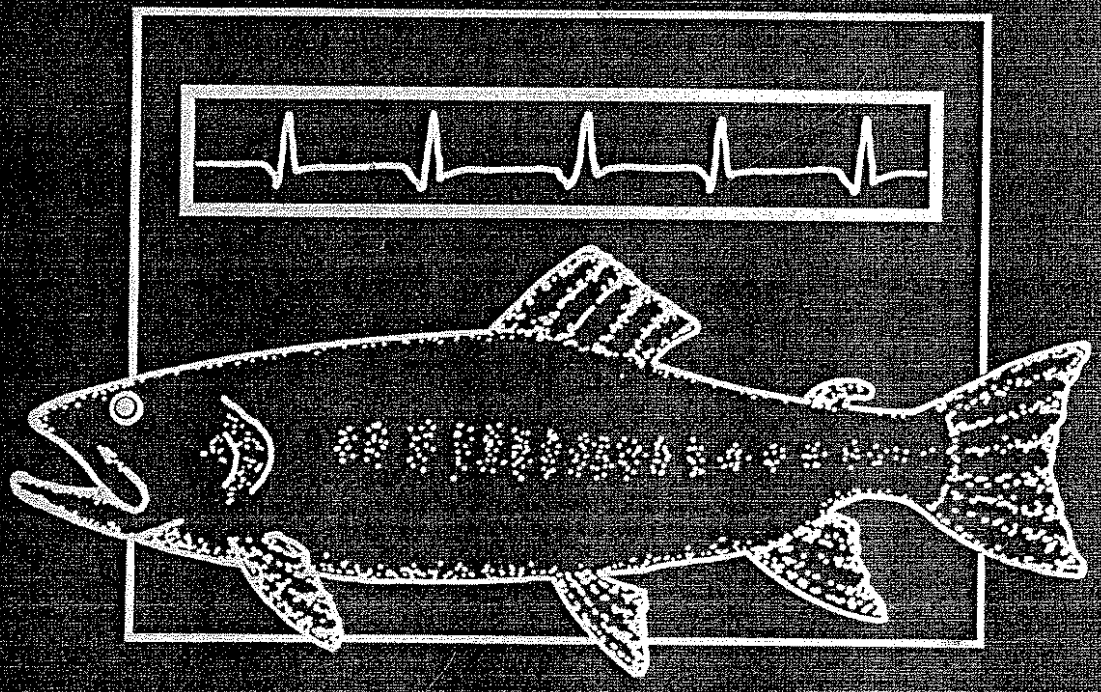
METHODS FOR FISH BIOLOGY



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Methods for Fish Biology

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Chapter 11

Growth

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11.1 PERSPECTIVES OF GROWTH

Growth may be viewed as an ongoing process or as something that has occurred in the past history of animals. The concept of growth implies many things and is too frequently presented in a misleading way. This imprecision can lead to misinterpretation of observations or experimental results.

Growth may be positive or negative, temporary or long-lasting. It may occur at all levels of biological organization: cells, tissues, organs, whole organisms, populations, communities. Depending on the level of organization, growth can be measured in terms of number, linear dimension, weight, volume, energy content, or the amount of a specific component such as protein. Furthermore, growth can be measured as an incremental change or as a rate of change. Finally, a variety of indices have been developed, such as glycine uptake by scales or RNA:DNA ratios, that are related to growth but in themselves are not direct measurements of growth. Thus, a precise universal definition of growth is precluded by the variety of processes that can be considered as growth and the variety of measurements that can be applied. However, it is imperative that growth be clearly defined within the context of specific investigations and that the method of measurement be appropriate for that definition.

For the purposes of this chapter, growth is defined as any change in size or amount of body material, regardless of whether that change is positive or negative, temporary or long-lasting. In the following sections, we examine techniques that are used in the study of growth and provide guidance to the appropriate uses of those methods.

11.2 NUMERICAL AND MATHEMATICAL EXPRESSIONS OF GROWTH

Numerical expressions of whole-body growth of fish may be based on absolute changes in length or weight (absolute growth) or changes in length or weight relative to the size of the fish being considered (relative growth). Measurements of growth expressed in terms of some interval of time (day, month, year) constitute a growth rate (Ricker 1979). For example, if t_1 is the time at the beginning of an interval and t_2 the time at the end, and if Y_1 and Y_2 are the respective fish sizes at those times,

$$\text{absolute growth} = Y_2 - Y_1;$$

$$\text{absolute growth rate} = (Y_2 - Y_1)/(t_2 - t_1);$$

relative growth = $(Y_2 - Y_1)/Y_1$; and

relative growth rate = $(Y_2 - Y_1)/[Y_1(t_2 - t_1)]$.

Relative growth and relative growth rate frequently are multiplied by 100 and expressed as percentages. These calculations imply a linear growth rate over the time interval of interest.

When growth rate is exponential, as it usually is over intervals of a year or less, growth should be expressed as an instantaneous rate. The instantaneous growth rate *(G)* is defined as the difference between the natural logarithms of successive sizes over a unit of time. This measurement has also been called the specific, intrinsic, exponential, or logarithmic growth rate. The working formula is

$$G = (\log_e Y_2 - \log_e Y_1)/(t_2 - t_1).$$

Instantaneous growth rate also is frequently multiplied by 100 and expressed as a percentage; then, it is more commonly called the specific growth rate.

Ricker (1975, 1979) provided detailed descriptions of these numerical expressions.

11.3 QUANTIFICATION OF CHANGES IN SIZE

11.3.1 Length

Measurements of body length give direct evidence for growth or lack of growth. Increases in length generally are retained, though a fish might shrink somewhat during starvation. Body length can be measured in many ways, although total length, fork length, and standard length are used most commonly for fish. Methods were described in depth by Ricker (1979), Anderson and Gutreuter (1983), and Weatherly and Gill (1986). Length can be easily and inexpensively measured in the field or laboratory, on live or preserved fish. Changes in length commonly occur with preservation but these changes are quantifiable and cease with time (Anderson and Gutreuter 1983).

11.3.2 Weight

Change in weight (mass) is probably the most commonly used assessment of whole-body growth of fish as well as of suborganismal growth. Weight also is the traditional measure for estimates of production—the elaboration of a group's or population's biomass—which are of interest to ecologists and resource managers.

Weight is relatively easy to measure and whole animals can be weighed without killing them. Scales and balances for weighing quantities in excess of 1 g generally are neither sophisticated nor expensive. Electronic balances that can weigh very small quantities or that can be used in an unsteady environment such as on a boat are costly by comparison.

