AN EVALUATION OF RELATIVE WEIGHT AS AN INDICATOR OF BODY COMPOSITION AND NUTRITIONAL STATUS IN WILD FISH

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Abstract

Condition indices are widely used to generate biological insight. However, purported relationships to indices are imprecise or inconsistent in the wild. I investigated factors influencing relative weight (W_r), a condition index commonly applied to fish.

I first examined the relationship of W_r to physiology in two bluegill *Lepomis macrochirus* populations over a year. I regressed tissue composition (percentages of lipid, protein and water) and organ indices (liver-, gonad-, and viscerosomatic indices) on W_r. The regression model had little explanatory power (adjusted R^2 = 0.14). Lipid was most influential (partial R^2 = 0.11), but correlation strength fluctuated by season and population.

To test the generality of these results, I performed a similar regression on a bluegill population with higher average W_r. Again, variables were not well correlated to W_r (adjusted R^2 = 0.13). Combining comparable data sets increased W_r range 64% but explanatory power was low (adjusted R^2 = 0.41) Both studies showed that expected correlations of physiological variables to W_r can be confounded in natural environments.

To examine differences between natural and laboratory environments, I manipulated initial W_r and ration of juvenile bluegills. Although organ indices and tissue composition of all groups changed in time (Wilks' $\Lambda \geq 0.387$, $P \geq 0.03$), no temporal pattern matched to W_r. At termination, all variables showed high correlations to W_r ($r^2 \geq 0.64$). Correlation strength increased with time in the laboratory. Both ration and environment influenced correlations.

Lastly, I examined differences in interpretation of W_r for chain pickerels *Esox niger*, largemouth bass *Micropterus salmoides*, and black crappies *Pomoxis nigromaculatus*. Regression models were compared to concurrent bluegill models. Piscivore models fit well (adjusted $R^2 > 0.50$), whereas bluegill models had the lowest explanatory power (adjusted $R^2 = 0.13$ and 0.14). Ecological specialization affected correlations to W_r.

Theoretically, condition index values are determined by resource acquisition versus expenditure. Exact physiological expression is determined by life history and performance. Condition indices are imprecise predictors but track net somatic investment with great generality. Ancillary data, such as growth or length-at-maturity, may clarify interpretation. Condition indices should be used as qualitative monitoring tools, not omnibus physiological predictors.

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The Gentle Reader may not know, but, in May 2001, I was diagnosed with chronic myelogenous leukemia. This disease is fatal without expensive treatment. The response of my friends and colleagues was inspiring. The people of the Fish & Wildlife department and CMI helped me research my options and kept my spirits up. A group was started to raise funds to cover my medical expenses through the National Foundation for Transplants. This effort was spearheaded by Melanie Culver (now at Arizona State) and Scott Klopfer (CMI). With such unselfish assistance, I not only survived but thrived. My heartfelt thanks belong to all who helped.

Finally, I am eternally indebted to my family. Perhaps the original impetus for this work can be traced back to my parents, Ed and Marilyn Copeland, who encouraged my curiosity towards the natural world as a child (or at least tolerated it). They put their lives on hold and came out of retirement to help with my medical care. In a very real sense, my brother Rich gave me the life I am now living. And I owe so much to Sylvia, who stayed by me in sickness and in health.

Table of Contents

List of Tables

List of Figures

CHAPTER 1. DISSERTATION INTRODUCTION

Condition is the ability of an animal to cope with its present and future needs (Owen and Cook 1977), i.e., the physiological well-being of an individual (Anderson and Gutreuter 1983). A condition index is a variable that is used as a surrogate for individual condition, usually with reference to nutritional status (Harder and Kirkpatrick 1996). On an organismal level, the fate of an individual is determined in large part by its physiology and nutritional reserves; on a population level, vital rates (e.g., natality and mortality) are influenced by the nutritional status of the individuals that comprise the population (Kirkpatrick 1975). Therefore, condition indices are routinely included in fish and wildlife investigations to offer insight into environmental quality and population dynamics (Goede and Barton 1990; Harder and Kirkpatrick 1996; Blackwell et al. 2000).

In theory, condition is directly related to resource acquisition and indirectly to lifetime production of offspring (Jakob et al. 1996). Fitness, defined as lifetime reproductive success, can be thought of as having two components: acquisition of resources and conversion of them into offspring (Brown et al. 1993). The ability to survive, exploit resources, and grow increases reproductive potential. Condition may be thought of as an aggregate of complementary characteristics indicative of reproductive potential (Rolff and Joop 2002); consequently, it is a multidimensional characteristic of the individual. Such a theoretical construct is impossible to measure directly. Instead, its expressions or correlates are measured as indices of condition. These indices may involve aspects of the

complete organism or specific parts of its physiology. A condition index is a simplified measure designed to capture most of the variation in physiological well-being. If measured appropriately, condition indices can have strong implications for individual fitness and population dynamics.

Standardized measures of body mass are frequently used as indices of condition. Organismal functions, such as reproduction, growth, and storage, and their correlates (e.g., fecundity, growth rate, or lipid content) may bear more closely on fitness but are hard to measure directly. Body mass is often related to fecundity and resistance to starvation (Grubb 1995). It also has the intuitive appeal that the whole is greater than the sum of the parts. Body mass (i.e., weight) is easily and cheaply measured, doesn't require destruction of the animal, and, when corrected for size (i.e., length in some physical dimension), provides a basis for comparison of animals of varying size (Johnson et al. 1985; Jakob et al. 1996). This derived variable is assumed to reflect a complex physiological state. As such, the value of a whole-body condition index is as a manifestation of biochemical and physiological processes expressed at the organismal level (Goede and Barton 1990). In practice, size-specific body mass is used as an indicator of a myriad of organismal traits. There is continuing debate on the use of body mass as a condition index and the proper way to correct for size (e.g., Johnson et al. 1985; Bolger and Connolly 1989; Cone 1989; Ormerod and Tyler 1990; Springer et al. 1990; Hayes and Shonkwiler 2001; Rolff and Joop 2002).

Whole-body condition indices have a long history of application to fish, the first being proposed in the early 1900's (Bolger and Connolly 1989). They have been used as indicators of many things, such as fish health, stress, body composition, growth, energetic content, fecundity, consumption, population/community structure, and habitat quality (Goede and Barton 1990; Anderson and Neumann 1996; Blackwell et al. 2000). While certainly not an exhaustive list, these uses give some perspective on the importance of condition indices in aquaculture, biology, ecology, and management. Refinements in fish condition indices have been driven largely by statistical concerns regarding the proper way to incorporate body size into the index (e.g., Cone 1989; Springer et al. 1990).

The use of whole-body condition indices is problematic because of the tenuous connection to fitness (Rolff and Joop 2002). Experiences in other areas of biology and ecology have demonstrated the dangers of over-generalization and misapplication of intuitively appealing but poorly supported theory (Rieman and Dunham 2000).These lessons apply to condition indices. Many factors may influence size-specific weight (Le Cren 1951; Ormerod and Tyler 1990) and interpretation of the index in the desired terms may be confounded. Hayes and Shonkwiler (2001) provided a broad indictment against condition indices and offered three criticisms concerning their use: 1) the intended meaning is nebulously defined; 2) indices are rarely validated; and 3) direct analysis of the parameter of interest is better than a surrogate index. I would add to Hayes and Shonkwiler's (2001) first point that the underlying theoretical basis for use of

condition indices in fish is vaguely defined. The criticisms listed above must be addressed for rigorous and valid use of condition indices. Unlike Hayes and Shonkwiler (2001), I do not advocate general abandonment of condition indices for fish and wildlife investigations. Their value as generators of biological insight seems evident in their widespread use across many taxa. By definition, any index is an indirect measure of another parameter, and as such, some degree of imprecision should be expected. Condition indices for fish were constructed from empirical patterns (Anderson 1980), not from a mechanistic theory. I believe prerequisites for use of an index are an awareness of the expected level of imprecision and a mechanistic understanding of how important factors influence index values. This knowledge would provide generality and some expectation of appropriate usage.

My goal for this dissertation was to provide guidance for rigorous and valid use of condition indices in fish by investigating factors that introduce variation into the relationship between size-specific weight and its components, thus influencing interpretability of whole-body condition indices. I addressed the criticisms listed above with a series of investigations. Before describing the individual studies, three topics need to be addressed in this Dissertation Introduction: 1) definition of the terminology and conceptual model of condition used in this dissertation; 2) the overall strategy and modes of inquiry; and 3) description and justification of the variables in common among studies, including the specific condition index employed.

Concepts of Condition

Application of theory requires clarity in regard to the concepts being applied; therefore condition should be clearly defined and the area of interest delimited. This addresses the first criticism of Hayes and Shonkwiler (2001). Such a definition should be precise, operational, and quantifiable (Murphy and Noon 1991) and clearly related to life history and evolutionary theory to establish its importance. Individual status can be evaluated in many ways (e.g., health, nutrition, age, social standing); hence, it is necessary to choose a particular focus. Given the influence of nutrition on physiology, population dynamics, and management (Kirkpatrick 1975), I used a nutritional emphasis because of its practical implications and relationship to fitness (Jakob et al. 1996).

Grubb (1995) explicitly defined nutritional condition as the state of body components controlled by nutrition that influence an animal's fitness. Nutrition is the rate of assimilation of nutrients and energy and is obviously part of Brown et al.'s (1993) definition of fitness. These related concepts are clearly implicit in most uses of whole-body condition indices because the body components frequently used to index condition (e.g., lipid reserves) are influenced by consumption and resource use. Typically, body component indices are the same as, or correlated to, morphological or biochemical components that influence fitness (Grubb 1995). Saltz et al. (1995) incorporated this definition into a conceptual model (Figure 1.1). In this model, condition is a state variable controlled by the interaction of nutrition and stress. Stress is defined as the rate at which energy and resources are depleted. In this context, condition represents

the storage of energy and nutrients within the animal's body for later mobilization to meet the demands of various life processes.

This conceptual model of condition has several advantages. It is related to individual fitness. Resource storage is one of the elements (with growth, maintenance, and reproduction) that influence life history evolution (Reznick and Braun 1987; Zera and Harshman 2001). The connection of this concept to accepted usage of condition indices is well established. Therefore, the nutritional condition model gives a definite context for the evaluation of condition indices. Throughout this dissertation, "condition" is used synonymously for "nutritional condition" or "nutritional status" and the physiological processes that influence condition are viewed as nutritional processes.

Operationalizing the model for empirical use requires several assumptions. These link index values (or changes in them) to occurrences of physiological or nutritional changes sufficient to affect the behavior and fitness of the individual (Murphy and King 1991). Assumptions should also connect the model to parameters related to fitness or its components. I propose the following. 1) The optimal fitness strategy requires investment in the individual's body; this assumption is required of any somatic index. 2) At least some resources are allocated to storage, which is reflected by plumpness; this is necessary for whole-body indices. 3) Index values change instantaneously with level of investment at some constant rate (Delahunty and DeVlaming 1980); hence, the index is a measure of the animal's current status. 4) Changes in condition are linearly related to the index, for precise prediction by standard parametric

statistics. 5) The relationship between condition and the index is constant for all individuals compared (i.e., controlling factors are similar); therefore, variables are in equilibrium (DeVlaming et al.1982). Divergence from these assumptions will cause confounding variation in correlations between index and fitness components. Rigorous use of a condition index should include evidence that all assumptions are satisfied.

Approaches to Understanding Condition

My analytic strategy to understand the components of fish condition has two common themes throughout this dissertation. First, I took a model validation approach (Rykiel 1996) to provide guidelines for rigorous and valid use of condition indices. In common with other ecological phenomena, the interpretation of patterns becomes problematic without understanding underlying processes; a test for boundary conditions is needed to determine when the postulated processes become unimportant (Van Horne 2002). In this sense, I focused on cases where condition indices begin to break down in terms of their ability to track fitness components. Secondly, I attempted a holistic examination. Condition is a multidimensional characteristic of the entire organism. By measuring multiple indicators of condition, I hoped to get a more complete picture of recent individual history (Weber et al. 2003) and its effects on whole-body condition. Such a broad understanding should allow better assessment of environmental effects on individuals (Beckman et al. 2000), a common goal for the use of condition indices.

For a condition index, I used relative weight (W_r). Like other whole-body condition indices for fish, W_r is derived from the relationship of length and weight, $W = aL^b$, where W is weight, L is length, a and b are empirically derived constants. Condition is parameter a. Rearranging to solve for condition gives $a = W/L^b$. Hence, fish condition is observed weight divided by weight predicted from length. Fulton's condition factor (K) is calculated as $K = (W/L^3)*10^5$. Because b varies from 2.5 to 4.0 according to species body form (Le Cren 1951), K is biased by length and not comparable among species. Le Cren (1951) developed relative condition (K_n), computed as $K_n = (W/W')*100$, where W' is predicted from the length-weight regression for a particular population. Because of its population-specific basis, K_n is not comparable among populations and is not sensitive to limitations in the environment of a particular population. Wege and Anderson (1978) proposed the index of relative weight, $W_r = (W/W_s)*100$, where W_s is the 75th percentile weight expected at a particular length based on length-weight regressions from all parts of a species' range (Murphy et al. 1990). As a condition index, W_r is robust, tractable, and statistically and biologically consistent (Murphy et al. 1990). If an adequate sample was used to calibrate W_s, W_r does not have a consistent length bias. Ideally, W_s removes genetic influences on weight such that local environmental factors control W_r (S. Gutreuter in Springer et al. 1990). In theory, W_r is comparable among lengths and species. In practice, W_r is a convenient and objective yardstick against which to measure individual plumpness. Currently, W_r is the condition index most widely used by fisheries management agencies in the United States (Blackwell et al. 2000). Empirical

relationships of many variables to W_r have been exhaustively reviewed by several authors (e.g., Murphy et al. 1991; Anderson and Neumann 1996; Blackwell et al. 2000) so I will not belabor specifics here. As with other condition indices, W_r proceeds from the assumption that "fatter is fitter" (*sensu* Glazier 2000). In each study, I regressed several variables related to nutrition on W_r. The overall multivariate model was then compared to a correlation matrix of all the variables to interpret the effects of physiology on nutritional status in a manner analogous to following an analysis of variance with multiple comparisons.

To provide a view of several facets of nutritional status, I used six variables commonly measured in studies of growth and resource storage. On a whole-body scale, I measured the relative quantities (percentage by weight) of lipid, protein, and water (Busacker et al. 1990). Lipid is the main energetic reserve in fish (Shul'man 1974). Protein is the main component involved in structural growth (Gerking 1952). Water content comprises the majority of a fish's body and is inversely related to lipid content (Iles and Wood 1965) and energy content (Hartman and Brandt 1995). To provide perspective on allocation of nutritional resources, I also measured the relative masses of three organs (also as percentages, i.e., organosomatic indices, Goede and Barton 1990). Liver-somatic index (LSI = liver weight/body weight*100) is related to energy flux within the body and is commonly used as an index of short-term growth (Bulow et al. 1978; Adams and McLean 1985). Gonadosomatic index (GSI = gonad weight/body weight*100) represents energy diverted away from the body for reproductive purposes (Tyler and Dunn 1976; DeVlaming et al. 1982). Finally, the

viscerosomatic index (VSI = gastrointestinal weight/body weight*100) is related to intraperitoneal lipid deposits, which are long-term energy stores for many fishes (Jensen 1980; Adams and McLean 1985).

All variables were expressed on a relative basis as the ratio of observed to expected body weight or percentages of observed weight with the intention of placing all individuals on the same scale of measurement regardless of body size. Because the relative size of body components does not change appreciably in a steady environment once adult form is attained (Weatherley and Gill 1983), comparison among lengths is justifiable and noticeable differences should be related to environmental fluctuations. However, adjustments for size may have unintended statistical effects and should be done with care (Packard and Boardman 1988). I do so here for several reasons related to the criterion of practicality. First, ratios and percentages are commonly used and thus comparison to the literature will be facilitated. Secondly, comparisons are often used to generate biological insight and there is a need to compare individuals of widely differing size. Lastly, the correct interpretation of the adjusted variables regards changes in the separate components relative to the whole (Dodson 1978), i.e., the relative importance of each variable in contributing to changes in W_r. If one accepts that the regressors express different aspects of fitness (i.e., resource acquisition and allocation), then their aggregate influence on W_r should indicate the level of nutritional investment in the somatic tissues. This is an important function of condition indices and a main focus of this dissertation.

My work in this dissertation proceeded in several stages, each detailed in a separate chapter. I used both inductive and deductive modes of inquiry. Two inductive field studies were conducted to seek correlative patterns of component variables to W_r that might suggest potentially important controlling factors. In Chapter 2, I examined how seasonal changes and population differences influence the interpretation of W_r. The results showed some of the limitations of whole-body condition indices. In Chapter 3, I probed some empirical explanations for these limitations and considered how general they might be. To deductively test potential causes for unexplained variation in W_r, I conducted a manipulative laboratory experiment, documented in Chapter 4. As well as testing mechanistic explanations, the experiment was intended to link theory and data, and laboratory results to field observations. Lastly, I conducted another field study, this time examining differences in interpretability of condition indices among species (Chapter 5). The comparisons were chosen to examine phylogenetic and ecological influences on nutritional status. As with the other chapters, Chapter 5 has implications for the generality of condition indices. I concluded this dissertation with a synthesis of my results with selected literature and current life history theory. In the final section, I presented my thoughts regarding the validity and proper use of condition indices and suggested fruitful avenues for further research. Condition indices are an important tool for fisheries management and ecological research, but their interpretation in biologically meaningful terms suffers from a lack of theoretical rigor. It is my hope that this dissertation will

inject some credible theory into their use and perhaps stimulate thought where there is now a “business as usual” mentality.

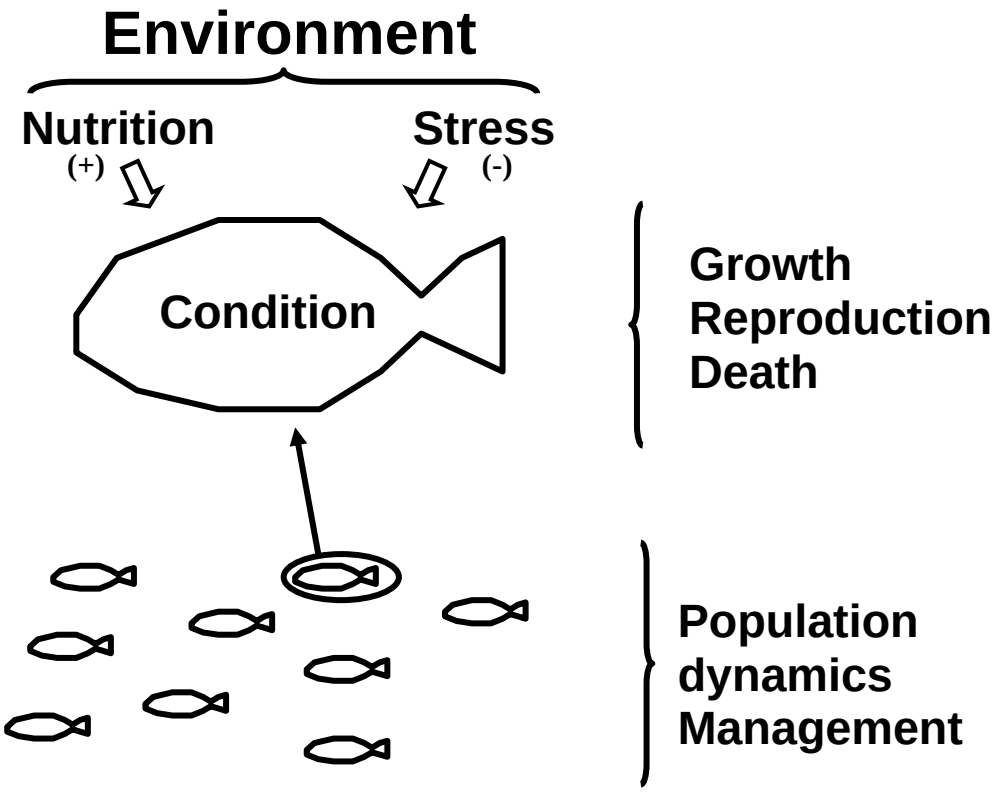

Figure 1.1. Schematic diagram showing the conceptual model of condition of Saltz et al. (1995) and its influence on individual and population processes. Condition represents the interaction of nutrition and stress in individuals, which influences the biological processes of growth, reproduction, and death. The sum of these processes over individuals determines population vital rates, thus influencing population dynamics and management.

CHAPTER 2. INTERPRETATION OF RELATIVE WEIGHT IN TWO POPULATIONS OF WILD BLUEGILLS

Introduction

Condition indices are intended to represent individual well-being (Anderson and Gutreuter 1983). Whole-body condition indices in fish, e.g., relative weight (W_r), are measures of relative plumpness, traditionally calculated as the ratio of weight observed to weight predicted from a function of length. One of the chief uses of such indices is to infer nutritional status, nutrition being the acquisition and storage of energy and nutrients. Biologists commonly use indices of condition as a simplified means to view nutritional information on the individual and population levels (Harder and Kirkpatrick 1996). Condition indices have been used as indicators of past and future ecological performance, i.e., how well the individual has related to its surroundings and its capacity to do so in the future (Owen and Cook 1977). But many variables can influence fish condition (Le Cren 1951; Sutton et al. 2000) and the intended meaning of condition often is not articulated clearly with reference to its application (Hayes and Shonkwiler 2001).

Relationships of condition indices to several physiological and environmental variables have been explored (see Goede and Barton 1990; Anderson and Neumann 1996; and Blackwell et al. 2000 for reviews). Attempts to correlate condition indices to physiological processes using energy and nutrients have met with limited success. For example, the observed relationship of W_r to measures of growth is inconsistent. Brown and Murphy (1991) found strong correlations between growth rate and W_r over a 12-week laboratory

experiment with juvenile striped bass (*Morone saxatilis*) and hybrid striped bass (*M. saxatilis* x *M. chrysops*). In wild populations of largemouth bass (*Micropterus salmoides*), northern pike (*Esox lucius*), and white crappie (*Pomoxis annularis*), comparison of W_r to length at age or backcalculated length increment within age classes resulted in strong and significant correlations (Wege and Anderson 1978; Willis and Scalet 1989; Gabelhouse 1991). However, such findings are not universal in field studies (e.g., Gutreuter and Childress 1990; DiCenzo et al. 1995; Liao et al. 1995). Schneider (1999) found low W_r in two populations of bluegills (*Lepomis macrochirus*) with lengths-at-age above the Michigan state average. He attributed this to time of sampling. Gabelhouse (1991) suggested that seasonal growth pattern could affect the usefulness of W_r as a growth indicator, e.g., fall W_r would be a poor growth index if most growth occurs early in the season but is followed by a late summer depression. Given the complexity of the growth process and the multitude of potential influences (e.g., temperature, diet, activity or size), it is not surprising that a connection to W_r may be confounded.

Similarly, correlations of W_r to all major components of body composition have been demonstrated in the laboratory (Rose 1989; Brown and Murphy 1991). Proximate composition is usually defined as the proportion by weight of water, protein, lipid, and ash in the body of a fish (Pearse 1925). Composition reflects and influences growth, reproduction, and nutritional status of the individual. However, Simpkins et al. (2003) found that correlations of W_r to lipid content were not very strong ($r^2 \leq 0.41$) in laboratory-reared rainbow trout

(*Oncorhynchus mykiss*). Similarly, Jonas et al. (1996) found that W_r was not a robust indicator of energy density (kcal/g) in wild muskellunge (*Esox masquinongy*). In two Texas reservoirs, Neumann and Murphy (1992) found seasonal fluctuations in the strength of correlations of relative organ weights and body composition to W_r in white crappie (*Pomoxis annularis*). The correlations generally explained <50% of the variation in W_r. Gershanovich et al. (1984) found no relationship between lipid content and condition factor in juvenile striped bass and sturgeon (*Acipenser nudiventris*) reared in ponds. In the wild, seasonal variations in condition seem to be positively related to gonad size (Le Cren 1951; Mann 1973; Mann 1976; Htun-Han 1978; Neumann and Murphy 1992). The annual reproductive cycle strongly affects body composition of fish (Iles 1984; Dygart 1990; Neumann and Murphy 1992; Encina and Granado-Lorencio 1997) and thus condition index as well. Other factors doubtlessly have influence (e.g., diet and environment, Shearer 1994) and may confound the relationship of W_r to body composition in wild fish.

Mathematically, a condition index consists of the sum of the relative weights of the body components (Iles 1984). Body condition includes more than lipid (Van der Meer and Piersma 1994; Breck 1998). The components of a fish's body (major tissue types and organs) interact to determine index value. However, important changes in energetic content may be obscured if one component increases while another decreases, as in the tradeoff between amounts of water and lipid. Viewing several components simultaneously should enable detection of confounding interactions. Changes in the weight of specific depots, such as

energetically active organs (e.g., liver, gonads, and viscera), can aid in interpretation of the whole-body measure. For example, translocation of resources from portions of the soma to the ovaries in English sole (*Parophys vetulus*) explained 33% of somatic mass lost over winter (Dygart 1990). Many past investigations linked condition index to single variables, typically lipid content. A more holistic approach is necessary if the relationship of condition index to body composition is to be understood, especially for individuals inhabiting complex, natural environments.

Biologists commonly use condition indices to assess the physiological status of wild fish; therefore, there is a need for a better understanding of how these indices represent condition in the wild. Potential sources of variation in a condition index should be considered before using it (Le Cren 1951; Sutton et al. 2000). In short, nutritional status of wild fish is inherently variable in time and space and investigators need to understand how this variation affects the interpretation of W_r. This study addressed the effects of inter-population and seasonal differences on interpretation of W_r. My strategy was to conduct a comparative field study examining body composition and energetically-active organs. Specifically, the objective was to describe the relationship of W_r to several indicators of nutritional status in two bluegill populations through an annual cycle.

I chose bluegill as the study species because they are the most abundant fish in Virginia impoundments and there is an established and validated standard weight (W_s) equation for bluegills (Hillman 1982; Murphy et al. 1990). The bluegill

was one of the first species for which a W_s equation was developed; the concept of W_r was originated using centrarchid communities. Bluegills are important to anglers and as prey for important sport fishes such as largemouth bass; therefore, bluegills receive much management attention. There is also good information on bluegill growth and proximate composition in the laboratory (McComish 1971) to aid in interpretation and identification of influential factors. Using McComish's (1971) data and differences between the study populations in selected life history parameters, I offer explanations for the patterns I observed.

Methods

Study Sites

The study sites were Lower Travis Lake (LT) and Smoots Pond (SM), two small (<20 ha) impoundments on the Fort A. P. Hill military reservation in Caroline County, Virginia. Both ponds are located in the Coastal Plain portion of the Chesapeake Bay drainage. The watersheds are forested with sandy soils and swampy bottoms; consequently, both ponds are infertile and slightly dystrophic (total alkalinity = 4 - 6 mg/l as $CaCO_3$, surface conductivity = 20 µS; T. Copeland, unpublished data). Smoots Pond is accessible only by boat via a small launch at the dam and most of the shoreline is off-limits to the general public. Conversely, at LT there are several cabins nearby available for general use, the entire shoreline is open to public access, and recreation is promoted in the surrounding area. Large-scale abiotic influences and bluegill genetic composition should be similar between impoundments due to their proximity (13 km straight-line

distance). However, accessibility has helped create bluegill populations with very different characteristics. Management inventories conducted in April 1999 showed differences in bluegill population density, size structure, and W_r characteristics in the two ponds (Table 2.1). Bluegills in LT were more abundant and smaller than in SM. Mean W_r was significantly different (LT = 90, SM = 102; t = 7.27, $p < 0.0001$). In LT, bluegill W_r was not correlated to length ($r^2 = 0.04$, p = 0.07) and was more variable at smaller lengths. In SM, bluegill W_r also was not correlated to length ($r^2 = 0.002$, p =0.75), but variability appeared similar regardless of length. Population pressures and per capita resource availability should be different between the two ponds. I wanted to see how these differences influenced W_r.

Data Collection

Each population was sampled quarterly during 1999 (March 23, June 7-9, August 17-18, November 18) to examine physiological status before and after the stresses of spawning and winter. The minimum length of fish collected was 80 mm, the minimum for application of W_r to bluegills (Murphy et al. 1991). Each population was stratified into three length categories (Copeland et al. 1999); the 33rd and 66th percentile lengths delimited the categories, based on preliminary data and concurrent management inventories. A sample of bluegills from each length category was collected by electrofishing so that the entire length range of each population was represented. Graphical comparison of variance in W_r versus sample size from several bluegill populations at Fort A. P. Hill showed that

variance apparently stabilized at sample sizes near 20 (T. Copeland, unpublished data). Hillman (1982) also found a sample size of 20 bluegills was necessary to get a 95% confidence interval of $W_r \pm 5$. A target was set of 20 fish from each third of each population, a total of 60 per pond per sample period. Bluegills were measured to total length and assigned to a category in the field. A running tally was kept and sampling continued until sample targets for each category were met. During the March collection in LT, the middle category was extremely narrow and I was able to collect only 13 bluegills in that category. During the June and August collections in SM, large and medium bluegills were not in the littoral zone in ample numbers and only 52 and 45 of the 60 fish desired, respectively, were collected despite multiple efforts.

Fish were put on ice for transport back to the laboratory, where they were stored on ice in a sealed container and processed within a week. Total length was measured again (nearest whole mm), the fish patted dry and weighed with an electronic balance to the nearest 0.001 g. Each individual then was dissected. Otoliths were extracted and examined to determine age. Liver, gonads, and viscera (the gastrointestinal tract with attached tissue) were removed and weighed to the nearest 0.001 g. Prior to weighing the viscera, any stomach or intestinal contents were extruded. All organs were replaced within the body cavity, the carcass chopped into small bits, placed on a pre-weighed aluminum dish, weighed, and dried at 37^{o}C in a drying oven. The dried pieces were ground with a coffee bean grinder. The resultant powder was replaced in the oven on the same pan until weight stabilized for the largest fish. Then, all were re-weighed.

Water content was the difference between wet and dry weights. Protein and lipid content were determined by the Forage Testing Laboratory at Virginia Tech using the macro-Kjeldahl procedure and ether extraction (AOAC 1990).

Statistical Analysis

I used multiple linear regression (MLR) to examine the relationship of the variables to W_r. All variables were standardized by weight. Lipid and protein content were expressed as percent of dry weight (the actual quantity analytically determined). All others were expressed as percent of total body (wet) weight. For the organ weights, this procedure transformed them into commonly-used organosomatic indices (Goede and Barton 1990): liver-somatic index (LSI), gonadosomatic index, (GSI), and viscerosomatic index (VSI). Data were examined to detect the presence of outliers and highly influential observations using standard diagnostics (Studentized residuals, diagonal elements of the hat matrix, DFFITS, and DFBETAS; Montgomery and Peck 1992). Obviously erroneous observations, such as impossible weights, were deleted from the data set. As a result, 32 individuals were removed from the data set. The remaining data were used to construct a model relating physiological variables to whole-body condition. First, I inspected the data for interactions among the regressors by constructing a correlation matrix. Then, I fit the data to a MLR model, W_r being modeled as a function of body components and organosomatic indices. Examination of model residuals and bivariate scatterplots did not reveal any obvious departures from homoscedasticity; therefore, I did not transform any

variables. I used the correlation matrix of the regressors to help interpret the MLR model. Significance of individual variables was determined by *t*-test (H_0: $\beta_i = 0$, $\alpha = 0.05$). In the following, r^2 is used to denote the coefficient of determination in a bivariate regression, whereas R^2 denotes the coefficient of multiple determination in a multivariate regression.

This analysis was designed to provide the broadest physiological interpretation of W_r. The most important variable was lipid content but the relationship was not strong (see Results); therefore, I examined the differences between samples in season and population in terms of the W_r-lipid relationship. Lipids are the chief energetic store in fish (Shul'man 1974) and a quantity that W_r should track closely (Anderson and Neumann 1996). To further aid with interpretation, I compared my data to those from McComish (1971). He manipulated bluegill growth in several laboratory experiments. Body composition was determined for many of these fish. I used his raw data to calculate W_r for all bluegills >80 mm in his experiment and regressed the lipid contents he determined (% dry weight) on them.

