exceed the tabular value, hence the null hypothesis of equal variance is not rejected; therefore, the assumption can be made that the variances are equal because the computed value (1.34) is less than the tabulated F_{max} statistic (5.34) at the 5% level.

When homogeneity of variance is lacking, parametric tests can still be used with caution if the calculated F_{max} value is less than or equal to 5. If a parametric test cannot be used on the data as is, an appropriate nonparametric test can be selected or attempts made to transform the data so that a parametric test can be used (see Sokal and Rohlf 1969).

STATISTICAL TESTS FOR COMPARING DIFFERENCES BETWEEN DATA SETS

Two-sample t-test

Problem: In an area where grazing occurred, the temperature of a small stream was determined by sampling with a hand-held thermometer to determine the effects of grazing on stream temperature. Temperature measurements were taken at site 1 on the stream within an area where grazing was restricted and at site 2 on the stream where grazing was not restricted. The two-sample t-test is used to test for differences when the samples are independent, the data are assumed to be normally distributed, and the variances are assumed to be homogeneous.

	Site 1		Site 2	
	X_i	X_i^2	X_i	X_i^2
	10.5	110.25	11.0	121.00
	10.3	106.09	11.2	125.44
	10.7	114.49	10.9	118.81
	10.9	118.81	10.8	116.64
	10.7	114.49	11.1	123.21
$\Sigma X_i^2 =$	53.1		55.0	
$\bar{X} =$	10.62		11.0	
$\Sigma X_i^2 =$		564.13		605.10

$$s_1^2 = \frac{\Sigma X_i^2 - (\Sigma X_i)^2/n}{n-1}$$

$$= \frac{564.13 - 563.92}{4}$$

$$= \frac{0.208}{4} = 0.052$$

$$s_2^2 = \frac{605.1 - 605}{4}$$

$$= \frac{0.100}{4} = 0.025$$

Solution:

1. $H_o: \mu_1 = \mu_2$

   $H_a: \mu_1 \neq \mu_2$

2. Select α; e.g., $\alpha = 0.05$.

3. Calculate the standard error (se) of the difference in the means, $\bar{X}_1 - \bar{X}_2$

$$= \sqrt{\left(\frac{n_1 + n_2}{n_1 n_2}\right)\left(\frac{(n_1-1)s_1^2 + (n_2-1)\, s_2^2}{n_1 + n_2 - 2}\right)}$$

$$= \sqrt{\left(\frac{5 + 5}{25}\right)\left(\frac{(4)\ (0.0520) + (4)\ (0.025)}{8}\right)}$$

$$= \sqrt{0.4\left(\frac{0.208 + 0.100}{8}\right)}$$

$$= \sqrt{(0.4)\ (0.0385)} = \sqrt{0.0154} = 0.124$$

4. Calculate $t = \frac{\bar{X}_1 - \bar{X}_2}{se} = \frac{(10.62 - 11.0)}{.124} = \frac{-0.38}{0.124} = -3.06$

5. Look up the tabular t value for $n_1 + n_2 - 2 = 5 + 5 - 2 = 8$ df:

   $t_{0.05,(n_1 + n_2 - 2)} = 2.306.$

6. The null hypothesis is rejected because the test statistic t = -3.06, which is less than the tabular critical value of t = -2.306 (for a two-tailed test, the tabular value is ±). The conclusion, with a 95% confidence level, is that the stream temperatures are significantly different at the site where grazing was restricted compared to the site where grazing was not restricted.

7. Assume that the management objective was to lower the stream temperature by 2° C at the restricted grazing site and that temperatures over the past several seasons (without any restricted grazing) averaged 11.5° C. μ becomes 11.5° C - 2° C = 9.5° C, and a one-tailed t-test can be used to test H_o: $\mu_o = 9.5$ versus H_a: $\mu \geq 9.5$.

$$t = \frac{\bar{X} - \mu_o}{s_1} = \frac{10.62 - 9.5}{0.0520} = \frac{1.12}{0.0520} = 21.54$$

$t_{0.05,n_1-1} = 2.132$ (Rohlf and Sokal 1979:Table Q).

The calculated t of 21.54 is greater than the tabular value of t = 2.132. Therefore, the null hypothesis is rejected with 95% confidence that stream temperatures in the area with restricted grazing were not lowered by 2° C. Note that the α level in Table Q is divided by 2 for a one-tailed test; e.g., if $\alpha = 0.05$ in a one-tailed test, select a value in the column $\frac{0.1}{2} = 0.05$.

The t'-test (Sokal and Rohlf 1969)

Problem: Stream temperatures (°C) were taken (15 readings) at a stream site before a management program was initiated to increase bank cover. Temperature readings (10 readings at the same time of the year) were also taken after the management program was initiated. The data are:

Before management		After management	
X_i	X_i^2	X_i	X_i^2
15.0	225.00	10.0	100.00
15.0	225.00	10.5	110.25
14.5	210.25	9.5	90.25
14.0	196.00	10.0	100.00
14.5	210.25	14.5	210.25
13.5	182.25	13.0	169.00
15.0	225.00	14.0	196.00
14.5	210.25	12.5	156.25
15.0	225.00	10.5	110.25
13.5	182.25	14.5	210.25
14.5	210.25	$\Sigma X_i = 119$	$\Sigma X_i^2 = 1452.5$
15.0	225.00		
14.5	210.25	$\bar{X}_2 = 11.9$	
14.0	196.00		
14.0	196.00		
$\Sigma X_i = 216.5$	$\Sigma X_i^2 = 3,128.75$		

$\bar{X}_1 = 14.43$

Solution:

1. H_o: Temperatures were the same before and after the management action or $\mu_1 = \mu_2$ versus H_a: $\mu_1 \neq \mu_2$.

2. The level of significance chosen is $\alpha = 0.01$.

3. The assumptions for a parametric test are not all met. In particular, the sample sizes and the variances are not equal. Therefore, the t'-statistic is used to test for differences:

$$s_1^2 = \frac{\Sigma(X_i)^2 - (\Sigma X_i)^2/n}{n-1}$$

$$= \frac{3{,}128.75 - \frac{(216.5)^2}{15}}{19} \qquad s_2^2 = \frac{1452.5 - \frac{(119)^2}{10}}{9}$$

$$= \frac{3{,}128.75 - \frac{46{,}872.25}{15}}{14} \qquad = \frac{1{,}452.5 - \frac{14161}{10}}{9}$$

$$= \frac{3{,}128.75 - 3{,}124.817}{14} \qquad = \frac{1{,}452.5 - 1{,}416.1}{9}$$

$$= \frac{3.933}{14} = 0.2810 \qquad = \frac{36.4}{9} = 4.0444$$

4. Compute the critical level, $t_\alpha' = \dfrac{\frac{s_1^2}{n_1}(t_{1,\alpha}) + \frac{s_2^2}{n_2}(t_{2,\alpha})}{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}$

where $t_{1,\alpha}$ has n_1-1 df = 14 df and $t_{2,\alpha}$ has n_2-1 df = 9 df.

$t_{1,\alpha} = t_{0.01,14} = 2.977$

$t_{2,\alpha} = t_{0.01,9} = 3.250$

$$= \frac{\frac{0.2810}{15}(2.977) + \frac{4.0444}{10}(3.250)}{\frac{0.2810}{15} + \frac{4.0444}{10}}$$

$$= \frac{\frac{0.8365}{15} + \frac{13.1443}{10}}{0.0187 + 0.4044} = \frac{0.0558 + 1.3144}{0.4231}$$

$$= \frac{1.3702}{0.4231} = 3.238$$

5. Calculate the t'-statistic:

$$t' = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{s_1^2}{n_1} + \frac{s_2^2}{n_2}}} = \frac{14.43 - 11.9}{\sqrt{\frac{0.2810}{15} + \frac{4.0444}{10}}}$$

$$\frac{2.53}{\sqrt{0.0187 + 0.4044}} = \frac{2.53}{\sqrt{0.4231}} = \frac{2.53}{0.6505}$$

$$= 3.89$$

6. The computed test statistic t' = 3.89 is greater than the critical value of 3.238. Therefore, the null hypothesis is rejected, and the conclusion, with 99% confidence, is that the mean temperatures are different.

7. Use the same computational procedure if $n_1 = n_2$.

Paired t-test

Problem: Ten transects were sampled in order to estimate the width of a stream along the 100 m length of a managed site. The following width measurements (meters), taken perpendicular to the flow of the water, were obtained prior to the management activity:

7.1, 6.3, 7.6, 5.2, 4.3, 4.0, 5.6, 5.2, 4.9, and 6.1

The following measurements were taken at the same 10 transects after the management action was implemented:

6.3, 5.9, 5.2, 3.7, 4.2, 3.1, 5.6, 3.8, 4.2, and 4.9

The paired t-test is used to determine if the stream width changed significantly after the management activity.

Solution:

1. The null hypothesis is that there is no difference in stream width before and after management: $H_o: \mu_1 = \mu_2$ versus $H_2: \mu_1 \neq \mu_2$.

2. The level of significance chosen is $\alpha = 0.05$.

3. The assumptions for parametric tests are met and the data are paired; therefore, a paired t-test (Snedecor and Cochran 1968) is used.