Frequently, length and weight of a fish are both measured. One may be calculated from the other, though with statistical error, if the population's length–weight relationship is known. With small numbers of fish, it is more precise to measure weight as well as length to estimate growth rates. With large numbers of fish, it may be as accurate to measure only length and to convert it to weight. The length–weight relationship is generally expressed by the equation

$$W = aL^n;$$

W = weight, L = length, a is a constant, and n is an exponent. Values of n are often near 3.0 and generally fall between 2.5 and 3.5. Values outside of this range are generally erroneous (Lagler 1956; Carlander 1969, 1977; Weatherly 1972; Ricker 1975, 1979; Pauly 1984). Interspecific or interstock comparisons should not be based on values of a unless values of n are the same for the groups under consideration (Pauly 1984). Also, weights determined by calculation are generalized and cannot be used for determinations of condition factor or for intraspecific comparisons (Lagler 1956; Weatherly 1972; Ricker 1975; Pauly 1984).

As a fish grows, changes in weight are relatively greater than changes in length, due to the approximately cubic relationship between fish length and weight. Thus, measurement of change in weight may provide greater precision over short periods of time.

In contrast to a change in length, a change in weight may be a very transient indicator of growth, so weight may not be a suitable measure in some growth studies. This is particularly true if wet weight is measured. A change in water content generally is not considered to be growth, but if a fish gains water because its water balance mechanisms fail, its weight will increase even though it has not added new body tissue. Weight can also increase due to change in body lipids, which may or may not be considered as growth. Weight varies with development of the gonads and with fullness of the stomach. If the purpose of the study allows, these problems can be surmounted by measuring dry weight or changes in mass of specific body components such as protein, or by eliminating the weight of gonads or stomach contents from the measurement.

11.3.2.1 Wet Weight

The simplest way to assess changes in weight is to periodically weigh the animals of interest; the individuals may represent a sample from a large population. If variations in individual weights are not of interest, the sampled fish can be weighed en masse and an average individual weight can be computed. These values are compared with weights determined previously to calculate growth or growth rates. Obvious sources of error in measurements of wet weight include retention of excess water on the surface or in the buccal cavity of live fish and dehydration of dead fish. Balances of appropriate sensitivity should be used. Anesthesia can quiet agitated fish during weighing, but its use may not be desirable or practical (see Chapter 8). Live fish may be less stressed if they are weighed in water; the weight of the water and container (tare) are subtracted from the total. Wet weights of tissue and organ samples also may be determined, but dehydration may be a problem if samples are stored in areas of low relative humidity and if they are not weighed quickly.

11.3.2.2 Dry Weight

When a whole fish or tissue sample is dried under reasonably benign conditions, it reaches an asymptotic or "constant" weight: the dry weight. The procedure removes internal water, which is a transient material (Snell and Biffen 1964; Dowgiallo 1975), and eliminates the errors due to excess external water or dehydration that plague wet-weight determinations. In most cases, dry weights

can be accepted if changes in weight between successive weighings are less than 0.1% (Snell and Biffen 1964). A temperature of 60°C for 24 h is usually adequate for samples of small size (0.5 g or less), but larger quantities may require higher temperatures or longer time periods. Bound water can be eliminated at 100–110°C, but volatile oils and lipids may also be lost. Oxidation reactions may either add weight to the sample or add to the apparent loss of volatile materials; these problems can be avoided if samples are dried to a constant weight at reduced pressure in a vacuum oven. At higher temperatures, large pieces of fresh material should be dried slowly at first, because rapid heating may burst steam pockets and cause loss of material. Freeze drying removes most of the water from tissue samples, but it is a slow process requiring special equipment, and up to 5% of the water is very difficult to remove (Crisp 1971). Dry weight is often expressed as a percentage of wet weight.

Tared weighing containers for dry weight determinations should be made of aluminum foil, platinum, ceramics, glass, or heat-resistant plastic that will not react with the samples (Snell and Biffen 1964). Most plastics will not tolerate 110°C without deforming.

11.3.2.3 Ash Weight

Ash weight, which may be the measurement least influenced by transient growth, is the weight of mineral residues left after organic materials have been burned away. Combustion temperatures are required for this measurement, and suitable sample containers are made of platinum, high-silica glass (Vycor, Pyrex, or equivalent), and some ceramics (Snell and Biffen 1964). Samples and containers are placed in a muffle furnace at 450–500°C for 30 min for samples of 0.1 g or less and up to 24 h or longer for larger quantities. Temperatures above 550°C may cause calcium carbonate to decompose and should be avoided (Dowgiallo 1975). Ash weight is determined by subtracting the tare weight. The difference between the dry weight and the ash weight is the amount of organic material lost to ignition, often called ash-free dry weight. Ash weight is often expressed as a percentage of dry weight or wet weight.

11.3.3 Proximate Analysis

Proximate analysis, the determination of categories of compounds in a mixture, is done when changes in absolute or relative amounts of body materials are of interest in defining the growth process. Information about the probable growth rate of an individual fish can be obtained from the composition of muscle or of the entire animal. Fish experiencing low energy intake have to use energy stored in the body tissue as lipid and protein, much of which is mobilized from the muscle tissue (Love 1980). After a time, the concentration of lipid and protein in the muscle tissue will decrease, as will the energy content (joules per gram, J/g). As these substances are removed from the tissues, much of their volume will be replaced by water. As a result, as shown by controlled laboratory studies, percent water content is inversely correlated with growth rate; conversely, the protein, lipid, and caloric contents of fish are strongly, and positively, correlated with growth rate (Brett et al. 1969; Elliot 1976). In natural populations of fish, tissue composition reflects the nutritional state of the individual (Parker and Vanstone 1966; Adams et al. 1982), and therefore is an indirect index of growth rate.

Materials most commonly measured under the heading of proximate analysis are (1) carbohydrates such as glucose, glycogen, and mucopolysaccharides; (2) proteins of molecular weights greater than 10,000; and (3) lipids. Often the material of interest must be physically separated from the others or broken down into its components to prevent analytical interferences (Snell and Biffen 1964; Dowgiallo 1975). For example, high levels of lipid interfere with the biuret protein reaction and usually must be extracted before the protein can be analyzed (Dowgiallo 1975).

Tissue for proximate analysis should, if possible, be removed from freshly killed animals and, if necessary, preserved for later analysis. However, preservation may present special problems. Samples should be processed for storage so they do not degrade and decompose. In the field situation, samples can be rapidly frozen in dry ice or liquid nitrogen, and stored for short periods therein. For long-term storage, samples should be kept in freezers capable of maintaining temperatures at −80°C. Freezers with automatic defrosting cycles are unsuitable for general long-term storage because their daily temperature variations cause water to translocate out of samples. At the normal temperature of common household freezers, −20°C, subcellular components may not freeze completely, allowing some localized biochemical reactions to continue. Cross-contamination can be prevented by wrapping samples individually. Even under proper storage conditions, tissues have limited shelf lives. Storage techniques can be examined by analyzing some samples at once, storing the rest by the chosen method, and removing some samples for analysis at regular intervals.