I employed multivariate analysis of variance (MANOVA) to detect differences in variables related to population and season, including the interaction. To determine which variables contributed to observed differences, the MANOVA was followed by univariate analysis of variance (ANOVA) with an interaction term to assess whether seasonal changes were different between populations. Use of the MANOVA protects the nominal error rate of the ANOVAs (Rencher 1995). To analyze differences in GSI, I also included a model with sex

among the main effects, the three two-way interactions, and a three-way interaction term (sex, season, and population). Tukey's post-hoc test was used to determine the nature of the differences. If the interaction term was significant, the comparisons were among the individual cells, i.e., each population-season sample. There were obvious differences among samples in variance (see Results), so I tried a logarithmic transformation but it did not change model interpretation except for GSI. Risk of Type I error was set at $\alpha = 0.05$.

I examined patterns of growth and maturity to consider what kinds of factors might cause inter-population differences in trends of W_r to lipid content. I plotted GSI versus length to determine length at first maturity. Bluegills were considered to be mature if GSI > 4.0 for females or if GSI > 0.5 for males. Novinger (1973) found well-defined groups of mature ova in female bluegills with GSI > 4.0. Ehlinger et al. (1997) determined GSI of reproductively mature male bluegills was >0.5. To assess age and growth, I examined the otoliths extracted. Age in years was determined as the number of opaque bands seen in whole otoliths. I calculated the median and 95th percentile ages for each population. Age was plotted versus length at capture to examine population growth characteristics. Patterns in W_r with length are often used as evidence to intuit important factors influencing population structure (Anderson and Neumann 1996), so I plotted W_r versus length for each population by month and computed coefficient of determination (r^2).

Results

Differences among Populations and Months

I collected and analyzed 215 bluegills from LT and 199 from SM (Table 2.2). Population differences were evident. Size structure was consistent with preliminary data; mean lengths were 118 mm and 157 mm and maximum lengths were 173 mm and 214 mm in LT and SM respectively. Means were greatly influenced by sampling design, i.e., minimum size constraints and collection evenly across lengths defined by the preliminary data. However, maximum lengths did not change greatly, so I believe samples were representative of all sizes within each population. Sex ratio was slightly skewed towards males in LT and towards females in SM. Minimum length at maturity differed among populations. Several females <100 mm were found to be sexually mature in LT, based on GSI, whereas the smallest mature female from SM was 155 mm (Figure 2.1). There were few mature males in LT samples; the smallest was 115 mm. In preliminary samples from LT, mature males were as small as 84 mm (T. Copeland, unpublished data). I collected more mature males from SM and they were larger ($\geq$ 184 mm). There were also differences in growth and age structure. Bluegills from LT did not live as long or grow as fast as those from SM, particularly females (Figure 2.2). Median age in the LT sample was 2 years versus 4 years in SM. As an indicator of longevity in each population, the 95th-percentile age was 5 years in LT and 8 years in SM.

Correlations of length to W_r differed, although mean W_r in the two populations was lower than in the preliminary data and data ranges broadly

overlapped (Figure 2.3). There was no correlation between length and W_r in SM during any month. In LT, W_r was inversely related to length, although correlation strength varied among months. The trends between the populations converged at smaller lengths. Thus, larger bluegills from LT tended to have lower W_r than similar-sized bluegills from SM, while the populations overlapped in W_r at smaller sizes.

There were differences among sample collections in terms of the physiological variables. Differences were related to month (MANOVA, Wilks' Λ = 0.31, $P < 0.0001$) and the population of origin (Wilks' Λ = 0.61, $P < 0.0001$). Response through the year depended on population; the interaction of population and month was significant (Wilks' Λ = 0.82, $P < 0.0001$). On a univariate basis, there were significant differences among collections in each variable (Table 2.3). Population differences were significant in VSI and water content. Monthly differences were found in lipid content, protein content, and VSI. The interaction between month and population was significant for lipid content. Main effects and the interaction were significant in W_r and LSI.

Values of W_r, organosomatic indices, and body components differed considerably among sample collections (Table 2.4). In terms of W_r and LSI, populations changed differently through the year (Table 2.4). Mean W_r peaked during June in SM and during August in LT. In SM, LSI was depressed in the summer but increased through the year in LT. Both populations changed similarly in VSI with a low in August but VSI was higher throughout the year in LT. Water content did not change through the year and was higher in LT. Protein and lipid

content did not differ between populations but cycled through the year. Population lipid cycles peaked at different times.

An additional problem was posed by GSI because of large differences between sexes. First, I analyzed GSI with both sexes together. Both month and population had significant effects (Table 2.3). In this analysis, GSI peaked in June and was always higher in SM. I then added sex to the model and log-transformed the values. All main effects were significant in this model, as well as two interactions. The main outcomes of the lumped model were also plain in the gender-specific model: GSI peaked in June and tended to be higher in SM bluegills. However, sex explained the largest amount of variation (F = 483.85), followed by month (F = 37.02) and population (F = 6.43). The significant interactions indicated a finer interpretation. Populations were different in August; SM had fish of both sexes with higher GSI than in LT at that time. Because of the large amount of variation, male GSI in LT did not differ significantly through the year. The annual reproductive cycle was much more apparent in SM.

Physiological Relationships to W_r

I pooled all 414 bluegills to consider the most general interpretation of W_r in terms of body composition and organosomatic indices. The regressors had many inter-relationships; 11 of 15 possible correlations were significant (Table 2.5). This was chiefly because of the large sample size and correlations generally had low explanatory power (R^2 < 0.50). The highest correlation was between water and lipid content and was negative. Energetically-important variables (LSI,

VSI, and lipid content) were positively correlated. Water was significantly related to all other variables; these relationships were negative except for protein.

The MLR model relating W_r to the regressors was highly significant (F = 11.97, $P < 0.0001$) but did not explain most of the variation in the data (R^2 = 0.15). All variables except GSI and VSI were significant in the model (Table 2.6). All variables except LSI contributed positively to W_r. The significant regressors included variables that were inversely related, e.g., water and lipid content. I attribute the significance of most of the variables to the large sample size rather than their explanatory power. Only lipid content explained more than 2% of the variation in W_r.

Given the lack of an obvious relationship of W_r to most variables, I focused on the relationship of W_r to lipid content. The simple linear regression relating lipid content to W_r did not explain much variation either (r^2 = 0.09). In general, the correlation was positive, but its strength changed through time and changes were not synchronized between populations (Figure 2.4). The SM population showed significant positive trends in March and August. The LT population exhibited a significant trend only in November. Scatter in the data did not seem to vary much (Table 2.4); it was the increase in slope that influenced model fit.

In contrast, there was a strong relationship (r^2 = 0.70) between W_r and lipid content in the bluegills reared by McComish (1971; Figure 2.5). The data depicted in Figure 2.5 included bluegills with lengths from 80 - 192 mm TL, so the studies were comparable in that respect. However, distributions of W_r and lipid content were very different (Figure 2.6). McComish's laboratory manipulations

achieved a relatively even distribution of values. In comparison, the values I measured were much more clustered about the average. Also, average values for W_r and lipid content were lower in this study.

Discussion

Population Characteristics

The study populations were not as well separated in W_r as the preliminary data indicated and neither population had high values at any time during 1999. In Virginia, the population-level median W_r of bluegills in the spring and fall is 93, while the 25th percentile W_r is 86 (John Kauffman, VDGIF, unpublished data). For Virginia, the LT population had low W_r, while SM bluegills were average to low. The seasonal cycle of W_r was different between populations, being higher in June in SM and August in LT. Seasonally, W_r tends to be highest just before spawning (Pope and Willis 1996), so the LT cycle is unusual from that viewpoint. Interestingly, the inverse trend of W_r with length in LT was consistent through the year, as was the lack of trend in SM.

Body composition was comparable to other studies of wild bluegills. Fischer et al. (1996) measured individual water content 71.3% - 79.5% versus a range of 71.9% to 80.7% in this study. Savitz (1971) collected bluegills that averaged 63.4% protein (dry weight) and then 67.1% after 29 days of starvation. Monthly means in this study varied from 61.4% to 66.2%. Bluegills in Par Pond, South Carolina, had a relatively stable mean lipid content of about 6% - 10%, with peaks before spawn and in early fall near 10% and lows in midsummer and

late fall (Belk and Hales 1993; Fischer et al. 1998). In Lake Opinicon, Ontario, mean lipid content in juvenile bluegills increased from 7% to 14% during the growing season (Booth and Keast 1986). Lipid cycles were different between LT and SM in terms of timing but the range of monthly means (6.4% - 9.7%) was within limits for wild bluegills in the literature.

Several studies have examined bluegill LSI under natural conditions. Bulow et al. (1978) found LSI in two populations was maximum in spring (maximum monthly average LSI 0.8 - 2.0), low during summer (minimum monthly average LSI 0.4 - 1.0), and gradually increased after late summer; however, timing was different between populations. The population cycles in this study differed from each other and the general pattern proposed by Bulow et al. (1978), but within the limits they described. Similarly, LT and SM were within or above the growing season means found by Cook (1994) for populations with good to poor growth characteristics (average LSI 0.53 - 1.03). Breck (1996) manipulated bluegill densities in a summer pond experiment that produced monthly W_r means close to those I observed (76.9 - 96.0, his normal and slow growth treatments), yet monthly LSI means (0.90 - 2.09) were higher than June and August averages from SM and LT. It is clear that LSI responds to factors that differ among populations. My results were typical in that regard.

Mean VSI of both populations was highest at beginning and end of the growing season but was generally higher in LT. Long-term energy storage should be highest before winter but become depleted during summer as resources stored in the viscera are used elsewhere, e.g., for growth or gonadal

development. Evidently, more stored energy is needed by LT bluegills. Normally, one would expect smaller fish, such as in LT relative to SM, to have fewer reserves because of the emphasis on growth in smaller fish (Weatherley and Gill 1987). Hence, I suspect that higher VSI in LT was biologically significant in terms of life history characteristics and resource allocation decisions.

The reproductive cycle, as tracked by GSI, was also different between populations. Both populations had the highest GSI for both sexes in June. But reproduction was more extensive in SM. During August, reproductively-active individuals of both sexes were collected in SM but none were observed in LT. Two other observations are relevant. First, more individuals in reproductive state were captured in SM. Second, cuckolders, non-nesting reproductive males of small size and high GSI (Gross and Charnov 1980), were collected in LT. Cuckolders allocate more resources to testicular development such that GSI > 3.0, whereas parental males, which have invested in somatic growth, have lower GSI (<3.0, Ehlinger et al. 1997).

The populations differed in physiological cycles although within ranges typical of wild bluegills. Each variable changed significantly among months, except water content. Timing of changes differed between LT and SM for W_r, LSI, GSI, and lipid content. Changes did not appear to be synchronized. Relative organ size should be sensitive to both input and distribution of energy within the individual (Goede and Barton 1990). Lipid content and W_r also seem malleable, given the effect of population and month interactions on their values. These changes should be influenced by how individuals interacted with the

environment and how each used the resources acquired. Hence, population characteristics, life history, and inferences on selective pressures should help explain observed patterns. Population density in LT was much higher, based on preliminary surveys, yet water fertility was similar. Also, population age and size structure was different. In LT, bluegills matured sooner, grew more slowly, and died sooner than those in SM. Females in LT disappeared before growing larger than 130 mm. These characteristics are typical of exploited bluegill populations (Coble 1988). Exploitation in LT should be facilitated by easy access for the public. This evidence leads me to conclude that there is higher adult mortality and fewer resources per capita in LT. Excessive adult mortality helps promote the occurrence of cuckoldry in bluegills (Drake et al. 1997). Alternative reproductive strategies may redirect energy via early maturation schedules, sacrificing growth for fitness (Deacon and Keast 1987). Thus, energy allocation strategy is likely to be different between the populations, genders, and among individuals within LT. Differences in maturity schedule can cause differences in nutritional status (Justus and Fox 1994); therefore, these population differences have implications for correlations to W_r.

The assumption that heavier fish at length are in better condition implies that changes in body weight are affected by energetically important tissues (Sutton et al. 2000). In this sense, whole-body condition indices are indirect, general measures that sum the action of specific components. Relative weight did not explain much of the variation in the variables. Lipid content was most highly correlated to W_r, but even that was weak. Lipid content fluctuated

seasonally, but population cycles were not synchronized. There was a great deal of inter-individual variation within both populations. This was evident in the scatter about the central trends in Figure 2.4, resulting in low r^2 even when a correlation was significant. The magnitude of such variation limits the usefulness of W_r as an index of physiological well-being for populations of wild bluegills.

The weak correlations of W_r to physiological variables were unexpected. Brown and Murphy (1991) found strong correlations ($r^2 \geq 0.585$) of W_r to body composition (e.g., lipid and protein content) in juvenile moronids. Neumann and Murphy (1992) did proximate analyses of white crappies in two Texas reservoirs. They found seasonal fluctuations and correlations were strongest in the fall: r^2 = 0.587 for GSI, and r^2 = 0.245 for lipid. Rose (1989) found strong relationships of W_r to body composition in juvenile walleyes (*Sander vitreus*, $r^2 \geq 0.518$). Simpkins et al. (2003) found lower correlations of W_r to lipid content (r^2 = 0.41 and 0.38 in sedentary and active juvenile rainbow trout), but these were still much stronger than those I observed. However, these species are all at least partially piscivorous and only Neumann and Murphy's (1992) study was done on wild fish.

Comparison to McComish (1971)

A better standard for comparison was needed to aid interpretation and guide development of likely explanations. McComish (1971) reared bluegills in the laboratory and manipulated growth in tightly-controlled experiments. Lipid content was highly correlated to W_r in these fish (Figure 2.5). This relationship

gives a straightforward interpretation: every 3 point increase in W_r produced a 1% increase in lipid content, on average. I assume this is the functional relationship of W_r to lipid content in growing, sedentary bluegills. Why was this pattern not evident in wild bluegills? Wild bluegills were much thinner and less fatty than those reared in the laboratory (Figure 2.6). In the laboratory, energetically-demanding behaviors are reduced, such as competing with congeners, avoiding predators, and searching for food (Aday et al. 2000). Gerking (1962) found that protein conversion efficiency was 17.6% lower in a wild population of bluegills than in the laboratory. Thus, limitations are placed on wild bluegills so W_r and lipid content do not range as widely as when manipulated in the laboratory. A lack of range in the data may cause important relationships to be statistically insignificant (Neter et al. 1996). To illustrate, I compared the strongest monthly correlation of lipid content to W_r in this study to McComish's data confined to $W_r < 98$, a range similar to those I observed (Figure 2.7). Regression slopes are parallel but the trend is shifted downward in the wild bluegills. Hence, wild bluegills had less lipid than laboratory-reared bluegills, even at a similar W_r. Increased energetic demands associated with life in natural environments should reduce lipid reserves. Additionally, fish under feed restrictions put on lean mass upon re-feeding (Johansen et al. 2001). The interaction of increased energetic demands and chronically reduced consumption should cause wild bluegills to have less lipid than well-fed laboratory fish, even when W_r is similar.

Another possibility is that wild bluegills have more variability about any relationship that may exist. There was more unexplained variation associated with the relationship of W_r and lipid content in wild bluegills than in laboratory-reared bluegills, even when trends were similar (Table 2.7). McComish (1971) kept fish individually in homogeneous tanks; natural environments are much more heterogeneous. The fish collected from SM likely had different diets and energetic output compared to McComish's (1971) bluegills and to each other. If energetic history is important to the correlation of W_r to lipid content, then omitting it from the regression model will increase the amount of variation not explained by the regression.

Explanatory Hypotheses

The variables that I compared to W_r are commonly used to assess the nutritional status of fish. Several previous studies found significant correlations between those variables and W_r. Why were correlations to W_r so weak in this study? I developed five explanatory hypotheses.

Statistical Limitations. – Physiological variables in wild fish are clustered by environmental conditions. This seems evident in the comparison of data from this study to the laboratory data of McComish (1971). Lack of range in the data reduces the ability of regression to detect and describe any real relationship (Neter et al. 1996). In essence, observational data inadequately sample the region of interest, while a designed experimental manipulation, such as done by McComish (1971), will reveal the putative relationship (Snee 1977). The problem

is relating mechanisms that operate in an artificial environment to a natural scenario. Combining data from populations with widely differing W_r may provide the needed range in the data to detect a functional relationship. Another approach would be to conduct an experimental manipulation with a natural environmental control against which treatment effects may be checked.

Species Bias. – Bluegills are a generalist species with a plastic morphology. Most previous research was done on more specialized species, especially piscivores. Piscivory places more restrictions on life history and is potentially more energetically profitable than other trophic strategies (Keast 1985). These traits may reduce variability about W_r trends and increase range within a population.

Differences in body shape may also be influential. Standard weight is exclusively a function of length. Body shape logically affects the chances of weight varying from changes in a dimension other than the main body axis. A compressed species, such as bluegill, should be more likely to exhibit changes in shape not related to length than more cylindrical species. Shape changes would be manifested as extra variation about the W_s curve. Additionally, habitat-related differences in morphology within bluegill populations have been demonstrated (Layzer and Clady 1987; Ehlinger and Wilson 1988). These differences may affect the ability of a single W_s equation to remove the size component of total weight in bluegills. A comparison of how nutritional variables correlate to W_r among species that differ in trophic strategy and body plan may suggest factors that influence interpretability of W_r.

Genetics. – Bluegills may be genetically inclined to be thin or plump and gradations between the extremes could be found in any population. In this case, W_r would carry limited information on nutritional status. Maceina and Murphy (1988) found that northern and Florida strain largemouth bass had different W_r although they were in a common environment and eating a similar diet. This result raises an important question about the assumption that standardizing weight by W_s effectively removes genetic influences so that environmental effects on weight are revealed (see Gutreuter's comment in Springer et al. 1990). Stunted bluegills sometimes do not perform as well as non-stunted conspecifics when transferred to a common environment (Cook 1994; Breck 1996; but see Heath and Roff 1987). It would be difficult to disentangle genetic from environmental effects and would take a series of long-term experiments involving artificial selection, environmental manipulation, and a common-garden design.

Energetic History. – Feeding history may influence the relationship of W_r to body components. Fed and starved fish have different energetic strategies and both are likely in the same population, because of resource patchiness and differences in individual ability. Under normal conditions, fish increase activity in order to locate food (Madon and Culver 1993). Conversely, starved fish become torpid at some point in order to reduce energetic expenses (Beamish 1964). Energetic history also influences growth, i.e., fish with ample energetic reserves grow differently than those with depleted reserves (Broekhuizen et al. 1994). Fasted fish will typically grow more quickly after re-feeding than individuals that have been fed continuously, a phenomenon termed compensatory growth

(Weatherley and Gill 1981; Hayward et al. 1997). Fish undergoing compensatory growth tend to produce more protein than normally growing fish (Johansen et al. 2001); therefore, differences in body composition may be induced by differences in energetic history. Resources in natural environments are not evenly distributed in time or space; hence, periods of starvation and restricted feeding are inevitable. Bluegill growth in the wild is often food-limited (Anderson 1959; Osenberg et al. 1988; Nibbelink and Carpenter 1998). Food limitation leads to inter-individual variation in growth response upon re-feeding (Jobling and Koskela 1996) with concomitant variation in relative amounts of protein and lipid accumulated. These circumstances have implications for the body composition of individuals with different environmental experiences, especially when comparing between simplified laboratory environments and the wild.

A multi-faceted approach is necessary to investigate the impact of energetic history on wild fish. Most previous research on body composition was done in the laboratory. In a typical feeding experiment, a constant ration is offered. As demonstrated by studies of compensatory growth (e.g., Hayward et al. 1997), a different physiological response is evoked when feeding regime is changed. Few studies have examined the impact of feeding regime changes on body composition and I am not aware of any that examined the influence of such changes on interpretation of condition indices. Design should include the effects of starvation, re-feeding, and interactions with nutritional reserves. Information from a wild population would provide context.

Population Ecology. – Energy allocation decisions are influenced by environmental conditions, resulting in different energy allocation strategies (Deacon and Keast 1987; Belk and Hales 1993; Drake et al. 1997). Allocation strategy can affect the nutritional state of individuals as energetic investment and expenditures are adjusted to maximize fitness (Justus and Fox 1994; Post and Parkinson 2001). One potential effect of alternative strategies would be to decouple W_r from lipid storage in the individual if the majority of energy is channeled into maturation and reproduction. On a population level, if several strategies are pursued within the population, the net effect would be wide variation about the relationship of W_r to nutritional variables as differences in fitness strategy mandate changes in somatic investment. Because cuckoldry in bluegills is a frequency-dependent fitness strategy (Gross 1982), males will pursue different allocation strategies in populations where cuckolders are present. Females may pursue their own strategy as well (Roff 1992).

Environmental conditions may influence energy allocation in a manner independent of reproductive strategy (Rogers and Smith 1993). The benefits of increased energy storage and body mass are well known. However, increased body mass has potential concomitant costs due to predation risk, metabolic costs, injury risk, increased foraging, and health effects of obesity (Witter and Cuthill 1993). I would add foregone growth and reproductive opportunities. Lipid storage and relative body mass are thus life history traits to be optimized in terms of survival (Rogers 1987). The optimal body mass (OBM) an individual should maintain represents the tradeoff of the costs versus benefits (Lima 1986). The

foregoing work was focused on birds, which have more obvious costs in terms of carrying extra mass. However, it is naïve to expect that lipid accumulation does not carry costs for fish as well. Models based on OBM principles have successfully predicted foraging behavior in salmonids (Bull et al. 1996; Biro et al. 2003). Young rainbow trout can switch allocation strategy from maximizing growth to maximizing lipid storage depending on growth rate; at intermediate growth rates, both strategies may be present (Post and Parkinson 2001). Habitat selection based on tradeoffs between predation risk and energetic profitability has been demonstrated for bluegills (Werner et al. 1983; Werner and Hall 1988). These tradeoffs can explain population differences in growth and size structure (Nibbelink and Carpenter 1998) and have implications for lipid storage and W_r.

The two theories above are not mutually exclusive and I believe that together they challenge the "fatter is fitter" paradigm (*sensu* Glazier 2000) implicit in the use of condition indices. A problem for testing these theories is that they are tautological, true by definition if one accepts their premises. Hence, differences from predictions can always be explained away as costs that have not been included in the model (Witter and Cuthill 1993). Still, models such as OBM offer an alternative to explain variation that makes no sense in condition theory and have the potential to link behavior, physiology, and ecology. An examination of the effects of reproductive strategy could include comparison of cuckolder versus parental males. A potential test of non-reproductive effects is to duplicate the pond experiment of Werner et al. (1983) to see if the predation risk/energetic profitability ratio influences lipid deposition and W_r.

Conclusions

The relationship of W_r to nutritional status was not straightforward in wild bluegills. I found: 1) lipid content had the strongest relationship to W_r, but 2) strength varied seasonally and was not similar between populations. The former result is in accordance with laboratory results (e.g., McComish et al. 1974; Rose 1989; Brown and Murphy 1991), but the latter implies that laboratory results may be confounded in more complex environments. Condition indices are not reliable indicators of nutritional status although seasonal index cycles may correspond to changes in composition in some cases (Strange and Pelton 1987; Wicker and Johnson 1987).

The foregoing results offer a cautionary tale for the use of W_r as a field tool for nutritional assessments. In this regard, I offer several recommendations for the use and interpretation of W_r: 1) Do not assume all populations have the same energetic cycle. Similarly, W_r cycles are often different among populations (Liao et al. 1995). Certain seasons have been proposed as the optimal times to sample for W_r interpretation; this will not be universally true. Optimal time for sampling will depend on purpose. 2) The definition of homogeneous groups for comparison may help interpretation. Attention should be paid to probable similarities in energy allocation. The use of ancillary life history data, such as sex-specific size and age at first maturity, will be invaluable for this purpose. 3) I suggest that a nutritional interpretation of W_r will be more clear and precise when and where the environment promotes somatic growth. For example, correlations were stronger and more frequent in SM. Shearer (1994) cautioned that

relationships predicting body composition should be applied only to growing fish. Beckman et al. (2000) observed that patterns of condition, LSI, growth, and lipid content in wild juvenile chinook salmon (*Oncorhynchus tshawytscha*) most closely correspond during periods of rapid growth. The effect of a benign environment seems apparent when considering the results of laboratory studies (e.g., McComish et al. 1974), which are usually less energetically demanding than natural conditions (Aday et al. 2000). 4) Given the unexplained variability in my data, W_r seems to be more of a general indicator of nutritional status in wild fish and not a precise predictor of body composition. To conclude groups are physiologically different based on W_r, one should have large sample sizes or observe a large difference. Such findings should be followed up by more specific investigations. 5) Lastly, most physiological work on fishes has been done in the laboratory with immature individuals. Biologists then apply results to the study and management of wild fishes. Findings should be extrapolated to natural settings with extreme caution, if at all. Research needs to be done on fish physiology in natural environments to verify such extrapolations.

Table 2.1. Preliminary estimates of population density (electrofishing catch per hour, CPH), proportional stock density (PSD, Anderson and Neumann 1996), average total length (TL, mm; standard deviation in parentheses), maximum total length (MTL), and average relative weight (W_r) in bluegills collected from Lower Travis Lake (LT) and Smoots Pond (SM) during management inventories in April 1999 (T. Copeland, unpublished data).

Water	CPH	PSD	TL	MTL	W_r
LT	227.3	25	113.8 (24.8)	168	90
SM	27.6	62	160.7 (37.9)	214	102

Table 2.2. Numbers of female and male bluegills analyzed for this study, their mean total length (TL, in mm; standard deviation in parentheses), and maximum total length (MTL) by population (Lower Travis Lake, LT; Smoots Pond, SM).

Water	Females	Males	TL	MTL
LT	97	118	117.6 (22.9)	173
SM	118	81	156.8 (37.9)	214

Table 2.3. *F* ratios of each factor from ANOVA testing of relative weight (W_r), organosomatic indices (LSI, GSI, VSI), and body components (water, protein, lipid). Factors entered into the ANOVA model were population, month, and their interaction (model df = 7, error df = 406).

	Factor		
Variable	Population (df = 1)	Month (df = 3)	Interaction (df = 3)
W_r	56.26***	26.35***	6.42***
LSI	41.47***	38.44***	8.56***
GSI	8.31**	26.36***	1.17
VSI	24.65***	10.40***	0.74
Water	12.92***	2.11	1.09
Protein	3.83	35.16***	2.37
Lipid	0.45	8.53***	3.79*

* $P < 0.05$, ** $P < 0.005$, *** $P < 0.0005$

Table 2.4. Mean W_r, organosomatic indices (LSI, GSI, VSI), and body composition (water, protein, lipid) for each sample. Female and male GSI are presented separately. All variables were expressed as percentage of body weight except protein and lipid content (% dry weight). Standard deviations are in parentheses. Samples are separated by month and population (Lower Travis Lake, LT; Smoots Pond, SM).

Sample	W_r	LSI	m GSI	f GSI	VSI	Water	Protein	Lipid
LT – March	81(6)	0.78(0.21)	0.82(0.18)	0.07(0.04)	3.29(0.62)	76.2(0.7)	61.4(2.9)	8.9(1.8)
June	85(6)	0.90(0.18)	2.60(2.06)	0.70(1.09)	3.02(0.67)	76.0(1.5)	66.1(4.4)	8.1(3.7)
August	87(6)	0.91(0.20)	0.64(0.19)	0.13(0.19)	2.82(0.59)	76.1(1.3)	64.5(2.4)	9.0(3.1)
November	82(6)	1.22(0.32)	0.75(0.15)	0.09(0.04)	3.14(0.56)	76.3(0.7)	66.2(2.4)	7.2(3.5)
SM – March	86(6)	0.83(0.27)	0.69(0.13)	0.06(0.09)	2.87(0.53)	75.5(1.6)	61.8(3.7)	8.9(3.6)
June	94(6)	0.72(0.22)	2.67(2.12)	0.40(0.43)	2.81(0.62)	75.3(1.7)	66.2(4.3)	9.7(3.6)
August	89(7)	0.73(0.18)	1.50(1.18)	0.35(0.33)	2.51(0.56)	76.0(1.8)	62.8(3.5)	7.4(4.6)
November	85(5)	0.95(0.21)	0.71(0.15)	0.11(0.08)	2.94(0.45)	75.9(1.5)	64.9(3.2)	6.4(3.7)

Table 2.5. Correlation matrix of organosomatic indices (LSI, GSI, VSI), and body composition (water, protein, lipid) to W_r. Elements above diagonal are correlation coefficients (r), elements below are P-values. Sample size is 414. All variables were expressed as percentage of body weight except protein and lipid content (% dry weight).

	LSI	GSI	VSI	Water	Protein	Lipid
LSI	--	0.0833	0.1464	-0.1441	0.0089	0.1104
GSI	0.0904	--	-0.0206	-0.2056	0.0377	-0.0417
VSI	0.0028	<0.0001	--	-0.1337	-0.1647	0.2714
Water	0.0033	<0.0001	0.0064	--	0.3936	-0.6014
Protein	0.8569	0.4438	0.0008	<0.0001	--	-0.3196
Lipid	0.0247	0.3976	<0.0001	<0.0001	<0.0001	--

Table 2.6. Parameters of the regression model relating W_r to organosomatic indices (LSI, GSI, VSI), and body composition (water, protein, lipid). All variables were expressed as percent of body wet weight except protein and lipid (% dry weight). Student's *t* value and associated probability are from the test of null slope (H_o: $\beta_i = 0$). Model adjusted $R^2 = 0.137$, error df = 407.

Parameter	Coefficient Estimate	Standard Error	*t*	*P*-value	partial R^2
Constant	7.23	23.26	0.31	0.756	--
β_{LSI}	-3.654	1.200	-3.04	0.002	0.0103
β_{GSI}	0.5531	0.3019	1.83	0.068	0.0016
β_{VSI}	0.3565	0.5711	0.62	0.533	0.0080
β_{water}	0.7000	0.3089	2.27	0.024	0.0025
$\beta_{protein}$	0.3145	0.0937	3.35	0.001	0.0168
β_{lipid}	0.8441	0.1159	7.28	<0.0001	0.1107

Table 2.7. Regression of W_r on lipid content (% dry weight) for three data sets. McComish 1 includes bluegills that had lipid content determined by McComish (1971) with lengths >80 mm. McComish 2 includes those above with W_r = 75-98. MSE is mean squared error.