4. The pairs are established:

Transect	Before	After	Difference	Deviation	
i	X_1	X_2	$d_i = X_1 - X_2$	$d_i - \bar{d}$	$(d_i - \bar{d})^2$
1	7.1	7.3	0.8	-0.14	0.0196
2	6.3	5.9	0.4	-0.54	0.2916
3	7.6	5.2	2.4	1.46	2.1316
4	5.2	3.7	1.5	0.56	0.3136
5	4.3	4.2	0.1	-0.84	0.7056
6	4.0	3.1	0.9	-0.04	0.0016
7	5.6	5.6	0.0	-0.94	0.8836
8	5.2	3.8	1.4	0.46	0.2116
9	4.9	4.2	0.7	-0.24	0.0576
10	6.1	4.9	1.2	0.26	0.0676
Total	56.3	46.9	9.4	0.00	4.6840

$\bar{X}$ = 5.63 4.69 $\bar{d}$ = 0.94 s^2 = 0.5204

$$s_{\bar{d}}^2 = 0.5204/10 = 0.0520, \quad s_{\bar{d}} = 0.2280$$

where $\bar{d} = \frac{9.4}{10} = \frac{\Sigma d_i}{n}$

$$s^2 = \frac{4.684}{9} = \frac{4.684}{n-1} = \frac{\Sigma d_i^2}{n-1}$$

$$s^2_{\bar{d}} = \frac{0.5204}{10} = \frac{s^2}{n}$$

$$s_{\bar{d}} = \sqrt{0.0520} = 0.2280$$

5. t is computed as:

$$t = \frac{\bar{d}}{s_{\bar{d}}} = \frac{0.94}{0.228} = 4.123$$

6. From the t table, $t_{0.05,9}$ is 2.26. n-1 = nine degrees of freedom.

7. The computed t of 4.123 is greater than the critical value. Therefore, the null hypothesis is rejected, and the conclusion, with a 95% confidence level, is that the means are different and that the management actions decreased the stream width.

Wilcoxon Signed-Rank Test

The Wilcoxon signed-rank test is the nonparametric analog of the paired t-test.

Problem: Average depth measurements in tenths of meters were taken in a stream, at the same sites, before and after management to determine the effect of the management action on the stream depths:

Sample	Average depth After	Average depth Before	Difference	Signed rank
1	2.0	1.3	0.7	3
2	1.2	1.1	0.1	1
3	0.5	0.9	-0.4	-2
4	1.9	0.8	1.1	5
5	2.1	1.2	0.9	4
6	4.0	1.0	3.0	6
7	4.5	1.0	3.5	7
ΣX_i =	16.2	7.3		
$\bar{X}$ =	2.31	1.04		
s^2 =	2.08	0.03		

Solution:

1. The null hypothesis is that the median (M) of the differences between before and after depth measurements equals zero; the alternative hypothesis is that this median is greater than zero. Thus, this is a one-sided test:

   H_o: $M = 0$

   H_a: $M > 0$

2. The level of significance chosen is $\alpha = 0.05$.

3. Three of the assumptions for parametric tests have been met; however, a nonparametric test will be used because the variances of the before and after measurements are significantly different. The measurements are paired, so the Wilcoxon signed-rank test is used to calculate the test statistic (T).

4. The differences between paired samples are ranked from smallest to largest, without regard to sign.

5. Sum the positive and negative ranks separately and determine their absolute values:

T+ = 26

T- = 2

6. Look up the tabular value for a one-tailed test in Appendix B of this manual. This value is obtained by letting n equal the number of pairs with nonzero differences (Wilcoxon and Wilcox 1964).[11] In this case, n = 7 and α = 0.05. The smaller T value (2) is less than the tabular value of 4; therefore, the null hypothesis is rejected. The conclusion with a 95% confidence level is that stream depths were greater after the management practices occurred. Another approach for using the Wilcoxon signed-rank test is discussed in Sokal and Rohlf (1969).

Mann-Whitney U-test

The Mann-Whitney U-test is the nonparametric analog of the unpaired t-test.

Problem: In a stream that was greatly affected by logging activity, a management objective was to improve the spawning habitat by increasing the substrate size. Average spawning gravel size was chosen as the variable to measure before and after management actions were initiated.

Before improvement	After improvement
11 mm	12 mm
6	13
1	10
4	11
10	12
$\bar{X}$ = 6.4	11.6
s^2 = 17.3	1.3

[11]This reference can be obtained from Lederle Laboratories, Pearl River, NY.

Solution

1. The null hypothesis for the one-tailed test is that the average spawning gravel diameters before management are equal to or greater than the diameters after management has occurred; the alternative hypothesis is that diameters after management has occurred are greater than the diameters before management.

2. The level of significance selected is $\alpha = 0.05$.

3. In testing the data for meeting parametric assumptions, it was found that the variances were not homogeneous. The most commonly used nonparametric test for comparing two independent (unpaired) samples is the Mann-Whitney test. For this test, it is assumed that the data consist of two independent random samples of continuous variables. If $n > 20$, refer to Sokal and Rohlf (1969) for the proper procedure.

4. Rearrange the data by ranking each sample separately:

Rank	A (before improvement)	B (after improvement)	Number of observations in A less than each B value
1	1	10	3.5
2	4	11	4.5
3	6	12	5
4	10	12	5
5	11	13	5
			C = 23

The last column is calculated as follows, starting with the first value:

A. There are three values in A less than 10 (the first value in B) and one value in A that equals 10; therefore, the first number in the last column is 3.5.

B. There are four values in A less than 11 and one value in A that equals 11; therefore, the second value in the column is 4.5.

C. There are five values in A less than 12 and five values in A less than 13; therefore, the last three numbers in the last column are 5.

5. The Mann-Whitney statistic U_s is the greater of C or n_1n_2-C. For this example, n_1n_2-C = (5)(5)-23 = 2. Therefore, U_s = 23.

6. Locate $U_{\alpha,(n_1,n_2)}$ for a one-tailed test in Rohlf and Sokal (1979: table cc): $U_{0.05,(5,5)} = 21$. U_s of 23 exceeds the tabular value of 21. Therefore, the null hypothesis is rejected and the conclusion, with a 95% confidence level, is that average substrate diameter increased as a result of management actions.

One-way Anlysis of Variance

Problem: The velocity of a stream was determined to be too low for good fish spawning habitat. Stream improvement devices were installed on a section of the stream in an attempt to increase velocity. Velocity measurements were taken at one site within the stream improvement area before the management actions occurred and at two different sites within the area after sufficient time lapsed for management actions to be effective.

Replicates	Before management	After management	
i	Site 1	Site 2	Site 3
1	0.4 (m/sec)	0.6 (m/sec)	0.7 (m/sec)
2	0.3	0.7	0.5
3	0.2	0.5	0.6
4	0.3	0.9	0.9
5	0.1	1.0	0.9
6	0.5	0.8	0.6
7	0.4	0.7	0.8
ΣX_i	2.2	5.2	5.0
$\bar{X}$	0.314	0.743	0.714
s^2	0.0181	0.0295	0.0248

The grand total of all observations is 12.4; the grand mean = 0.590.

Solution:

1. The null hypothesis (H_o) is that the means at all sites are equal: H_o $\mu_1 = \mu_2 = \mu_3$. The alternative hypothesis (H_a) is that the mean of at least one site is different from the means of the other sites; in particular, $\mu_2 = \mu_3 \neq \mu_1$.

2. The level of significance chosen is $\alpha = 0.05$.

3. All of the assumptions for parametric tests have been met, and the parametric anlysis of variance ANOVA test will be used to test for differences.

4. Calculate the grand total for all of the observations squared:

$$(0.4)^2 + (0.3)^2 + \ldots + (0.6)^2 + (0.8)^2 = 8.56$$

5. Divide the sum of the squared site totals by the number of replicate samples:

$$= \frac{(2.2)^2 + (5.2)^2 + (5.0)^2}{7} = \frac{4.84 + 27.04 + 25.0}{7}$$

$$= \frac{56.88}{7} = 8.126$$

6. Calculate correction term CT = grand total squared and divided by the <u>total</u> sample size:

$$CT = \frac{(12.4)^2}{21} = \frac{153.76}{21} = 7.322$$

7. SS_{Total} = quantity from Step 4 - CT

   = 8.56 - 7.322 = 1.238

8. SS_{Groups} = quantity from Step 5 - CT

   = 8.126 - 7.322 = 0.804

9. $SS_{Within} = SS_{Total} - SS_{Groups}$

   = 1.238 - 0.804 = 0.434

10. Prepare the ANOVA Table:

Variation	df	SS	MS	F-value
Between sites	a-1 = 2	$SS_{Groups} = 0.804$	$\frac{SS_{Groups}}{a-1} = 0.402$	$\frac{0.402}{0.024} = 16.75$
Within sites (error)	a(n-1) = 18	$SS_{Within} = 0.434$	$\frac{SS_{Within}}{a(n-1)} = 0.024$	

where a = number of sites
n = number of samples within each site

Tabular	$F_{0.05,(2,18)} = 3.55$	$F_{0.01,(2,18)} = 6.01$

11. The null hypothesis is rejected because the computed F test statistic of 16.75 is greater than the tabular F value of 3.55. The conclusion, with at least a 95% confidence level, is that the mean velocities for the three sites are unequal. (In this example, this test is significant at a greater than 1% confidence level).

12. The next step is to determine which sites differ from which other sites. It was assumed that Site 1 would be different from Sites 2 and 3 and that Sites 2 and 3 would be the same; therefore, an *a priori* comparison is used.

13. The level of significance chosen is $\alpha = 0.05$.

14. Determine the specific pair-wise comparisons. In this case, there are three comparisons: Site 1 vs. 2; Site 1 vs. 3; and Site 2 vs. 3.