11.3.3.1 Protein Analysis

Change in protein content is considered a measure of sustainable growth, although body protein is readily used as a source of energy during starvation (Brett and Groves 1979). Tissue samples homogenized for protein analysis can be treated with trichloroacetic acid to quantitatively precipitate the protein and separate it from the soluble materials (Mendel and Hoogland 1950). The precipitates can then be subjected to short-term alkali digestion to denature the protein structure; this ensures that the amino acids important for colorimetric analysis are not masked by quaternary molecular structure (Peterson 1979).

There are three general colorimetric methods for protein analysis: Kjeldahl nitrogen method (Snell and Biffen 1964; Dowgiallo 1975), biuret reaction method (Gornall et al. 1949; Richterich 1969), and Lowry or Folin phenol method (Lowry et al. 1951; Richterich 1969; Peterson 1977, 1979). The Kjeldahl nitrogen method measures total protein nitrogen if protein has been isolated from other nitrogenous compounds, or total nitrogen if there has been no such separation. (Nitrogen is an integral part of the molecular structure of amino acids, and some amino acids have more than one nitrogen atom. Only amino acid nitrogen will be measured by the Kjeldahl process if proteins are isolated from other cellular and tissue constituents.) The biuret reaction attaches a color-developing substance to the free amino groups on polypeptide chains. The Lowry reaction depends upon the presence of the amino acids tyrosine and tryptophan for color development. None of these methods give results that are strictly proportional to the total quantity of amino acids because the proportion of amino acids that produce color varies from protein to protein. It is convenient to standardize the colorimetric method being

used by doing a single Kjeldahl nitrogen determination on a tissue (Dowgiallo 1975) so that others using the biuret or Lowry reaction on the same tissue can make more accurate comparisons.

Macro- or microprocedures are available for conducting Kjeldahl nitrogen analyses (Snell and Biffen 1964; Jacobs 1965). Samples are digested in sulfuric acid to convert organic nitrogen to ammonia, which is then held as ammonium sulfate. The solution is refluxed or distilled, and the acid-neutralized concentrations of ammonia are measured. The micromethod allows samples with as little as 2 mg total nitrogen in a 200-mg sample to be analyzed (Snell and Biffen 1964). For the macromethod, samples should contain 30–40 mg of nitrogen.

The Lowry reaction is more sensitive to false positive color interferences from several substances than is the biuret reaction. Bensadoun and Weinstein (1976), Cookson (1978), and Peterson (1979) listed interfering materials as well as methods for coping with them. Lipid extraction is generally not required if tissues have low lipid content, although it may be required for the biuret reaction. The Lowry reaction is more sensitive than the biuret method, giving good results with protein concentrations as low as 5–25 μg/mL; the biuret reaction is typically effective for concentrations of 0.5–3 mg/mL. Both techniques have been adapted for automated chemical analysis (Huemer and Lee 1970; Honn and Chavin 1975).

11.3.3.2 Lipids

Lipids may be very transient body materials, but they are an important source of potential chemical energy, and their presence or absence reflects the physiological capacity of fish. Lipids are readily separated from proteins, carbohydrates, and other cellular compounds by their solubility in nonpolar solvents such as ethyl ether, chloroform, methanol, and methylene chloride (Entenman 1957a). Total lipid content may be determined gravimetrically following extraction and evaporation of the solvent (Dowgiallo 1975). "Total lipids" also have been estimated by colorimetric procedures (Holland and Gabbott 1971; Barnes and Blackstock 1973). Lipid classes such as triglycerides and long-chain fatty acids can be assayed by enzymatic or colorimetric methods (Entenman 1957b; Holman 1957) and kits for these types of analysis are commercially available (e.g., Sigma kits 336 and 405; Sigma Chemical Co., St. Louis, Missouri). Fatty acids may be determined specifically by chromatography or generally by oxidative and other chemical procedures (Snell and Biffen 1964). In addition, lipid class analysis can be accomplished relatively easily with a system involving thin-layer chromatography and a flame ionization detector (Ackman 1980; Fraser et al. 1985). Proper sample storage is important to avoid decomposition and oxidation. Samples should not be dried with heat and are better stored frozen without exposure to oxygen.

11.3.3.3 Carbohydrates

Quantities of carbohydrates in fish are generally low and not typically analyzed in growth studies. Carbohydrate stores, particularly as liver and muscle glycogen, are important sources of energy for intense activity by fish, and may be of interest in certain investigations.

Most reactions for determining carbohydrates are colorimetric (Dische 1955; Ashwell 1957). Typical analytical procedures for carbohydrates first require homogenization of tissues, then fractionation of the slurry to simple hexoses and

pentoses, glycoproteins, glycogen, or total sugars. Hexoses and pentoses are soluble in alcohol and trichloroacetic acid. Glycogen can be precipitated with 80% alcohol, dried, and then reacted with phenol and concentrated H_2SO_4 (Montgomery 1957). Total sugars can be separated from protein by alkali digestion followed by acid hydrolysis and final testing for hexoses and pentoses (Dubois et al. 1956). Hexoses react with anthrone and H_2SO_4, pentoses react with orcinol–$FeCl_3$ (Dowgiallo 1975). Glucose may be determined by a simple enzyme-coupled colorimetric reaction (Blaedel and Uhl 1975) available in kits (e.g., Sigma kit 510). Glycogen can be estimated by the same procedure if the homogenate is first incubated with amyloglycosidase to convert glycogen to free glucose (Carr and Neff 1984; Gould et al. 1985). Glycoproteins alone are difficult to separate because of their diverse makeup and solubilities, which generally do not allow quantitative separation by precipitation techniques. They can be separated by column chromatography (Wold and Selset 1977; Uskova et al. 1981).

11.3.4 Calorimetric Analysis of Energy Content

Calorimetry measures the heat given off by various processes such as chemical reactions. It is a general analytical tool that may be used to determine energy contents of organic materials or the energy used for metabolism of living organisms. The heat given off by the combustion of samples is related to the total available energy in the sample. For biological samples, this heat is the theoretical energy available for work if the sample were to be completely metabolized and no losses occurred during the transformations. Calorimetry is important for bioenergetic determinations because it defines growth in terms of energy equivalents and allows partitioning of energy intake into anabolic and catabolic processes. Many bioenergetic models use energetic units of measurement because many dissimilar materials (protein, lipids, carbohydrates) and processes (ingestion, assimilation, growth, catabolism, etc.) can be described in terms of units of energy required or contained (Warren and Davis 1967). See Chapter 12 for more details on bioenergetics.