Data set	n	r^2	slope	MSE
McComish 1	62	0.71	0.33	8.26
McComish 2	30	0.26	0.28	7.71
Smoots (8/99)	43	0.23	0.34	14.70

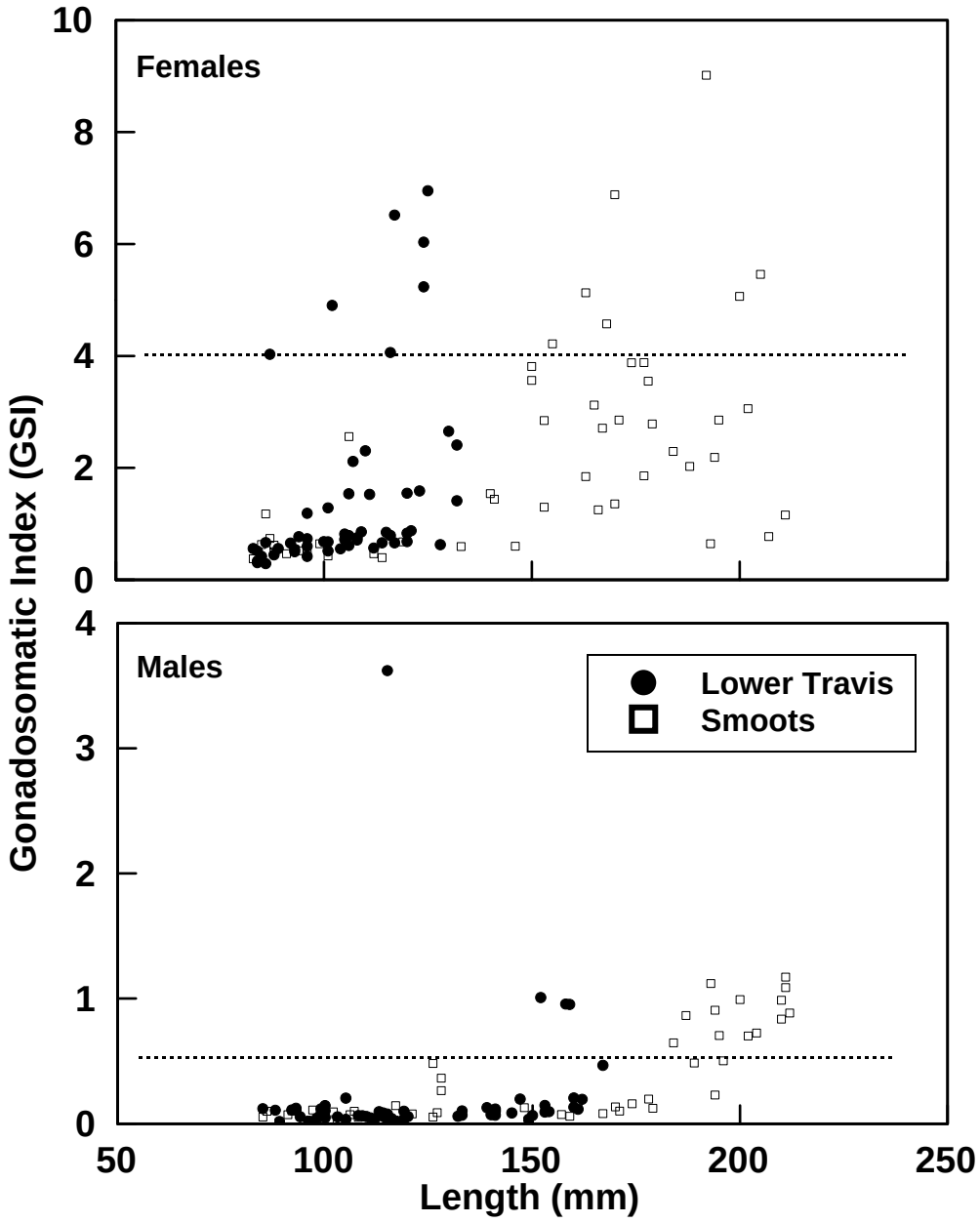

Figure 2.1. Gonadosomatic index (GSI) plotted against length by gender for bluegills captured from Lower Travis Lake and Smoots Pond in June and August, 1999. Dotted lines indicate GSI level above which fish were considered mature: 4.0 for females, 0.5 for males.

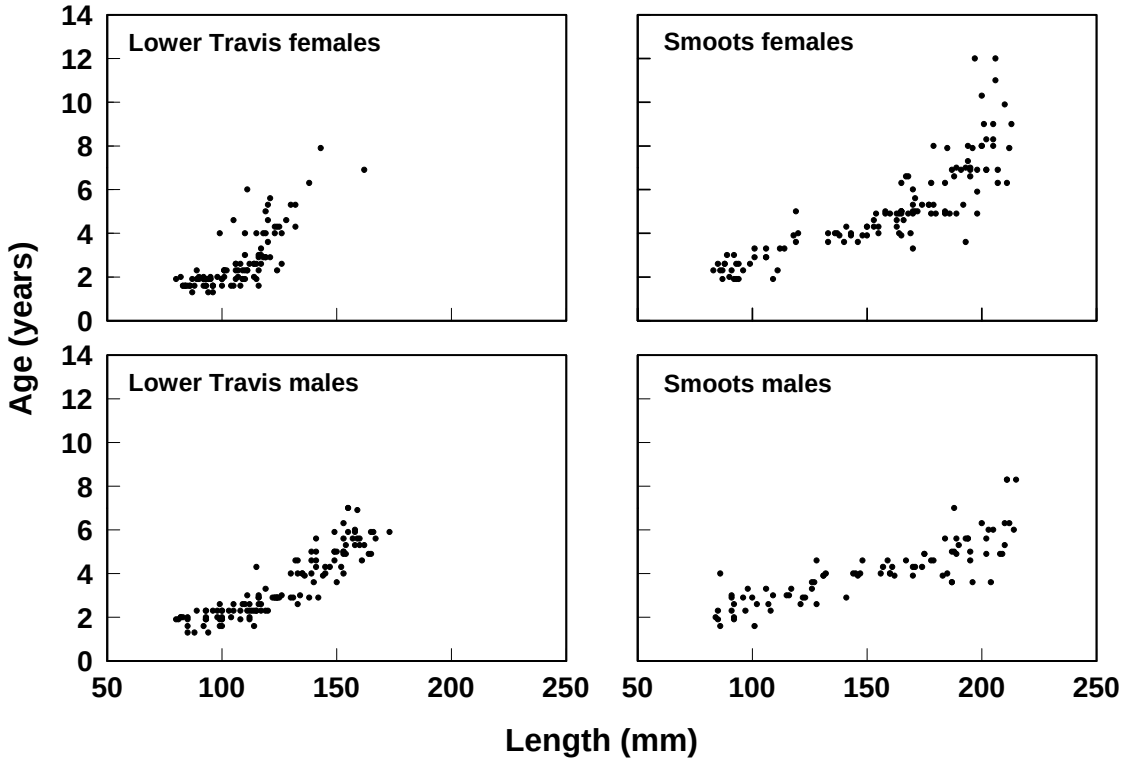

Figure 2.2. Length at age by population and gender. To pro-rate growth during the growing season, 0.3 was added to age for June samples, 0.6 for August samples, and 0.9 for November samples.

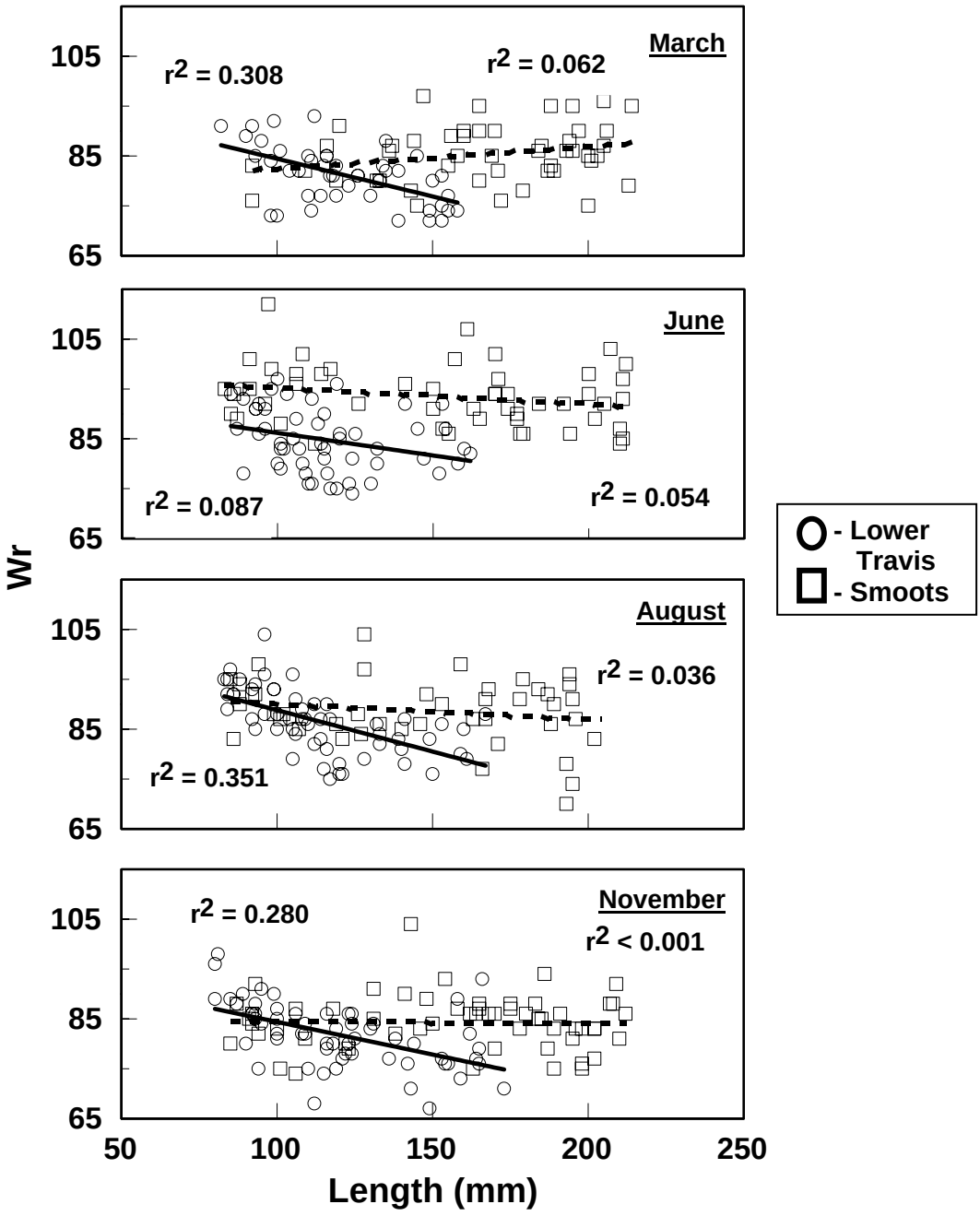

Figure 2.3. Relationship of relative weight (W_r) to length by month. Circles are Lower Travis and squares are Smoots Pond bluegills. Coefficients of determination (r^2) for Lower Travis are on the left in each panel; those for Smoots are on the right.

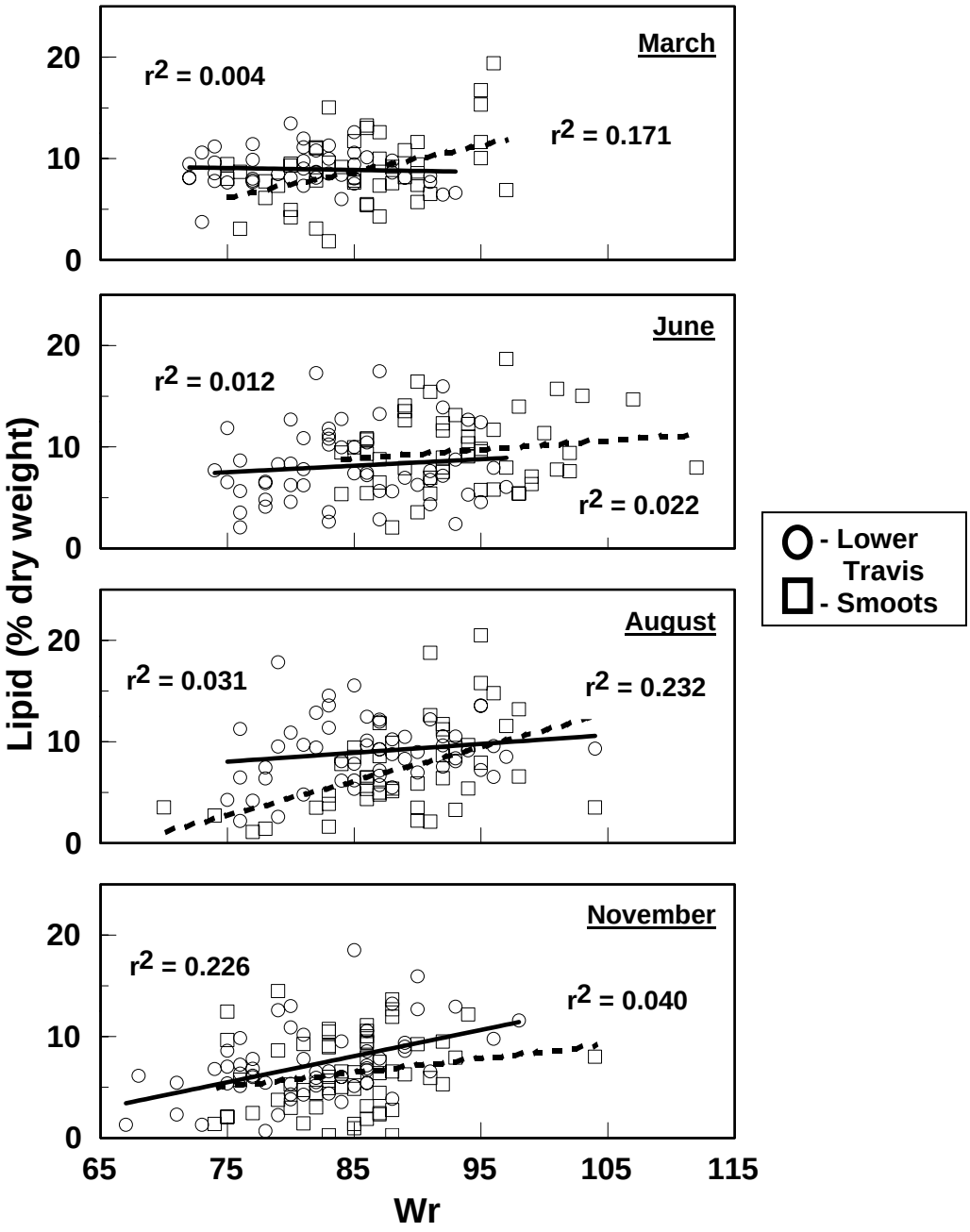

Figure 2.4. Relationship of relative weight (W_r) to lipid content by month. Solid line is regression for Lower Travis. Dashed line is regression for Smoots Pond. Coefficients of determination (r^2) for Lower Travis are on the left in each panel; those for Smoots are on the right.

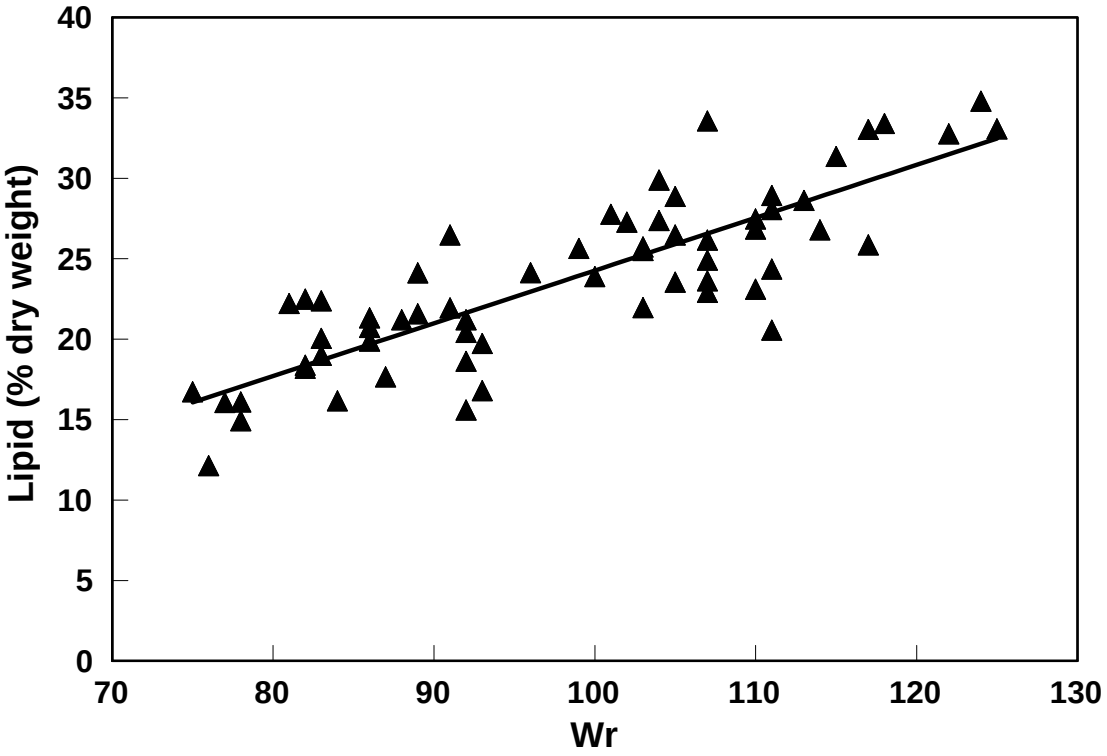

Figure 2.5. Relationship of W_r to lipid content in bluegills reared by McComish (1971).

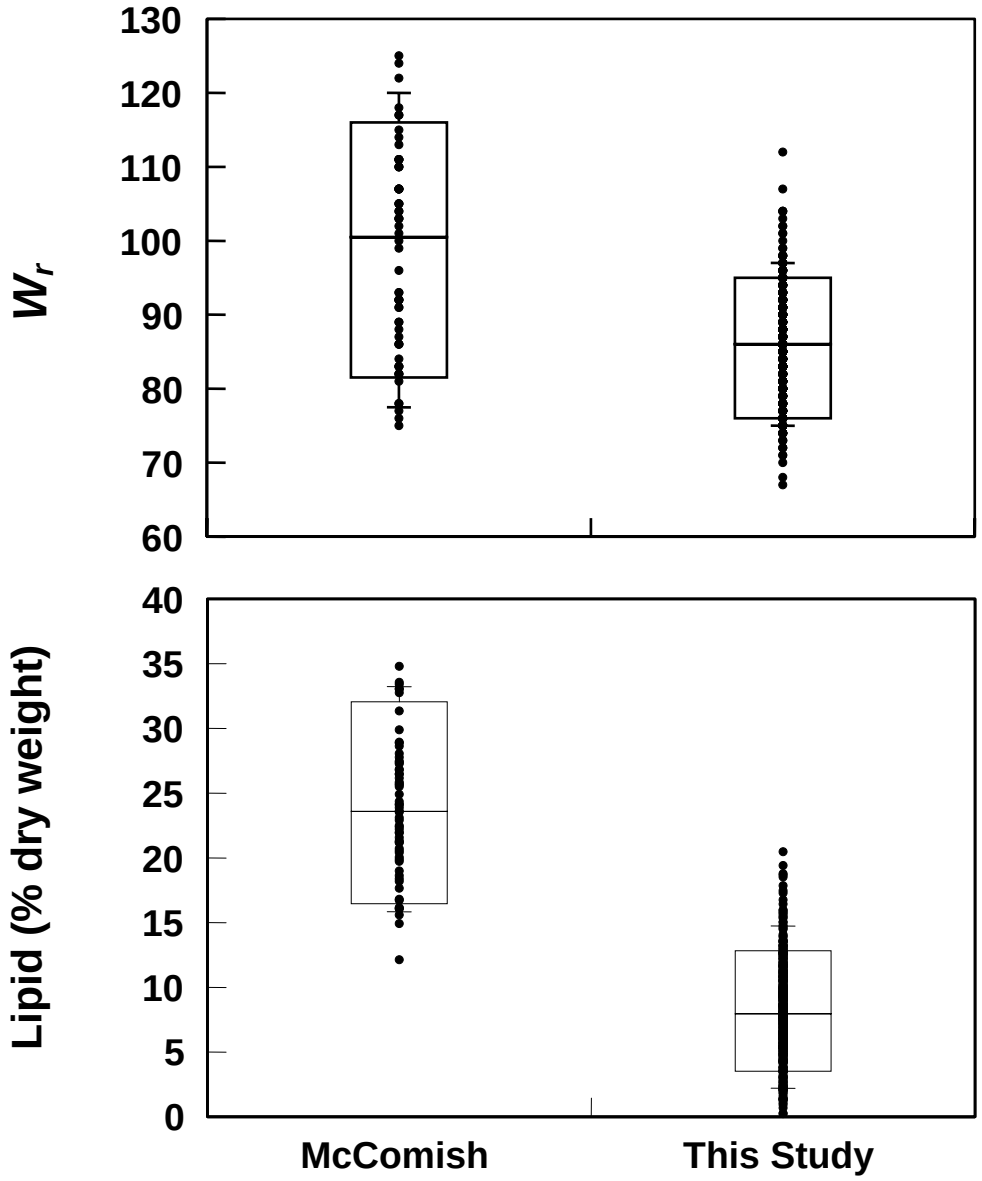

Figure 2.6. Distribution of relative weights (W_r) and lipid content from McComish (1971) and the present study. The box represents the 10th and 90th percentiles with the median shown as a line. The whiskers show the 5th and 95th percentiles. Individual data are superimposed.

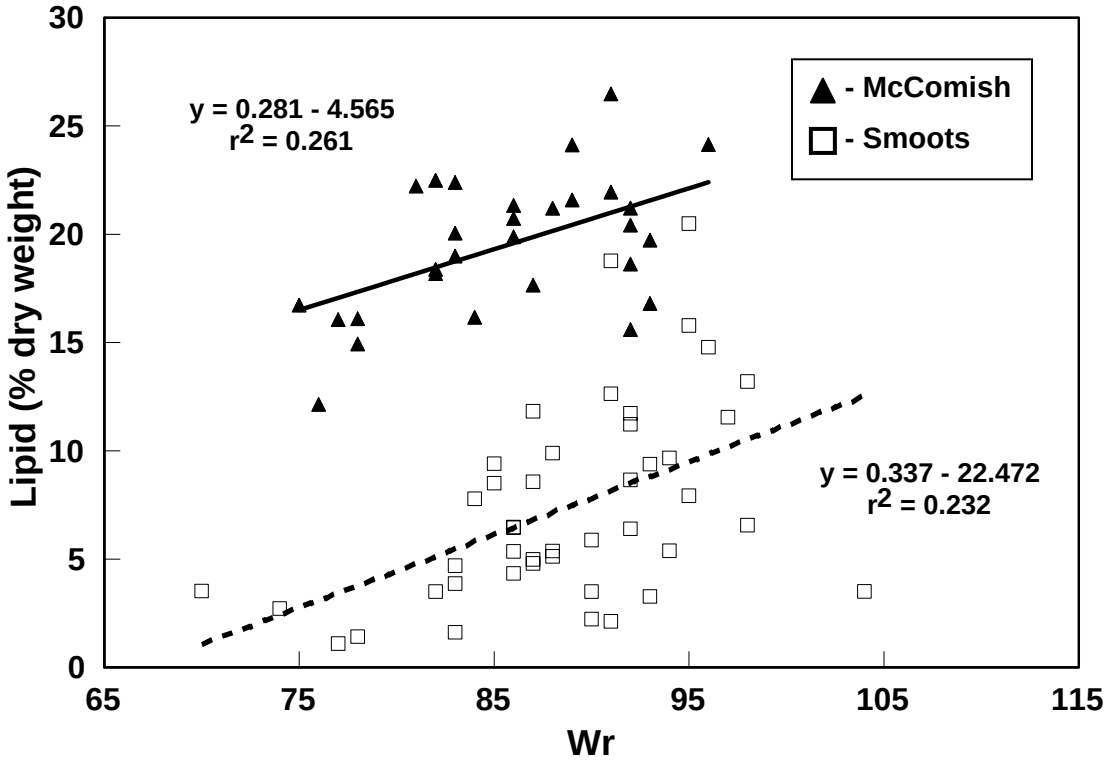

Figure 2.7. Relationship of lipid content to W_r in bluegills collected from Smoots Pond in August 1999compared to that of bluegills reared by McComish (1971) with $W_r \leq 98$. Solid line is regression of McComish's data (parameter estimates in upper left). Dashed lined is regression of Smoots data (parameter estimates in lower right).

CHAPTER 3. LIMITATIONS OF RELATIVE WEIGHT AS AN INDICATOR OF NUTRITIONAL STATUS IN WILD BLUEGILLS

Introduction

Whole-body condition indices for fish are measures of relative plumpness that are used as surrogates for physiological well-being (Anderson and Gutreuter 1983). However, interpretation of condition indices in wild fish is often problematic because the underlying mechanisms that determine index values are poorly understood (Rice et al. 1983). Condition indices are influenced by many factors (Le Cren 1951; Sutton et al. 2000), such as environmental stress, consumption, reproduction, and life history (Goede and Barton 1990). Index values are influenced by both internal and environmental factors (Busacker et al. 1990). The opportunity for masking or opposing effects is great because net nutritional income fluctuates with an animal's environmental experience and degree of somatic allocation may differ among individuals. The interaction of multiple constraints produces a pattern in which variance increases as more constraints act on a variable (Huston 2002). Such interactions would preclude unambiguous interpretation of condition indices, e.g., index value x equates to y percentage lipid.

Whole-body condition indices should reflect the sum of the parts (Iles 1984), the major body components being water, lipid, and protein. In laboratory studies, strong correlations between body composition and condition have been demonstrated (e.g., McComish et al. 1974; Rose 1989; Brown and Murphy

1991). However, a straightforward physiological interpretation of condition in wild fish has been elusive (e.g., Salam and Davies 1994; Jonas et al. 1996).

In previous work on wild bluegills (*Lepomis macrochirus*), I found explanatory power of regression models relating W_r to nutritional variables was low in two wild populations (Chapter 2). In contrast, condition of laboratory-reared bluegills was well correlated to body composition (McComish et al. 1974). How general are my previous results? The most stringent test of a model is by the application of new data (Montgomery and Peck 1992). The new results may corroborate, refute, or suggest limitations of previous conclusions.

One likely hypothesis for the weak correlation between W_r and other variables concerns the nature of observational data. Because environmental conditions limit the values a variable may take, observational data may inadequately sample the region of interest (Snee 1977). Variables that are influential at other scales appear statistically insignificant when confined in range (Neter et al. 1996). An inadequate range in the data may also exacerbate the apparent influence of confounding factors (e.g., varying amounts of somatic investment), which may spread or bias values. Hence, lack of an apparent relationship may be real or due to action of some confounding factor. I termed this potential explanation the statistical limitation hypothesis (Chapter 2).

Several characteristics of my previous study populations suggest that statistical limitations may be obscuring influence of physiological variables on W_r. Lipid content and W_r in wild bluegills (Chapter 2) were much more clustered than those from the laboratory population reared by McComish (1971). The two wild

populations were not well-separated in terms of W_r; monthly means were <10 points apart (Chapter 2). Lastly, both populations inhabited infertile, slightly dystrophic, ponds with high summer temperatures. A harsh environment may limit ability of bluegills to store energy compared to more salubrious waters.

The contrast between my findings and previous work needs corroboration and explanation. My first objective was to verify that the correlation of W_r to variables related to nutritional status is not precise in wild bluegills (Chapter 2). Second, I wanted to test the hypothesis of statistical limitation as an explanation of previous results. Will data from a population with higher W_r show a clearer correlation of W_r to physiology? To investigate this question, I examined data from another population of wild bluegills alone and in combination with comparable data from the previously-studied populations (Chapter 2).

Methods

Study Site

The study site was Rural Retreat Lake (RR), a small (36 ha) impoundment in Wythe County, Virginia. The outlet stream is a minor tributary of the New River. In previous work, mean bluegill W_r was >100 in RR (John Copeland, Virginia Department of Game and Inland Fisheries [VDGIF], personal communication). The lake is owned by VDGIF and is easily accessed.

I chose RR to provide a contrast to my previous study sites, Lower Travis Lake (LT) and Smoots Pond (SM). Rural Retreat Lake is located in the Ridge and Valley physiographic province, whereas the other two are on the edge of the

Coastal Plain. Local land use in the RR drainage is largely agricultural and consequently the water is fertile (total alkalinity = 33.4 mg/l as $CaCO_3$, VDEQ 1990; surface conductivity = 183 µS, John Copeland, personal communication). The other sites are located on the Fort A. P. Hill military reservation, a forested area over sandy soils. Consequently, LT and SM are very infertile (total alkalinity $\leq$ 6 mg/l as $CaCO_3$, surface conductivity = 20 µS; T. Copeland, unpublished data). Biological characteristics were very different among the bluegill populations. Electrofishing catches during the most recent management inventories were 118.2/h, 227.3/h, and 27.6/h for RR, LT, and SM respectively. Proportional stock densities, the proportion of quality-sized fish in the catchable population (Anderson and Neumann 1996), were 17, 6, and 69, for RR, LT, and SM respectively. The RR population was intermediate in terms of population density and size structure but much higher in mean W_r (>100 versus monthly means of 81 to 94 in LT and SM, Chapter 2).

Data Collection

Field sampling was conducted April 1-2, 2002. Collections were made in cooperation with VDGIF spring field work. The minimum total length (TL) of fish collected was 80 mm, the minimum for application of W_r to bluegills (Murphy et al. 1991). Based on previous length data, the entire population length range was stratified into thirds delimited by the 33rd and 66th percentile lengths, and samples were taken evenly within strata as per Copeland et al. (1999). The overall target sample size was 60 individuals, 20 within each length category.

Bluegills collected were measured to TL in order to assign each individual to a category in the field. A running tally was kept and sampling continued until targets for each category were met.

Fish were put on ice for transport back to the laboratory, where they were stored on ice in a sealed container and processed within a week. Length was measured again (nearest whole mm). Fish were patted dry and weighed to the nearest 0.001 g. Each individual was dissected and the liver, gonads, and viscera removed and weighed (nearest 0.001 g). Otoliths were extracted and examined to determine age. Prior to weighing the viscera, stomach or intestinal contents were extruded. All organs were replaced within the body cavity. For logistical reasons, body composition was determined for only 29 bluegills evenly and randomly chosen across length strata. All subsequent proximate analyses were based on those 29 individuals. Each carcass was chopped into small bits, placed on a pre-weighed aluminum dish, weighed, and dried at 37^{o}C in a drying oven. The dried pieces were ground with a coffee bean grinder. The resultant powder was replaced in the oven on the same pan until weight stabilized for the largest fish then all were re-weighed. Difference between wet and dry weights was used to determine water content. Protein and lipid content were determined at the Virginia Tech Aquaculture Center laboratory using the macro-Kjeldahl procedure and chloroform:methanol extraction (AOAC 1990).

Statistical Analyses

I used multiple linear regression (MLR) to examine the relationship of variables (whole-body water, protein, and lipid content, and weights of liver, gonads, and viscera) to W_r. All were standardized by size; lipid and protein content were expressed as percent of dry weight, all others were expressed as percent of wet weight. For the organ weights, this procedure transforms them into commonly used organosomatic indices (Goede and Barton 1990): liver-somatic index (LSI), gonadosomatic index, (GSI), and viscerosomatic index (VSI). Data were examined using standard diagnostics (Studentized residuals, diagonal elements of the hat matrix, DFFITS, and DFBETAS) to detect the presence of outliers and highly influential observations for inspection (Montgomery and Peck 1992). Obviously erroneous observations, such as impossible weights, were deleted from the data set. Remaining data were used to construct a regression model relating physiological variables to whole-body condition. To reduce the complexity of the model and provide for the most general interpretation, I did not include gender effects. Then I fit the data to a MLR, W_r being modeled as a function of body components and organosomatic indices. Examination of model residuals and bivariate scatterplots did not reveal any obvious departures from homoscedasticity; therefore, I did not transform any variables. Significance of individual variables was determined by *t*-test (H_0: $\beta_i = 0$, $\alpha = 0.05$). To help interpret the MLR model, I constructed a simple linear regression (SLR) of each regressor variable to W_r alone. For this second analysis, I separated male and female GSI. In the following, r^2 is used to denote

the coefficient of determination in a bivariate regression, whereas R^2 denotes the coefficient of multiple determination in a multivariate regression. To establish the generality of my previous conclusion, that W_r was weakly related to body composition and the selected organosomatic indices (Chapter 2), I then compared the RR MLR model to those similarly constructed using data from bluegills captured from LT and SM on March 23, 1999 (Chapter 2). Model fits were compared using adjusted R^2 (Montgomery and Peck 1992).

I employed multivariate analysis of variance (MANOVA) to detect differences among RR, LT, and SM samples in all variables. To determine which variables contributed to the observed differences, the MANOVA was followed by univariate analysis of variance (ANOVA). Use of the MANOVA protects the nominal error rate of the ANOVAs (Rencher 1995). To analyze differences with respect to GSI, I separated the sexes. Tukey's post hoc test was used to determine population differences. Risk of Type I error was set at $\alpha = 0.05$. To consider how addition of RR data would increase variable ranges, I subtracted the range of SM and LT data from the range of RR, SM, and LT data combined and expressed this increase as percentage of the SM and LT range.

To test the statistical limitation hypothesis, I examined the effect of extending the range of W_r on the strength of the correlation of W_r to each regressor variable. I considered three mutually exclusive predictions. First, that extending W_r range by combining data sets would result in a stronger correlation because of the greater range in W_r (Figure 3.1A). Second, that the correlation would not be substantially stronger because the relationship had shifted upward

(Figure 3.1B). Third, the correlation would not be stronger because the combined slope was approaching zero (Figure 3.1C). Because no correlations from LT were significant in March 1999 (Chapter 2), I consider each prediction by combining SM with RR, as well as combining all three data sets.