15. Calculate the Least Significant Different Term (any pair-wise difference in means that exceeds this term is considered significant):

$$LSD = t_{\alpha,(df)}\sqrt{\frac{2}{n} MS_{within}}$$

where $\alpha = 0.05$

$df = a(n-1) = 18$

$$LSD = 2.101\sqrt{\frac{2}{7} (0.024)}$$

$$= 0.174$$

16. Calculate the differences between means and compare these differences to the LSD value:

$$\bar{X}_2 - \bar{X}_1 = 0.429$$
$$\bar{X}_3 - \bar{X}_1 = 0.400$$
$$\bar{X}_2 - \bar{X}_3 = 0.029$$

In this example, the first two sets of means are significantly different because the differences exceed the LSD value of 0.174. The conclusion is that, for both sites, the means are significantly different than the mean for the "before" management condition. Means for the two sites after management actions occurred were not significantly different from each other. The Student-Newman-Keuls test (Sokal and Rohlf 1969) can also be used for multiple comparisons of means.

17. More complex comparisons are also possible; in this example, the average of Site 2 and 3 means are compared to the mean of Site 1:

$$\text{diff} = \left[(\bar{X}_2 + \bar{X}_3)/2 - \bar{X}_1\right]$$

$$= (\tfrac{1}{2})\ \bar{X}_2 + (\tfrac{1}{2})\ \bar{X}_3 - \bar{X}_1$$

This is a linear combination of means, as are the pairwise comparisons. The variance of each mean is $s^2 = (MS_{Within})/n$. The variance of a linear combination is the sum of the squared coefficient multiplying each mean times the variance of that mean. In this example:

$$\text{var(diff)} = (\tfrac{1}{2})^2\ \text{var}(\bar{X}_2) + (\tfrac{1}{2})^2\ \text{var}(\bar{X}_3) + (-1)^2\ \text{var}(\bar{X}_1)$$

$$= (.25)\ \frac{MS_{Within}}{n} + (.25)\ \frac{MS_{Within}}{n} + (1)\ \frac{MS_{Within}}{n}$$

$$= [(.25) + (.25) + 1]\ \frac{MS_{Within}}{n}$$

$$= 1.5\ \frac{MS_{Within}}{n}$$

$$= 1.5\ \frac{0.024}{7} = 0.005143$$

The test statistic [it has a t-distribution with a(n-1)df; this is the df of the MS_{Within}] is:

$$t = \frac{\text{diff}}{\sqrt{\text{var(diff)}}}$$

$$= \frac{(0.743 + 0.714) - 0.314}{\sqrt{0.005143}}$$

$$= \frac{0.729 - 0.314}{0.0717} = 5.788$$

The critical level (two-tailed) is $t_{0.05,(18)} = 2.101$. The computed test value of 5.788 exceeds 2.101; therefore, the conclusion is that the average of Sites 2 and 3 differs from the average for Site 1. Because the averages for Sites 2 and 3 do not differ significantly from each other, the assumption can be made that all the significant difference suggested by the F-test represents before vs. after management conditions. Note that, in the absence of a control site, the conclusion that management caused the increased velocity cannot be made on the basis of statistics alone.

Kruskal-Wallace Nonparametric Test for One-Way ANOVA (Sokal and Rohlf 1969).

Problem: The problem is the same one used to illustrate the one-way analysis for variance but it is assumed that requirements for a parametric test are not met. Assemble the data from all three sites in one array, starting with the lowest value and ending with the highest:

Velocity measurement	Rank	Velocity measurement	Rank	Velocity measurement	Rank
0.1	1	0.6	11	0.9	19
0.2	2	0.6	11	0.9	19
0.3	3.5	0.6	11	0.9	19
0.3	3.5	0.7	14	1.0	21
0.4	5.5	0.7	14		
0.4	5.5	0.7	14		
0.5	8				
0.5	8	0.8	16.5		
0.5	8	0.8	16.5		

Ranks for equal data values are determined by averaging the positions of the equal values; e.g., the ranks for the third and fourth values are:

$$\frac{3rd + 4th}{2} = 3.5$$

The $\underline{X|}$ values indicate the number of tied observations. These are denoted as t_j in the following equations. Prepare a table with ranks replacing the original observations in each data set:

i		Before management Site 1	After management Site 2	Site 3
1		5.5	11.0	14.0
2		3.5	14.0	8.0
3		2.0	8.0	11.0
4		3.5	19.0	19.0
5		1.0	21.0	19.0
6		8.0	16.5	11.0
7		5.5	14.0	16.5
ΣX_i	=	29	103.5	98.5
$\bar{X}$	=	4.143	14.786	14.029

Solution

1. H_o: The expected means for the three sites are the same.

   H_a: The expected means for the three sites are different.

2. Select $\alpha = 0.05$.

3. Compute $H = \frac{12}{(N)\ (N+1)}\left[\frac{\text{Sum of squared column totals}}{n}\right] - 3(N+1)$

where N = total number of observations for all data sets

n = number of observations per sample site

$$= \frac{12}{(21)\ (22)}\left[\frac{(29)^2 + (103.5)^2 + (98.5)^2}{7}\right] - 3(22)$$

$$= 0.0260\ \left[\frac{841.00 + 10{,}712.25 + 9702.25}{7}\right] - 66$$

$$= (0.0260)\left[\frac{(21{,}255.50}{7}\right] - 66$$

$$= \frac{552.64}{7} - 66 = 12.949$$

4. Compute correction term for H to compensate for tied values:

$$D = 1 - \frac{\text{Sum of } (t_j - 1)\ t_j(t_j + 1) \text{ for each set of tied values}}{(N-1)\ (N)\ (N+1)}$$

where t_j = number in each set of tied values, shown as, e.g. $\underline{2}$. In this example, there are seven sets of tied values.

$$= 1 - \frac{(1)(2)(3) + (1)(2)(3) + (2)(3)(4) + (2)(3)(4) + (2)(3)(4) + (1)(2)(3) + (2)(3)(4)}{(21-1)(21)(21+1)}$$

$$= 1 - \frac{6 + 6 + 24 + 24 + 24 + 6 + 24}{9240}$$

$$= 1 - \frac{114}{9240} = 1 - 0.01233 = 0.9877$$

5. Adjusted $H = \frac{H}{D} = \frac{12.949}{0.9877} = 13.11$

6. Because H is approximately distributed as a chi-square variable, the table value of $x^2_{0.05,a-1}$ is obtained where a = number of columns or data sets $x^2_{0.05,2} = 5.991$.

7. Because the computed value of H = 13.11 is greater than $x_{0.05,2} = 5.991$, the null hypothesis is rejected, and the conclusion, with at least a 95% confidence level, is that the velocity increased after management actions occurred. Again, without a control site, the conclusion that the increased velocity resulted from the management action cannot be reached on a purely statistical basis. This conclusion may be, however, quite reasonable from a biological viewpoint.

Parametric Two-Way ANOVA Without Replication

Problem: Pool-riffle ratios were measured in three locations in a stream. Two sites were spatial controls and the third site received special management designed to increase the number of pools. The sample data taken after management occurred are summarized below:

	15 May		16 Jun		14 Jul		17 Aug		13 Sep		15 Oct		
Site	X_i	X_i^2	X_i	X_i^2	X_i	X_i^2	X_i	X_i^2	X_i	X_i^2	X_i	X_i^2	ΣX_i
1(Control)	15	225	20	400	20	400	25	625	30	900	30	900	140
2(Managed)	35	1225	35	1225	40	1600	40	1600	45	2025	55	3025	250
3(Control)	15	225	15	225	20	400	25	625	25	625	30	900	130
Totals	65	1675	70	1850	80	2400	90	2850	100	3550	115	4825	

$\Sigma X_i = 520$

$\Sigma X_i^2 = 17150$

Row means:

$$\text{Control 1} = \frac{140}{6} = 23.333$$

$$\text{Management} = \frac{250}{6} = 41.667$$

$$\text{Control 2} = \frac{130}{6} = 21.667$$

Mean of Control Means = 22.5

Solution:

1. H_o: Sampling periods and treatments have no affect on pool-riffle ratios.

   H_a: Sampling periods or treatments or both affect pool-riffle ratios.

2. The level of significance is $\alpha = 0.05$. All assumptions for a parametric test are met and the two-way ANOVA test is selected.

3. Sum the values for all measurements; i.e., 15 + 20 + 20 + . . . + 25 + 30 = 520.

4. Sum all the squared measurements; i.e., 225 + 400 + . . . + 625 + 900 = 17,150.

5. Sum the squared column totals, and divide the sum by the sample size for the columns (i.e., the number of "treatments"):

$$= \frac{(65)^2 + (70)^2 + (80)^2 + (90)^2 + (100)^2 + (115)^2}{3}$$

$$= \frac{4225 + 4900 + 6400 + 8100 + 10,000 + 13,225}{3} = \frac{46,850}{3}$$

$$= 15,616.667$$

6. Sum the squared row totals and divide by the sample size for the row (i.e., the number of sampling times):

$$= \frac{(140)^2 + (250)^2 + (130)^2}{6}$$

$$= \frac{19,600 + 62,500 + 16,900}{6} = \frac{99,000}{6}$$

$$= 16,500$$

7. Compute the correction term, CT, by squaring the grand total and dividing the square by total sample size:

$$= \frac{(520)}{(6)(3)} = \frac{270,400}{18} = 15,022.222$$

8. Compute SS_{Total} = Quantity 4 - CT

   = 17,150 - 15,022.222 = 2,127.778

9. Compute $SS_{Columns}$ = Quantity 5 - CT

   = 15,616.667 - 15,022.222 = 594.445

10. Compute SS_{Rows} = Quantity 6 - CT

    = 16,500 - 15,022.222 = 1,477.778

11. Compute $SS_{Error} = SS_{Total} - SS_{Columns} - SS_{Rows}$

    = 2,127.778 - 594.455 - 1,477.778 = 55.545

12. Prepare ANOVA Table

Source of variation	df	SS	MS	F-value
Days (Column SS)	c-1 = 5	594.44	118.89	21.38***
Treatments (Row SS)	r-1 = 2	1,477.78	738.89	132.89***
SS error	(c-1)(r-1) = 10	55.56	5.56	
SS non-additivity[a]	1	6.50	6.50	1.19[b]
Residual SS	9	49.06[c]	5.45	

$F_{0.05,(2,10)} = 4.10$ $F_{0.05,(1,9)} = 5.12$ for SS_{Nonadd} and $SS_{Residual}$

[a]The F-value for nonadditivity is insignificant when compared to $F_{0.05,(1,9)} = 5.12$. This test confirms that the effects of time and treatments are additive, which is a prerequisite for the ANOVA test. If significance is detected, it may mean that a data transformation is necessary (Snedecor and Cochran 1968). Computations for the $SS_{Nonadditivity}$ are in Appendix C.