The energy contents of whole fish or their food, organs, and tissues can be measured by direct combustion in a calorimeter. In some cases, interference during combustion requires that sample components be separated before calorimetry. For example, large amounts of inorganic bone may prevent complete combustion of a sample in the short time necessary for accurate measurements. However, most of the problems of calorimetry are not in the actual measurements but making the appropriate decisions about what the resulting data actually mean (Brown 1969; Beezer 1980). In part this is because tissues are complex mixtures of carbohydrates, lipids, and proteins and the ratios of these constituents can vary dramatically according to the general physiological condition of the fish. Also, there is not necessarily a direct correspondence between energy content and physiological fitness. Therefore, caution must be exercised when judgments about an animal's physiological condition are based upon the results of calorimetry.

Metabolic energy of living organisms can be measured by direct calorimetry—whereby the heat produced by organisms is measured—or by indirect methods (Brown 1969; Prus 1975; Beezer 1980). Live fish are generally unsuitable for direct calorimetry because they produce heat at a low rate and the heat capacity of their aquatic medium is high (Brett and Groves 1979). Indirect calorimetry, which

involves measurement of respiratory gas production, is typically used to determine fish metabolism (Chapter 10). Energetic equivalents for oxygen uptake and carbon dioxide production by fish are based on the heat of combustion of different energy substrates such as proteins, lipids, or carbohydrates. These equivalents have been measured by direct calorimetry and values are available in the literature (Brett and Groves 1979; Nestle 1981). Biological data from calorimeters traditionally have been expressed as total kilocalories (kcal) per unit wet weight, dry weight, or ash-free dry weight. The conversion factor for calories to joules (J), the current international standard unit of energy, is 1 cal = 4.2 J (1 kcal = 4.2 kJ). Kilocalories are also equivalent to Calories (Cal).

11.4 PHYSIOLOGICAL AND BIOCHEMICAL INDICES OF GROWTH

The previously discussed methods of measuring growth require changes in a fish's size, amount of material, or energy content over time. Several methods have been developed to estimate the growth rate of a fish at the time it is sampled. These methods are largely indirect measurements of growth rate because they do not measure a change in size. They measure either a rate at which body tissue is elaborated, as is the case with protein synthesis, or something that is correlated with the rate of growth of the fish, such as the RNA:DNA ratio, glycine uptake by scales, or nutritional status. The value of some of these methods is in their ability to measure changes in growth rate over very short periods of time, particularly growth in response to changing environmental conditions. Other indices reflect the growth rate of the fish during preceding weeks or months.

Aside from the influence of growth rate, all the indices can be affected by internal and environmental variables such as species, sex, age, season, acclimation temperature, and reproductive state. Their value is greatest in studies for which the experimental design and sampling regime provide some control over these extraneous sources of variability.

11.4.1 Protein Synthesis

Because protein synthesis plays a major role in the growth of fish (Haschemeyer and Smith 1979; Haschemeyer et al. 1979), the rate of protein synthesis should be correlated with whole-body growth rate. Typical procedures for measuring protein synthesis involve administering a radioactive amino acid to the fish and subsequently analyzing the amount of radioactivity incorporated into proteins. In vivo methods include injection or infusion of the amino acid into the fish and subsequent analysis of its incorporation into protein, usually in muscle tissue if the study concerns growth rate (Haschemeyer and Smith 1979; Fauconneau et al. 1981; Smith 1981; Pocrnjic et al 1983). In vitro determinations of protein synthesis have measured amino acid incorporation into mitochondria of isolated liver cells and slices (Kent and Prosser 1980), into isolated hepatocytes (Saez et al. 1982), and into ribosomes isolated from muscle tissue (Lied et al. 1982).

The theoretical and methodological pitfalls in measuring protein synthesis are numerous and failure to follow appropriate procedures easily produces erroneous results (Haschemeyer 1973, 1976). The methodology is complex, time consuming, and not easily applied to large numbers of animals or tissue samples.

11.4.2 Glycine Uptake by Fish Scales

Based on traditional practices of measuring distances between annuli on fish scales to determine annual growth rates and the fundamental assumption that growth rate of the scale is proportional to growth rate of the fish, Ottaway and Simkiss (1977) proposed that the rate at which a fish scale takes up glycine in vitro would provide a relative index of the rate at which protein is synthesized in the scale. An assumption upon which the method is based is that living cells associated with scale formation remain viable after they are detached with the scale from the fish; thus, the rate of uptake of the amino acid reflects the growth rate of the fish.

The methodology involves removing one or more scales from a fish, incubating the scales for 2 h in physiological saline containing ^{14}C-glycine (glycine containing an atom of radioactive carbon-14), rinsing the scales in saline, drying and weighing the scales, then counting the radioactivity taken up by the scales after the scale is digested in a tissue solubilizer. Busacker and Adelman (1987) provided detailed methods. Radioactivity is usually expressed in terms of scale surface area.

Highly significant correlation coefficients between glycine uptake by scales and growth rate of individual fish have been found for the sea bass *Dicentrarchus labrax* (Ottaway and Simkiss 1979), for white suckers, yellow perch, bluegills, and common carp (Adelman 1980; Goolish and Adelman 1983a), and for largemouth bass (Smagula and Adelman 1982).

As with the more direct analysis of protein synthetic rate, glycine uptake by scales responds in the expected manner to growth-influencing factors such as temperature, meal size, low dissolved oxygen, and handling stress (see review by Adelman 1987). The methodology is nonsacrificial and is relatively easy to perform, and large sample sizes can be handled quickly. Acclimation temperature of the fish and temperature of the scale incubation medium influence glycine uptake, and these factors must be considered during the analysis and interpretation of results (Goolish and Adelman 1983b).

11.4.3 Nucleic Acids

The ratio of RNA (ribonucleic acid) to DNA (deoxyribonucleic acid) content of various tissues has been used as a measure of growth rate. Because DNA content is relatively constant within a cell and RNA content varies with rate of protein synthesis, the ratio of RNA to DNA provides an index of protein synthetic activity and growth (Bulow 1987). In addition to the RNA:DNA ratio, RNA content and concentration have also been used in growth studies. Both DNA content and DNA concentration in tissue samples have been used to indicate cell number and thus may be used to indicate growth in terms of cell number. The protein:DNA ratio has been used as a relative index of cell mass and thus may be used to indicate growth in cell size (Bulow 1987).