Results

I collected 26 female and 34 male bluegills from RR. Female age ranged 2-9 years and male age ranged 2-5 years. Median age was 3 years for both sexes. Mean length was 134 mm TL. Maximum TL was similar between sexes; 222 mm for females and 215 mm for males. Males grow somewhat faster than females (Figure 3.2). There was no trend of W_r with length ($r^2 < 0.01$).

Comparison of populations showed that RR bluegills were different from bluegills collected from LT and SM in terms of their body composition and organosomatic indices (MANOVA, Wilks' $\Lambda = 0.1517$, $P < 0.0001$; Table 3.1). Mean W_r was highest in RR and lowest in LT; all three populations were significantly different (Table 3.1). The interpopulation pattern of W_r was reflected by liver and protein; RR was highest and LT was lowest, although values from LT were not significantly different than those from SM. Mean values of all variables were highest in RR, except water content. Differences were statistically significant between RR and at least one of the other populations in all variables except for lipid content. Male GSI was highest in RR. Female GSI was similar in RR and LT.

The MLR of body components and organosomatic indices to W_r was not significant using data from RR (F = 1.68, P = 0.17; Table 3.2). The variables with the largest influence on W_r (as measured by partial R^2) were VSI and LSI. Model fits were not very good for any single population (Table 3.3). The set of regressors did not explain a significant amount of variation in W_r for any single population. In general, SLR of each variable to W_r corroborated MLR results (Table 3.4). No SLR was significant in LT bluegills, but two were in RR and SM each. Female GSI was inversely correlated to W_r in RR. This was lost in the MLR when the sexes were combined. There were significant correlations of VSI to W_r in both RR and SM, while lipid content was correlated to W_r only in SM.

Combining data sets increased MLR fit (Table 3.3). Regressions based on RR and SM data and all data combined were highly significant (F = 12.84 and 15.07; P < 0.0001). Data combination greatly increased the influence of LSI; partial R^2 increased from <0.10 to >0.40. Protein content was significant in the RR/SM and combined models but did not have a large influence. Effects on other variables were non-significant or more ambiguous. For example, VSI was significant in the RR/SM model but not in the combined model. More variables were correlated to W_r on a one-on-one basis than were significant in the MLR model but the major features of the combined MLR models were corroborated (Table 3.4). All variables except for overall GSI and water content were significant in at least one of the aggregations. Protein content and LSI were more highly correlated to W_r when data sets were combined. The lipid content-W_r correlation evident in SM bluegills was reduced upon combination with data from

other populations. The VSI-W_r correlation in RR was reinforced in the RR/SM combination but reduced when LT data were added. Male GSI was positively correlated to W_r only when all data were included. Curiously, the negative correlation of female GSI to W_r in RR bluegills was reversed upon combination with SM data.

Combination of all data provided a greater range than any population alone (Table 3.5). However, intra-population variation was not substantially greater in RR as evidenced by standard deviations (Table 3.1) and data ranges (Table 3.5). Further evidence was provided by examination of the determinants of the covariance matrices, a measure of total multivariate variation in the data. These values were 45.7, 62.7, and 65.9 in LT, SM, and RR, respectively. The largest increases in range of values were in W_r and LSI. Only in water and lipid content did data combination fail to add to the range of values.

Because of the imprecision of the relationship of W_r to all variables, evaluation of the statistical limitation hypothesis was made on a qualitative basis. There was support of the hypothesis for LSI and VSI (Figure 3.3). For VSI, RR lined up well with Chapter 2 data as in Figure 3.1A. In the case of LSI, the relationship was more like that in Figure 3.1B but the slope increased in RR data, suggesting a break point below which LSI did not change greatly. The relationship in protein content and female GSI among populations was dissimilar from that of individuals within any population. Water and lipid content were the only variables that did not show a trend with W_r after the addition of RR data to SM/LT data.

Discussion

Population Comparisons

Bluegills from RR provided a good contrast to those from LT and SM. Compositional characteristics were different among populations (Table 3.1). Except for water content, RR bluegills had the highest values, although differences in lipid content were not large enough to be statistically significant. Within-population ranges were generally similar (Table 3.5). Differences were not reflected in population growth trends (Figure 3.2; see Chapter 2, Figure 2.1 for comparison). Bluegills from RR were similar to those from SM in growth pattern, W_r - length trend, and maximum TL. However, there were differences in their overall nutritional status and the factors affecting nutritional variables.

In my previous study (Chapter 2), the probability of failing to detect a genuine relationship (Type II error) between W_r and nutritional variables seemed likely because of a lack of range in the variables. Aggregation of LT and SM data did not result in an appreciable extension of range for any variable over the range in either population. Addition of RR data extended range of all but water and lipid content. The extended ranges of W_r and LSI were >50% larger than for single populations. I concluded range extension provided by RR fish made this an appropriate case to test whether environmental limitations mask the relationship of W_r to physiology among populations of wild bluegills.

Interpretation of W_r in Wild Bluegills

Precise quantitative interpretation of W_r was inconclusive using data from any single population. No single-population MLR was significant, although some simple regressions were significant in SM and RR. Error degrees of freedom in the single-population MLR models were reduced (relative to SLR) because other variables were included but there was no accompanying increase in explanatory power. Protein content, LSI, and VSI became significant in the MLR upon aggregation. All variables except water content had some influence on SLR models of the aggregated data. Comparison of MLR to SLR results provided some clarification. However, all correlations were weak ($r^2 < 0.50$). Presumably, intra-population factors not included in the analysis design interacted to preclude an easily-interpreted linear relationship of W_r to GSI or lipid content. These factors could include differences in reproductive schedules or energy storage costs.

I compared correlations in three populations as they emerged from winter, typically a time of reduced foraging when individuals draw on reserves. My interpretation of the aggregated data is that RR bluegills were beginning to feed and grow at this time, thus increasing LSI with W_r. Average protein content was higher in RR than in LT or SM, yet lipid contents were similar, indicating that protein growth was occurring in RR. Higher W_r values among populations were associated with higher VSI and protein content. Visceral fat is a long-term energy buffer (Adams and McLean 1985); therefore, individuals with higher W_r had more remaining long-term energy storage. Higher relative protein content could result

from the use of carcass lipids or from the tendency of fish emerging from feed restriction to allocate energy to protein synthesis (Johansen et al 2001). Little energy had been allocated to more short-term lipid storage or gonad maturation. I expect that more energy would be allocated to the gonads as the spawning season drew closer. Lipid storage should not begin until post-spawn.

Validation of Previous Results

Validation is testing if a model is appropriate for its intended use (Rykiel 1996). Relative weight is intended for use throughout a species' range. Previously, I had found that energetically important physiological variables were not strongly correlated to bluegill W_r (Chapter 2). Variables measured are commonly used to index energetic status of fish and many previously have been correlated to condition indices (Busacker et al. 1990; Goede and Barton 1990). I conducted this investigation to ascertain the validity and generality of my previous conclusions. The most rigorous method of validation is by collection of new data, as I have done here. Correlations of nutritional variables to W_r were low again.

The major difference between these results and Chapter 2 was the increased importance of LSI. Detection of this trend required aggregation of data from different populations within the same season. Phenological changes in energetic allocation may obscure this effect if data from different seasons are combined as done in Chapter 2. It is well established that W_r and its

interpretation vary seasonally (Pope and Willis 1996). Seasonal changes should occur in the processes controlling W_r and variables likely to be correlated to it.

The correlation between W_r (or other whole-body condition indices) to constituent variables has rarely been very strong except in the laboratory. Most reported correlations explain <50% of the variation in the data, whereas predictive power of regressions is low for $r^2 \leq 0.65$ (Prairie 1996). Individual variation is likely to be high even under controlled conditions (Wang et al. 1998). Even so, McComish et al. (1974) found that body composition of laboratory-reared bluegills could be reliably predicted from length, weight, and condition index. My results suggest the relationships McComish et al. (1974) documented are confounded in natural environments. In general, results of this work support my previous conclusion: W_r is more valid as a general indicator than a precise predictor of nutritional status for wild bluegills (Chapter 2).

Statistical Limitation Hypothesis

The statistical limitation hypothesis was weakly supported. Only a few variables showed increased model fit. There are several potential explanations why only some variables responded to the aggregation across the three populations. First, the phenological cycle of bluegills may emphasize action of certain variables in the early spring, such as LSI or GSI. Second, the three populations may not differ enough to allow detection of slighter, but meaningful, trends. Or lastly, functional relationships of W_r to some variables are non-existent or confounded in natural environments. I will discuss each individually.

Phenological cycles, driven by reproduction, have large effects on physiology (Miller 1979). There should be more variation among individuals in organ and tissue compartments that are involved in energy translocation and allocation at a particular point in the seasonal cycle, thus increasing range of related variables (Piersma and Lindstrom 1997; Secor and Diamond 2000). Increased range of regressors makes detection of a relationship more likely (Neter et al. 1996); this is the essence of the statistical limitation hypothesis. For bluegills, winter is a period of reduced feeding when individuals draw on accumulated energy reserves (Booth and Keast 1986). In Virginia, bluegills spawn beginning during May (Jenkins and Burkhead 1993), well after my collections. During early spring, bluegills should have reduced energy reserves but should be feeding and storing energy for the spawning period. However, the lipostatic growth regulation hypothesis (Johansen et al. 2001) says that individuals emerging from food restriction would preferentially synthesize protein over lipid. Therefore, greater data range should be expected in LSI, which should be active as energy becomes available, and in long-term reserves such as VSI, which have just been drawn on, but not in more easily mobilized short-term reserves such as muscle lipid. This explains the trends I observed but not why they were so weak.

Differences in W_r are commonly interpreted as differences in lipid content; however, such a relationship was not evident in wild bluegills (Chapter 2). Perhaps the range of data was insufficient to detect this relationship. I specifically chose the Rural Retreat population to increase the range of W_r available.

Addition of the Rural Retreat data increased the range of W_r values 64%. However, range of lipid content did not increase. Fischer et al. (1996) collected Par Pond bluegills in May and June with lipids ranging 1.5% - 10.4%. Later work at Par Pond documented lipids averaging near 7% with monthly peaks fluctuating from 2% to 10%, lowest during January and peaked at spawning (Fischer et al. 1998). Booth and Keast (1986) found lipids in immature bluegills increased from 7% to 14% during the growing season. Savitz (1971) collected bluegills from the wild in June that averaged 14.82% lipid. I conclude that the lipid contents I observed were typical of wild bluegills.

The foregoing review leads to the possibility that expected correlations were not detected because they are confounded in wild bluegills. This is different from the phenological explanation because environmental complexity blocks the expression of correlations, regardless of season. For example, the correlation of condition to lipid in laboratory–reared bluegills (McComish et al. 1974) may be confounded by pandemic food restriction and detectable only in populations that can accumulate lipid. Nutritional interpretability of condition indices varies between aquacultural and natural environments and among natural populations (Rikardsen and Johansen 2003). This may result from differences in environmental stress and complexity, e.g., differences in conclusions regarding lipid content and condition indices reached by studies on juvenile walleyes (*Sander vitreus*) under laboratory conditions (Rose 1989) versus aquacultural ponds and natural environments (Copeland and Carline 2004).

Interpretation of W_r in precise nutritional terms was not possible based on data from single populations. Each population was limited in the observed ranges of variables, presumably because of environmental conditions. I found a logical interpretation can be made by aggregating data from disparate populations, although precision was too low for this relationship to be predictive. This interpretation assumes that all populations were responding similarly to the gradient represented by the three environments and that phenological stage was similar among them.

Conclusions

My objectives in this chapter were to corroborate the findings of Chapter 2 and to test the statistical limitation hypothesis as an explanation for those findings. My results from Chapter 2 were validated in that no relationship, whether multivariate or bivariate, explained >50% of the variation in W_r. An unambiguous nutritional interpretation of W_r for wild bluegills remains elusive. The populations examined differed in biological and ecological aspects; consequently, they also differed in values of the physiological variables measured. Thus, data from these populations provided an appropriate setting to test the statistical limitation hypothesis. Yet there was still a great deal of unexplained variation about the relationship of W_r to the variables I measured, even with the extended ranges provided by aggregating data from three populations. I conclude that the weak correlations observed were real and not a statistical artifact.

Bluegill W_r should be interpreted cautiously because of its imprecise relationship with nutritional variables and the many potentially confounding factors. The factors influencing W_r and its precise relation to nutritional variables will differ among seasons and populations. Conclusions in regard to body composition will only be justified in the presence of large differences or large sample sizes (Chapter 2). Quick temporal changes may also indicate a change in body composition (Brown and Murphy 2004). I suggest knowledge of the biology and ecology of the population at hand will help discern likely confounding factors (Chapter 2). The lack of a difference in W_r will not be informative; large differences signal qualitative differences in nutritional status but additional data will be required for more precise interpretation.

My results here and in Chapter 2 suggest that the complex natural environment masks or confounds the relationship of physiological variables to W_r. This concept could be tested empirically by comparison of wild and laboratory populations. Manipulations should attempt to create an energetic history typical of the natural environment as well as duplicating previous laboratory experiments. Properly designed, such an experiment could corroborate McComish et al.'s (1974) results while probing their limitations. As such, starvation or food limitation should be included as a treatment, as those are conditions that wild fish typically experience. The application of these results to other species in natural environments should be examined also.

Table 3.1. Mean and standard deviation of relative weight (*Wr*), organosomatic indices (LSI, GSI, VSI), and body components (water, protein, lipid). All variables are expressed as percent wet weight except protein and lipid content (% dry weight). *F* ratios comparing populations are reported in the last column (* $P < 0.05$). Degrees of freedom were 2, 118 except for female GSI (2, 55) and male GSI (2, 60). Values with different letters are significantly different (Tukeys post hoc test).

Variable	Rural Retreat	Lower Travis	Smoots	*F*
W_r	98 (7)x	81 (6)y	86 (6)z	66.96*
LSI	1.63 (0.41)x	0.78 (0.21)y	0.83 (0.27)y	88.64*
☐ GSI	0.97 (0.28)x	0.82 (0.18)xy	0.69 (0.13)y	9.85*
☐ GSI	0.12 (0.07)x	0.07 (0.04)y	0.06 (0.09)y	5.20*
VSI	3.50 (0.62)x	3.29 (0.62)x	2.87 (0.53)y	11.57*
Water	76.07 (1.20)xy	76.23 (0.74)x	75.46 (1.56)y	4.86*
Protein	67.56 (2.43)x	61.43 (2.86)y	61.81 (3.72)y	39.61*
Lipid	9.08 (2.67)x	8.95 (1.82)x	8.90 (3.57)x	0.04
N	29	45	47	

Table 3.2. Parameters (constant and coefficients, β) of the multiple regression model relating relative weight to organosomatic indices (LSI, GSI, VSI), and body components (water, protein, lipid) from bluegills captured in Rural Retreat Lake. Model adjusted R^2 = 0.127.

Parameter	Estimate	Std dev	*t*	*P*-value	partial R^2
Constant	116.3	143.1	0.81	0.425	--
β_{LSI}	4.663	3.716	1.25	0.223	0.0947
β_{GSI}	1.088	3.795	0.29	0.777	0.0044
β_{VSI}	4.431	2.460	1.80	0.085	0.2002
β_{water}	-0.117	1.725	-0.07	0.947	0.0004
$\beta_{protein}$	0.472	0.697	-0.68	0.505	0.0130
β_{lipid}	-0.148	0.714	-0.21	0.838	0.0013

Table 3.3. Model fit (adjusted R^2) and contributions of each variable (partial R^2) to models relating relative weight to organosomatic indices (LSI, GSI, VSI), and body components (water, protein, lipid) for three single and two combined data sets (Rural Retreat, RR; Lower Travis, LT; Smoots, SM). *N* is sample size.

Parameter	RR	LT	SM	RR & SM	All data
N	29	45	47	76	121
Adj R^2	0.127	-0.036	0.132	0.486**	0.413**
LSI	0.095	0.007	0.028	0.411**	0.402**
GSI	0.004	0.039	0.058	0.009	<0.001
VSI	0.200	0.002	0.064	0.071**	0.008
Water	<0.001	0.044	0.023	<0.001	0.001
Protein	0.013	0.004	0.001	0.024*	0.027*
Lipid	0.001	0.008	0.070	0.013	0.003

* $P < 0.05$, ** $P < 0.005$

Table 3.4. Correlation coefficients (*r*) of organosomatic indices (LSI, GSI, VSI), and body components (water, protein, lipid) to relative weight. Coefficients are reported for three single and two combined data sets (Rural Retreat, RR; Lower Travis, LT; Smoots, SM).

Variable	RR	LT	SM	RR & SM	All data
LSI	0.308	0.087	0.169	0.641**	0.634**
GSI	0.082	0.214	-0.213	-0.076	0.039
Female	-0.632*	-0.145	0.096	0.342*	0.194
Male	0.184	-0.306	<0.001	0.319	0.249*
VSI	0.470*	0.017	0.315*	0.577**	0.292**
Water	0.051	0.125	-0.160	0.083	-0.026
Protein	-0.334	-0.046	-0.145	0.341**	0.370**
Lipid	0.107	-0.061	0.413**	0.232*	0.157

* $P < 0.05$, ** $P < 0.005$

Table 3.5. Ranges of relative weight (W_r), organosomatic indices (LSI, GSI, VSI), and body components (water, protein, lipid) for each data set (Rural Retreat, RR; Lower Travis, LT; Smoots, SM) and percent increase with addition of Rural Retreat data over the previous data combined. All variables expressed as percentage of wet weight except lipid and protein (% dry weight).

Variable	RR	LT	SM	LT & SM	All data	Increase
W_r	21	22	27	25	41	64.0
LSI	0.94	1.53	1.63	1.53	2.45	60.2
GSI	1.11	0.90	1.31	1.11	1.36	22.7
VSI	3.13	2.58	2.66	3.31	3.64	10.1
Water	3.43	7.10	4.11	7.10	7.10	0.00
Protein	13.40	13.34	10.07	13.78	17.12	24.2
Lipid	9.72	17.57	11.36	17.57	17.57	0.00

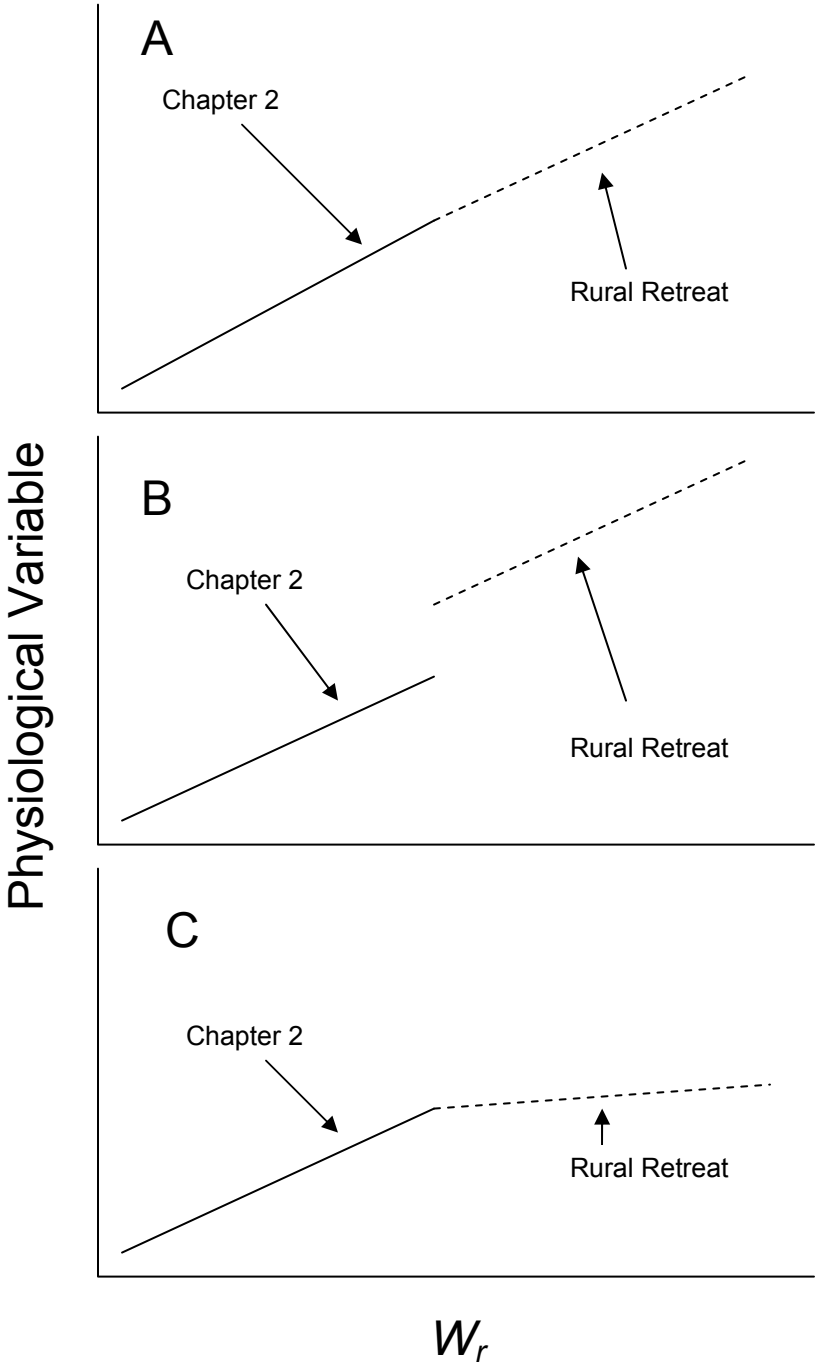

Figure 3.1. Potential comparisons between data sets in the relationship of selected variables to W_r.

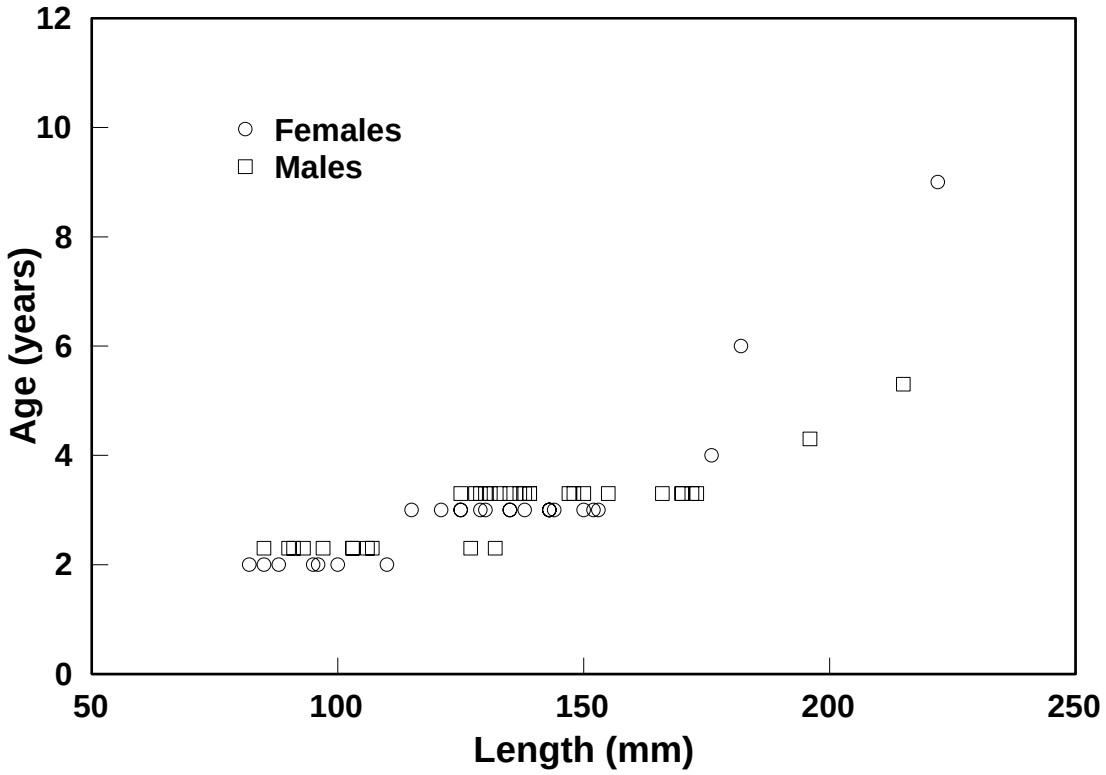

Figure 3.2. Lengths at age of female and male bluegills from Rural Retreat Lake. Male ages are offset by 0.3 years to facilitate comparison between sexes.

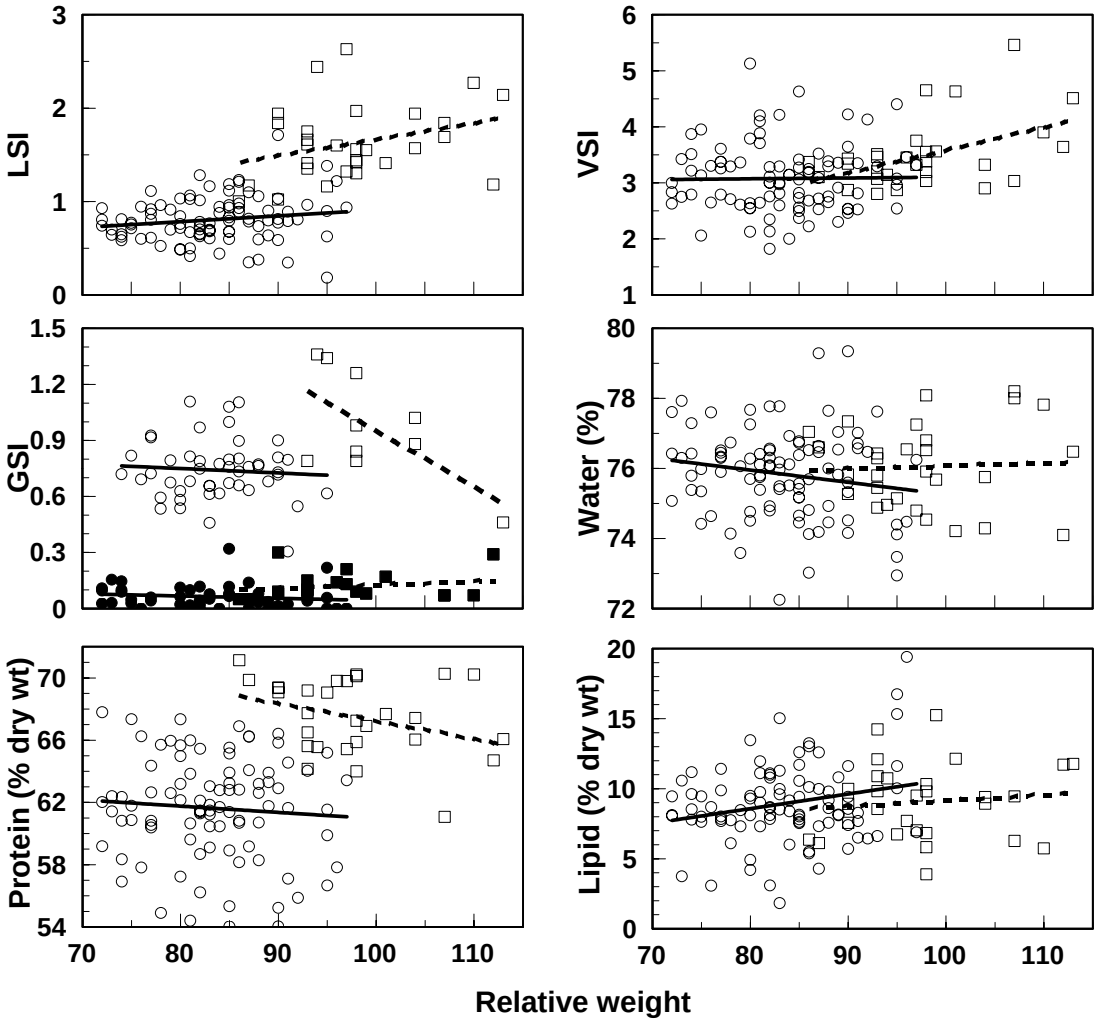

Figure 3.3. Comparison of trends in organosomatic indices (LSI, GSI, VSI), and body components (water, protein, lipid) with relative weight (W_r) between bluegills from Rural Retreat (squares) and Lower Travis Lake and Smoots Pond combined (circles). For GSI, males are indicated by filled symbols. Regression lines are dashed for Rural Retreat and solid for combined Lower Travis and Smoots data. Correlation coefficients are reported in Table 2.4.

CHAPTER 4. THE EFFECTS OF FEEDING HISTORY AND ENVIRONMENT ON CONDITION, BODY COMPOSITION, AND GROWTH OF BLUEGILLS

Introduction

Growth and body composition are important considerations in the biology and ecology of fishes but are logistically difficult to measure with precision. Many investigators use a condition index as an indicator of nutritional status, i.e., the state of body components controlled by nutrition which influence an animal's fitness (Grubb 1995). Condition indices are measures of relative plumpness that are used as surrogates for physiological well-being (Anderson and Gutreuter 1983). Condition indices are widely used by managers and researchers (Murphy and Willis 1991; Blackwell et al. 2000). Many laboratory studies have documented strong correlations between condition indices and variables describing growth and body composition (e.g., McComish et al 1974; Caulton and Bursell 1977; Rose 1989; Brown and Murphy 1991). However, many factors can influence fish condition (Le Cren 1951; Sutton et al. 2000). Studies seeking to verify the relationship of condition indices to growth or body composition in natural environments have met with mixed success. In general, the relationships documented in the field have been imprecise for both growth (e.g., Gutreuter and Childress 1990) and body composition (e.g., Neumann and Murphy 1992; Salam and Davies 1994).

What factors cause differences between laboratory and natural environments in the quality of information provided by condition indices?

Laboratory experiments on growth, such as those cited above, typically apply a constant ration throughout the study. In wild populations, fish of disparate feeding histories are present simultaneously. Positive correlations have been demonstrated between condition and presence or abundance of appropriate forage (Wege and Anderson 1978; Lemly and Dimmick 1982; Mosher 1984; Hubert et al. 1994; Liao et al. 1995; Marwitz and Hubert 1997; Porath and Peters 1997); therefore, feeding rate is an important factor in determining condition index values. Simpkins et al. (2003) found the relationship of lipid to condition index was imprecise in rainbow trout *Oncorhynchus mykiss* over a 147-d starvation experiment, r^2 = 0.38 and 0.41 for active and sedentary treatments. Those results suggest that poor correlations can be induced by starvation or food restriction. Resources in natural environments are not evenly distributed in time or space; hence, periods of starvation and restricted feeding are inevitable. These circumstances have implications for the body composition of individuals with different environmental experiences, especially when comparing between simplified laboratory environments and the wild.

Fed and starved fish have different energetic strategies with implications for growth and body composition. Fish with adequate energy reserves increase activity in order to locate food; activity costs tend to increase with consumption (Madon and Culver 1993). Conversely, starved fish become torpid at some point to reduce energetic expenses (Beamish 1964). Additionally, fish with ample energetic reserves grow differently than those with depleted reserves (Broekhuizen et al. 1994). Fasted fish typically will grow more quickly after re-

feeding than continuously-fed individuals, a phenomenon termed compensatory growth (e.g., Hayward et al. 1997). Differences in duration and severity of restricted feeding may produce several responses in growth and body composition upon re-feeding (Miglavs and Jobling 1989). Fish undergoing compensatory growth produce more protein and can have a different body composition than normally growing fish (Johansen et al. 2001). Clearly, differences in body composition may be induced by previous feeding history and therefore initial energetic status for the time period considered.