[b]1.19 = 6.50/5.45.

[c]49.06 = 55.56 -6.50.

13. The null hypothesis is rejected, and the conclusion, with a 99.9% confidence level, is that sampling periods and treatments both affect pool-riffle ratios. Therefore, the management actions increased the pool-riffle ratios, and the improvement in the ratio persisted over time.

14. Calculate the management effect by subtracting the mean of the control means from the management mean; i.e., 41.667 - 22.500 = 19.167. This represents the magnitude by which management actions increased the pool-riffle ratio (approximately doubled in this example).

15. A t-test can be applied to confirm the conclusion that management affected the pool-riffle ratio.

A. Calculate the variance of the management effect:

$$= \frac{1}{n}\left(\frac{1}{m} + \frac{1}{s}\right) \text{ MS}$$

where n = number of observations at each sampling site

m = number of treatment ("managed") sites

s = number of control sites

MS = Error MS from the ANOVA table

$$= \frac{1}{6}\left(1 + \frac{1}{2}\right) 5.56$$

$$= \frac{1}{6}(1.5)(5.56)$$

$$= 1.390$$

B. Standard error of the management effect = $\sqrt{1.390} = 1.179$.

C. Calculate $t = \frac{\text{Management effect}}{\text{Standard error of management effect}}$

$$= \frac{19.167}{1.178} = 16.27$$

The degrees of freedom of this, or any, t-test are the same as the degrees of freedom associated with the estimate of the standard error used in the denominator. Degrees of freedom are

given in the ANOVA table for this test; in this example, there are 10 df. From a t-distribution table, the 5% critical value for 10 df is $t_{0.05,10} = 2.288$. Because 16.25 exceeds 2.228, it is confirmed, with at least 95% confidence, that the management actions improved pool conditions (actual significance level of this test is much better than 5%).

Nonparametric Two-Way ANOVA Without Replication

Problem: The problem is the same as the above example which used the parametric two-way ANOVA without replication.

The summarized data and their ranks within each period are:

Period	Site 1 Control	Rank	Site 2 Management	Rank	Site 3 Control	Rank
15 May	15	1.5	35	3	15	1.5
16 Jun	20	2.0	35	3	15	1.0
14 Jul	20	1.5	40	3	20	1.5
17 Aug	25	1.5	40	3	25	1.5
13 Sep	30	2.0	45	3	25	1.0
15 Oct	30	1.5	55	3	30	1.5
Rank sums over periods		10.0		18		8.0

The data are presented by period and by treatment (sample site), exactly as in the parametric analysis. Each value is ranked across treatments within periods ("blocks", in statistical terminology). In this example, there are three sample sites, and ranking is easy. These ranks replace the original data. When ties occur within

periods, the ranks are averaged. For example, in the period 15 May the two controls are tied for ranks 1 and 2. Therefore, both ranks equal 1.5.

Next, sum the ranks within each sample site. For example, the sum of the ranks for the management site is 18.

Solution:

1. H_o: Pool-riffle ratios for the three sites are the same.

   H_a: Pool-riffle ratios for the three sites are not the same.

2. Let $\alpha = 0.05$. Friedman's method (Sokal and Rohlf 1969), which employs a chi-square (χ^2) test statistic, will be used.

3. Compute χ^2 as:

$$\left[\frac{12}{(a)(b)(a+1)}\right] \cdot \left[\begin{array}{c}\text{Total of the squared} \\ \text{rank sums}\end{array}\right] - 3b(a+1)$$

where a = number of treatments (sample sites = 3)

b = number of sample sites (i.e., blocks)

In this example, this test statistic is:

$$\left[\frac{12}{(3)(6)(4)}\right] [(10)^2 + (18)^2 + (8)^2] - 3(6)(4)$$

$$= \frac{12}{72} (100 + 324 + 64) - 72$$

$$= 0.1667 (488) - 72$$

$$= 9.35$$

4. This test statistic has a chi-squared distribution with a-1 df under the null hypothesis. In this example, using $\alpha = 0.05$, the critical level is $\chi^2_{0.05,a-1} = \chi^2_{0.05,2} = 5.99$. Because the calculated value of $\chi^2 = 9.35$ is greater than the critical value, the null hypothesis is rejected, and the conclusion, with a 95% confidence level, is that there is a difference in the pool-riffle ratios among the three sites. The assumption is made, based on the study design and an inspection of the means, that the change in ratios resulted from the management actions.

Parametric Two-Way ANOVA with Replication

	Before	After	
	19	44	
Management	15	40	
	14	39	
Totals	48	123	171
	25	36	
Control	21	30	
	23	33	
Totals	69	99	168
Grand totals	117	222	339

1. H_o: Management had no effect on biomass changes.

   H_a: Management affected biomass changes.

2. The level of significance is $\alpha = 0.05$.

3. Sum all the data values; e.g., $19 + 15 + 14 + \ldots + 33 = 339$.

4. Sum the squares of all of the data values; e.g., $19^2 + 15^2 + 14^2 + \ldots + 33^2 = 10{,}719$.

5. Square and add the sums of all of the values in each data set and divide the square of the sums by n, where n = the number of observations per cell.

$$\frac{(48)^2 + (123)^2 + (69)^2 + (99)^2}{3}$$

$$= \frac{2304 + 15{,}129 + 4761 + 9801}{3}$$

$$= \frac{31{,}723}{3} = 10{,}665$$

6. Compute the correction term, CT:

$$CT = \frac{(\text{Grand total})^2}{rcn}$$

where r = number of rows

c = number of columns

n = number of observations per cell

$$= \frac{(339)^2}{12}$$

$$= \frac{114{,}921}{12} = 9{,}576.75$$

7. SS_{Total} = Quantity from Step 4 - CT

= 10,719 - 9,576.75

= 1,142.25

8. $SS_{Subgroup}$ = Sum from Step 5 - CT

= 10,665 - 9,576.75

= 1,088.25

9. SS_{Within} = SS_{Total} - $SS_{Subgroup}$

= 1,142.25 - 1,088.25

= 54

10. Prepare preliminary ANOVA table:

Variation	df	SS	MS	F-ratio
$SS_{Subgroup}$	rc-1 = 3	1,088.25	362.75	53.74
SS_{Within}	rc(n-1) = 8	54.00	6.75	
	rcn-1 = 11	1,142.25		

The tabular $F_{0.05,(3,8)}$ = 4.07. Because 53.74 > 4.07, it is very reasonable to assume that some effect is influencing subgroup means and that additional testing is necessary.

11. Square the row totals for the treatments and controls, sum these squares, and divide this sum by cn

where c = columns

n = observations per cell

$$= \frac{(171)^2 + (168)^2}{6}$$

$$= \frac{29,241 + 28,224}{6}$$

$$= \frac{57,475}{6} = 9,577.5$$

12. Square the column totals for before and after periods and divide the square by nr

where r = number of rows = 2

$$= \frac{(117)^2 + (222)^2}{6}$$

$$= \frac{13,689 + 49,284}{6}$$

$$= 10,495.5$$

13. SS_{Rows} (SS due to treatment vs. control)

= Quantity 11 - CT

= 9,577.5 - 9,576.75

= 0.75

14. $SS_{Columns}$ (SS due to time)

= Quantity for Step 12 - CT

= 10,495.5 - 9,576.75

= 918.75

15. $SS_{Interaction}$ [SS due to time X (treatment + control)]

= $SS_{Subgroup} - SS_{Rows} - SS_{Columns}$

= 1,088.25 - 0.75 - 918.75

= 168.75

16. Completed ANOVA Table

Variation	df	SS	MS	F-value
Subgroup	rc-1 = 3	1,088.25	263.75	
Rows	r-1 = 1	0.75	0.75	
Columns	c-1 = 1	918.75	918.75	
Interaction	(r-1)(c-1) = 1	168.75	168.75	25.00*
Error	rc(n-1) = 8	54.00	6.75	

Tabular F for interaction = $F_{0.05,(1,8)} = 5.32$

17. Because the computed F for interaction > 5.32, the null hypothesis is rejected, and it is concluded that the management actions did affect the biomass.

18. Estimate the effects of natural environmental changes over time (T), the natural between-site variation (S) of biomass, and the effects resulting from management action (M).

A. Environmental changes

H_o: The naturally occurring environmental changes over time did not affect biomass.

H_a: The naturally occurring environmental changes over time did affect biomass.

Test at $\alpha = 0.5$; $t_{0.05,8df} = 2.306$.

where there are 8 df for the error in the ANOVA Table (Step 16).

The environmental effect = $E = \bar{X}_{CA} - \bar{X}_{CB} = 33 - 23 = 10$

where $\bar{X}_{CA}$ = the mean for the control site after management

$\bar{X}_{CB}$ = the mean for the control site before management

Therefore, the biomass was changed by 10 units as a result of environmental effects.