Of the various tissues that might be used for determining RNA:DNA ratios, white muscle is the best indicator of somatic growth (Bulow 1987). Red muscle is more active metabolically (Mustafa 1977b) and liver metabolic activities often are not directly related to growth (Emmersen and Emmersen 1976; Buckley et al. 1985). In larval fish it is possible to use the whole-body RNA:DNA ratio (Barron and Adelman 1984; Buckley 1984). Size, stage of maturation, temperature acclimation of the fish, contaminants, and species all affect the RNA:DNA ratio

(Mustafa 1977a; Lone and Ince 1983; Rosenlund et al. 1983; Barron and Adelman 1984; Buckley 1984; Goolish et al. 1984).

At present there is no standard technique for extraction and quantification of fish nucleic acids. Buckley and Bulow (1987) briefly reviewed analytical techniques for nucleic acids and made recommendations for analysis of larval, juvenile, and adult fish tissues. Typically, two different assays must be run following nucleic acid extraction from the tissue sample. The RNA can be quantified by the orcinol method of Schneider (1957) and DNA by the diphenylamine method (Burton 1956). The Schmidt–Thannhauser (1945) technique, as modified by Munro and Fleck (1966), has been used for both RNA and DNA in fish tissue (Buckley 1979; Barron and Adelman 1984). Fluorometric methods can offer greater sensitivity and ease of use (Buckley 1980; Rosenlund et al. 1983), but fluorometric spectrophotometers presently are not common laboratory instruments. Other methods are based upon detection of nucleic acids by ultraviolet and visible light spectrophotometers (Schmidt and Thannhauser 1945; Burton 1956; Schneider 1957; Bulow 1971; Buckley 1979).

11.4.4 Liver–Somatic Index

Fish store energy in muscle tissue, but they also accumulate energy in the liver during periods of high energy intake; much of this stored energy is in the form of glycogen. Therefore, the relative size of the liver should be correlated with the nutritional state of the fish and with the growth rate. This indirect measure of growth rate is referred to as the liver–somatic index (LSI) and is expressed as

$$\text{LSI} = (\text{liver weight/body weight}) \times 100.$$

This index is often used in studies of the seasonal and yearly changes in growth of natural populations of fish (Bulow et al. 1978; Adams and McLean 1985).

11.4.5 Condition Factor

During periods when fish have high energy intake, the growth of tissues and the storage of energy in muscle and liver can cause an individual to have a greater-than-usual weight at a particular length. This excess is usually revealed by the coefficient of condition (K) or by Fulton's condition factor (Bagenal and Tesch 1978; also see the exponent n in the length–weight equation, Section 11.3.2) and is calculated as

$$K = \text{weight}/(\text{length})^3.$$

The condition factor reflects the nutritional state or "well-being" of an individual fish, and, by the same reasoning as in Section 11.4.4, is sometimes interpreted as an index of growth rate.

11.5 ESTIMATION OF GROWTH IN NATURAL POPULATIONS

Methods for estimating growth of fish in natural populations fall into three general categories: length-frequency analysis of fish in identifiable cohorts (age-groups) that are followed through time; back-calculation of previous fish lengths

Table 11.1 Key for selection of options for methods of estimating growth of fish in natural populations.

1. Sample representative of the population 2
   Not representative 5

2. Fish can be aged accurately 3
   Cannot be aged accurately 4

3. Options
   A. Identify cohorts
      a. Determine numbers at age in sample
      b. Examine lengths at current age
      c. Growth rates
   B. Length-frequency analysis to confirm ages and to apportion length-classes to ages
   C. Back-calculations
      a. Identify cohorts at previous ages
      b. Estimate size at previous age
      c. Growth rates
   D. Mathematical models
      a. Growth rate
      b. Estimate of maximum size

4. Options
   A. Validate ages (may then be possible to go to choice 3)
      a. Mark and recapture
   B. Assign cohorts
      a. Length-frequency analysis to assign ages and to apportion length-classes to assigned ages
      b. Determine numbers at assigned ages in sample
      c. Examine lengths at assigned ages
      d. Calculate growth, assuming increments represent average growth
   C. Mathematical models
      a. Growth rate
      b. Estimate of maximum size

5. Sampling bias can be corrected 2
   Bias cannot be corrected 6

6. Recognize bias effect on final analysis 2
   Redesign study or abandon analysis

by reference to permanent growth records in hard body parts; and recapture of marked individuals whose size at a previous time is known. The success of all these methods depends strongly on proper sampling procedures.

11.5.1 Collection of Data

The method for estimating growth of wild fish in a natural population should be chosen after a consideration of possible biases in the sample. If the sample is not representative of the population, sampling biases could lead to erroneous conclusions about the true nature of the population. Technical problems, such as the ability to accurately identify cohorts, also must be considered. A decision key for considerations of this type is presented in Table 11.1.

Accurate and complete records of sampling procedures and data collection will help to eliminate subsequent uncertainties about the data base. Generalized archival requirements are given below; see Lagler (1956) for examples of data sheets. Items chosen from the list will vary with the intent of the study (Bagenal and Tesch 1978).

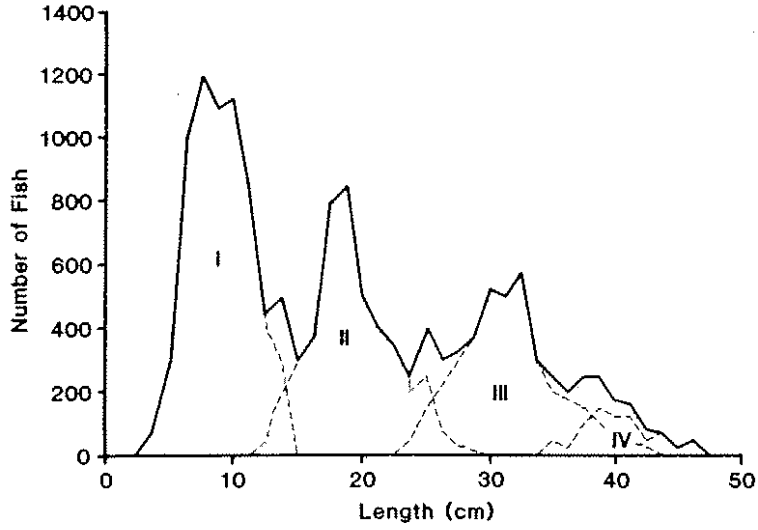

Figure 11.1 Frequency distribution of a hypothetical fish population showing numbers of fish versus fish length. The dashed lines indicate numbers at each age where there is overlap between age-classes. Age-classes are designated by Roman numerals.

(1) Record field conditions, date, place, and method of capture.
(2) Record—for each individual—length and weight, sex, gonadal maturity, and obvious deformities or pathological conditions.
(3) Collect scales or bony structures to be used for aging and store them in labeled coin envelopes.
(4) Complete laboratory measurements for each individual if fish are preserved.
(5) Begin numerical analysis using appropriate procedures.