Previously, I conducted two comparative field studies examining correlations between condition index and body composition in wild bluegills *Lepomis macrochirus* (Chapter 2, Chapter 3). In those studies, I measured liver-somatic index (LSI), gonadosomatic index (GSI), viscerosomatic index (VSI), and percentages of water, protein, and lipid in the body. These variables have been used to index growth and energetic status of fish (Busacker et al. 1990; Goede and Barton 1990). Correlations between these variables and relative weight (W_r, Blackwell et al. 2000) were weak ($r^2 \leq 0.50$).Those results contradict the findings of McComish et al. (1974) concerning laboratory-reared bluegills. In short, wild bluegills are much more variable about the trend of body composition with W_r than those reared in the laboratory. Bluegill growth in the wild is often food-limited (Anderson 1959; Seaburg and Moyle 1964; Osenberg et al. 1988; Nibbelink and Carpenter 1998); hence my previous results raise questions regarding the applicability of laboratory findings to fish in the wild.

Breck (1998) developed a model predicting dynamics of growth, body composition and W_r of bluegills during starvation and growth. In this model, energetic dynamics are related to changes in length and weight, and hence condition index. A key assumption is that body tissues fall into two categories: structural components that cannot be metabolized and storage components that comprise an energetic reserve (Broekhuizen et al. 1994). Energy may be allocated to structure, i.e., length and associated weight changes, or storage, which would affect weight only. Some of the other assumptions of the model are: 1) when reserve energy is high relative to structural energy, more is allocated to growth in length; 2) when reserves are low, more is allocated to bolstering storage; and 3) during starvation, initial losses from reserves will be rapid until a threshold below which metabolic expenses decrease. If size and metabolic demands, in terms of temperature and activity, are held constant, using this model we may predict four extreme W_r trajectories in time, based on ration (fed or starved) and initial state (high or low reserves; Figure 4.1). The relationship of W_r to nutritional variables should differ between diverging trajectories and become similar between converging trajectories because of the differences or similarities in energetic strategy of the individuals on those trajectories.

The goal of this study was to examine the effect of feeding history and initial condition index on the correlation of body composition and organosomatic indices to W_r in wild bluegills, using Breck's (1998) model as a framework. My intention was to identify potential confounding factors and thus define a domain of applicability for the use of condition indices in bluegills. Objectives were

threefold. 1) Examine concurrence of condition and nutritional status by manipulating ration and examining growth and changes in W_r and variables related to energy storage. It is crucial for interpretation to know the time period reflected by tissue-composition indices (Arndt et al. 1996); therefore, I observed response time in the variables and determined whether interpretation changes with initial state. 2) Determine correlations between W_r and body composition and organosomatic indices over all treatments. This facilitated comparison of this study to that of McComish (1971) and other previous work. 3) Compare laboratory correlations to those in a wild population. During data analysis, it became obvious that correlations progressively changed through the experiment. I described these changes with reference to time in the laboratory, using wild samples as a baseline. My strategy to address these objectives was to vary ration and initial W_r of the laboratory fish, while controlling energetic output and variations in allocation due to maturation by confining fish within a common environment and using juveniles. This experiment is unique in that the treatments mimic natural (albeit extreme) feeding fluctuations and in the use of the source population as a real-world check and environmental control.

Methods

Experimental System

This experiment was conducted in a recirculating system at the Virginia Tech Aquaculture Center. Four circular tanks were linked to a common sand filter and sump with biofilter media. Aeration was provided by airstones and influent

flow. Each tank had a single influent pipe, a central stand pipe for effluent, and 2000 L capacity (183 cm diameter, 76 cm depth). To prevent the bluegills from being unduly stressed by operations in the building, each tank was covered with a framed cloth. Temperature, dissolved oxygen (DO), pH, and total ammonia nitrogen (TAN) were checked every morning. Nitrate and nitrite levels were periodically checked. A natural summer photoperiod was maintained. Average temperature was 24.6^{o}C (range 23.4 - 26.2), within the optimal range for growth in bluegills (Lemke 1977). Temperatures were within 0.2^{o}C among all tanks and did not fluctuate more than 1.0^{o}C within a day. Minimum DO was 7.21 mg/L, mean pH was 8.14 and mean TAN was 0.09 mg/L.

After culling and pre-experimental manipulations (see *Study Design*), all bluegills were held individually in chambers suspended within the circular tanks. Confinement facilitated individual identification, diet tracking, and reduced activity costs. The chambers were modified clear plastic water containers, 18.9 L capacity. The top was cut wider so that fish were easily accessible. Holes 12 mm diameter were drilled 25 - 37 mm apart evenly around the middle of the container so it would easily submerge, ensuring adequate flow through the chamber. A short length of PVC pipe, 100 mm inside diameter, was provided for shelter in the bottom of each chamber. Fifteen chambers per tank were suspended so that each was filled with approximately 17.0 L of water. The top was covered with mesh so food could be dropped into the chamber but the bluegills could not escape.

Study Design

This experiment featured manipulation of initial W_r (low versus high) and ration (fed versus starved) to produce four condition trajectories: high condition increasing, high condition decreasing, low condition increasing, and low condition decreasing (Figure 4.1). I assumed energetic expenses were equivalent among experimental fish because all were confined, all were exposed to the same temperature and water quality regime, and immaturity was confirmed by post mortem examination of the gonads (see Results). Criteria for maturity were based on GSI (Chapter 2). To serve as an environmental control, samples were taken from the wild parent population at the initial collection and just before termination of the experiment. Condition was indexed by W_r (Blackwell et al. 2000) and five variables were used as indicators of energetic depots (LSI, VSI, and the percentages of water, protein, and lipid). I did not incorporate GSI in the design as previously (Chapter 2, Chapter 3) because I was trying to control maturity state. Power analysis of McComish's (1971) data showed that, for two samples of 10, mean W_r should differ by 14 to detect a significant difference in lipid content (% dry weight). I used the results of this analysis to set targets for sample sizes and initial W_r differences. After the target difference was reached via ration manipulation, a portion of the fed group continued to be fed (FF treatment), while another portion was then starved (FS treatment). Conversely, a portion of the starved group continued to be starved (SS treatment), while another portion was re-fed (SF treatment). The experiment ended when the fed treatments (FF and SF) converged in W_r.

All bluegills used in the experiment were collected from Claytor Lake, Pulaski County, Virginia, on June 12 and 14, 2002. Approximately 310 bluegills 85 - 120 mm total length (TL) were electrofished from the same stretch of shoreline on both days. On June 14, 10 bluegills were sacrificed to provide a baseline for comparison and treated as described in Data Collection. Fish were put in an aerated tub and transported to the Virginia Tech Aquaculture Center. There they were placed indoors in a standard circular production tank and acclimated for 10 d. An external fungal infection was treated with formalin, NaCl, and $KMnO_4$. Mortalities and obviously infected fish were removed quickly. There were no mortalities after June 22. During acclimation, bluegills were offered small mealworms (*Tenebrio molitor*) twice daily, a total of 4% of their initial body weight per day (bw/d). Fish began taking mealworms within a day.

Manipulations began after acclimation. On June 24, bluegills were graded into high and low W_r groups and placed into two separate tanks. The high W_r group was fed 4% bw/d of mealworms, while the low W_r group was starved. Measurements on July 7 showed the difference in mean W_r between groups was 15. All fish were culled to retain specimens for individual treatment during the experiment. Specimens with lesions or eroded fins were rejected, as were any <85 mm or >120 mm. Starved fish were retained if $W_r \leq 90$ and fed fish were retained if $W_r > 95$. A sample of 10 fish from each group was sacrificed for analysis. Individual chambers were installed and numbered. Each chamber was randomly assigned a treatment without replacement (10 FF, 20 FS, 20 SF, and 10 SS). After culling, fish were assigned to a treatment or sacrificed based on a

pre-determined random draw until all chambers were occupied and 20 individuals had been sacrificed.

After culling, length, weight, and ration consumed were individually monitored. Consumption was determined by comparing weight of mealworms offered each day versus weight (after air drying) of those removed from the chamber prior to morning feeding the next day. By July 17, some bluegills were consuming their entire ration, so daily offerings were increased to 6% bw/d. Weight and TL were measured on July 22 and August 5. On July 22, FS and SF groups had similar W_r (t = 0.10, P = 0.92). A sample of 10 was sacrificed from each of these two groups. Individuals were chosen so that mean W_r was equivalent between groups and between sacrificed and remaining bluegills (analysis of variance (ANOVA), F = 0.02, P = 0.99). The experiment was terminated on August 13, when FF and SF mean W_r was similar (t = 1.19, P = 0.25). At this point, the remaining bluegills had been in the laboratory 60 d, 37 d in individual confinement. Treatment effects were uniform within groups; there were no tank effects on weight changes during the three times that measurements were checked (F < 1.63, P > 0.192).To provide a reference from the source population, a sample of 10 bluegills 90 - 110 mm TL was electrofished from the same Claytor Lake shoreline on August 8.

Data Collection

Samples of 10 per group were taken for measurement of selected variables at three points during the experiment. Initial samples were randomly

collected from fed and starved groups during culling on July 7. Samples were collected from FS and SF groups on July 22, when mean W_r was equivalent. The remainder were sacrificed at termination. To establish the state of the Claytor Lake population, samples were collected on June 14 and August 8. In total, 100 bluegills were subjected to laboratory analysis.

Laboratory procedures closely duplicated those used previously (Chapter 2, Chapter 3). Fish were placed on ice and transported to the laboratory for processing. I measured TL to nearest mm, patted the fish dry with a paper towel, and weighed them to nearest 0.01 g. Each individual was dissected. The liver, gonads, and viscera were removed and weighed to nearest 0.01 g. Stomach and intestinal contents were extruded prior to weighing the viscera. Organs were replaced within the body cavity and the carcass frozen. Later, carcasses were removed from the freezer, chopped into small bits, placed on a pre-weighed aluminum dish, weighed, and dried at 37^{o}C in a drying oven. Dried pieces were ground with a coffee bean grinder. The resultant powder was replaced in the oven on the same pan until weight stabilized for the largest fish; then all were re-weighed. The difference between wet and dry weights was water content. Protein and lipid content were determined from dried samples using the macro-Kjeldahl procedure and chloroform:methanol extraction (AOAC 1990). All variables were standardized by size. Lipid and protein content were expressed as percentage of dry weight. Water content, LSI, and VSI were expressed as percentage of wet weight.

Growth and consumption were calculated from measurements made during the course of the experiment. There were four measurements made after initiation of individual treatment; hence, there were three periods: July 7-22 (Period 1), July 23-August 5 (Period 2), and August 6-13 (Period 3). Daily consumption was calculated as explained previously. Average consumption (*C*, % bw/d) was computed for each period as $C = (\sum DC)/(W_i * t_i)$, where DC is daily consumption, W_i is initial weight, and t_i is time of period *i* in days. Growth (*G*, %/d) was calculated as $G = \ln (W_f / W_i)/t_i * 100$, where W_f is final weight. Absolute changes in length (d*L*), weight (d*W*), and relative weight (dW_r) were determined for the entire experiment.

Data Analysis

I used multivariate analysis of variance (MANOVA) to detect differences among groups to: 1) verify differences in fed and starved fish going into the individual chambers on July 7, 2) compare FS and SF groups at July 22, 3) compare among all groups at the end of the experiment, including the lake sample, and 4) to detect changes of each group through time. The latter comparison included the baseline sample from Claytor Lake collected on June 14. Acceptable risk of Type I error for the experiment was set at 0.05. Variables used in MANOVA were W_r, LSI, VSI, and water, protein, and lipid contents. Significant differences were followed by univariate ANOVA and Tukey's post hoc test to detect which variables contained the differences and how the groups were separated. This procedure protected the experiment-wise error rate (Rencher

1995). I also compared experimental groups for differences in growth and consumption. Because the informative differences were within fed (FF and SF) and starved (FS and SS) treatments, I compared only within those groups. This examination tested the effect of initial W_r level; growth differences between fed and starved groups are of trivial importance in this case. I used a two-sample *t*-test to compare dL, dW, and dW_r. To test for differences in G and C in each period, I used two-way ANOVA with an interaction term, blocking for period. Because of small sample sizes and non-normality, I performed nonparametric tests but the results were the same, so only parametric outputs are reported.

The aggregated terminal samples were used to describe the form and precision of the relationship of W_r to other variables. I regressed W_r on LSI, VSI, and body composition (water, protein, and lipid content) and plotted W_r versus G computed for the entire experiment. To examine performance of the fish through the experiment, I plotted G versus C and dW_r. To compare this study to previous research, multivariate analysis of covariance (MANCOVA) was used to compare the results to those of McComish (1971). Following the global test, I calculated the regression of W_r to each variable and compared regressions with analysis of covariance (ANCOVA, Zar 1996). To test for homogeneity of slopes, I modeled each variable as a function of time, W_r, and their interaction; if the interaction term was significant, regression slopes differed.

As the experiment progressed, correlations changed consistently with time in the laboratory. To test this, I performed a MANCOVA using time indoors as category (0 d, 23 d, 39 d, and 60 d) and W_r as the covariate. Both lake samples

were combined to show the initial relationship, i.e., 0 d indoor experience. Based on ANCOVA results, I used multiple contrasts to compare slopes among times and used Tukey's post hoc test to compare means adjusted for differences in W_r. To consider magnitude of laboratory-time effects versus treatment effects, I repeated the ANCOVA and included treatment group in the model. I used the sequential *F* ratio from this test as a relative measure of effect size. Data from FF and SS groups sampled on July 7 and August 13 were used for this comparison (n = 40). Given the increased variation of LSI with increased W_r, I tried a logarithmic transformation, but this did not change the results, so only the untransformed results are reported.

Results

Treatment Effects

There were differences among treatments in absolute and relative changes in size and condition (Table 4.1). Fed groups (FF and SF) were similar in d*L* and d*W* (10.6 mm versus 13.0 mm and 13.6 g versus 14.4 g, respectively; $t \leq 1.77$, $P \geq 0.10$) but SF bluegills gained 6 percentage points more W_r (t = 2.36, P = 0.04) and had an average of 0.49% bw/d greater growth and 1.03% bw/d more consumption than the FF fish ($F > 16.0$; df = 1, 54; $P < 0.001$). There were no time or interaction effects on consumption ($F \leq 2.20$, df = 2, 54, $P \geq 0.12$), but there was a time effect on growth (F = 10.23; df = 2, 54; $P < 0.001$); both fed groups grew slowest August 6-13. Starved groups (FS and SS treatments) lost weight differently; FS bluegills lost 1.8 g more weight (t = 3.00, P = 0.01) and 7

points more W_r (t = 3.67, P = 0.003) than the SS fish. Rate of weight loss was similar between starved groups, an average of -0.49 % bw/d and -0.45% bw/d, respectively (F = 0.002; df = 1, 54; P = 0.82), but time and interaction effects were significant ($F \geq 6.97$; df = 2, 54; $P \leq 0.002$). Both groups lost weight at similar rates but lost more August 6-13. While SS fish lost less than the FS group during July 7-22 and during August 6-13, these differences were not significant.

Manipulation of diet changed W_r as expected (Figure 4.2). The switched groups, FS and SF, changed fastest. Handling stressed the bluegills, especially after separation into individual chambers (personal observation). For example, W_r of the FF group declined slightly after confinement. Consumption typically declined for a few days after measurements, which greatly influenced growth during the last period because of its brevity.

The initial manipulations (June 24 – July 7) produced large differences between fed and starved groups (Wilks' Λ = 0.075, $P < 0.0001$). Univariate ANOVAs showed there were differences in all variables ($P \leq 0.0016$). The fed group had higher W_r, LSI, VSI, and lipid content, while the starved group had higher water and protein content (Table 4.2). By July 22, W_r of the FS and SF treatments had become equivalent but the two groups were different (Wilks' Λ = 0.265, P = 0.003) because LSI was much higher in the SF group (Table 4.2). At the end of the experiment, all groups were significantly different and there were differences between some of the laboratory groups and the wild bluegills (Wilks' Λ = 0.013, $P < 0.0001$). The fed groups (FF and SF) had higher W_r, LSI, VSI, and lipid content, while the starved groups (FS and SS) had higher water and protein

content (Table 4.2). The pattern in wild bluegills was not as consistent. They were equivalent to FF and SF in W_r, overlapped all groups except SS in LSI, were similar to SS in water and protein content, were between SS and FS in lipid content, and equivalent to FS and SS in VSI.

All groups changed through time (Table 4.3), including the lake samples (Wilks' $\Lambda \leq 0.387$, $P \leq 0.03$). All groups had significant changes in water, protein, and lipid content ($F \geq 13.49$, $P \leq 0.002$). The Claytor Lake samples did not change significantly in W_r ($F = 3.41$, $P = 0.08$), but all experimental groups did ($F \geq 9.42$, $P \leq 0.0001$). Similarly, LSI was similar for both lake samples ($F = 2.72$, $P = 0.12$). The SS group did not change in LSI or VSI ($F < 2.80$, $P > 0.08$). Temporal differences in LSI were significant for FF, FS, and SF ($F \geq 14.71$, $P < 0.0001$). Temporal differences in VSI were significant for FF, FS, SF, and the lake samples ($F \geq 15.87$, $P < 0.0001$). At the initiation of individual treatment, LSI was highest for FF and FS, but was highest in SF two weeks after re-feeding (July 22). Lipid content and VSI continued to rise for fed groups throughout the experiment, while water and protein content steadily fell. Lipid content and VSI rose with W_r, while protein and water content showed an inverse tendency (Table 4.3). However, the pattern of significant differences among sample times for any physiological variable did not precisely mirror the pattern of significant changes in W_r. For example, fish continuously starved through the experiment (SS group) maintained W_r from June 14 through July 7, after which W_r dropped significantly. Concurrently, LSI for this group also fell, but none of the differences were significant because of intra-group variability.

Correlations to W_r

There were significant correlations to W_r in all but the July 22 sample (Table 4.4). All variables were correlated to W_r in the July 7 and August 13 samples. In the lake samples, correlations to LSI, water content and lipid content were significant. All correlations to water and protein content were negative, whereas those to LSI, VSI, and lipid content were always positive. Correlations in the July 22 samples were not significant because of a lack of range in W_r. Variance increased in LSI and VSI as W_r became larger (Figure 4.3). For these two variables, there was a change in the relationship near W_r = 80, because LSI and VSI were low and relatively constant for the SS group, regardless of W_r.

Correlations of W_r to body composition in the data of McComish (1971) were different from those at the end of this experiment (Wilks' $\Lambda = 0.333$, $P < 0.0001$; Figure 4.4). All slopes were significantly steeper in my data ($P \leq 0.013$) and the regression explained more of the variation (Table 4.5). My treatments generated a greater range of W_r. In comparison to McComish's (1971) data, the correlation between W_r and water content was similar but the present data had lower water content at high W_r. In this study, protein content also was lower at high W_r but lipid content was lower at low W_r. The SS group had very low lipid content and was well below the line described by McComish's (1971) data. The FS group stretched between the SS group and McComish's data, with lipid content in the group rising with W_r in a sigmoid pattern.

The relationship of W_r to growth was different between fed and starved groups (Figure 4.5). The FF group appeared to have a linear relationship of W_r to

growth rate but the SF group did not. The latter had higher *G* than FF, but d*W* was similar (Table 4.2). Growth was linearly related to consumption. Starved fish lost weight at a fairly constant rate as indicated by the clustering on the y axis in Figure 4.6. Growth was linearly related to change in W_r for fed fish but not for starved groups because all starved fish were losing weight at approximately the same rate (Figure 4.7). Hence, differences in dW_r between starved fish were driven by initial state because FS fish lost more W_r than did SS bluegills.

Laboratory Effects on Correlations

The change in environment from Claytor Lake to the aquaculture laboratory caused a progressive, directional change in correlations (Wilks' Λ = 0.210, $P < 0.0001$). In general, the strength of correlations was less in the wild bluegills (Table 4.4) because the relationship of W_r to the other variables had changed between June and August. Excluding the July 22 (39 d) sample for lack of range, all correlations were weakest in 0-d samples and strongest in 60-d samples. Regression slopes and adjusted means tended to increase in absolute value with amount of time in the laboratory (Table 4.6). Adjusted means calculated by date over all treatments (starved and fed) changed in a progressive manner between the wild (0 d in the laboratory) and August 13 samples (60 d in the laboratory). In general, the 60-d adjusted mean was different from other times even though the 60-d W_r range encompassed all other samples. Slopes increased with greater amount of laboratory experience (Figure 4.8). The exception was the July 22 sample (39 d in the laboratory); because the range of

W_r in the combined sample was so small, all regressions were insignificant (Table 4.6) and the slope estimates suspect. The correlation between lipid and water content also changed (Figure 4.9), indicating a general change in nutritional balance after transfer between wild and laboratory environments. There were also differences in the relationship of W_r to LSI and VSI (Figure 4.10). Changes in the correlation to VSI mimic those in the relationship to lipid content. Variance increased in the regression to LSI, while slopes and adjusted means remained similar (Table 4.6).

Physiological adjustments to the laboratory had important effects that could influence interpretation of results. For all but LSI, variability ascribed to changes with time was of similar magnitude to those associated with ration level (Table 4.7). In the case of water content, differences between July 7 and August 13 samples were greater than differences between FF and SS treatments. For VSI, protein content, and lipid content, ration effects were larger than time effects.

Discussion

Experimental manipulations induced a wide range in the variables measured but did not push the limits of which bluegills are capable. Breck (1996) recorded individual W_r as low as 48 and means in the lower 60s in bluegills from an extremely dense experimental population. Gabelhouse (1987) found mean W_r as high as 125 in a Kansas pond; the highest individual W_r in my experiment was 123. Mean W_r of the FF group remained steady despite being offered a high

ration. Condition index of channel catfish (*Ictalurus punctatus*) fed to satiation remained steady for 98 d, despite continual growth and lipid accumulation (Gaylord and Gatlin 2000). Perhaps some stimulus that would promote greater plumpness was lacking or there was a low level of stress associated with the artificial environment. Peak LSI during rapid growth can exceed 2.0, as in the SF group on July 22. Mean LSI near 0.4 has been documented during periods of depressed growth in the wild (Bulow et al. 1978) and the laboratory (Cook 1994). For short-term growth, LSI is a good within-population indicator, but absolute values are not easily compared among populations (Bulow et al. 1978). I previously observed a lower VSI (0.9, Chapter 2). The lowest VSI from the June Claytor Lake sample was 2.0, while the lowest SS VSI was 2.4. Similarly, the lowest lipid content in the June Claytor Lake sample was 2.11% compared to 2.52% in the August 13 SS sample. Anderson (1959) reared bluegills with lipid contents in excess of 45% compared to a maximum of 31.5% in this study. Handling of wild bluegills can cause variation in digestion and absorption of food as well as other physiological and psychological characteristics (Windell 1966). I did not attempt to acclimate experimental fish to handling; therefore, it is not surprising that greater responses were not observed.

Growth response to starvation and feeding depended on initial state. The SS group lost less weight and W_r than the FS group, despite longer starvation, presumably because of a conservative energetic strategy (Beamish 1964). The SS group also seemed more stressed than the others after the last measurements because SS fish lost the most percentage of body weight of any

group during the final period. Their reserves should have been close to depletion at the end of the experiment as LSI, VSI, and lipid content were very low and relatively invariable. At this point, they were likely using protein for an energy source (Savitz 1971; Niimi 1972; Jobling 1980) and losing associated water, whereas the FS group still had relatively high lipid content and no need to catabolize extra protein. Conversely, the SF group seemed to be undergoing compensatory growth, which should continue until reserves were replete (Broekhuizen et al. 1994). Growth, consumption, and LSI were highest in the SF group. By the end of the experiment, SF lipid content was the same as in the FF group, which should end the compensatory response (Johansen et al. 2001). However, the SF fish were still eating and growing more than the FF bluegills, which raises the possibility that overcompensation (Hayward et al. 1997) might have occurred if the experiment continued. However, protein content declined, which is not the norm for fish undergoing compensatory growth (Johansen et al. 2001). This decline was related to the change from a natural environment to the aquaculture laboratory (Table 4.6).

The comparison of FS and SF when mean W_r was equivalent was designed to see if initial state (high versus low condition) would influence body composition. On the surface, it did not; the two groups were similar in every compositional variable except LSI, which was lower for the FS group. The similarities were not due to equal changes such that the groups met at some intermediate state; rather, the FS group maintained its lipid content while the SF group caught up to it. The FS group dropped 9 percentage points in W_r yet was

the same as the July 7 sample in every variable except LSI. For this group, change in W_r did not reflect a change in body composition; proportions stayed the same and losses came equally from all components. For the SF group, the other variables changed with W_r as expected, e.g., lipid increased with W_r. Therefore, initial W_r affected subsequent body composition.

All variables were influenced by two factors, ration level and the change in environment. The two factor effects were of similar magnitude. Body composition and VSI were significantly influenced by both factors, whereas W_r and LSI were more affected by ration. The brief period of feeding during acclimation enabled the experimental fish to double their lipid reserves. The starved group on July 7 had more than twice the lipid content of the bluegills collected from Claytor Lake on June 14. At this point, they had been fed for at least 9 d and then starved for 12 d. By the end of the experiment, after another 36 d of starvation, SS lipid content was similar to the initial Claytor Lake sample. The benign laboratory environment enabled them to conserve reserves after 9 - 11 d of feeding for 48 d of starvation. Muscle lipid content of channel catfish remained relatively constant during 28 d of starvation and was similar to lipid level in control fish fed to satiation (Gaylord and Gatlin 2000). Thus the laboratory environment mitigated starvation effects. These results are consistent with the concept that condition is determined by a tradeoff between nutrition and stress (Saltz et al. 1995).

Concurrence of W_r with Growth and Nutritional Variables

The relationship of growth to W_r was not straightforward. Starved and fed groups exhibited different patterns in growth rate to W_r (Figure 4.5) and dW_r (Figure 4.7). For fed groups, W_r was added linearly with growth (Figure 4.7). However, because of the initial low SF W_r, the growth patterns to W_r in the fed groups had not converged (Figure 4.5). Thus, even though the SF group was undergoing compensatory growth, they were not able to attain the highest W_r found in the continuously growing FF group. Starved groups had similar average rates of weight loss over the course of the experiment (Table 4.1). Thus W_r and dW_r appeared to vary without relationship to *G* (Figure 4.5; Figure 4.7). The FS group lost more W_r than the SS group during the experiment (Figure 4.7) but the initial low condition of the SS group ensured that the mean W_r of that group stayed the lowest. The energetic strategy of starved fish is to conserve body condition by reducing metabolic costs (Beamish 1964; Jobling 1980); therefore, convergence of W_r in starved groups would take much longer than the course of this experiment.

Similar temporal changes occurred in LSI and W_r except in the SS group. For SS fish, LSI was not correlated to W_r because there was no growth by this group during or immediately prior to the experiment. In general, starved fish had LSI < 1.50, although some FS fish had higher LSI. This may indicate growth from accumulated reserves after feeding had ceased for those individuals. Generally, LSI increased with W_r above 80, resulting in a positive correlation at the end of the experiment. There is a fan-shaped relationship above the inflection point

(Figure 4.10a); at high W_r, LSI may increase to varying degrees. Liver weight can change quickly and thus is a short-term measure of growth (Adams and McLean 1985). A whole-body condition index has more inertia inherent because it does not vary as much (Tyler and Dunn 1976; Arndt et al. 1996). Initial state should have greater impact on W_r because it changes more slowly than LSI. This is evident in the scatter among fed groups in the relationship of growth rate to W_r (Figure 4.5). When excess resources are present such that fish grow, a proportion of energy will go to increasing W_r, but differences in initial W_r and individual responses will cause variation around the trend of W_r with growth.

The period covered by this experiment was considerably less than a growing season. Storage and associated weight changes can occur on a short time scale such as 8 to 10 days (Arndt et al. 1996). Structural growth takes longer. Although the treatments induced major differences in weight change in less than 14 days, growth in size, i.e., length and associated weight changes, occurs over a longer period. Therefore, time scale of changes in W_r will not necessarily reflect seasonal growth patterns. Hence, use of W_r to infer annual growth is problematic (Gutreuter and Childress 1990; Schneider 1999). The order of inputs and stresses is therefore important to interpretation of W_r in terms of annual growth in length (Gabelhouse 1991). Variability will also be important; if growth rate is steady, then W_r should reflect growth over a longer period. As variability in growth rate increases, W_r will be more influenced by index value at the start of the growing season and will not be useful as a growth index.

Nutritional condition is connected to growth because fish with excess resources for storage should also be devoting energy towards growth and vice versa (Calow 1994). During this experiment, interpretation of W_r trends in terms of body composition depended on focus. At the end of the experiment, VSI and body composition were strongly correlated to W_r. Continuity in the environment and feeding regimes produced very precise relationships. But changes with time compromised the utility of these relationships to a certain extent because the average gram of fish had different composition, depending on time in the laboratory (see *Comparison to Wild Bluegills* below). This violates the assumption that the relationship between index and variable is constant for all individuals compared (Chapter 1). Hence, comparison becomes quantitatively imprecise when data from all sample dates are included.

Laboratory growth studies have generated precise relationships between condition index and body composition. McComish's (1971) work provides a specific reference for bluegills. My experiment likewise generated very precise relationships by the end of the study but they diverged from correlations present in McComish's (1971) data. The largest difference was in the relationship of W_r to lipid content. Only the fed groups (FF and SF) were centered on the trend from McComish's (1971) data. For his experiments, McComish manipulated photoperiod and temperature. No fish were starved and individual consumption ranged approximately 1.5% - 5.0% bw/d. Range of consumption of fed groups in my experiment was approximately 2.0% - 5.5% bw/d. The differences between experiments were due to the starvation treatments. Bluegills catabolize more lipid

than protein during starvation (Savitz 1971). Teleost lipid is a light-weight tissue, typically in the range of 0.91 - 0.93 g/mL (Brawn 1969). Very little water is associated with lipid, whereas about 3 g of water is associated with 1 g of protein (Gerking 1955). Therefore, starving bluegills lose lipid content faster than W_r. Similarly, overwintering largemouth bass *Micropterus salmoides* frequently drop in energy density while maintaining or even increasing mass (Fullerton et al. 2000). Body composition may change without affecting mass (Niimi 1972). Thus, starved groups (FS and SS) diverged from the trend in McComish's (1971) data with time of starvation; i.e., their lipid content was lower than would have been predicted from his data.

Comparison to Wild Bluegills

Environmental conditions can have large effects on the relationship of body composition to condition index. Anderson (1959) reared bluegills with lipid content far in excess of either my study or McComish (1971), yet the Fulton's condition factors Anderson calculated were within the range of those calculated by McComish. Fish from natural environments typically have less lipid and more lean body mass than those reared in laboratory or aquacultural environments (McDonald et al. 1998; Rikardsen and Johansen 2001; Alasalvar et al. 2002; Grigorakis et al. 2002; Orban et al. 2002; but see Craig et al. 2000). Differences between wild and laboratory fish were evident at the end of the experiment. Wild bluegills had very little visceral fat and were similar to the SS group in terms of water and protein content. However, their LSI and lipid content in August was

higher than SS fish or the June 14 sample, showing that they were growing. In August, LSI and W_r of wild fish were similar to the fed groups, but their energetic reserves were less. These symptoms are characteristic of feed restriction (Miglavs and Jobling 1989) and contribute to lower correlations in wild fish.

I documented a change in body composition progressing from removal of the experimental fish from Claytor Lake. Gerking (1955) found that wild bluegills grew and increased in lipid content when brought into the laboratory and fed a maintenance diet calculated from energy expenditures in other laboratory experiments. Changes in my experiment were evident within three weeks (June 14 – July 7) and approached correlations in the data of McComish (1971), taken from fish spawned and reared in the laboratory. Means and slopes increased in an orderly fashion with time. This conclusion is conservative because feeding during the acclimation period enabled subsequently starved fish to accumulate reserves that were still apparent after 2 weeks. Apparently, with the removal of environmental stressors, the energetic equilibrium of the experimental fish was changing. These included changes in the water - lipid relationship, which is supposed to be very stable (Iles and Wood 1965; Niimi 1972). This is evidence of an overall shift in the nutritional balance of the fish. Because my treatments included starvation, I concluded that the change is due to removal of important stressors that act on wild bluegills. Additional evidence is that the LSI – W_r correlation in the SS group was similar to that seen in the combined wild sample. This correlation was influenced only by ration level and not time in the laboratory.