Variance for $E = \frac{2\ EMS}{n} = \frac{2(6.75)}{3} = 4.5 = var(E)$

where EMS = MS for the error in the ANOVA Table (Step 16)

2 = number of means considered

Standard error for E is $se(E) = \sqrt{var(E)} = \sqrt{4.5} = 2.12$

Compute t statistic for test: $\frac{E}{se(E)} = \frac{10}{2.12} = 4.72$

Because the computed t of 4.72 > 2.306, the null hypothesis is rejected, and the conclusion is that environmental changes over time, unrelated to the management actions, did affect biomass.

B. Natural between-site variation

H_o: Site differences did not affect biomass.

H_a: Site differences did affect biomass.

Test at $\alpha = 0.05$; $t_{0.05,8df} = 2.306$

Site effect = $S = \bar{X}_{MB} - \bar{X}_{CB}$

where $\bar{X}_{MB}$ = the mean for the treatment site before management

$\bar{X}_{CB}$ = the mean for the control site before management

$= 16 - 23 = -7$

Variance for $S = \frac{2\ EMS}{3} = \frac{2(6.75)}{3} = 4.5$

Standard error for $S = \sqrt{4.5} = 2.12$

Compute t statistic for test: $\frac{S}{se(S)} = \frac{-7}{2.12} = -3.30$

Because the computed t statistic of $-3.30 < -2.306$, the null hypothesis is rejected, and it is concluded that natural site variation did affect biomass.

C. Management effects

H_o: Management actions did not affect biomass over time.

H_a: Management actions did affect biomass over time.

Use the same α and tabular t as for the previous tests; i.e., 2.306.

Management effect = $M = (\bar{X}_{MA} - \bar{X}_{MB}) - (\bar{X}_{CA} - \bar{X}_{CB})$.

In this example, M = (41-16) - (33-23) = 25-10 = 15. M can also be computed as:

$$(\bar{X}_{MA} - \bar{X}_{MB}) - E \quad \text{or as} \quad (\bar{X}_{MA} - \bar{X}_{CA}) - S.$$

where $\bar{X}_{MA}$ = the mean for the management site after management

Therefore, there was a 15 unit increase in biomass due to management actions.

$$\text{Variance for M} = \frac{4\ (EMS)}{n} = \frac{4(6.75)}{3} = 9$$

where 4 is a factor indicating that four means are being compared

the standard error for M = se(M) = $\sqrt{9}$ = 3.

Compute t statistic: $\frac{M}{se(M)} = \frac{15}{3} = 5.$[12]

The null hypothesis is rejected, and the conclusion is that management actions did result in an increase in biomass. Because there are control samples, it is valid to conclude that management had a causal effect on biomass changes.

For this test, the effects of management, environment, and site variation were evaluated. The following three study designs can be used to estimate effects, as indicated below:

---

[12]Note that this t^2 = the F-value for interaction.

	Premanagement	Postmanagement	Estimatable effects
Management site	Yes	Yes	Management, environment, and site.
Control site	Yes	Yes	

	Premanagement	Postmanagement	
Management site	No	Yes	The sum of management and site effects (no premanagement sampling done).
Control site	No	Yes	

	Premanagement	Postmanagement	
Management site	Yes	Yes	The sum of management and environmental effects (no control sites sampled)
Control site	No	No	

Fixed-site, Pre-, and Postevaluation of Management Actions

This is a very useful type of study design. Assume eight stream sites are evaluated. The eight sites should be selected randomly from a larger set of possible sites in the area of interest so that valid inferences can be made for this larger area. The sites can be on eight different streams of the same type in the same general area, on one stream, or as sets of control and treatment sites on four streams. Management (treatment) activities should be applied to four randomly selected sites out of the eight sites.

Assume that the study objective is to increase the population of catchable sport fish. Therefore, a premanagement estimate of population size must be made at each site before management actions occur. Control sites are established so that any natural changes in fish numbers can be documented. After sufficient time has passed for management effects to occur, the eight sites are resampled.

Accurate population estimates are assumed. Acceptance of this assumption means that the within-site sampling variances of these estimates are not considered relevant.

(The data is arranged by sample site order):

	Site	Premanagement	Postmanagement	Difference
Control	1	100	132	32
	2	132	140	8
	3	157	185	28
	4	205	230	25
Treatment	5	80	123	43
	6	121	186	65
	7	165	203	38
	8	225	277	52

1. Compute the difference for each pair as the post- minus the premanagement abundance. These differences reflect time plus management effects for treatment sites. For the control sites, the differences reflect only time effects. Compute the means and standard deviations for these two sets of values:

	Mean	s^2	s
Control, $\bar{X}_C$	23.25	111.58	10.56
Treatment, $\bar{X}_T$	49.50	140.33	11.84

2. The null hupothesis, H_o: there was no treatment effect, is tested against the one-sided alternative H_a: treatment resulted in an increase in the number of catchable fish. A one-sided t-test is used:

The treatment effect = $\bar{X}_T - \bar{X}_c = 49.50 - 23.25 = 26.35$

The standard error of this treatment effect is:

$$se = \sqrt{\left(\frac{(n_c-1)s_c^2 + (n_T-1)s_T^2}{n_c + n_T - 2}\right)\left(\frac{1}{n_c} + \frac{1}{n_T}\right)}$$

where n_c = number of control sites

n_T = number of treated sites
($n_c = n_T = 4$).

In this example:

$$se = \sqrt{\left(\frac{3(111.58) + 3(140.33)}{6}\right)\left(\frac{1}{4} + \frac{1}{4}\right)}$$

$$= \sqrt{62.97} = 7.93$$

3. The t-test statistic is:

$$t = \frac{\bar{X}_T - \bar{X}_c}{se} = \frac{26.25}{7.93} = 3.31.$$

The df = $n_c + n_T - 2 = 6$ in this example. The critical level for an $\alpha = 0.05$ level one-tailed t-test is:

$$t_{0.05,6} = 1.943$$

The computed value of 3.31 exceeds the tabular value of 1.943. Therefore, H_o is rejected, and the conclusion is that management actions resulted in an increase in the catchable fish population. (The actual significance level of this test is much better than $\alpha = 0.05$).

4. The test for a time effect is also a t-test (two-sided) with $n_c + n_T - 1$ df: (recall that X_c is the mean of the differences in fish abundance in the control sites before and after management actions):

$$t = \frac{\bar{X}_c}{se}$$

$$se = \sqrt{\frac{(n_c-1)s_c^2 + (n_T-1)s_T^2}{n_c + n_T - 2}\left(\frac{1}{n_c}\right)}$$

$$= 5.61$$

$$t = \frac{23.25}{5.61} = 4.14$$

The critical level is $t_{0.05,6} = 2.447$. Therefore, the conclusion is that there were significant time effects on the size of the catchable fish population.

Even if the management treatment had no effect on fish populations, the pre- and postcomparison of responses of the four treated sites would have shown a significant increase in catchable fish due to time effects. This example illustrates the need for controls in long term environmental studies.

5. Given random assignment of treatments, there should be no difference between the expected abundance in the premanagement control sites and in the treated sites. This is tested with an unpaired, two-sided t-test, computed the same as was the test in Steps 2 and 3, above. Relevant summary statistics use only premanagement data:

	Mean	s^2	s
Control (n=4)	148.5	1963.0	44.30
Treatment (n=4)	147.8	3856.9	62.10
pooled (n=8)	148.1	2494.4	49.94

It is clear there is no difference in means between the two groups of sites (the actual t value is 0.02; 6 df).

6. Given that the control and treated sites are, on the average, identical with respect to the abundance of catchable fish, prior to management activities, it is valid to just compare the postmanagement measurements to estimate, and test for, treatment effects. The problem with this approach is that it lacks sensitivity because the benefits of using fixed sites (i.e., the pairing of the pre- and postmanagement measurements) are lost. The large, natural, site-to-site variation obscures the significance of any management effect.

From the above, the pooled estimate of the standard deviation of the pre- and postmanagement differences is:

$$\sqrt{\frac{3(111.58) + 3(140.33)}{6}} = 11.2$$

The standard deviation in premanagement measurements across all eight sites is 49.94. The "pairing" effect of pre- and postmeasurements on the same site greatly reduces the variation in the experiment results.

The unpaired t-test, which does not involve the use of the pretreatment data, uses the following statistics (based on postmanagement data only):

	Mean	s^2	s
Control ($\bar{X}_c$)	171.8	2052.3	45.3
Treatment ($\bar{X}_T$)	197.3	4010.9	63.3

The valid, but very inefficient, t-test for a treatment effect is:

$$\frac{197.3 - 171.8}{38.9} = \frac{25.5}{38.9} = 0.66$$

This calculation has 6 df and is one-sided, but it is not significant. Even though management significantly increased the abundance of catchable fish, this fact would not be proven without the inclusion of pretreatment data.

7. In this example, the estimated treatment effect is 26.25 more catchable fish. This relative increase may not be applicable to other areas because the management effect often depends on the initial size of the population. A better way to express the treatment effect may be as the percent change relative to "baseline" conditions. Baseline condition is the average number of fish in the treatment site prior to treatment (147.8 in this example). If it is known, or assumed, that there is no difference between control and treatment sites prior to treatment, the estimate of relative treatment effect is based on the average pretreatment value (148.1 in this example).

   The estimated percent relative increase in catchable fish in this example is:

$$\frac{26.25}{148.1}\ (100\%) = (0.177)100\% = 17.7\%$$

Point 8 below further illustrates the benefits of fixed sites (i.e., pre- and post- "pairing"); this material requires use of a more complex statistical concept.