11.5.2 Cohort Identification

Cohorts may be identified by grouping members of common length-classes, then using the modal lengths of these groupings to represent the lengths of those cohorts. Generally, these groupings will identify fish of common age-classes, but there will be some overlap (Figure 11.1). Cohorts can also be identified by mark-and-recapture methods—the marked group being the cohort—and by aging individual fish from annular marks on scales or other bony structures.

Cohorts identified in one sample may be followed over several years or over intervals shorter than 1 year. Growth or growth rates can be calculated with the equations in Section 11.2.

11.5.3 Growth Estimates

11.5.3.1 Length-Frequency Analysis

Lengths of fish are easily and quickly determined in field investigations and then are available for a preliminary examination of sample data. Because lengths of fish of a single age tend to form a normal frequency distribution, ages may be determined from a length-frequency distribution as in cohort analysis (Figure

11.1). However, cohort distributions increasingly overlap as fish become older, and the method generally is unreliable for assigning ages to older fish. If ages and median length can be determined for each cohort (age-group) in the population, a growth rate for that cohort from hatch till time of sampling can be calculated. Graphical and statistical approaches for separating polymodal size frequency distributions into cohorts are available (Bagenal and Tesch 1978; Jearld 1983). Even if age cannot be precisely determined, the growth rate of a distinct cohort within the population may be estimated by following that cohort in subsequent samples (Ebert 1973; Pauly 1984).

11.5.3.2 Back-Calculation

There are several methods available for back-calculation of size at a given age. Although we describe them in terms of scales, they apply equally to any body part (otolith, bone, spine, etc.) that carries a record of growth episodes.

(1) The Fraser–Lee method (covered in detail below).
(2) Regression methods. Simple regression equations can be used to relate scale radius (distance from the scale's focus to its edge) to fish length. Then, measurements along a scale radius from focus to annual marks can be applied to the equation to give a fish's length at past ages (Carlander 1981).
(3) The covariance method (Bartlett et al. 1984). A modification of the direct regression approach, covariance analysis can address some problems that invalidate simple regression, such as nonlinearity and different body–scale relations among age-groups or year-classes.
(4) The linear model approach (Weisberg and Frie 1987). Linear models accept growth increments directly from the scale measurements, and they allow age-group and year (environmental) effects to be distinguished. Comparisons of growth after good and bad years can be made and statistically tested to assess age effects. The method may be used to describe effects on growth from environmental manipulations that fishery managers might use (Weisberg and Frie 1987).

In general, back-calculation only allows growth to be resolved at yearly intervals. Growth rates over fractions of a year can be determined by sampling a single cohort on several occasions, by using back-calculation techniques on scale margin from the edge to the last annulus, or by measuring the daily growth increments that often are recorded in the hard parts of young fish.

If a population can only be sampled once, back-calculations can be used to determine previous growth rates for individual fish and provide an estimate of the actual growth rate of all fish in the population. The actual instantaneous growth rate for individuals can be determined by the equation for G (Section 11.2), and a method for determining the population instantaneous growth rate was given by Bagenal and Tesch (1978). As an alternative, the apparent population growth rate can be used if the fish are aged but back-calculation is not done. Using the identified age for each fish, one can determine the mean size at each age represented in the population sample. The growth interval is 1 year, and size differences between successive age-classes constitute an estimate of annual

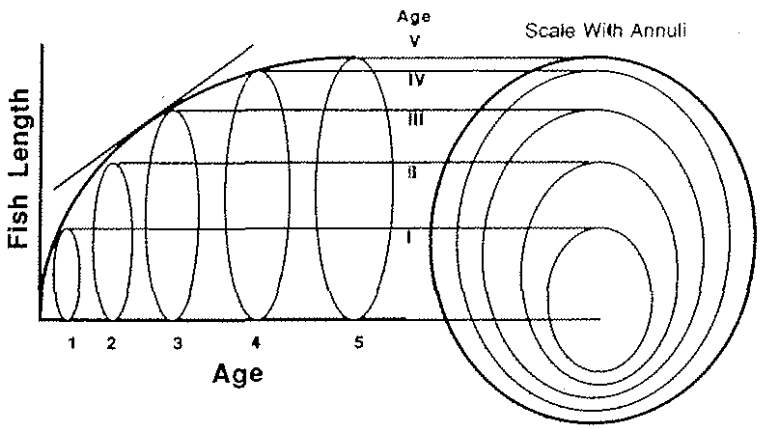

Figure 11.2 Proportionality between length of a fish and "radius" (distance from focus to edge) of its scale. The growth history of an individual fish can be back-calculated from scale annuli, or the growth rate for the population can be estimated from mean fish size at each age.

growth for that age interval. If it can be assumed that an age-class always has the same growth rate—that 3-year-olds always grow the same amount, for example—the apparent population rate can be determined from a single sample estimate. The use of apparent population growth rate can cause spurious results, however, particularly with short-lived fish species subject to size-selective mortality and large annual variation in growth rates due to climatic and other variables.

If fish age can be established from scales or other body structures, the proportionality between size of these structures and size of the whole fish permits the calculation of growth in previous years (Figure 11.2). The increments of growth for individuals in those years can be determined by back-calculation techniques, discussed in more detail below (also see Lagler 1956; Carlander 1981, 1982; Frie 1982; Jearld 1983; Newman and Weisberg 1987; Weisberg and Frie 1987). For the use of otoliths to determine growth over short time increments such as a day, see Panella (1971), Mayo et al. (1981), and Campana and Nielson (1985). Recently, there has been increased concern about the accuracy of aging methods applied to older fish, particularly when scales are used (Casselman 1983).

Back-calculated lengths are often based on the traditional Fraser–Lee method, which describes the body–scale relationship as the rectilinear regression

$$L = a + bS;$$

L = body length, S = scale length (radius) from the focus to the anterior edge, and a and b are constants (Lagler 1956; Hile 1970; Carlander 1981, 1982). This body–scale relationship is typically developed by measuring scales on a subsample of fish from all size-groups. The lengths at previous ages (L_i) are then calculated by a modification of the Fraser–Lee equation:

$$L_i = a + (L_c - a)(S_i/S_c);$$

L_c = length of fish at capture, L_i = calculated length at age i, S_c = distance from focus to edge of scale, S_i = distance from focus to annulus i, and a = intercept of

the body–scale regression or a standard value from the literature (Carlander 1981, 1982; Frie 1982). These increments of growth over annual time periods can be used to calculate growth rates in different years for each individual represented by a scale sample. Average increments for all individuals in the sample will suggest actual population growth during these years. Microcomputers can markedly reduce the effort needed to calculate the body–scale relationship and to back-calculate previous lengths (Frie 1982). Various transformations of regression equations are sometimes used to linearize estimates of the body–scale relationship (Bagenal and Tesch 1978).