Ration level and stress may have different effects. Differences in nutritional intake and initial W_r can cause variability about any trends between W_r and lipid content. Differences in feeding move the individual on the curve. Starvation causes movement off of the curve, evidence that the harsher, more complex, natural environment is capable of suppressing correlations. When environmental controls are released, such as in a controlled laboratory setting, the correlation observed by McComish et al. (1974) develops. Release of confounding factors allows expression of the W_r – lipid correlation in a manner similar to release of other limiting factors allowing the effect of a single factor on animal abundance to be seen (Cade et al 1999). What are these factors that might limit energy and resource storage? Output factors might include activity, maturation, or stress. Generalized stress responses are non-specific in terms of cause (Wedemeyer et al. 1990; Harder and Kirkpatrick 1996) and may result from multiple sources not present in a laboratory tank, e.g. social hierarchy, predator avoidance. Also, gonadal maturation may remove energy from storage; several bluegills sampled directly from the lake were in various stages of maturation while only one in the experiment was.

Other factors influencing consumption can also contribute to variability about trends between W_r and lipid content. Feed restriction is likely a pandemic condition in the wild. Cycling back and forth from feast to famine would limit the amount of energy available for lipogenesis without noticeable effect on W_r. Thus environmental variability would translate into a suppressed condition – body composition relationship. Several factors may interact to influence correlations in

natural environments. The ratio of available energy to metabolic demands should be important. The nature of constraints also may be important, e.g., feed deprivation versus restriction (Miglavs and Jobling 1989). Finally, there are the effects of fitness-related allocation decisions. It is adaptive for organisms inhabiting variable environments to store energy but also to make up for lost growth opportunities. The importance of storage versus growth changes with local selective pressures (Post and Parkinson 2001), generating variability within and among populations.

Implications for Interpretation of W_r

Changes in condition-related variables occurred quickly, as also shown by Arndt et al. (1996). This malleability should tend to reduce dependence on initial W_r, which would increase the responsiveness of W_r to changes in energetic status. However, this depends on the strength and consistency of the changes. Sudden changes in W_r should be physiologically significant (Brown and Murphy 2004). Oscillation, such as that caused by a continual cycle of feeding and fasting, should not only cause W_r changes to cancel out but also induce variability about correlations to W_r as energetic strategy changes with nutritional condition. Slower-growing fish tend to have less lipid than faster growing fish of the same mass (Post and Parkinson 2001). I suggest that the clearest interpretation in terms of body composition should occur when conditions promote relatively constant growth, as for the FF and SF treatments. Knowledge of type and degree of environmental variability present would be invaluable in

assessing the validity of this assumption. This may be possible only in aquacultural environments.

This work also confirms the lack of clear concordance of condition with structural growth. Elaboration of tissue in bluegills during a 50-d experiment was first expressed as weight and then length (Cook 1994). Hence, the time scale on which W_r changes is less than that on which structural growth occurs. Seasonal growth patterns in length develop over months and may not be unimodal. Mid-summer growth slumps are common in bluegill populations (Legler 1977). Given the short response time of condition indices, interpretation in terms of structural growth is ambiguous unless initial index values are known and growth is constant for the period. This is a similar conclusion to that of Gabelhouse (1991).

Differences in energetic history affect how W_r changes and how body composition relates to it. Energetic strategy changes with feeding regime and state of reserves. Starvation mandates conservation of weight so that fish do not drop rapidly in W_r, even though they are catabolizing lipids. Rapid weight loss should only occur once lipid reserves are exhausted. Fish should be torpid before this point in an attempt to husband their reserves (Beamish 1964). On the other hand, compensatory growth caused by fasting or feed restriction allows individuals to make up for lost growth opportunity somewhat but degree of compensation is inconsistent (Jobling and Johansen 1999) and full or over compensation seems rare (e.g., Gaylord and Gatlin 2000; Hayward and Wang 2001; Johansen et al. 2001). It may take longer to restore equality of W_r among groups than the compensatory response allows. Even then, fish that have had a

compensatory growth response should have lower amounts of lipid than those growing steadily (Johansen et al. 2001). Status of initial reserves influences how quickly physiology adjusts to changes in feeding. For example, the quickest W_r changes took place in the switched treatments (SF and FS). The presence of different energetic histories can induce variability in condition - composition correlations. The strategies used under different energetic regimes should influence whether energy is incorporated into the body as lipid or protein. Hence, predictive power of W_r as an indicator of body composition is reduced by differences in energetic trajectories. Regressions predicting body composition should only be used for growing fish (Shearer 1994). For predictive uses, regressions should be evaluated with each use and interpolations used for the population from which the sample was drawn (Hayes and Shonkwiler 2001). Extrapolations beyond the sample in time or space should be avoided for quantitative predictions.

Laboratory results are used in the methodological literature as evidence to validate the use of W_r in wild populations (Anderson and Neumann 1996; Blackwell et al. 2000). But comparison of experimental results to the lake samples shows that a strict compositional interpretation of W_r can be problematical. High W_r values in the laboratory mean increased energetic reserves are possible but the coincident comparison to the parent population shows that the complex natural environment tends to limit storage reserves regardless of condition index. Likely limiting factors include variable feeding, maturation costs, activity, and general stress levels. In the experiment, I

controlled the latter three factors to concentrate on feeding. Costs incurred as a result of additional limiting factors will reduce the energy available for growth or storage. For example, energetic deficits in the wild may result from increased metabolic costs due to predator avoidance and prey pursuit, rather than a shortage of prey. These additional factors will exacerbate the influence of food shortages. Lipostatic control of growth acts to reduce the amount of reserves stored as lipids in the wild in favor of increased growth (Johansen et al. 2001). Food limitation, such as is common in the wild, leads to inter-individual variability in growth patterns (Jobling and Koskela 1996), which then leads to variability in amounts of lipid and protein added. Limiting factors (e.g., environmental stress) may also change the relationship of condition index to body composition. It is the balance of energetic intake versus expenditures that determines growth response (Jobling and Johansen 1999); hence stressors are as important as feeding history in controlling growth and composition. Rose (1989) pointed to differences in stress between experiments as shifting the inflection point of the W_r-lipid relationship of juvenile walleyes (*Sander vitreus*). In the field, ecological costs mandate that animals maintain body mass and energetic reserves at sub-maximal levels and differential cost:benefit ratios among individuals can generate different correlations between condition indices and lipid content (Witter and Cuthill 1993). These considerations militate against extrapolation among environments of any correlations between condition indices and variables reflecting nutritional reserves.

Conclusions

Several assumptions are implicit in the use of condition indices to indicate somatic investment (Chapter 1). Bluegills can facultatively alter their somatic investment depending on circumstances. This adaptability is important for life in an uncertain environment and is a central tenet of life history theory. Presumably, changes are adaptive; that is, they increase fitness of the individual. The ability to store more energy in anticipation of increased demands is thus adaptive. Life history characteristics and nutritional status are linked (Justus and Fox 1994). Population ecology has influence on tradeoffs involved in life history decisions for bluegills, e.g., effects of population size structure on age and size at maturity (Aday et al. 2002). Tradeoffs can influence how energy is acquired, used, and the excess allocated. These factors should either suppress or allow the expression of the condition – body composition correlation. Therefore, the assumptions of whole-body indices need to be evaluated relative to intended use (Rykiel 1996), which should be explicitly defined for each investigation (Hayes and Shonkwiler 2001).

Gross biochemical relationships can change among disparate environments (Rikardsen and Johansen 2003). This observation leads to three conclusions. First, environmental similarity should promote similarity in the W_r – nutritional condition relationship. Increased amount and variability of metabolic demands relative to consumption can overwhelm expression of the relationship. Therefore, interpretation is clearest when the environment promotes growth, as pointed out by Shearer (1994). Even then, predictive precision is unlikely except

in highly controlled situations. Second, basic assumptions should be evaluated in the absence of analysis of body composition. Form of the seasonal growth pattern would be useful but may be logistically difficult to determine. I recommend examination of age and growth data, as well as determination of basic life history parameters such as age at first maturity. Such data can usually be collected with standard methods (e.g., Murphy and Willis 1996) and will allow assessment of comparability. Lastly, even in the absence of validation, the detection of large differences or quick changes should be an indication of nutritional state, albeit in a general manner. In such a case, more specific variables should be examined and likely mechanisms checked to determine effects on population dynamics. Thus, I recommend condition indices should be used as general monitoring tools and not as omnibus physiological variables.

Table 4.1. Mean changes in length (d*L*, mm), weight (d*W*, g), relative weight (dW_r) of experimental fish alive from July 7 to August 13. Consumption and growth rate (% body weight per day) for each measurement period (Pd) and mean for the experiment are reported. Period 1 is July 7-22, Period 2 is July 23-August 5, and Period 3 is August 6-13. Groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); and continuously starved (SS).

				Consumption				Growth			
Group	d*L*	d*W*	dW_r	Pd1	Pd2	Pd3	Mean	Pd1	Pd2	Pd3	Mean
FF	10.6	13.6	5	2.75	3.15	2.40	2.85	1.13	1.08	0.42	0.99
FS	0.2	-4.0	-16	--	--	--	0.00	-0.45	-0.41	-0.80	-0.49
SF	13.0	14.4	11	3.53	4.29	3.78	3.88	1.53	1.60	1.09	1.48
SS	-0.9	-2.2	-9	--	--	--	0.00	-0.25	-0.45	-0.99	-0.45

Table 4.2. Comparison of relative weight (W_r), organosomatic indices (LSI, VSI), and body components (water, protein, lipid) among the experimental groups. Values within samples with the same letter are not significantly different (Tukeys post hoc test). Groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); continuously starved (SS); and from the wild (Lake).

	Sample								
	Initial		Crossing		Final				
Variable	Fed	Starved	FS	SF	FF	FS	SF	SS	Lake
W_r	108a	89b	99c	99c	104x	86y	101x	70z	98x
LSI	1.98a	1.05b	1.22c	2.10d	1.69x	1.09yz	1.60x	0.88z	1.40xy
VSI	5.23a	3.16b	5.34c	5.94c	8.69x	4.25y	7.38x	2.93y	3.57y
Water	72.5a	74.1b	72.0c	72.7c	69.1x	72.5y	69.8x	75.3z	75.4z
Protein	60.6a	65.5b	59.0c	58.9c	52.5x	61.2y	54.0x	64.3yz	66.6z
Lipid	18.2a	9.7b	17.9c	17.6c	25.3x	14.9y	24.1x	4.2z	10.4w

Table 4.3. Variable changes in time within each group. Groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); continuously starved (SS); and from the wild (Lake). Times of samples are June 14 (1), July 7 (2), July 22 (3), and August 8 or August 13 (4). Values within each group-variable combination with the same letter are not significantly different.

		Variable					
Group	Time	W_r	LSI	VSI	Water	Protein	Lipid
FF	1	92x	1.15x	2.62x	77.4x	70.0x	4.3x
	2	108y	1.98y	5.23y	72.5y	60.6y	18.2y
	4	104y	1.69y	8.69z	69.1z	52.4z	25.3z
FS	1	92x	1.15x	2.62x	77.4x	70.0x	4.3x
	2	108y	1.98y	5.23yz	72.5y	60.6y	18.2y
	3	99z	1.22x	5.34y	72.0y	59.0y	17.9y
	4	86x	1.09x	4.25z	72.5y	61.2y	14.9y
SF	1	92x	1.15x	2.62x	77.4x	70.0x	4.3x
	2	89x	1.05x	3.16x	74.1y	65.5y	9.7y
	3	99y	2.10y	5.94y	72.7z	58.9x	17.6z
	4	101y	1.60z	7.38z	69.8w	54.0w	24.1w
SS	1	92x	1.15x	2.62x	77.4x	70.0x	4.3x
	2	89x	1.05x	3.16x	74.1y	65.5y	9.7y
	4	70y	0.88x	2.93x	75.3y	64.3y	4.2x
Lake	1	92x	1.15x	2.62x	77.4x	70.0x	4.3x
	4	98x	1.40x	3.57y	75.4y	66.6y	10.4y

Table 4.4. Correlation coefficients (*r*) of relative weight to LSI, VSI, water, protein, and lipid content from all experimental samples by date and all wild fish combined.

	Sample			
Variable	July 7	July 22	August 13	Wild
LSI	0.70*	0.21	0.80*	0.52*
VSI	0.71*	-0.10	0.80*	0.33
Water	-0.50*	0.15	-0.90*	-0.46*
Protein	-0.53*	0.03	-0.87*	-0.10
Lipid	0.69*	-0.08	0.91*	0.54*

* $P < 0.05$

Table 4.5 Comparison of slope and coefficient of determination (r^2) of regressions of W_r to water, protein and lipid content calculated from the data of McComish (1971) and this study.

	McComish (1971)		This study	
Variable	Slope	r^2	Slope	r^2
Water	-0.124	0.67	-0.168	0.81
Protein	-0.237	0.63	-0.330	0.76
Lipid	0.328	0.71	0.560	0.83

Table 4.6. Comparison of regression slopes (β) of W_r on organosomatic indices (LSI, VSI), and body components (water, protein, lipid) and slope-adjusted means ($\bar{x}$) among samples with different amounts of time in the laboratory. Slopes and means with the same letter are not significantly different.

	Sample			
Variable	Wild (0 d)	July 7 (23 d)	July22 (39 d)	August 13 (60 d)
β_{LSI}	0.027x	0.036x	0.029x*	0.023x
$\bar{x}_{LSI}$	1.27x	1.41x	1.54x	1.43x
β_{VSI}	0.034x*	0.080x	-0.025x*	0.152y
$\bar{x}_{VSI}$	3.08x	3.87x	5.76y	6.50y
β_{water}	-0.103xy	-0.059y	0.045y*	-0.168x
$\bar{x}_{water}$	76.44w	73.53x	72.13y	70.91z
$\beta_{protein}$	-0.037x*	-0.166x	0.021x*	-0.330y
$\bar{x}_{protein}$	68.31x	63.74y	58.83z	56.52z
β_{lipid}	0.330xy	0.347y	-0.052y*	0.560x
$\bar{x}_{lipid}$	7.25x	12.54y	17.99z	19.63z

* Slope not significantly different from 0 ($P > 0.05$).

Table 4.7. Sequential *F*-ratios and associated *P*-values of the relative effects of ration treatment (FF or SS) and sample time (July 7 or August 13) with the differential effects of time on the relationship of variables to W_r partialed out. Sample size was 40.

	Factor	
Variable	Ration	Time
LSI	26.78 (<0.0001)	0.34 (0.56)
VSI	8.16 (0.007)	5.76 (0.02)
Water	14.56 (0.0005)	18.03 (0.0003)
Protein	27.48 (<0.0001)	14.14 (0.0006)
Lipid	25.02 (<0.0001)	16.00 (0.0003)

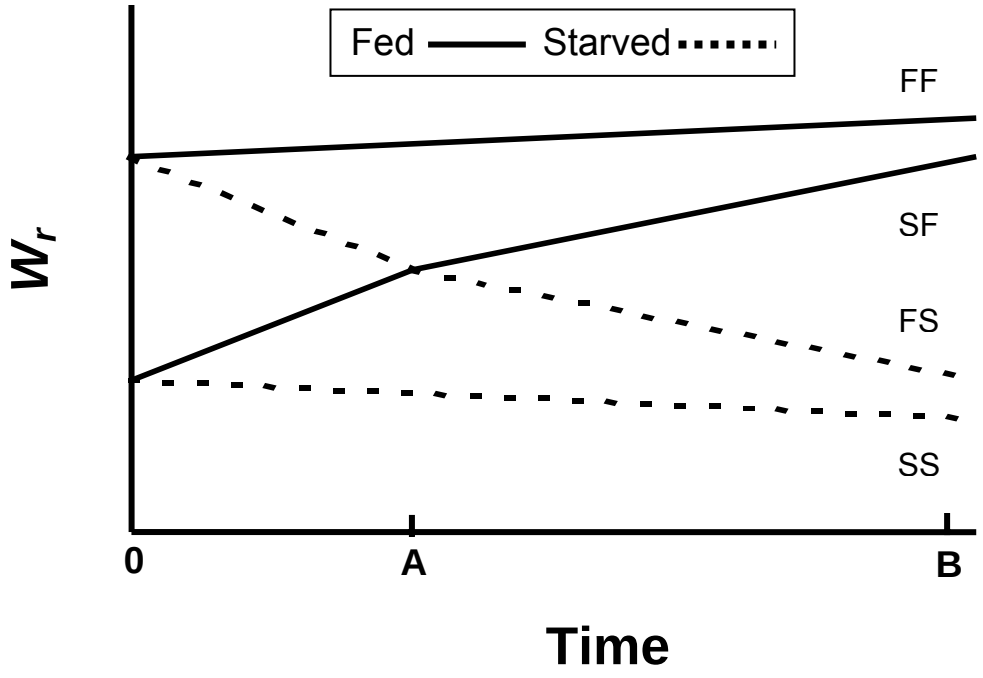

Figure 4.1. Projected trajectories of experimental treatments in relative weight (W_r) through time of fish with different initial condition and rations. Treatments were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); and continuously starved (SS). Sacrifices were planned at times 0, A, and B.

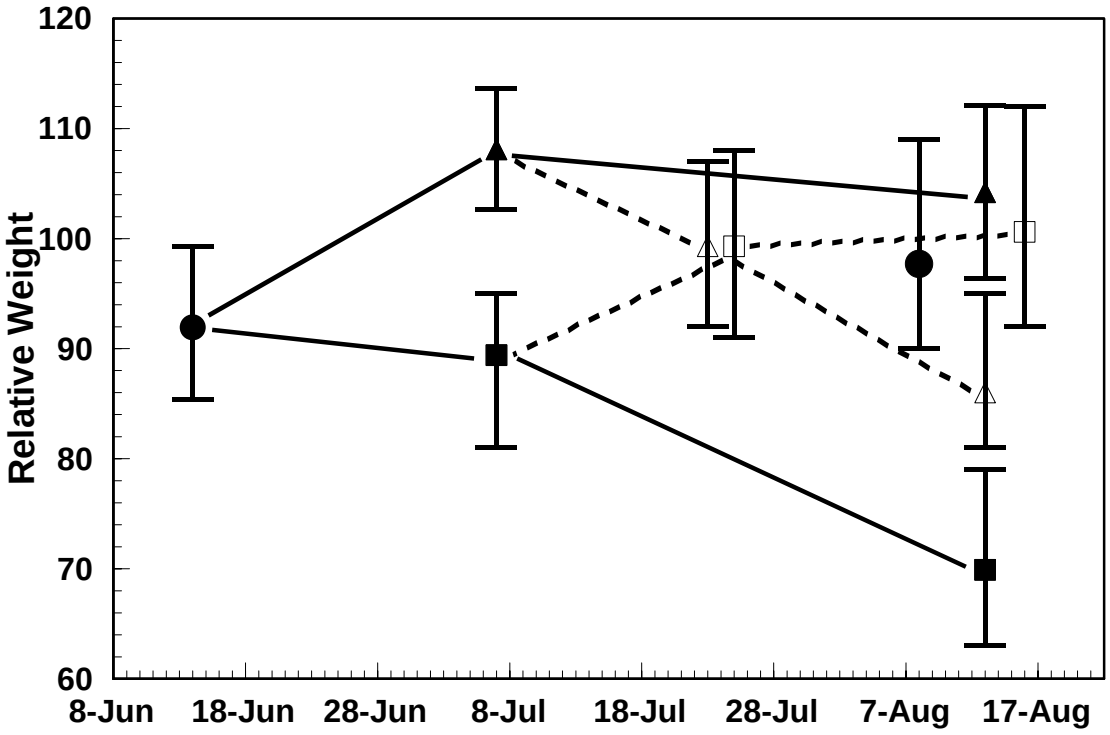

Figure 4.2. Mean relative weight (± 1SD) of all laboratory and wild bluegill at sacrifice with respect to date. Solid triangles represent the fed treatment, solid squares represent the starved treatment. Hollow triangles represent the fed group that was starved and the hollow squares represent the starved group that was fed. The solid spheres are samples from Claytor Lake.

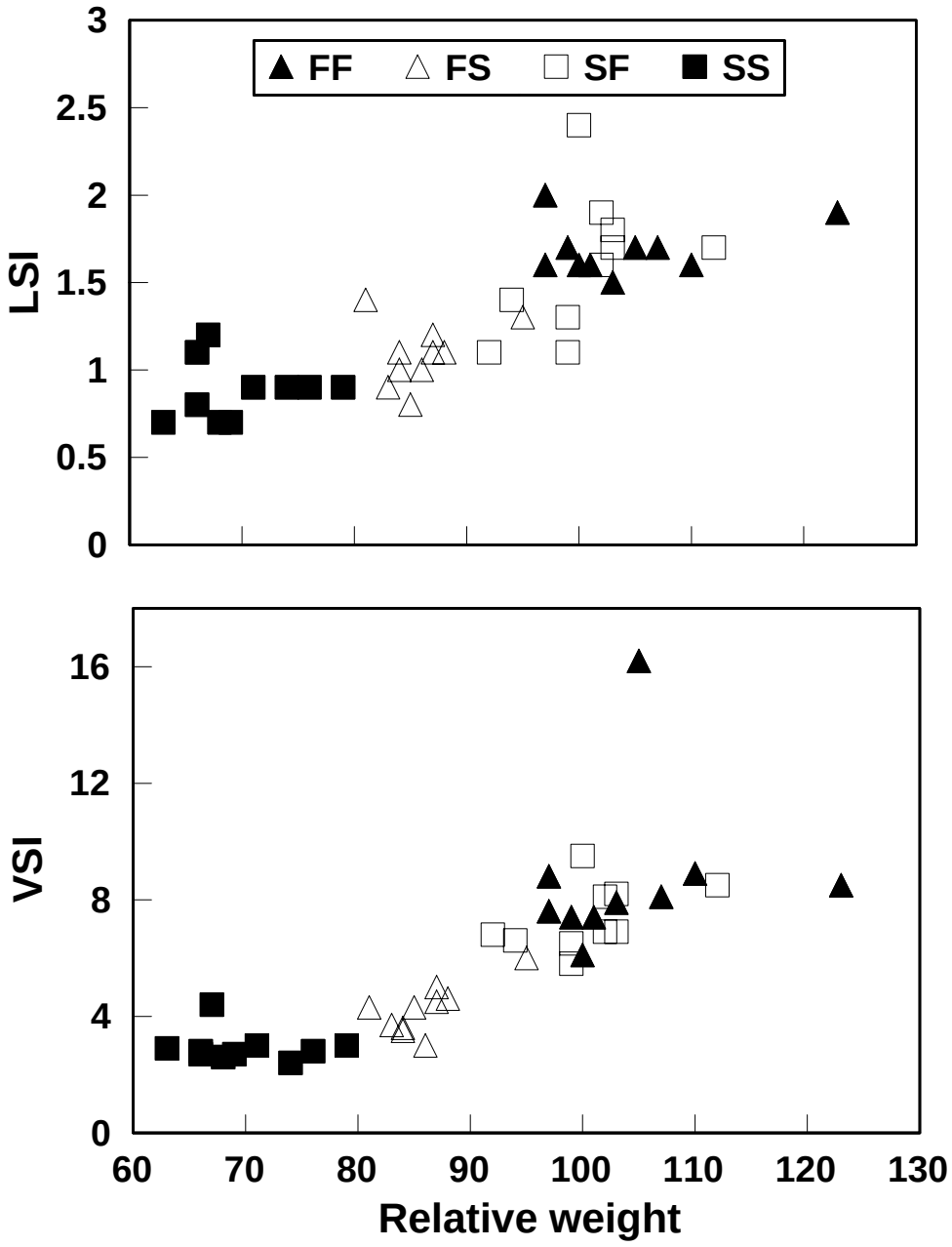

Figure 4.3. Relationship between relative weight and liver and visceral somatic indices (LSI and VSI) at the end of the experiment. Treatment groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); and continuously starved (SS).

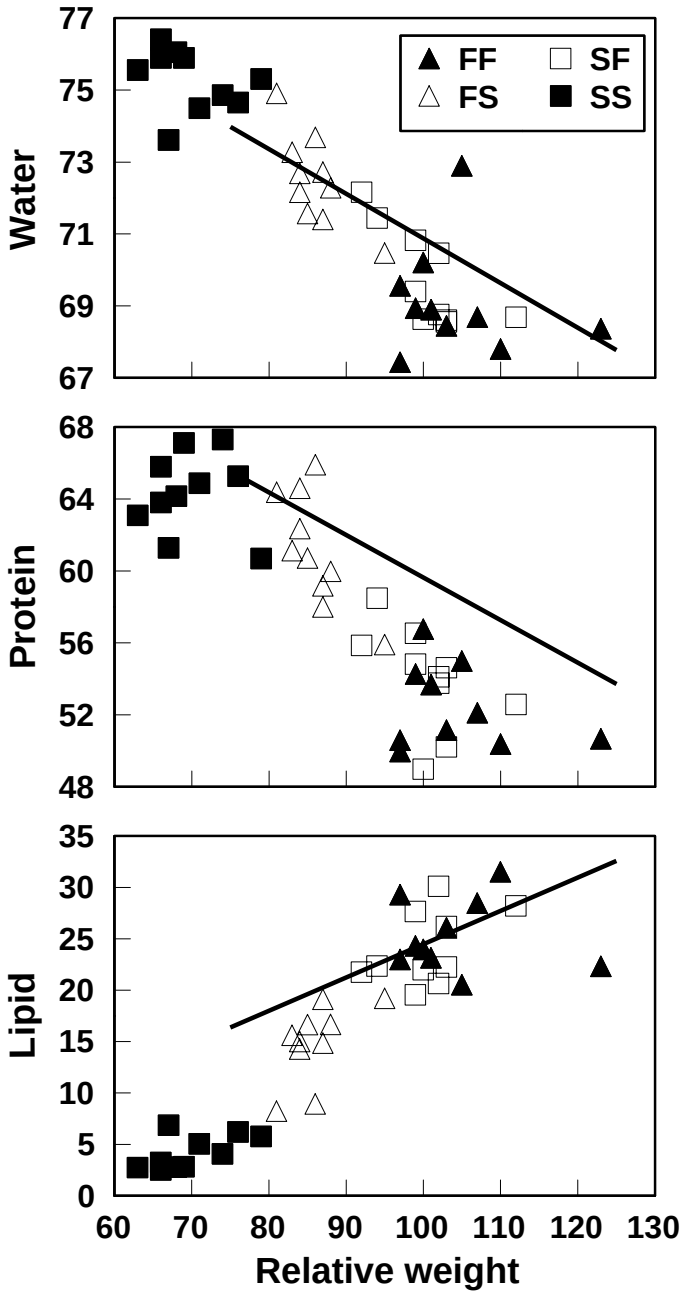

Figure 4.4. Relationship of relative weight to body composition at the end of the experiment as compared to regression lines from the data of McComish (1971). Water content is percent wet weight, protein and lipid content are percent dry weight. Treatment groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); and continuously starved (SS).

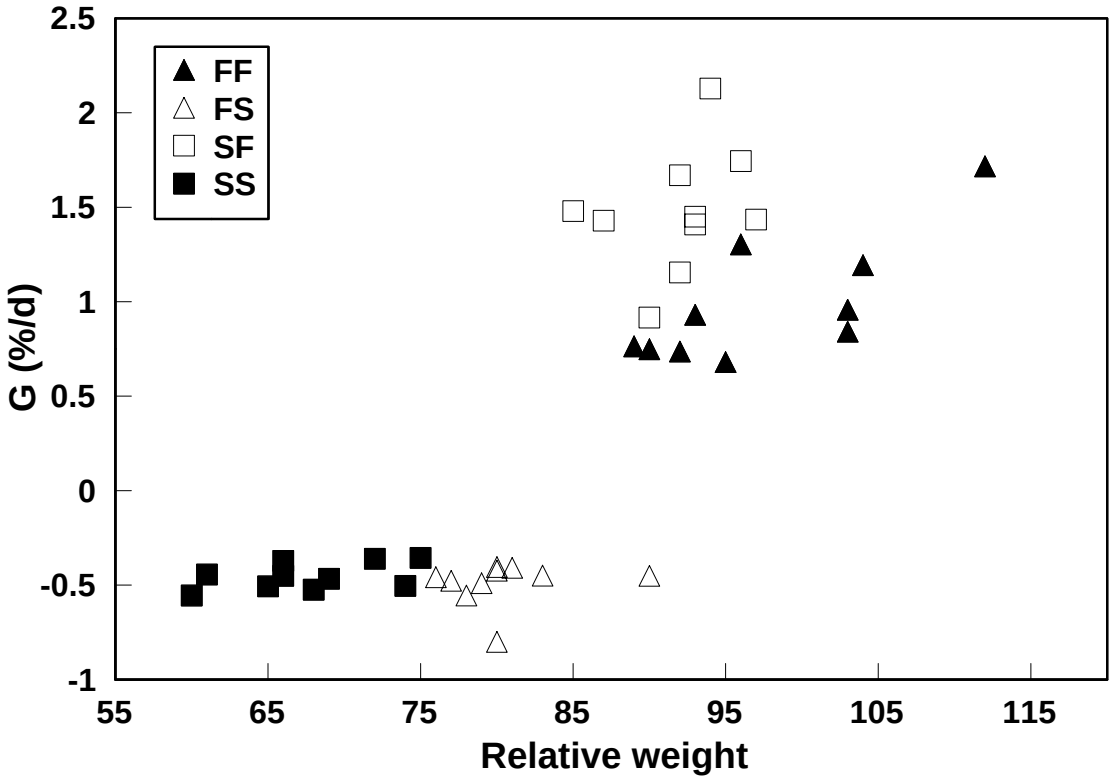

Figure 4.5. Relationship of final relative weight to growth rate (*G*) through the experiment. Treatment groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); and continuously starved (SS).

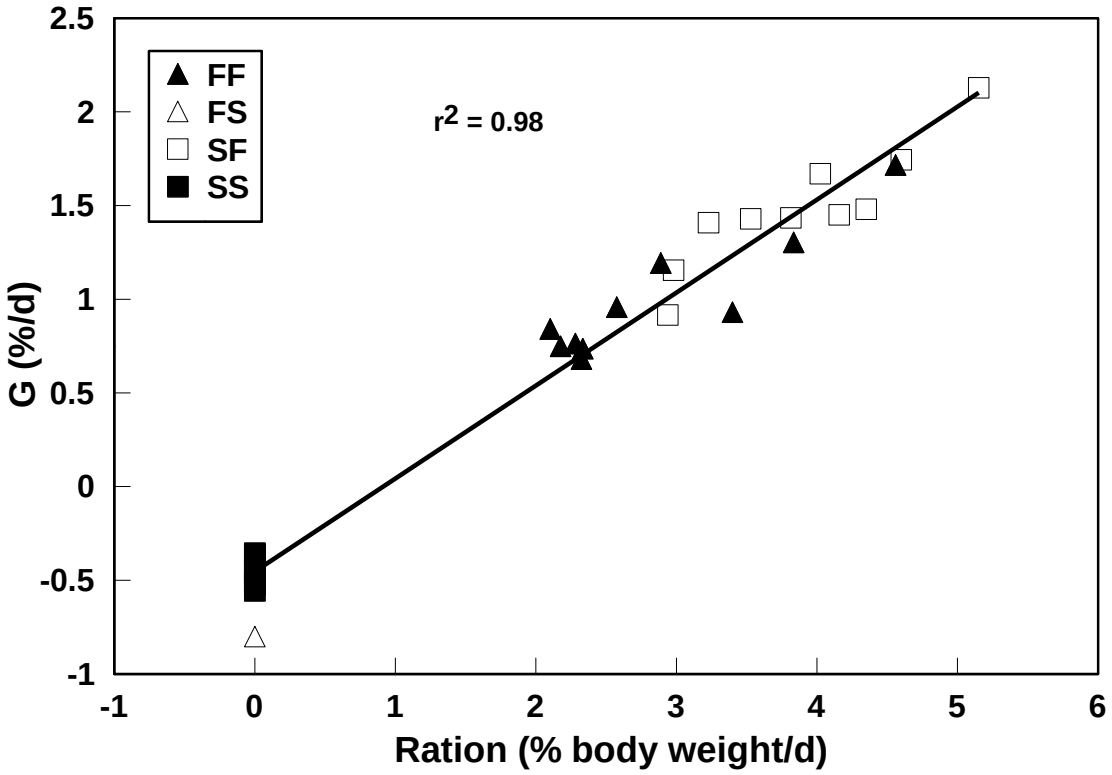

Figure 4.6. Average growth rate (*G*, %/d) and average ration consumed (% bw/d) of bluegills alive through the end of the experiment. Treatment groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); and continuously starved (SS).