8. First, consider what results from analyzing all of the data with a two-way ANOVA with replication. This analysis (illustrated earlier in this chapter) is appropriate when there are no fixed sites. In this case, a different set of sites would have been sampled after management in both the control and management areas. This is an inefficient study design. However, the reader may want to try computing the two-way ANOVA for these data. Results are:

Interaction SS = 689.063 (1 df)

Error SS = 35649.3 (12 df)

$$\text{F-ratio testing management effect}_{(1,12\ df)} = \frac{\text{Interaction MS}}{\text{Error MS}} = 0.23$$

In such a study design, the management effect is measured by the classical interaction term, expressed here as:

$$(\bar{X}_{TA} - \bar{X}_{TB}) - (\bar{X}_{CA} - \bar{X}_{CB})$$
$$= (197.30 - 147.80) - (171.75 - 148.50)$$
$$= 49.50 - 23.25$$
$$= 26.25$$

This is the same as the treatment effect previously computed. But, in a completely random two-way design (no fixed sites over time), the variance of this effect is based on the average within-site error mean square:

$$\text{se(treatment effect)} = \sqrt{(\text{Error MS})\ \frac{4}{r}}$$

where r = the number of replicate samples at each time, within each area (control or treatment). For this example, r = 4, and the t-test for a treatment effect is:

$$t = \frac{26.25}{54.50} = 0.4816 \quad (12 \text{ df})$$

It is an algebraic identity that the square of this t-test value equals the F-test value for testing interaction (i.e., in this case, $0.4816^2 = 0.23$).

Fixed Sites Combined with Paired Control-Managed Sites

The previous study design can be improved by pairing data for control and treatment sites. This type of pairing was not done in the above example, where pre- and postmanagement measurements on the same site were paired, because the sites were fixed over time. Pairs of fixed sites are selected to implement the more efficient study design. Paired sites should be in the same habitat type and near each other. Assume that there are n such pairs. The power of this study design is that each control-management pair results in a direct estimate of the management effect. If the previous example had been designed and tested this way, the data might look like (Note: to illustrate a point, these values are not the same as those used in the above example):

	Premanagement		Postmanagement		Management
Site pair	Control	Managed	Control	Managed	effect
1	100	80	111	133	42
2	132	121	162	176	25
3	157	165	217	244	19
4	205	225	194	245	31
Means	148.5	147.8	171.0	199.5	29.25
standard deviations	44.30	49.94	45.92	54.85	9.81

Each treatment effect is computed as:

$$\left[\begin{pmatrix}\text{managed}\\ \text{after}\end{pmatrix} - \begin{pmatrix}\text{control}\\ \text{after}\end{pmatrix}\right] - \left[\begin{pmatrix}\text{managed}\\ \text{before}\end{pmatrix} - \begin{pmatrix}\text{control}\\ \text{before}\end{pmatrix}\right]$$

For example, the calculation for the first pair is:

$(133-111) - (80-100) = 22 - (-20) = 22 + 20 = 42$

1. H_o: the average management effect = 0.

   H_a: the average management effect > 0.

   Sometimes the alternative hypothesis is 2-sided, but it is usually one-sided when the treatment is a deliberate management action to achieve some goal.

   A t-test (n-1 df) is used to test the H_o:

$$t = \frac{\text{average treatment effect}}{\text{se(average treatment effect)}}$$

$$\text{se(average treatment effect)} = \frac{\text{standard deviation of the treatment effect}}{\sqrt{n}}$$

$$= \frac{9.81}{\sqrt{4}} = 4.905$$

df = 3

$$t = \frac{29.25}{4.905} = 5.963.$$

For a one-sided test and an α-level of 0.01, $t_{0.01,3} = 4.541$. Therefore, the H_o is rejected, and the conclusion is that the management actions increased the number of catchable fish.

2. This result can be compared to the result obtained when the same data are analyzed as if the sites were fixed, but where no pairing of control and treatment sites was done. A t-test [2(n-1) = 6 df] is used, based on the sets of before and after differences (as explained in the preceding example):

site	Control differences	Managed differences
1	11	53
2	30	55
3	60	79
4	-11	20
$\bar{X}$ =	22.5	51.75
standard deviation =	30.09	24.24

The t-test statistic is:

$$t = \frac{51.75 - 22.5}{se}$$

$$se = \sqrt{\frac{3(30.09)^2 + 3(24.24)^2}{6}\left(\frac{1}{4} + \frac{1}{4}\right)}$$

$$= 19.32$$

$$t = \frac{29.25}{19.32} = 1.514.$$

For $\alpha = 0.05$, the one-sided critical value of $t_{0.05,6} = 1.943$. Therefore, the null hypothesis is not rejected. The failure to reject the null hypothesis is due to the inefficient study design. When possible, fixed sites with paired control-managed sites and before and after management measurements is the best study design (there should be at least four replicate pairs).

Regression Analysis[13]

The most common use of regression analysis in the context of fisheries studies is to relate fish weight to length. The relationship of weight to length is $E(W) = \mu L^b$, where L = fish length, W = fish weight, and E(W) = expected, or average, weight for the given length. Transforming the data to logs produces a linear regression problem:

$$\log(W) = a + b(\log L) + \varepsilon$$

where ($a = \log \mu$)

b = the slope of the line

ε = the uncertainty about the line

[13]When regression analysis is used to compare data, X values are for the independent variable and values of Y are random variables (dependent variables).

The average value of $(\varepsilon)^2$ is the "residual mean square error;" it is analogous to the error mean square in analysis of variance methods. Note that given estimates of the parameters a and b, the weight can be predicted given the length by the equation $W = \mu L^b$, where $\mu = e^a$.

The use of linear regression analysis can be illustrated with data from the study of Keller and Burnham (1982). In their sampling site "3U", 19 brook trout were captured by electrofishing, using two passes. Virtually all of the brook trout present were caught. The fish weights in grams and lengths in millimeters, the logs of these values, and the products of Y times X are presented below:

i	W	L	Y = log(W)	X = log(L)	YX
1	8	86	2.0794	4.4543	9.2623
2	10	97	2.3026	4.5747	10.5337
3	7	90	1.9459	4.4998	8.7562
4	10	95	2.3026	4.5539	10.4858
5	10	91	2.3026	4.5109	10.3868
6	9	102	2.1972	4.6250	10.1621
7	10	102	2.3026	4.6250	10.6500
8	18	116	2.8904	4.7536	13.7398
9	15	117	2.7081	4.7622	12.8965
10	17	119	2.8332	4.7791	13.5401
11	18	116	2.8904	4.7536	13.7398
12	15	114	2.7081	4.7362	12.8261
13	13	110	2.5649	4.7005	12.0563
14	58	171	4.0604	5.1417	20.8774
15	58	171	4.0604	5.1417	20.8774
16	49	170	3.8918	5.1358	19.9875
17	72	190	4.2767	5.2470	22.4398
18	83	206	4.4188	5.3279	23.5429
19	94	210	4.5433	5.3471	24.2935
		totals	57.2794	91.6700	281.0540
		means	3.0147	4.8247	-
		s^2	0.7792	0.0888	-

To compute a simple linear regression, tabulate Y, X, and YX and then compute the sum of the products YX; the means of Y and X; and the standard deviation s_Y^2 and s_X^2 of the Y and X variables. Most recently developed scientific calculators compute regression slopes and correlations automatically, once the basic X,Y data are entered.

Five basic items are required to compute linear regressions. The items needed in addition to the means X, Y, are:

$$SP = \sum_{i=1}^{n} (X_i - \bar{X})(Y_i - \bar{Y}) \qquad \text{(a sum of products)}$$

$$= \sum_{i=1}^{n} X_i Y_i - n\bar{X}\bar{Y}$$

$$SS_X = \sum_{i=1}^{n} (X_i - \bar{X})^2 \equiv (n-1)s_X^2 \qquad \text{(a sum of squares)}$$

$$SS_Y = \sum_{i=1}^{n} (Y_i - \bar{Y})^2 \equiv (n-1)s_Y^2$$

The only new quantity needed is the sum of the cross products, SP. It is computed by first summing all XY terms; 281.0540 in this example. Then subtract $n\bar{X}\bar{Y}$:

$$SP = 281.0540 - 19(3.0147)(4.8247)$$

$$= 281.0540 - 276.3554$$

$$= 4.6986$$

$$SS_Y = (n-1)s_Y^2 = 18(0.7792) = 14.0256$$

$$SS_X = 18(0.0888) = 1.5984$$

Given these statistics, the regression results can be computed.

1. Compute the regression coefficient, $\hat{b}$:

$$\hat{b} = \frac{SP}{SS_X}$$

$$= \frac{4.6986}{1.5984}$$

$$= 2.9396$$

2. Compute the Y-intercept, $\hat{a}$:

$$\hat{a} = \bar{Y} - \hat{b}\,\bar{X}$$

$$= 3.0147 - (2.9396)(4.8247)$$

$$= -11.1680$$

In this example, the equation for the regression line is:

$$\log(\hat{W}) = -11.168 + 2.9396[\log(L)]$$

To compute a predicted weight, insert log(L).

For example, if L = 120,

$$\log(\hat{W}) = -11.168 + 2.9396(4.7875)$$

$$= -11.168 + 14.0733$$

$$= 2.9053$$

Taking the antilog, $\hat{W} = e^{2.9053} = 18.3$ grams.

This calculation can be very useful when not all the fish at a site are both weighed and measured for length, because fish weights can be reliably predicted from length measurements.

3. Compute the correlation coefficient, r:

$$r = \frac{SP}{\sqrt{(SS_X)(SS_Y)}}$$

$$= \frac{4.6986}{\sqrt{(1.5984)(14.0256)}}$$

$$= \frac{4.6986}{4.7348} = 0.9924$$

The value of r is always between ±1. The closer r is to either of the extremes (+1 or -1), the better the linear relationship of the variables. In this example, r = 0.9924, indicating a nearly perfect linear relationship of log(W) and log(L). An r value of 0 indicates that no correlation exists; therefore, Y cannot be predicted from X.