Various problems or considerations are associated with back-calculations of length.

(1) Special care must be taken to insure that annuli are valid indicators of age (Bagenal and Tesch 1978; Beamish and McFarlane 1983; Jearld 1983).
(2) Wide variations in a, the intercept of the body–scale relationship, occur between populations of the same species due to problems associated with fish collection, scale sampling, and measurement. Standard intercept values from the literature may reduce this variance for common species (Carlander 1982), although these values may also cause problems when they are grossly incorrect.
(3) Individuals in the sample must provide an adequate representation of the body–scale relationship in the different size-classes (Carlander 1981).
(4) Variability in back-calculated lengths is likely to be higher among younger fish due to the nature of the variance associated with a regression equation used to describe the body–scale relationship (Carlander 1981). For least-squares regressions of the type $Y = a + bX$, the variance associated with predicted body length (Y) increases as the measured scale length (X) departs from the mean of all scale lengths in the population (Snedecor and Cochran 1967).
(5) Back-calculated lengths are more accurate if a key scale or scales from the same region of each fish are chosen. Newman and Weisberg (1987) presented methods to determine the number of scales to collect per fish and the number of fish to sample in order to attain a given precision.
(6) Larger fish in a year-class often have a mortality rate different from that of the smaller members of the same age. This may induce an artifact known as Lee's phenomenon, which occurs when back-calculated lengths of a given age-group are smaller when calculated from older fish than they are when derived from younger fish (Ricker 1975, 1979; for a reverse effect, see Frie 1982). This difference may also be due to biased (nonrandom) sampling, size-selective mortality, or improper back-calculation methods (Bagenal and Tesch 1978). Newman and Weisberg (1987) suggested that scale growth increments be examined to determine if Lee's phenomenon occurs independently of back-calculation techniques.

11.5.3.3 Mathematical Models

Several mathematical models can be used to describe increases in fish size with age. Empirical data usually can be represented adequately by the von Bertalanffy growth equation, which has the formula

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

The time units, t, represent the age of the fish, usually (but not necessarily) measured in years from a starting time, t_0. The length of the fish at age t, L_t, is a function of maximum (asymptotic) length L_∞, the growth coefficient K, and age; e is the base of the natural logarithm. An analogous formula can be used with weight data. In theory, t_0 is the age at which the fish would have had zero size if it had always grown as described by the equation. In practice, however, t_0 is a time at which the fish already has attained a finite size.

When the von Bertalanffy equation is fitted to size-at-age data, values can be calculated for maximum size, size at t_0, and K (Ricker 1979; Pauly 1984). Maximum size cannot be estimated directly from body–scale data and back-calculations. If true ages are unknown but growth over known time intervals can be determined by, for example, recapture of tagged fish, the von Bertalanffy formula can still be used. In this case, the equation gives maximum size and K, but not t_0, so size at t_0 cannot be calculated (Fabens 1965; Pauly 1984). Other methods can be used to estimate maximum size and the growth coefficient when data on size increases with time are available but ages and sizes at age are unknown (see Pauly 1984; Schnute 1985).

11.6 CONSIDERATIONS FOR LABORATORY GROWTH STUDIES

The major influences on fish growth are temperature and food consumption rate, but there are many other characteristics of fish and experimental design that can affect the outcome of a growth study. These should be considered early in an experiment to assure that results will be easily analyzed and interpreted, as well as comparable with results of other studies. It is beyond the scope of this chapter to examine in detail the effects of environmental and trophic factors that affect growth (see Brett 1979; Brett and Groves 1979; Tytler and Calow 1985).

11.6.1 Characteristics of the Fish

It is important to choose the species and life stage that best suit the purposes of the study and the facilities available. Younger fish are more easily domesticated than older ones and usually feed more readily in new surroundings. Immature fish also present none of the complications (for growth studies) of gonad development. Piscivorous species are often best for quantitative studies of food consumption because exact ration sizes can be easily measured; pelleted food often dissolves or is not eaten once it settles to the bottom of the tank. A species may have many strains and races that can display different growth potentials (Reinitz et al. 1979).

As poikilotherms, fish adapt to decreased ambient temperature through a variety of mechanisms, most of them associated with increased enzyme activity (Hochachka and Somero 1984), and growth rate at a particular temperature depends on a fish's thermal history. For this reason, a fish should be acclimated to the chosen experimental temperature for at least 1 week before a growth study begins. Acclimation may not occur in 1 week if the difference between ambient and experimental temperatures is large, but probably will in 3–4 weeks. Gradual changes in temperature, as might occur seasonally, generally result in the same

growth as would occur at the mean temperature over the interval, but exceptions have been noted. The effect of daily fluctuating temperature on growth is more complex, but there is some indication that fluctuations may stimulate growth if temperatures are below the optimum (Brett 1979; Spigarelli et al. 1982).

Growth rate also is affected by the fish's previous nutritional history (Weatherley and Gill 1981; Talbot et al. 1984). Prior starvation, even short-term deprivation, can result in changes in intestinal morphology and enzyme activity that influence subsequent food consumption and growth rates. Furthermore, if fish are obtained from the field, one may encounter populations that are "stunted" because of food shortages or a low genetic potential for growth (Murnyak et al. 1984).

Finally, fish in general (but wild fish in particular) have endogenous seasonal rhythms in growth potential (Baker and Wigham 1979). Thus, growth experiments conducted at different times of the year may result in different growth rates. The greatest growth can usually be expected in the spring and summer.

11.6.2 Experimental Conditions

Little attention is given to the feeding regime of growth studies, but the way in which a particular ration is presented can influence the response of the fish. For example, maximum ration size is often estimated as the maximum amount of food eaten in one or, at best, several feedings per day. However, the true maximum ration can only be presented when food is constantly available to the fish so they can feed ad libitum. Automatic feeders can be used to dispense feed at short, timed intervals. Demand feeders are useful for some fish, but many species cannot be trained easily to use them. Even when fish are fed the same ration size, the number of feedings per day can change the efficiency of food processing by the fish and therefore their growth rate (Yamada et al. 1981). In general, large rations are best presented as several smaller meals, usually 1% of body weight per meal. Regardless of the number of feedings, it is probably best to condition the fish by feeding them at the same times each day and, if possible, to match these times with the natural feeding times of the species. Finally, feeding should be stopped 1 or 2 d before fish are weighed so that gastric evacuation will be complete; the time necessary for intestines to empty depends on species and temperature.