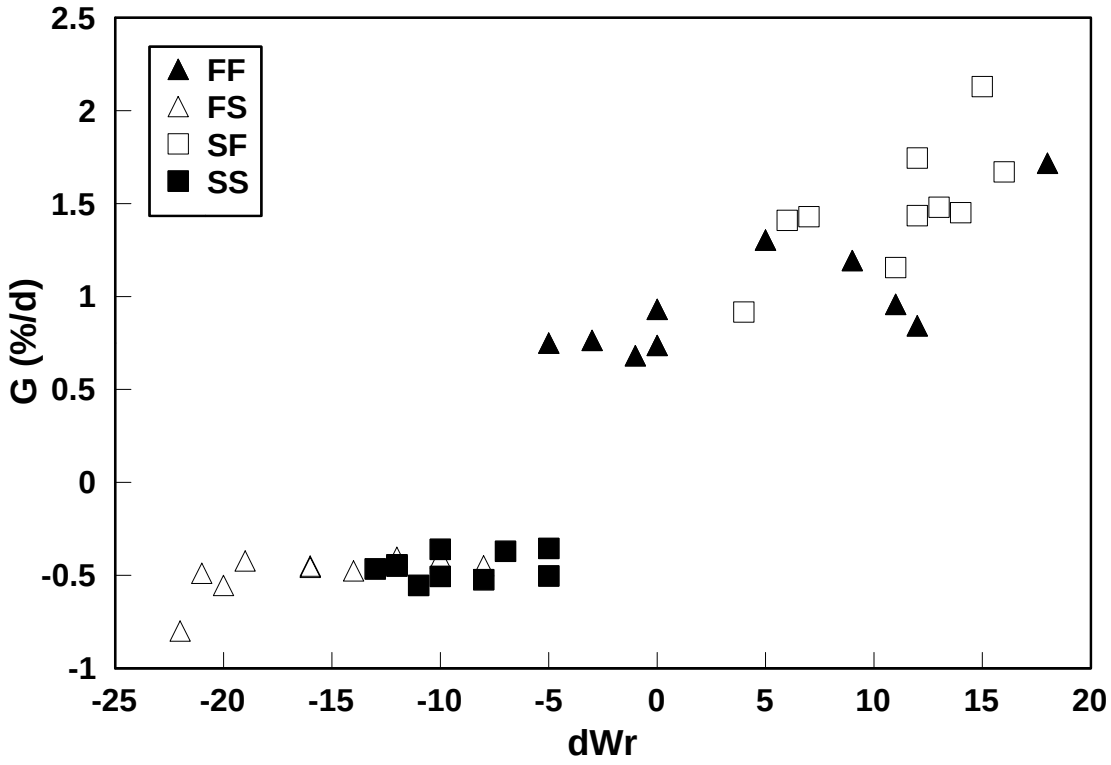

Figure 4.7. Growth rate (*G*) through the experiment versus change in relative weight (dW_r) from initial values (July 7) of bluegills at the end of the experiment. Treatment groups were continuously fed (FF); fed, then starved (FS); starved, then fed (SF); and continuously starved (SS).

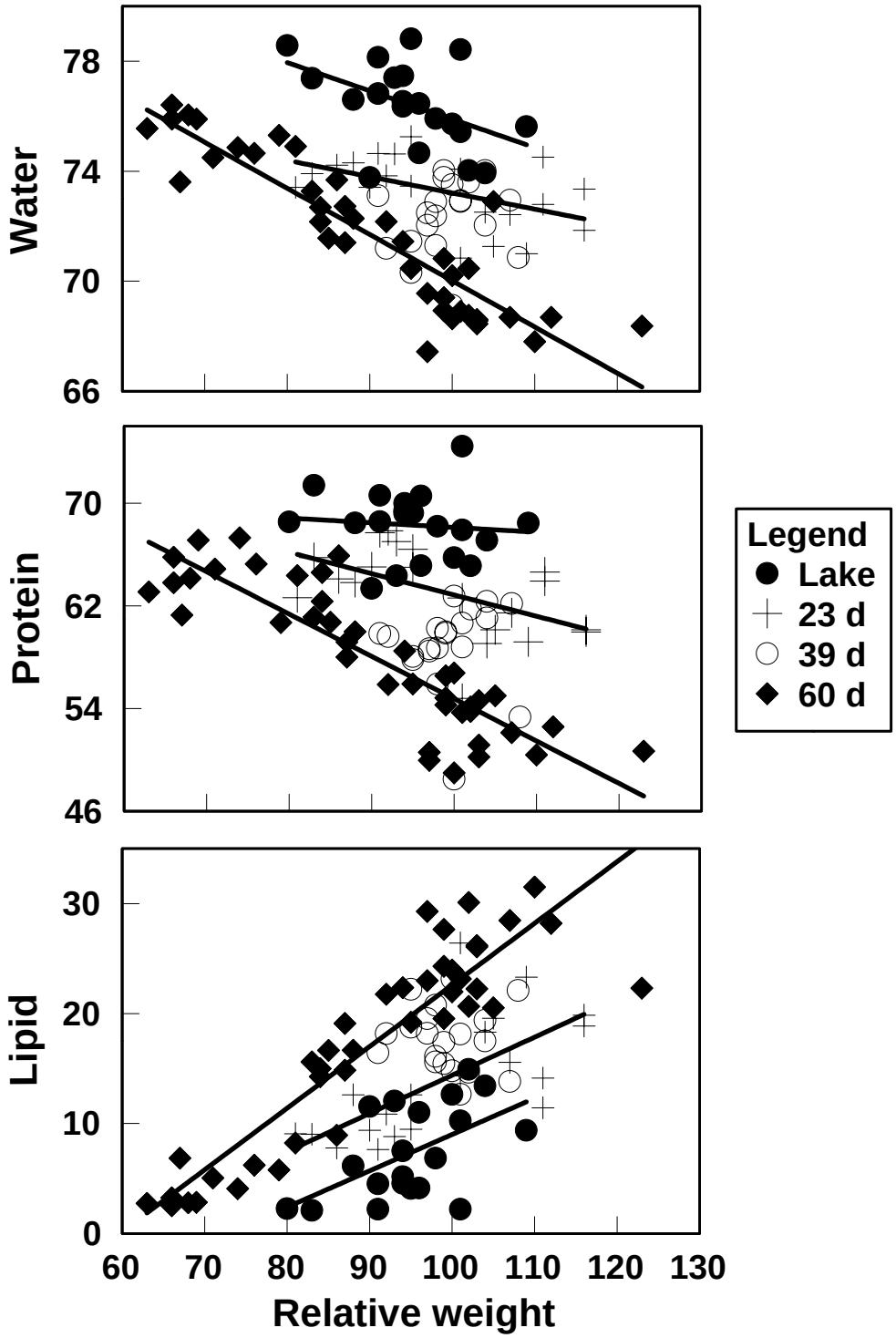

Figure 4.8. Correlations of W_r to body composition at 0 d (Lake), 23 d, 39 d, and 60 d in the laboratory. Regression line for the 39 d group is not shown.

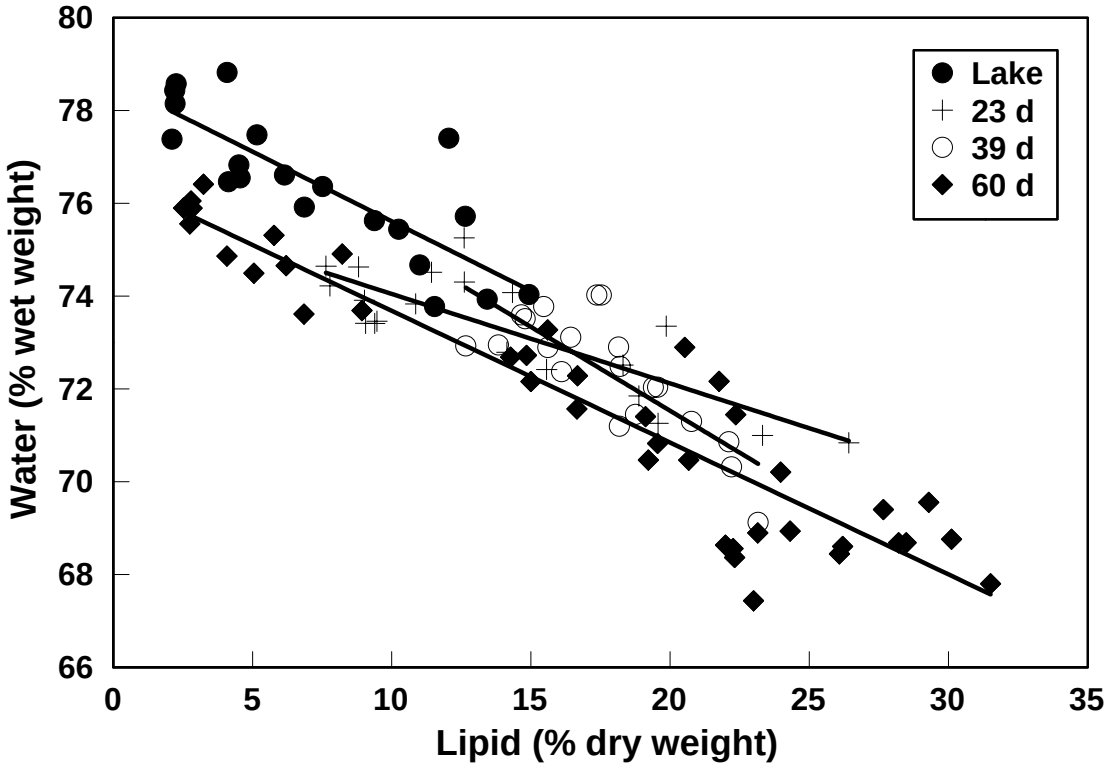

Figure 4.9. Changes in correlation of water to lipid content at 0 d (Lake), 23 d, 39 d, and 60 d in the laboratory. Regression lines for all groups shown.

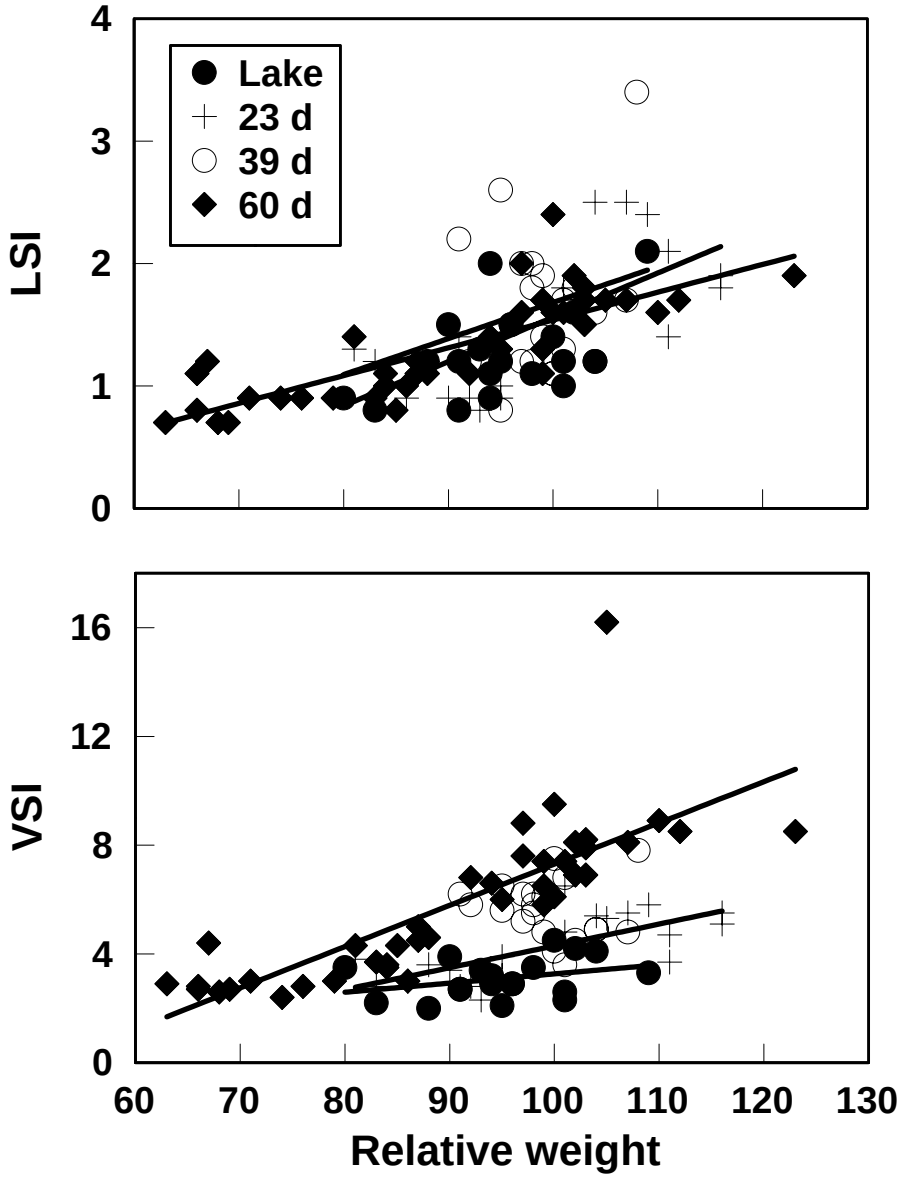

Figure 4.10. Changes in liver-somatic index (LSI) and viscerosomatic index (VSI) at 0 d (Lake), 23 d, 39 d, and 60 d in the laboratory. Trend lines are shown for Lake, 23 d, and 60d groups. Trend for the 39 d group is not shown.

CHAPTER 5. A COMPARISON OF RELATIVE WEIGHT AND NUTRITIONAL STATUS AMONG FOUR MEMBERS OF A FISH COMMUNITY

Introduction

Whole-body condition indices, such as relative weight (W_r), are often used to infer the ecological and nutritional circumstances of fish. These indices are calculated for each individual but are amenable to summarization over large groups (Ney 1993). Thus, researchers and managers are able to integrate individual physiological data at the population level, the scale on which management typically is focused (Harder and Kirkpatrick 1996).

Dietary history of a fish clearly influences condition index. It is also clear that condition may change rapidly following changes in feeding patterns. The indirect, positive, relationship of W_r and forage availability has proven rather strong and consistent (Wege and Anderson 1978; Mosher 1984; Liao et al. 1995; Marwitz and Hubert 1997; Porath and Peters 1997). Arndt et al. (1996) found that Fulton's condition factor (K) responded to reductions in feeding of Atlantic salmon (*Salmo salar*) within 4 - 8 d. Reductions in K of hatchery-reared brook trout (*Salvelinus fontinalis*) soon after stocking were due to their inability to efficiently feed and exploit their environment (Ersbak and Haase 1983). Hubert et al. (1994) found that mean W_r of lake trout (*Salvelinus namaycush*) populations was related to lake trophic status and prey base. Although these studies are quite consistent, most of them focus on species that are at least partially piscivorous.

Studies of physiology and condition indices have yielded inconsistent results. Wege and Anderson (1978) found strong correlations of condition to growth, but other studies have found weak (Adams and McLean 1985; Gutreuter and Childress 1990; Willis et al. 1991) or inconsistent relationships (Gablehouse 1991; Liao et al. 1995; DiCenzo et al. 1995). This inconsistency is puzzling. Strong correlations to body composition (relative amounts of water, protein, and lipid) have been shown in the laboratory for W_r (Rose 1989; Brown and Murphy 1991), relative condition (K_n, Caulton and Bursell 1977), and K (McComish et al. 1974). Other studies have found weak or imprecise correlations between condition index and body composition (e.g., Gershanovich et al. 1984; Neumann and Murphy 1992; Salam and Davies 1994; Simpkins et al. 2003). Most of these investigations, including all field studies, focused on species that are piscivorous to some degree. A reasonable conclusion from the foregoing trove of information is that acquiring energy is important in determining condition index but unspecified factors in natural environments may confound expected physiological expressions.

Some species may have less precise relationships of body composition to condition indices than other species. I conducted comparative field studies on bluegills (*Lepomis macrochirus*) examining the relationship of W_r to variables describing nutritional condition (Chapter 2, Chapter 3). There was considerable spatial and temporal variation in the correlation of W_r to constituent variables. In general, I found that relationships described in laboratory experiments were weak and hard to detect in the wild. The complexity of the natural environment and the

ability of bluegills to adapt their physiology to energetic regime (Chapter 4) suggest that there is intra-population heterogeneity in how wild bluegills allocate their energetic resources. Sunfishes (genus *Lepomis*) are known to be extremely variable in their life history strategies (Gross and Charnov 1980; Fox 1994; Bertschy and Fox 1999). Differences in strategies have physiological implications (Jennings and Philipp 1992; Justus and Fox 1994). Perhaps other species are less variable in their resource allocation strategies and hence there would be less ambiguous interpretation of their condition indices. Most previous research was done on more specialized species, especially piscivores. Piscivory constrains life history and is potentially more energetically profitable than other trophic strategies (Keast 1985). Certain life history characteristics and high net energy balances may reduce variability about W_r trends.

The use and application of W_r has increased greatly in recent years (Blackwell et al. 2000). The concept of W_r was developed in the mid 1970s to enable inter-population comparisons of condition without length bias (Anderson and Neumann 1996). It is calculated as the ratio of observed weight to length-specific standard weight. Standard weight (W_s) equations are empirically defined for each species as the 75th percentile of the weights predicted from a series of length-weight regressions from populations across a species range (Murphy et al. 1991). The first W_s equations were developed for largemouth bass (*Micropterus salmoides*, Wege and Anderson 1978) and bluegills (Legler 1977) and soon expanded to other centrarchids. The number of taxa covered by W_s equations has grown rapidly (Figure 5.1). The concept has been applied far

beyond the original taxa (centrarchids) to species known to be physiologically and morphologically plastic (e.g., common carp *Cyprinus carpio*; Corti et al. 1988; Loy et al. 1996) or little known in terms of their population biology (e.g., longnose gar *Lepisosteus osseus*; Johnson and Noltie 1997). Standard weight equations are being published without step-wise examination of their biological basis for interpretation. Important questions regarding the use of condition indices remain to be addressed. More research on wild fish should be undertaken and the role of species characteristics in determining the relationship between index and nutritional condition needs to be investigated.

The relationship of physiology to W_r is clearly not straightforward in the wild. The current state of knowledge requires considerable extrapolation to interpret W_r in many species. Do species characteristics influence whole-body expression of nutritional condition? Such has been demonstrated in birds, where winter lipid reserves varied by foraging guild among species within family Emberizidae (Rogers and Smith 1993).To my knowledge, no one has addressed this question for fish. My goal was to examine the ability of W_r to reflect the nutritional status of wild fish. Specifically, I compared the relationships of W_r to energetically influential variables among four species from a common environment. I qualitatively compared the amount and kind of information contained in W_r among largemouth bass (LMB), black crappies (BC, *Pomoxis nigromaculatus*), and chain pickerels (CP, *Esox niger*) to the relationships I previously determined for bluegills (BG; Chapter 2, Chapter 3). All four species have defined W_s equations (BG, Hillman 1982; BC, Neumann and Murphy 1991;

LMB, Henson 1991; CP, Neumann and Flammang 1997).The former three species were chosen to provide a range of phylogenetic distances from BG. These species also provide a range of life history characteristics in terms of trophic ecology and reproductive strategy (Table 5.1).

My previous studies of bluegills found that the correlation of W_r to energetic variables was weak and there was much variability about the trend when it was present (Chapter 2, Chapter 3). I expected that other species would have more distinct trends and less variability about them. Using the adjusted coefficient of determination (adjusted R^2) as a measure of trend strength and variability, I postulated four alternative hypotheses to explain potential patterns. The null hypothesis was that correlations will be low in all species, H_o: BG = BC = LMB = CP. The next hypothesis was that correlation strength is related to phylogeny. Because W_r was developed for centrarchids, it should work best for them. Therefore, while BG may be the most variable centrarchid among the study species, CP are from a different order and should be even more variable, H_1: BC, LMB > BG > CP. Another explanation for differences could be species ecology. Trophic ecology is linked to other life history characteristics such as reproductive strategy (Keast 1985). Bluegills are generalists, while the others are more specialized (Table 5.1). Bluegills are known to be variable in their life history (e.g., Gross and Charnov 1980; Aday et al. 2002), and these variations influence condition (Justus and Fox 1994). Based on degree of piscivory, I predicted that LMB and CP should exhibit the strongest correlations and bluegills the weakest, H_2: CP, LMB > BC > BG. Lastly, the four species differ in external

morphology. Bluegills are laterally compressed; BC are even more so (personal observation). The piscivores (CP and LMB) are cylindrical and fusiform. Body plan affects the chances of weight varying from changes in dimension besides the main body axis, length. Standard weight is exclusively a function of length. The predicted pattern under this hypothesis would be H_3: CP, LMB > BG > BC. I evaluated these hypotheses with data from a single community to control abiotic influences. Initially, I did not collect enough BC to clearly evaluate the hypotheses; therefore, a second community was sampled.

Methods

Study Sites

Field sampling was conducted on two small impoundments in different parts of Virginia. Smoots Pond (SM) is a 21-ha impoundment on the property of Fort A. P. Hill in Caroline County. The watershed lies in the Coastal Plain portion of the York River drainage. This is a forested area with sandy soils and swampy bottoms; therefore, the pond is infertile and slightly dystrophic (total alkalinity = 4 mg/L as $CaCO_3$, surface conductivity = 20 µS; T. Copeland, unpublished data). Recent surveys documented that 13 fish species were present (T. Copeland, unpublished data).The second study site was Rural Retreat Lake (RR), a 36-ha impoundment in Wythe County. The outlet stream is a minor tributary of the New River and drains a portion of the Ridge and Valley physiographic province. Local land use in the watershed is primarily agriculture; therefore, water quality is more fertile and eutrophic (total alkalinity = 33.4 mg/L as $CaCO_3$, VDEQ 1990; surface

conductivity = 183 µS, John Copeland, Virginia Department of Game and Inland Fisheries (VDGIF), personal communication). Recent management surveys documented eight fish species present (J. Copeland, unpublished data).

Populations were chosen to compare against concurrent studies of bluegills. Inventories conducted the spring prior to sampling for this study show several differences between the two communities (Table 5.2). Chain pickerel were not present in RR. Catch rates of BG, BC, and LMB were 3.8 times higher in RR. Proportional stock densities (PSD, Anderson and Neumann 1996) of BG and LMB were higher in SM but population mean W_r was higher in RR. Black crappie populations were similar in PSD but not in catch rate.

Data Collection

Field sampling was conducted at SM on June 8, 1999 and at RR on April 1-2, 2002. I collected BC, LMB, and CP at SM. Based on previous data, the population length range was divided at the median and samples were taken evenly above and below. The minimum length of fish collected was the minimum for application of W_r for each species (Blackwell et al. 2000). The overall target sample size was 40 individuals, 20 within each length category. Collections of LMB and BC at RR were made in cooperation with VDGIF spring field work. Largemouth bass were sampled as before. To ensure an adequate BC sample from RR, the population length range was divided at the 33rd and 66th percentiles and 20 individuals collected within each length category (n = 60).

These data were compared to a subset of data from more intensive studies on BG conducted simultaneously (Chapter 2, Chapter 3).

Fish were placed on ice and transported back to the laboratory, where they were stored on ice in a sealed container and processed within a week. Each fish was patted dry with a paper towel, measured to total length (TL, nearest mm), and weighed (nearest 0.01 g). I measured six variables indicative of stress and energy storage: liver-somatic index (LSI), gonadosomatic index (GSI), viscerosomatic index (VSI), and percentages of water, protein, and lipid in the body. These variables have been used in the past to index growth and energetic status of fish (Goede and Barton 1990). Each individual was dissected. The viscera (the gastrointestinal tract with attached tissue), liver, and gonads removed and weighed to nearest 0.01 g. Stomach or intestinal contents were extruded prior to weighing the viscera. After weighing, all organs were replaced within the body cavity. For logistical reasons, body composition was determined for only 30 BC collected at RR, 10 randomly chosen from within each length strata. Carcasses were chopped into small bits, placed on a pre-weighed aluminum dish, weighed, and dried at 37^{o}C in an oven. The dried pieces were ground with a coffee bean grinder. The resultant powder was replaced in the oven on the same pan until weight stabilized for the largest fish; then all were re-weighed. The difference between wet and dry weights was water content. For the SM data, protein and lipid content were determined at the Virginia Tech Forage Testing Laboratory using the macro-Kjeldahl procedure and ether extraction (AOAC 1990). For the RR data, protein and lipid content were determined at the

Virginia Tech Aquaculture Center laboratory using the macro-Kjeldahl procedure and chloroform:methanol extraction (AOAC 1990).

Data Analysis

I used multiple linear regression (MLR) to examine the relationship of the six sub-organismal variables to W_r, a whole-body measure. Lipid and protein content were expressed as percentage dry weight, all others were expressed as percentage wet weight. Data were examined to detect the presence of outliers and highly influential observations using standard diagnostics (Studentized residuals, diagonal elements of the hat matrix, DFFITS, and DFBETAS; Montgomery and Peck 1992). Obviously erroneous observations were deleted from the data set. The remaining data were used to construct a MLR model for each population. Examination of model residuals and bivariate scatterplots did not reveal any obvious departures from homoscedasticity; therefore I did not transform any variables. Collinearity was not a problem (all variance inflation factors <5, Montgomery and Peck 1992). Significance of individual variables was determined by *t*-test (H_0: $\beta_i = 0$, $\alpha = 0.05$). To measure the unique contribution of each variable to the model's explanatory power, partial R^2 was computed. To reduce the complexity of the models and provide for the most general interpretation, I did not include gender effects. Model fits were compared using adjusted R^2. I used this to measure correlation strength for evaluation of the hypotheses. In the following, R^2 is used to denote the coefficient of multiple determination in a multivariate regression, whereas r^2 denotes the coefficient of

determination in a bivariate regression. To help corroborate and interpret MLR correlations, I also used simple linear regression of each regressor versus W_r and a correlation matrix of the regressors. For these analyses, I separated male and female GSI. Patterns of W_r with length are often used as evidence to intuit important factors influencing population structure (Anderson and Neumann 1996), so I plotted this relationship for each population and computed r^2. Differences within species with respect to all variables measured were tested between populations with a multivariate analysis of variance (MANOVA) followed by two-sample t-tests. Use of the MANOVA protects the nominal error rate of subsequent univariate tests (Rencher 1995). Risk of Type I error was set at α = 0.05.

Results

I analyzed 216 fish for this study (Table 5.3). Length ranges were widest for CP and LMB. Sex ratio was highly skewed towards males in RR BC. Minimum sample size was 28, except for SM BC (n = 13). Concerns about the inadequacy of the latter sample prompted me to include RR as a study site. Populations had different correlations of W_r to length (Figure 5.2). Length was negatively correlated to W_r in CP, LMB, and BC from SM ($r^2 > 0.26$) but not for SM bluegills or any of the RR populations ($r^2 < 0.10$).

Populations of each species differed with respect to the variables measured (Table 5.4). The LMB populations were similar in W_r and female GSI. The BC populations were similar in VSI. The BG populations were similar in

protein and lipid content. All species captured from RR had higher LSI; apparently, all were growing at the time of sampling. Bluegill deviated from consistent trends exhibited by BC and LMB. Female GSI was higher for LMB and BC in the RR sample, whereas bluegill GSI was higher in the SM sample. Protein content was higher and lipid lower in SM for BC and LMB; whereas, for bluegills, protein and lipid content were similar between populations. Although I did not test among species, BG had consistently higher lipid contents than the other species. Chain pickerel had the lowest lipid content. Black crappie had the lowest VSI. For the SM samples, W_r followed the same pattern among species as LSI and VSI.

Models relating W_r to the other variables were significant for all piscivore populations and for RR BC (Table 5.5). The RR LMB model had the greatest explanatory power. Adjusted R^2 values of the CP and SM LMB models were close. The SM BC model was not significant because of small sample size but a large amount of the variance in W_r was explained by the model. Model precision was low for both BG MLR models; neither was significant.

Different combinations of variables were significant in each model (Table 5.6). For most models, one or two variables contributed explanatory power, although sometimes three variables were significant. For example, in the RR LMB model, LSI and VSI together contributed the most to the model's explanatory power; protein content was significant but only added 6% to the model's power. Of the seven MLR models, LSI and GSI were significant in three, VSI and water content were significant in two, and protein and lipid content were

significant in only one MLR each. Because of correlations among the regressors (Table 5.7), related variables, like lipid content, did not contribute much additional explanatory power to the model, although the correlations were not enough to influence parameter estimation.

Lipid content, VSI, and LSI tended to be inter-correlated in all samples (Table 5.7) and one of these three variables was significantly related to W_r for each population in which the MLR model was significant. Regressor variables that were significantly correlated tended to have correlations in the same direction for all populations. For example, all significant correlations between LSI and lipid content were positive. Water and protein content tended to be negatively correlated to all other variables except each other.

Individual correlations of variables to W_r varied among the species (Table 5.8). Lipid content, LSI, and VSI were positively correlated to W_r in four, three, and five populations, respectively. Female GSI was negatively correlated to W_r in four populations but the correlation was positive for RR BC. Male GSI was negatively correlated to W_r only for SM BG. Protein content was negatively correlated to W_r in RR LMB and BC. Water content was negatively correlated to W_r in RR BC, but positively in SM BC. I attribute the latter result to poor estimation caused by the low sample size. The RR BC population exhibited the most significant correlations to W_r with six. Both LMB populations had four significant correlations and the other populations had two. Correlations were consistent in the LMB populations: three were common to both. The bluegill populations had one correlation in common.

Discussion

Population Comparisons

Differences in the variables between RR and SM were related to each species' phenological cycle, especially in regard to reproduction. In the SM samples, only bluegills were in reproductive condition. The others were in a post-reproductive state; male GSI was low ($\leq$ 0.13) and similar for CP, LMB, and BC. Neumann and Murphy (1992) found GSI of non-reproductive white crappies (*P. annularis*) was < 2.0 for females and < 1.0 for males. Medford and Mackay (1978) found that female and male GSI of northern pike (*E. lucius*) were 0.34 and 0.15 before gonadal growth began. These were close to mean female and male CP GSI. Length was negatively correlated with W_r in CP, LMB, and BC in SM, perhaps as a consequence of spawning stress in larger fish. Conversely, in the RR BG sample, male GSI was similar to the non-reproductive level of the other species in SM. In RR, LMB and BC were in a pre-reproductive state, as indicated by GSI. Black crappie were on the verge of spawning, as shown by the intense black coloration of snout and cheeks in the males (personal observation), which is a sign of breeding condition (Jenkins and Burkhead 1993).

Site differences were also related to differences in the yearly growth cycle. It is not uncommon to see an early spring peak in growth and energy storage of temperate freshwater fish (Gerking 1966; Adams et al. 1982; Adams and McLean 1985). In RR, all species had elevated LSI relative to the SM populations. The RR populations of LMB and BC also had higher lipid contents. These fish should

have been feeding in preparation for spawning. All RR populations should have been growing and storing energy when they were sampled.

The correlations among the regressors were remarkably consistent among populations and species. Correlations were influenced by energy flow within the individual. Lipid content and VSI should be linked as energetically positive variables; VSI is a long term storage depot (Adams and McLean 1985), whereas total lipid content also includes more easily mobilized stores. The previous two variables were often tied to LSI; more excess energy for growth also means more energy for storage (Calow 1994). Water and protein content tended to be negatively related to lipid content and associated variables. As energetic content increased, these two variables became less important. There was a consistent inverse correlation of GSI with water and protein content but it had few significant correlations to other regressors.

Interpretation of W_r

Relative weight carried variable amounts of information as indexed by adjusted R^2. Comparison of MLR models with simple correlations clarified interpretation of differences in W_r within each population. The particular pattern of correlations among the regressors in each population affected which were significant in the MLR. Because the correlations were not particularly precise ($r^2 \leq 0.65$, Prairie 1996), relationships to W_r are not suitable for prediction.