The slope estimate, $\hat{b}$, and r are closely related:

$$\hat{b} = r \frac{s_Y}{s_X}$$

Because the standard deviations s_Y and s_X are not zero, testing the null hypothesis that the true b = 0 is equivalent to testing H_o : E(r) = 0 (i.e., the true correlation of Y and X is zero).

4. Compute the standard error of $\hat{b}$. The variance of $\hat{b}$ is:

$$\text{var}(\hat{b}) = \frac{s_Y^2(1-r^2)}{SS_X} .$$

In this example, $s_Y^2 = 0.7792$, r = 0.9924, and $SS_X = 1.5984$. Therefore:

$$\text{var}(\hat{b}) = \frac{(0.7792)(1-(0.9924)^2)}{1.5984}$$

$$= 0.007381$$

$$\text{se}(\hat{b}) = \sqrt{\text{var}(b)} = \sqrt{0.007381}$$

$$= 0.0859$$

The degrees of freedom associated with the standard error are n-2 because two parameters are estimated from the data (the intercept and slope). The numerator of $\text{var}(\hat{b})$, i.e., $s_Y^2(1-r^2)$, is the residual variance about the line. It can also be computed as:

$$\frac{\sum_{i=1}^{n} (Y_i - \hat{Y}_i)^2}{n - 2} = s_Y^2(1-r^2)$$

where $\hat{Y}_i = \hat{a} + \hat{b} X_i$. This equation is not as convenient a computation, but more clearly shows the nature of the residual variance and the fact that computing the residual variance first requires the estimation of the two parameters.

5. Test $H_o : b = 0$ vs. $H_a : b \neq 0$. A t-test is used; it has n-2 df:

$$t = \frac{\hat{b}}{\text{se}(\hat{b})} .$$

In this example, assume an $\alpha = 0.01$. The critical level of the test is (a two-sided test):

$$t_{\alpha,n-2} = t_{0.01,17} = 2.567$$

The computed t-value is:

$$t = \frac{2.9396}{0.0859} = 34.22$$

H_o is rejected, and the conclusion is that there is a highly significant relationship between X (length) and Y (weight).

6. A confidence interval on $\hat{b}$ is more appropriate than a test of H_o for fish length-weight data. The $1-\alpha$ confidence interval is:

$$\hat{b} - t_{\alpha,n-2}\, se(\hat{b}) = \text{lower limit}$$
$$\hat{b} + t_{\alpha,n-2}\, se(\hat{b}) = \text{upper limit}$$

Assume $\alpha = 0.05$. The n of $t_{0.05,17} = 2.110$. The lower limit is:

$$2.9396 - 2.110\ (0.0859) = 2.758$$

The 95% confidence interval on b is thus $2.758 < b < 3.121$.

7. The confidence limits for a predicted (estimated) value of $\hat{Y}$ for a given X value can also be calculated. The standard error, $s_{Y|X}$, of $\hat{Y}$, given X, is needed:

$$s_{Y|X} = \sqrt{s_Y^2(1-r^2)}\ \sqrt{\frac{1}{n} + \frac{(X - \bar{X})^2}{SS_X}}$$

In the above formula, all calculations are based on the sampled data, except X, which is specified.

Predict average fish weight at length L = 200 mm:

$$X = \ln(200) = 5.298$$

$$\hat{Y} = -11.168 + 2.9396(5.298)$$

$$= 4.406$$

$$W = e^{4.406} = 81.94 \text{ grams.}$$

The standard error of $\hat{Y}$ is:

$$s_{Y|X} = (0.1086)\sqrt{\frac{1}{19} + \frac{(X - 4.8247)^2}{1.5984}}$$

In this example, X = 5.298. Therefore:

$$s_{Y|X} = (0.1086)\sqrt{\frac{1}{19} + 0.140148}$$

$$= 0.04768$$

The standard error has n-2 df [it basically depends on $s_Y^2(1-r^2)$, which has n-2 df]. For a 95% confidence interval on the true expected value of Y at X = 5.298, use:

$$Y \pm t_{0.05,n-2}\,(s_{Y|X}).$$

In this example, the calculation is:

$$4.406 \pm (2.110)(0.04768) = 4.3054 \text{ to } 4.5066.$$

Taking antilogs, the 95% confidence interval on average fish weight at a length of 200 mm is 74.1 to 90.6 gm.

When confidence limits are calculated for the dependent variable (Y), the estimates are more accurate for X values that are close to the sample mean $\overline{X}$ (Figure 14).

8. When there is more than one sample site, such as control and treatment sites or different habitat types, the correct analysis is an analysis of covariance. This method allows testing equality of regression lines for several sites (Sokal and Rohlf 1969). A simple approach for visually comparing results is to plot actual length-weight data on log-log paper. Plots of each data set will be patterned in a straight line. Plotting is also useful when there is just one data set in order to determine if there are any nonconforming data points.

9. Nonparametric tests for the association of continuous variables are also available; e.g., Spearman's or Kendall's coefficient of rank correlation tests and Olmstead and Tukey's corner test for association. These methods are discussed in Sokal and Rohlf (1969).

Contingency Table

Problem: The following relative abundance of trout and nontrout fish was found after management activities (pre-management data showed no differences in control and to-be-managed sites) in a stream monitoring study:

Site	Trout	Nontrout
Control	34	65
Managed	41	59

The chi-square (χ^2) nonparametric test (Sokal and Rohlf 1969) is used to test if the relative abundance of trout and nontrout fish is related to management activities.

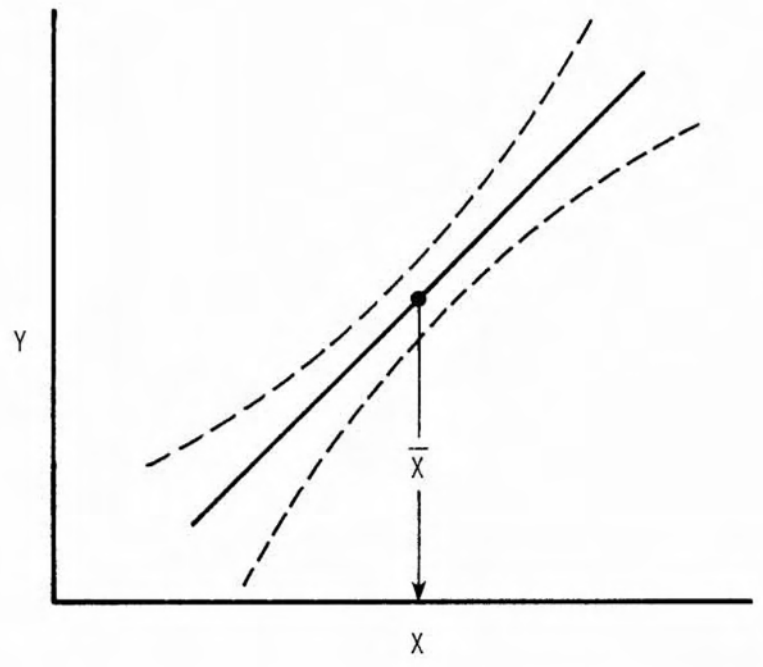


Figure 14. Confidence limits for values of Y given values of X (the curved lines). The interval widens as values of X deviate from the sample mean, $\overline{X}$.

Solution:

1. Null hypothesis: the relative abundance of trout and nontrout fish is unrelated to management activities.

2. Arrange the data for a two-way contingency test:

a	b	a + b
c	d	c + d
a + c	b + d	n

In this example:

34	65	100
41	59	100
75	125	200

3. Calculate x^2

$$x^2 = \frac{(ad - bc)^2 \; n}{(a + b)(c + d)(a + c)(b + d)}$$

$$x^2 = \frac{(34 \times 59 - 65 \times 41)^2 \; 200}{(100)(100)(75)(125)}$$

$$x^2 = 0.926$$

4. From a chi-square distribution table, the critical value for chi-square with one degree of freedom [df = (r-1)(c-1); r = rows and c = columns] and $\alpha = 0.05$ is 3.84.

5. Because the value of the x^2 test statistic (0.926) $\leq$ critical x^2 (3.84), the null hypothesis is not rejected. The conclusion is that management did not increase the relative abundance of trout.

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APPENDIX A. COMMON CONVERSIONS OF ENGLISH UNITS OF MEASUREMENT TO THEIR METRIC EQUIVALENTS

English units	Metric units
1 inch	2.54 cm
1 foot	30.48 cm
1 cfs	0.028 m^3/sec
°F = (C° x 1.7985) + 32°	°C = (°F - 32°) x 0.556
1 lb	453.592 g
1 gal	3.785 l
1 acre-foot	1233.49 m^3
1 acre-foot	1,233,342.25 l

Appendix B. Critical values for the Wilcoxon signed rank test[a]

N = 5(1)50

One-sided	Two-sided	N = 5	N = 6	N = 7	N = 8	N = 9	N = 10	N = 11	N = 12	N = 13	N = 14	N = 15	N = 16
P = .05	P = .10	1	2	4	6	8	11	14	17	21	26	30	36
P = .025	P = .05		1	2	4	6	8	11	14	17	21	25	30
P = .01	P = 0.2			0	2	3	5	7	10	13	16	20	24
P = .005	P = .01				0	2	3	5	7	10	13	16	19

One-sided	Two-sided	N = 17	N = 18	N = 19	N = 20	N = 21	N = 22	N = 23	N = 24	N = 25	N = 26	N = 27	N = 28
P = .05	P = .10	41	47	54	60	68	75	83	92	101	110	120	130
P = .025	P = .05	35	40	46	52	59	66	73	81	90	98	107	117
P = .01	P = .02	28	33	38	43	49	56	62	69	77	85	93	102
P = .005	P = .01	23	28	32	37	43	49	55	61	68	76	84	92

One-sided	Two-sided	N = 29	N = 30	N = 31	N = 32	N = 33	N = 34	N = 35	N = 36	N = 37	N = 38	N = 39
P = .05	P = .10	141	152	163	175	188	201	214	228	242	256	271
P = .025	P = .05	127	137	148	159	171	183	195	208	222	235	250
P = .01	P = .02	111	120	130	141	151	162	174	186	198	211	224
P = .005	P = .01	100	109	118	128	138	149	160	171	183	195	208

One-sided	Two-sided	N = 40	N = 41	N = 42	N = 43	N = 44	N = 45	N = 46	N = 47	N = 48	N = 49	N = 50
P = .05	P = .10	287	303	319	336	353	371	389	408	427	446	466
P = .025	P = .05	284	279	295	311	327	344	361	379	397	415	434
P = .01	P = .02	238	252	267	281	297	313	329	345	362	380	398
P = .005	P = .01	221	234	248	262	277	292	307	323	339	356	373

[a] From Wilconxon, F., and R. A. Wilcox. 1964. Some rapid approximate statistical procedures. Lederle Laboratories, Pearl River, New York. 60 pp.