The particle size of the food presented can be important in growth studies. In nature, fish actively choose prey of certain sizes such that food processing is energetically most efficient (Townsend and Hughes 1981; Mittelbach 1983). In the laboratory, food items that are too small or too large can result in less-than-maximum growth. In long-term studies during which fish grow appreciably, food particle sizes should be increased accordingly.

Fish density can influence growth by affecting social interactions and water quality. The rate of food consumption by a fish raised in isolation may be increased by "social facilitation" if the fish is placed with others. The number of fish present can determine the character of the interactions and change the feeding behavior of the fish. For example, if a hierarchy of fish dominance develops, a few fish will often feed more aggressively than others. The result will be high variability in the growth data (Koebele 1985) that will decrease the statistical resolution of growth trends or of differences between experimental treatments. Species of fish that form dominance hierarchies are not well suited for growth

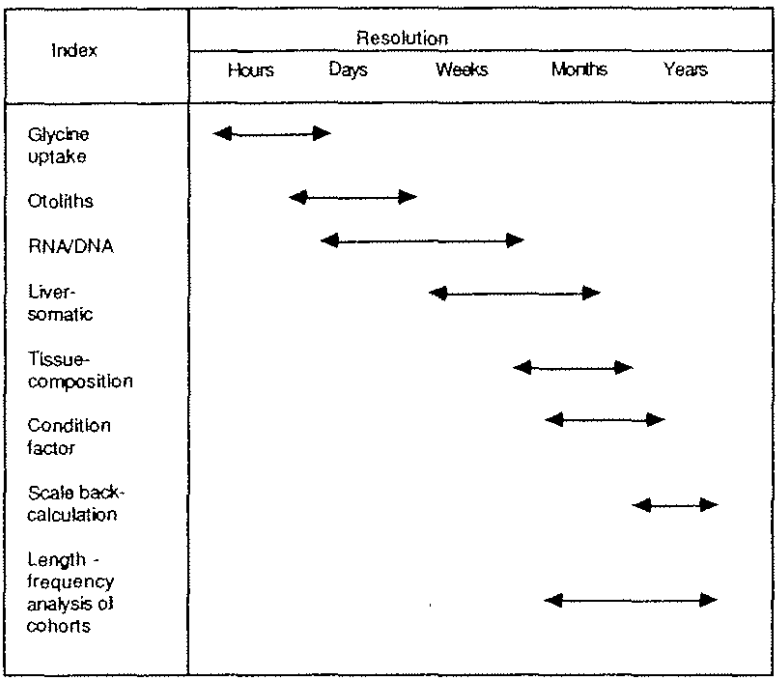

Figure 11.3 Time periods over which growth rate may be determined by various indices.

studies. In general, reducing the concentration of fish or increasing the frequency of feeding (Jobling 1985) reduces the effects of social interactions.

Photoperiod can act as a cue to fish and, by acting through hormonal mechanisms, influence their growth rates (Brett 1979). Responses to photoperiod are often very complex; the photoperiod effect interacts with those of temperature and endogenous rhythms. For most species, it seems that the greatest growth can be expected with long day lengths or with increasing day lengths (Brett 1979). For some species, longer day lengths may simply allow a longer feeding period. The intensity of light may also affect feeding behavior. Many fish species are less stressed and feed better under low-light conditions.

Water quality affects growth and optimum conditions should be maintained. If possible, dissolved oxygen concentrations should be maintained above 5 mg/L and preferably at saturation. Piper et al. (1982) provided suggestions for maintaining optimum water quality.

11.6.3 Statistical Considerations

To assure unbiased estimates of growth rate, fish should be randomly assigned to treatment groups. Proper methods for doing this were described by Snedecor and Cochran (1967). If fish are subsampled for weighing, efforts should be made to obtain representative fish. For example, chasing fish with a net should be minimized because this often leads to capture of the smaller or weaker individuals, which will bias the growth estimates. It is usually advantageous to mark each fish uniquely so individual growth rates can be obtained, allowing an estimate of variance. Methods for marking fish have been reviewed by Wydoski and Emery (1983).

Table 11.2 Logistic considerations for selection of indices of growth

Index	Complexity of analysis[a,b]	Cost[a,c]	Sample size[a,d]	Suitable location	Lethal	Individual or population measure	Special needs
Change in size							
Length	L	L	H	Lab, field	No	Ind, pop	Measuring board
Weight	L	L	H	Lab, field	No	Ind, pop	Balance
Proximate analysis							
Protein	M	M	L	Lab, field	Yes	Ind	Spectrophotometer
Lipid	M–H	M	L	Lab, field	Yes	Ind	Spectrophotometer
Carbohydrates	M	M	L	Lab, field	Yes	Ind	Spectrophotometer
Energy content	H	H	L	Lab, field	Yes	Ind	Calorimeter
Change in physiology or biochemistry							
Protein synthesis	H	H	L	Lab	Yes	Ind	Conclusions
Glycine uptake	H	H	M	Lab, field	No	ind	Liquid scintillation counter, sensitive balance (1 μg)
Nucleic acids	H	H	H	Lab, field	Yes	Ind	Centrifuge, spectrophotometer
Liver–somatic	L	L	H	Field	Yes	Ind	Balance
Condition factor	L	L	H	Field	No	Ind	Balance, measuring board
Growth in natural populations							
Cohort identification	L	L	M	Field	No	Pop	Graph paper
Growth estimates							
Length frequency	L	L	M	Field	No	Pop	Graph paper, computer
Back-calculation and daily increments	M	M	M	Field	No	Ind, pop	Scale projector, microscope, or scanning electron microscope
Mathematical models	H	L	H	Field	No	Pop	Calculator or computer

[a]L = low; M = medium; H = high.
[b]No previous experience assumed.
[c]Exclusive of specific equipment and capitalization costs.
[d]Number of fish required for estimates.

As fish become larger during a growth experiment, their physiological potential to grow decreases. Therefore, determination of maximum growth rate depends on the length of the experiment. Also for this reason, it is best to start an experiment with fish of similar size to minimize the variability in growth. Corrections have been proposed by Jobling (1983) for comparing the growth rates of fish of different sizes. These corrections may be of value in the analysis of experimental results.

11.7 CONCLUSION

The methods available for growth studies are numerous and the choice among them depends upon the focus of the study, the study's objectives, and the resources available. There is no single reference that gives all or even most of the decision points in the conduct of growth studies. Guidance for matching the appropriate methodology to the period of study is given in Figure 11.3. Other

characteristics of growth indices that may influence the choice of methods are given in Table 11.2. Further aspects of growth investigations are available in the cited literature.

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