APPENDIX C. TUKEY'S TEST FOR ADDITIVITY
(SOKAL AND ROHLF 1969)

Data from the example for the ANOVA test on Page 159 (refer also to page 162).

	Period j						Row	Row	
Site i	1	2	3	4	5	6	sums	means	dr_i
1	15	20	20	25	30	30	140	23.333	-5.566
2	35	35	40	40	45	55	250	41.667	12.778
3	15	15	20	25	25	30	130	21.667	-7.222
Column sums	65	70	80	90	100	115	520		
Column means	21.667	23.333	26.667	30	33.333	38.333	GM = 28.889		
dc_j	-7.222	-5.566	-2.222	1.111	4.444	9.444			

In the example, GM = the grand mean; i.e., the average of all observations (3 • 6 = 18, in this example). A set of differences is computed next:

dc_j = column mean j - GM

dr_i = row mean i - GM.

For example,

$dc_1 = 21.667 - 28.889 = -7.222$

$dc_6 = 38.333 - 28.889 = 9.444$

$dr_2 = 41.667 - 28.889 = 12.778$

Another table is prepared as an intermediate step to computing the sum of squares (1 df) for nonadditivity. In the above table, let X_{ij} be the response value at site (row) i and period (column) j; e.g., $X_{11} = 15$ and $X_{25} = 45$. The main entries in the intermediate table are the products $Y_{ij} = X_{ij}\ dr_i\ dc_j$. It is useful to also tabulate $(dr_i)^2$ and $(dc_j)^2$:

Site i	Period j 1	2	3	4	5	6	$(dr_i)^2$
1	601.88	617.38	246.91	-154.32	-740.73	-1574.13	30.869
2	-3229.90	-2484.81	-1135.71	567.85	2555.34	6637.15	163.277
3	782.36	601.88	320.95	-200.59	-802.36	-2046.14	52.157
$(dc_j)^2$	52.157	30.869	4.937	1.234	19.749	89.189	

Element $Y_{1,1}$, which is 601.88 in the above table, is computed as:

$$Y_{1,1} = 601.88 = 15(-5.556)(-7.222)$$

Similarily, element $Y_{2,6}$ (i = 2, j = 6) is:

$$Y_{2,6} = 6637.15 = 55(9.444)(12.778)$$

Compute three sums from the above table:

$Q = \Sigma\Sigma Y_{ij}$ = the sum of all main elements in the table

$R = \Sigma(dr_i)^2$ = the sum of the squared values of the dr_i values

$C = \Sigma(dc_j)^2$ = the sum of the squared values of the dc_j values

Many calculators can accumulate these sums directly from the original table, without recording the intermediate values. However, producing the intermediate table is a useful check for errors.

In the above example:

$$Q = 601.88 + 617.38 + \ldots - 802.36 - 2046.14$$

$$= 563.01$$

$$R = 30.869 + 163.277 + 52.157 = 246.303$$

$$C = 52.157 + 30.869 + \ldots + 89.189 = 198.135$$

The sum of squares for nonadditivity is:

$$SS_{\text{nonadditivity}} = Q^2/(RC)$$

$$= \frac{(563.01)^2}{(246.303)(198.135)}$$

$$= 6.4953$$

$$= 6.5$$

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U.S. Fish and Wildlife Service
Creekside One Building
2627 Redwing Road
Fort Collins, CO 80526-2899

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316 East Myrtle
Boise, ID 83702

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This publication contains information pertaining to monitoring programs which may be used to evaluate the effects of land use practices on small salmonid streams in the Western United States. Information includes an approach for designing a monitoring program, variables which may be used with field measurement techniques, and statistical tests for evaluating data.

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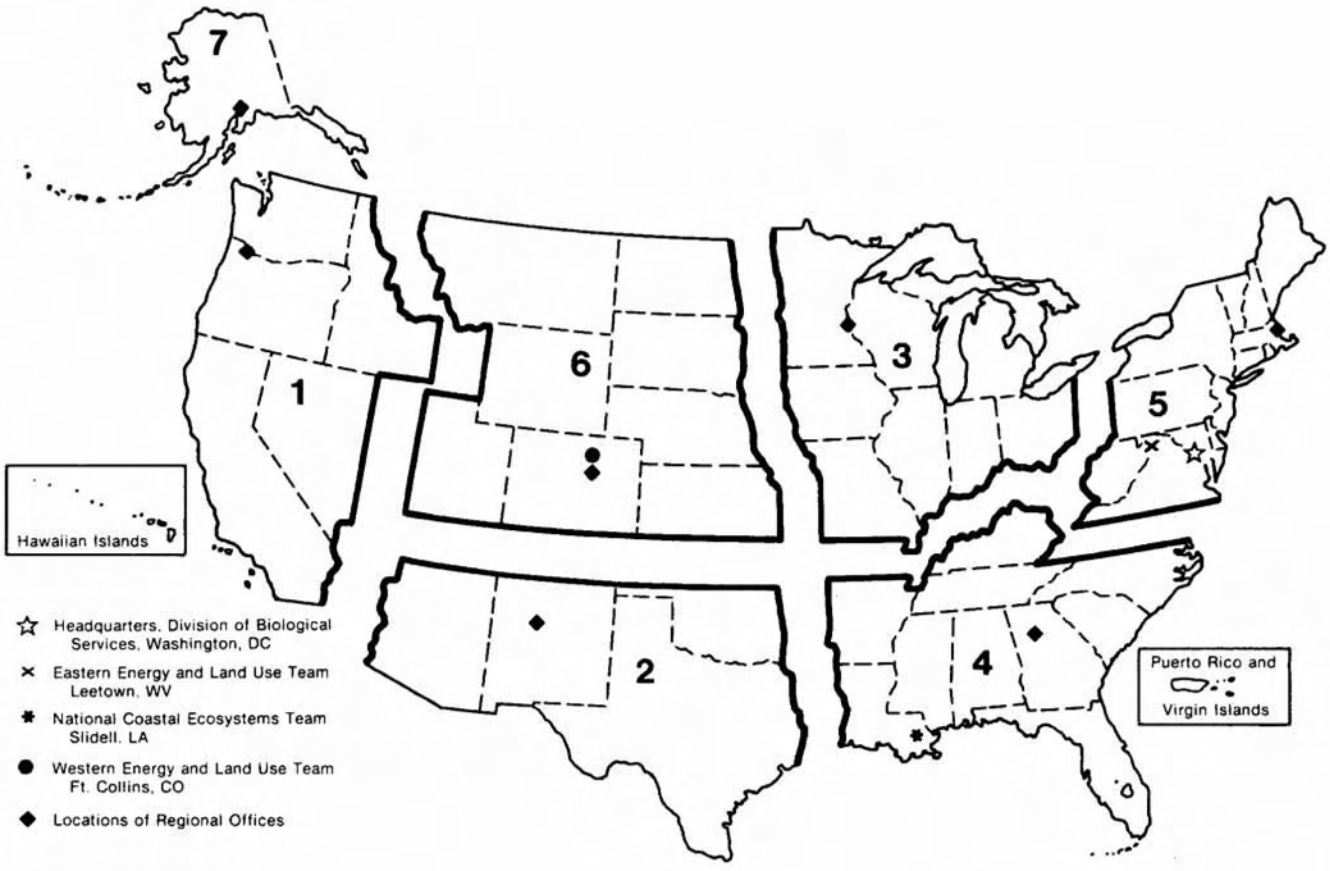

REGION 1
Regional Director
U.S. Fish and Wildlife Service
Lloyd Five Hundred Building, Suite 1692
500 N.E. Multnomah Street
Portland, Oregon 97232

REGION 2
Regional Director
U.S. Fish and Wildlife Service
P.O. Box 1306
Albuquerque, New Mexico 87103

REGION 3
Regional Director
U.S. Fish and Wildlife Service
Federal Building, Fort Snelling
Twin Cities, Minnesota 55111

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Regional Director
U.S. Fish and Wildlife Service
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75 Spring Street, S.W.
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REGION 5
Regional Director
U.S. Fish and Wildlife Service
One Gateway Center
Newton Corner, Massachusetts 02158

REGION 6
Regional Director
U.S. Fish and Wildlife Service
P.O. Box 25486
Denver Federal Center
Denver, Colorado 80225

REGION 7
Regional Director
U.S. Fish and Wildlife Service
1011 E. Tudor Road
Anchorage, Alaska 99503

DEPARTMENT OF THE INTERIOR

U.S. FISH AND WILDLIFE SERVICE

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering the wisest use of our land and water resources, protecting our fish and wildlife, preserving the environmental and cultural values of our national parks and historical places, and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to assure that their development is in the best interests of all our people. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

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