In the CP sample, W_r was influenced by GSI and lipid content, as shown by both MLR and SLR. The significance of water content in the MLR model

seems counterintuitive, especially because it was negatively correlated to lipid content. It was not significantly correlated to W_r on an individual basis but appeared to significantly add to the explanatory power of the MLR model, even though the actual increase in model adjusted R^2 was 9%. There was an inverse correlation of GSI to W_r; under conditions of food limitation, a greater investment in gonadal mass means less somatic mass (Glazier 2000). This investment was presumably greatest in mature females and was apparent well after spawning. This effect was driven by the strong association of GSI and W_r in females, even though they were less than half of the sample (16 of 38). Lipid content and GSI were not significantly correlated and hence explain different aspects of nutritional condition, storage versus reproductive expense.

In both LMB populations, W_r was most strongly associated with LSI. Both condition index and LSI have been positively correlated to LMB feeding rate (Heidinger and Crawford 1977), so it is not surprising the two are related here. Lipid content was not significant in either MLR model, but simple correlations to W_r were significant. Lipid content was significantly correlated to LSI, thus the latter explained variation in both variables in the MLR. There was a similar relationship of W_r to VSI, although this variable was significant in the RR model. As in the CP MLR model, the significance of GSI in the SM LMB model was driven by females. This was not the case in the RR sample, because females were at different stages of gonadal development (data not shown). Protein content was significant in the RR sample presumably because of its correlation to lipid content. Lipid content was not significant in the MLR but had a very

significant SLR for RR LMB. Comparison of the results from the two populations showed the positive influence of increased energetic activity on W_r, in terms of growth and storage. The negative correlation of W_r to GSI is evidence of the cost of spawning in SM females. All correlations were stronger in the RR sample, possibly because of growth and storage at this point in the phenological cycle. Similarity in changes of LSI, condition index, and lipid content was shown for juvenile chinook salmon (*Oncorhynchus tshawytscha*) during early spring growth (Beckman et al. 2000). Evidently, growth promotes correlation of body composition and LSI to condition index.

Comparison of the BC MLR models was complicated by small size of the SM sample. Low sample size can cause the model parameter estimates to become unstable as well as obscure functional trends (Montgomery and Peck 1992; Neter et al. 1996). The positive association of W_r with water content is puzzling and probably a statistical artifact. The RR BC model is significant and there were many significant correlations among the regressors (13 of a possible 15) such that only LSI was significant in the MLR model. Again, LSI explains much of the variation found in these other variables. The RR BC sample was the only one in which female GSI was positively correlated to W_r, presumably because BC had not yet spawned and so energy that would be lost during spawning was still present within their bodies. Correlations of W_r to LSI and lipid content in RR BC were similar to those Neumann and Murphy (1992) found for pre-spawn white crappies (r = 0.468 and 0.549). However, the correlations in their data to female GSI, water and protein content were much weaker (r = 0.293,

0.187, and 0.298). Note that their correlations to water and protein content were positive, which seems counter-intuitive. Although Neumann and Murphy (1992) did not present any *P*-values, the latter correlations were probably not significant.

Results from both BG populations were consistent despite difference in phenological stage. Neither MLR model was significant. Both had almost identical adjusted R^2 values. For the SM sample, there was a lack of significance despite the largest sample size in this study. The SLR models of W_r to VSI were significant and explained the same amount of variance in W_r for both populations. Energetic storage appeared to influence BG W_r but the relationship was imprecise. Male GSI was inversely correlated to W_r for SM BG, whereas female GSI was inversely correlated for RR BG. Wide variation in gonadal development likely obscured a similar correlation for SM females. Actual reproductive costs in male fish are directed more towards activity than in females (Craig 1977); this should be particularly true of nest-guarders like male BG. Differences in type of reproductive costs may influence the time they are incurred.

Comparison across species and populations showed consistencies in interpretation of W_r. Foremost was the positive relationship of variables describing energy storage and short-term growth to W_r. Lipid content, VSI, and LSI tended to be correlated in all populations, and at least one of these variables had a significant correlation to W_r in all populations. Secondly, the negative influence of female GSI in post-reproductive fish was common across species lines. Where this relationship was significant, W_r was inversely correlated to length. Apparently, the magnitude of energetic investment by females has a

lasting effect. The inverse correlation also could be explained by increased metabolic costs or decreased foraging opportunities but these should be unlikely as fish get larger. As a result of lesser reproductive investment by males, male GSI was not significantly related to W_r for most populations. For male centrarchids, major costs of reproduction should arise from nest guarding and competing for females and not from investment in the gonads. Lastly, comparison of the LMB samples suggests that conditions promoting increased growth and storage, such as present in the early spring, act to clarify relationships of the underlying variables to W_r.

Species Differences

Although the overall type of information was similar, W_r carried different amounts of nutritional information among species. The order of species by amount of variation in W_r accounted for by energetically influenced variables (adjusted R^2) was most consistent with the ecological hypothesis (H_2): RR LMB > CP, SM LMB > RR BC > SM BG, RR BG. I excluded SM BC because of the low sample size and concomitant problems for estimation of regression parameters such as adjusted R^2. Therefore, W_r provided the most information on nutritional condition for CP and LMB, very little on the generalist BG, and an intermediate amount for BC. This pattern is most consistent with the trophic role of the species examined.

One explanation for order of correlations may be the profitability of the piscivorous life-style. It may be easier for piscivores to acquire excess energy in

a short period of time. For example, LMB grow much more quickly on a fish diet compared to an invertebrate diet (Olson 1996). Piscivores tend to have empty stomachs more often than other trophic groups (Arrington et al. 2002). These two observations show that predators rely on stored energy more than non-predators (Secor and Diamond 2000) and that non-piscivores should forage more often (Bowen et al. 1995), thus using energy for activity instead of for somatic investment. Because piscivores tend to be larger, they have fewer predators and so may pursue their own prey freely. Bluegills are frequently confined to less profitable habitats by predation risk (Werner et al. 1983). Consequent starvation or feed restriction obscures the W_r - condition relationship (Chapter 4). However, this concept is hard to square with the observation that bluegills have consistently higher lipid level than piscivores. This may result from the tendency for lower trophic levels to be more limited by protein than energy (Bowen et al. 1995).

Trophic role also is tied closely to other traits that influence the acquisition and use of energy. For example, reproductive timing of piscivores is strict (Keast 1985) and may be restrictive in regard to allocation strategy. Piscivores must spawn such that their young are at a size to be effective predators when young of other species become available as prey (Adams and DeAngelis 1987; Buijse and Houthuijzen 1992; Olson 1996). The exigencies of piscivorous life therefore leave less room for differences in allocation strategy. Bluegills are fractional spawners with an extended reproductive season (Carlander 1977). Individual reproductive investment can cycle back and forth during an extended spawn (Wootton 1977),

creating variability in the W_r – condition relationship. Bluegills also have alternative tactics for reproduction (Gross and Charnov 1980) and variable body plans (Layzer and Clady 1987; Ehlinger and Wilson 1988). Such phenotypic plasticity can result in differences in resource allocation (Boggs 1994). Where fitness does not depend as heavily on somatic investment, whole-body condition may be sacrificed because of maintenance costs, which include activity and predation risk (Rogers 1987). In this case, lower somatic condition may be adaptive (Grubb 1995).

A final possibility has to do with how energy is stored. Flath and Diana (1985) found that for fatty fish like clupeids, wet weight is a poor indicator of energy content because of the tendency for water to replace lipids as they are catabolized, resulting in little change in weight with change in energy density. However, for lean fish such as esocids or LMB, they proposed that wet weight is good indicator of energy. This idea coincides with the species order in lipid content that I observed. Adult piscivores are able to maintain lower lipid content because their prey is available year-round and they are not as restricted in their choice of foraging habitat as are BG. Conversely, the trophic role and life history of BG may induce greater variability in how body weight is expressed.

Conclusions

There are differences among species that affect interpretation of W_r or other condition indices. Strength of correlation to nutritional variables varied among species but many of the same variables were influential for all. Depending on timing, the reproductive cycle can exert considerable influence (Le Cren

1951). Nutritional condition may be suppressed for a considerable period after the reproductive season. The greater onus is likely to be on females (Craig 1977; Jonsson and Jonsson 1997).

Specific interpretations of W_r were most warranted in piscivorous species. As the degree of piscivory increases, W_r should provide more precise information. This may be due to the profitability of piscivory or a by-product of the constraints on piscivores. Generalist life strategies evidently promote variability in nutritional relationships because of the wide range of life history tactics available to such species. This flexibility has implications for energy allocation and somatic investment (Jennings and Philipp 1992; Justus and Fox 1994). Only in RR LMB was any relationship precise enough for prediction ($r^2 > 0.65$, Prairie 1996). Conclusion of nutritional differences based on W_r requires large differences, large sample sizes, or both. I predict that clarity is enhanced when and where the environment promotes growth. The demand for growth in piscivores is consistent with this prediction.

The specific concepts behind W_r have been applied across many species, as evident in the number of published W_s equations. Without concomitant research into the biological basis of the index, this is an extrapolation and the validity of it needs to be tested, especially for non-piscivores. Until then, W_r or any other condition index should be regarded as a qualitative indicator. Research should be done on wild populations because the natural environment is where W_r is chiefly used. Environmental factors are likely to limit the whole-body expression of nutritional condition (Chapter 4) and should be considered. Factors

regulating the costs and benefits of maintaining higher W_r need to be elucidated. A comparative approach could enable testing of the mechanisms resulting from ecological differences (e.g., trophic structure) among and within populations. Divergence in population ecology within a species should be particularly informative. For example, if the profitability of piscivory is influential, I predict that relationships in BC would become clearer as degree of piscivory increases among populations. Such studies need to verify the assumptions behind condition indices by linking them to life history and evolutionary ecology (Hayes and Shonkwiler 2001; Rolff and Joop 2002).

Table 5.1. Trophic class and spawning period in Virginia of the four study species. Trophic classification is after Keast (1985). Spawning period is according to Jenkins and Burkhead (1993).

Species	Trophic class	Spawning period
Bluegill	Invertivore	May-August
Black Crappie	Secondary piscivore	April
Largemouth bass	Specialized piscivore	May-June
Chain pickerel	Specialized piscivore	February-March

Table 5.2. Mean electrofishing catch rate (CPUE, number/hour), proportional stock density (PSD), and mean relative weight (W_r) by site for all study species.

Site/Species	CPUE	PSD	W_r
Smoots Pond (T. Copeland, unpublished data)			
Bluegill	27.6	62	102
Black crappie	11.3	30	91
Largemouth bass	21.3	70	96
Chain pickerel	27.2	6	82
Rural Retreat Lake (Virginia Dept. Game & Inland Fisheries, unpublished data)			
Bluegill	118.2	17	121
Black crappie	90.7	26	90
Largemouth bass	81.1	28	104

Table 5.3. Mean total length (TL, mm), length range and number of males and females collected by site for all study species

Site/Species	Mean TL	Length Range	Males/Females
Smoots Pond			
Bluegill	154	83 - 212	19 / 31
Black crappie	191	105 - 249	8 / 5
Largemouth bass	262	134 - 374	13 / 15
Chain pickerel	275	160 - 411	22 / 16
Rural Retreat Lake			
Bluegill	135	90 - 182	19 / 10
Black crappie	195	142 - 242	22 / 8
Largemouth bass	242	178 - 357	14 / 14

Table 5.4. Means of relative weight (W_r), LSI, male and female GSI, VSI, water content (%), and protein and lipid content (% of dry weight) for each population (chain pickerel, CP; largemouth bass, LMB; black crappie, BC; bluegill, BG). Within species, values with different letters are significantly different between populations (*t*-test, $P \leq 0.05$).

	Smoots Pond				Rural Retreat Lake		
Variable	CP	LMB	BC	BG	LMB	BC	BG
W_r	93	101u	87w	94y	102u	96x	98y
LSI	0.72	0.87u	0.50w	0.72y	1.70v	1.69x	1.63y
m GSI	0.12	0.13u	0.12w	0.40y	0.21v	0.62x	0.12y
f GSI	0.22	0.71u	0.59w	2.67y	1.17u	7.24x	0.97y
VSI	2.63	3.35u	1.91w	2.81y	3.86v	1.89w	3.50y
Water	77.0	76.1u	76.5w	75.3y	75.1v	73.9x	76.1y
Protein	76.4	73.3u	70.5w	66.2y	66.9v	65.7x	67.6z
Lipid	3.4	5.5u	5.1w	9.7y	6.9v	8.2x	9.1z

Table 5.5. Output of analysis of variance testing and adjusted coefficient of multiple determination (adjusted R^2) for regression models relating relative weight to selected variables for each population.

Population/species	*F* ratio	df	*P*-value	adj R^2
Smoots Pond				
Chain pickerel	8.14	37	<0.001	0.54
Largemouth bass	5.21	27	0.002	0.48
Black crappie	4.21	12	0.05	0.62
Bluegill	2.31	49	0.05	0.14
Rural Retreat Lake				
Largemouth bass	27.37	27	<0.001	0.85
Black crappie	2.89	29	0.03	0.28
Bluegill	1.68	28	0.17	0.13

Table 5.6. Squared partial correlation coefficients and significance of variables included in regression models of relative weight in each population (chain pickerel, CP; largemouth bass, LMB; black crappie, BC; bluegill, BG). Significance is based on *t*-tests of estimated parameter coefficients (H_o: $\beta = 0$).

	Smoots Pond				Rural Retreat Lake		
Variable	CP	LMB	BC	BG	LMB	BC	BG
LSI	0.05	0.35*	0.02	0.04	0.58**	0.36*	0.09
GSI	0.24**	0.14*	0.48*	0.02	0.05	0.01	0.00
VSI	0.00	0.02	0.00	0.17*	0.17**	0.04	0.20
Water	0.09**	0.05	0.25*	0.01	0.03	0.00	0.00
Protein	0.06	0.01	0.00	0.00	0.06**	0.00	0.01
Lipid	0.18**	0.04	0.05	0.00	0.01	0.02	0.00

* $P < 0.05$, ** $P < 0.005$

Table 5.7. Significant correlations among LSI, male and female GSI, VSI, water content (%), and protein and lipid content (% of dry weight) by species (chain pickerel, a; largemouth bass, b; black crappie, c; bluegill, d; none, na). Correlations above the diagonal are from Smoots Pond, elements below are from Rural Retreat Lake. Correlation direction is indicated by superscript.

	LSI	f GSI	m GSI	VSI	Water	Protein	Lipid
LSI	--	d^+	d^+	a^+,b^+,d^-	d^-	c^-,d^-	a^+,b^+
m GSI	na	--	--	d-	na	d^-	na
f GSI	na	--	--	na	na	d^-	na
VSI	b^+	na	na	--	na	na	b^+,d^+
Water	c^-	na	c^-	c^-	--	d^+	a^-,d^-
Protein	b^-,c^-	na	na	b^-,c^-,d^-	c^+	--	d^-
Lipid	b^+,c^+	na	na	b^+,c^+	b^-,c^-,d^-	b^-,c^-,d^-	--

Table 5.8. Correlation coefficients (r) of relative weight (W_r) to LSI, male and female GSI, VSI, water content (%), and protein and lipid content (% of dry weight) for each population (chain pickerel, CP; largemouth bass, LMB; black crappie, BC; bluegill, BG).

	Smoots Pond				Rural Retreat Lake		
Variable	CP	LMB	BC	BG	LMB	BC	BG
LSI	0.22	0.59**	-0.14	-0.21	0.76**	0.60**	0.31
m GSI	0.17	-0.21	-0.52	-0.45*	0.04	0.11	0.19
f GSI	-0.65*	-0.48*	-0.91*	-0.01	-0.00	0.75*	-0.63*
VSI	0.02	0.40*	-0.32	0.47**	0.81**	0.37*	0.47*
Water	0.28	0.20	0.64*	0.10	-0.26	-0.48	0.05
Protein	0.21	0.05	0.40	0.09	-0.47*	-0.37*	-0.33
Lipid	0.38*	0.42*	0.32	0.15	0.72**	0.56**	0.11

* $P < 0.05$, ** $P < 0.005$

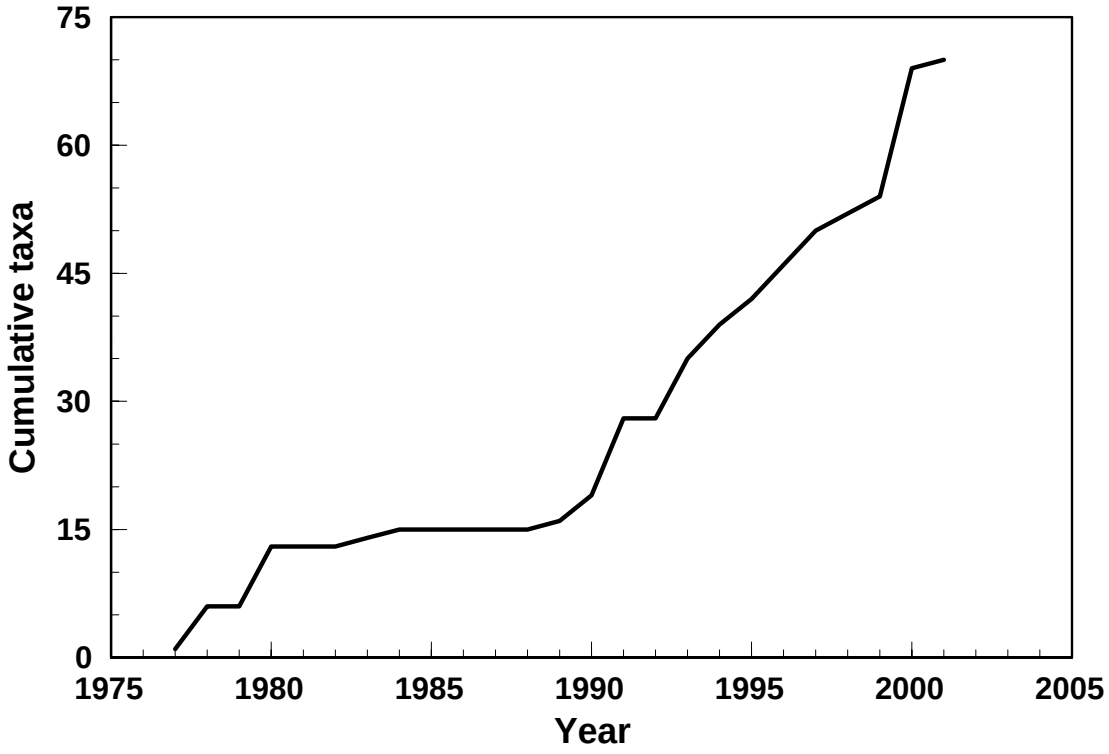

Figure 5.1. Cumulative number of taxa covered by published standard weight equations by year. Sources were Anderson (1980), Anderson and Gutreuter (1983), Murphy et al. (1991), Anderson and Neumann (1996), Blackwell et al. (2000), and Brenden et al. (unpublished manuscript). Taxa count does not include revisions of previously existing equations.

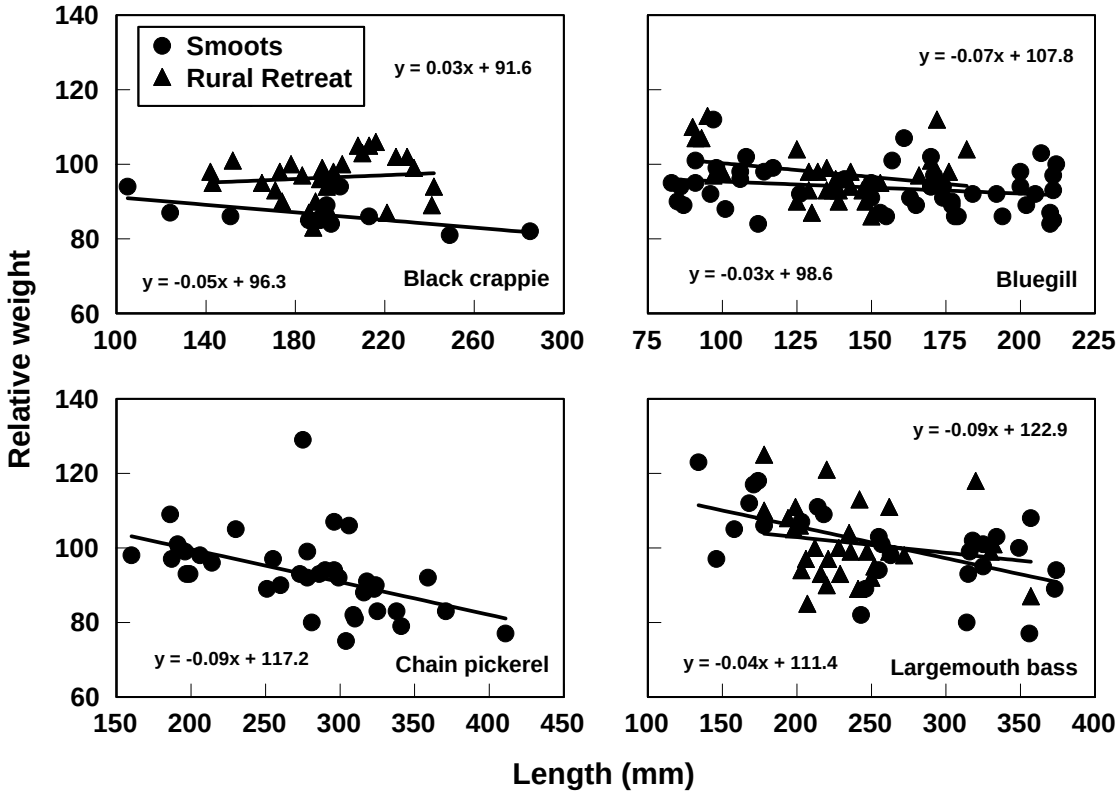

Figure 5.2. Relationship of relative weight to length by species. Regression equations for populations from Smoots Pond are in the lower left of each panel, equations for Rural Retreat Lake populations are in the upper right.

CHAPTER 6: A SYNTHESIS OF RESEARCH AND THEORY FOR INTERPRETATION OF CONDITION INDICES IN FISHES

Whole-body condition indices for fish are measures of relative plumpness that are used as surrogates for physiological well-being (Anderson and Gutreuter 1983). All such indices implicitly have the common assumption that fatter is fitter, i.e., plumpness is positively related to "well-being". Indices are interpreted with the belief that an animal will seek to maximize its fat stores. Life history theory suggests that this is not necessarily so. How do we reconcile the empirical use of condition indices with current theory? Here I consider the main conclusions of my research on nutritional interpretation of condition indices in fish, review relevant evolutionary and ecological theory, and offer recommendations for rigorous use and interpretation of condition indices.

Empirical Conclusions

The proximate interpretation of condition indices usually is made in terms of a correlation to gross tissue composition and energetic status (e.g., lipid content or energy density). What determines this correlation and how precise is it likely to be? My research has led me to three general conclusions. In the following section, I have integrated these conclusions with empirical results from the fisheries and ecological literature.

Effects of Feeding and Stress

Environmental stressors have an important influence on condition (Chapter 4). The proximate determinants of the numerical value of a condition index are resource acquisition and stress, i.e., depletion of energy and resources (Saltz et al. 1995). Much work has been done on the influence of food availability on condition index. Those results are fairly consistent. Increased system productivity promotes higher relative weight (W_r), if a wide range of trophic states (e.g., oligotrophic to eutrophic) are included in the analysis (Hubert et al. 1994; DiCenzo et al. 1995; Anderson and Neumann 1996). Similarly, I found mean W_r of bluegills from a eutrophic impoundment was higher than the means from dystrophic ponds (Chapter 3). The presence or abundance of appropriate-sized prey is related to trends in condition (Wege and Anderson 1978; Lemly and Dimmick 1982; Mosher 1984; Hubert et al. 1994; Liao et al. 1995; Marwitz and Hubert 1997; Porath and Peters 1997). Feeding studies in the laboratory have demonstrated the connection between ration and condition (Tyler and Dunn 1976; Rose 1989; Brown and Murphy 1991; Frischknecht 1993). Clearly, consumption can have a large effect on condition index.

Environmental stressors can modify this relationship. Relationship of drift abundance to condition in Green River rainbow trout (*Oncorhynchus mykiss*) was mediated through an interaction with temperature (Filbert and Hawkins 1995). Heidinger and Crawford (1977) found that temperature changes the relationships (slope and correlation coefficient) between condition measures and ration in largemouth bass (*Micropterus salmoides*). The relationship between W_r and lipid

content was different between active and sedentary rainbow trout during a starvation experiment (Simpkins et al. 2003). I conclude that the values of condition indices result from the interaction of nutritional intake and nutritional stress, as proposed by Saltz et al. (1995).

Animals reared in the laboratory often have differences in tissue composition compared to those from natural surroundings. I documented a progressive change in gross tissue composition of bluegills after transfer from a lake to an aquaculture laboratory independent of ration manipulation (Chapter 4). This change was related to the relaxation of environmental pressures typical of the wild. Hence, energy that normally would be expended in the wild became available for growth and storage in the laboratory. Maximum W_r did not increase greatly, but maximum lipid content did; added weight tended to have a greater percentage of lipid as time in the laboratory increased. Animals in a laboratory environment typically have more adipose tissue and different length - weight relationships than those in the wild (Tyler and Dunn 1976; Bohlin et al. 1994; Jakob et al. 1996; Jonas et al. 1996). Proximate composition of wild bluegills in my experiment (Chapter 4) came to resemble composition of laboratory-reared fish after transfer to an aquaculture facility. Therefore, expression of weight in terms of tissue composition is influenced by environmental stressors. This phenomenon needs theoretical explanation and will be addressed in subsequent sections of this synthesis.

Variation Among Groups

Correlations between condition index and constituent variables are variable in the wild. I documented differences among seasons (Chapter 2), populations (Chapter 2, Chapter 3), and species (Chapter 5). Some of this variation was due to the limited range of values that were expressed in a particular time and place (Chapter 3). However, because differences in correlations were ubiquitous throughout my studies, I conclude that the different correlations were due chiefly to differences in the nutritional and ecological processes determining resource allocation.

Processes determining condition index may not be synchronized among natural populations. Liao et al. (1995) observed asynchronous temporal cycles in W_r between pumpkinseeds (*Lepomis gibbosus*) and golden shiners (*Notemigonus crysoleucas*) within the same lakes. Cunjak and Power (1987) observed that timing of the winter nadir of condition in brook trout (*Salvelinus fontinalis*) and brown trout (*Salmo trutta*) populations was different between adjacent streams. The phenological cycle of a population can have large effects on condition, chiefly driven by reproductive demands and the need to store resources for winter (Miller 1979). Given that timing of important cues can change among sites and years, it is not surprising that condition index cycles differ.

Correlations of condition and body weight to constituent variables can change through time (Delahunty and DeVlaming 1980; Neumann and Murphy 1992; Sutton et al. 2000), among populations (Rikardsen and Johansen 2003;

Copeland and Carline 2004), and among species (Strange and Pelton 1987). The differences I documented among species within a common environment (Chapter 5) underscore the importance of ecological dissimilarities in altering correlations. As ecological circumstances change, relationships between whole-body condition and its constituents may change, promoting differences among species, among populations, and even within populations.

Correlation of Condition to Its Constituents

As a consequence of physiological malleability, whole-body condition is an imprecise predictor of its constituent components. All the correlations I documented in wild populations were marked by low coefficients of determination (r^2), i.e., there was much individual variation at all levels. This is a common result of field studies of condition and body composition. For example, although Neumann and Murphy (1992) found significant correlations to W_r, they caution that the relationships were too weak for predictive purposes. Laboratory studies typically document much more precise relationships and these are often referred to in the methodological literature on use of condition indices. However, laboratory settings control the extreme environmental variation typical of natural settings. Even then, individuals do not always respond similarly to nutritional changes (Jobling and Koskela 1996). I conclude that diversity of individual circumstances favors compositional variability, hence some level of imprecision should be expected. This diversity may change length-specific weight and it may also change how weight is allocated in terms of tissue composition. Energy

reserves are not necessarily all lipid (Van der Meer and Piersma 1994; Bonnet et al. 1998).

My overall conclusion from the above results *in toto* is that condition index values are determined by a tradeoff of resource acquisition versus expenditures as proposed by Saltz et al. (1995). The exact physiological expression is determined by individual circumstances; therefore, we should *expect* imprecise correlations. I believe that deviations from the mean response represent important information and not statistical "noise". However, we need relevant theory to guide the interpretation of these deviations. Explicit reference to life history theory has been lacking hitherto in condition studies.

Theory for Interpretation of Condition Indices

Ultimate Causes of Patterns and Variability

To be relevant in life history theory, condition indices must be linked to evolutionary fitness (Rolff and Joop 2002). I previously defined condition as representing the storage of energy and nutrients within an animal's body for later mobilization to meet the demands of various life processes (Chapter 1). Common usage is consistent with this definition, as condition indices are often correlated to health, growth, body composition, reproductive potential, and successful foraging (Anderson and Neumann 1996; Harder and Kirkpatrick 1996; Blackwell et al. 2000). Mechanistic models incorporating the concept of condition often define it as the ratio of weight of mobilizable tissues to the weight of structural tissues and use it as an indicator of the pool of available energy and materials

(e.g., Broekhuizen et al. 1994; Rowe and Houle 1996; Van Winkle et al. 1997). This pool is largely lipid but also includes carbohydrate, protein, and some minerals (Van der Meer and Piersma 1994; Bonnet et al. 1998).

Ricklefs (1991) parsed physiological processes influencing life history and fitness into five categories: reproduction, growth, storage, activity, and maintenance. Processes influence fitness by increasing survival or production of young (Roff 1984). Assimilated resources are allocated among the categories (Figure 6.1). Resources are limited in supply (Boggs 1994) and it is usually assumed that resources invested in one category are unavailable for other uses, hence allocation is balanced to maximize fitness (Ricklefs 1991; Sinervo and Svensson 1998). Storage is an exception in that stored materials can be mobilized to support other activities (Figure 6.1) if intake from the current environment becomes limiting (Perrin et al. 1990; Bradley et al. 1991; Calow 1994). Life history traits are optimized to increase fitness (Reznick and Braun 1987; Partridge and Harvey 1988; Ricklefs 1991; Perrin and Sibly 1993). The fitness advantage conferred by storage is a measure of independence from environmental fluctuations (Reznick and Braun 1987; Calow 1994).

The fitness of any allocation strategy is specific to ecological situation (Partridge and Harvey 1988; Sibly 1991). Resources are limited by system productivity and stability (Partridge and Harvey 1988) and the availability of resources to individuals is further reduced by competition and predation risk (Charnov et al. 1976; Basset and Glazier 1995; Welton and Houston 2001). Investment strategy is constrained by phylogeny (Partridge and Harvey 1988),

but in practice many organisms are phenotypically plastic and can change their allocation strategy as circumstances warrant (Perrin et al. 1990; Boggs 1994; Sinervo and Svensson 1998). Thus, the observed life history strategy may differ across a species' range (e.g., Schaffer and Elson 1975; Glebe and Leggett 1981).

Actual pattern of resource investment results from an interaction of resource availability and allocation strategy (Van Noordwijk and DeJong 1986). Priorities for allocation of assimilated resources are set according to the current status of the organism and the expectation of future conditions (Bull et al. 1996; Nicieza and Metcalfe 1997; Witter and Swaddle 1997). As conditions change, a determination is made whether to continue investing as previously or to change priorities. This selection is based on the marginal increase in fitness expected (Perrin and Sibly 1993; Metcalfe et al. 2002). Response to selective pressures depends on phenology and life stage (Boggs 1994). For example, ratio of adult to juvenile mortality is influential in determining onset of maturity (Partridge and Harvey 1988; Ylikarjula et al. 1999), which is when allocation priorities often change (Boggs 1994). Post-breeding survival and growth affects reproductive investment and timing (Schaffer and Elson 1975). Animals also adjust allocation depending on time of year. Metcalfe et al. (2002) found that young Atlantic salmon (*Salmo salar*) recovering from starvation increased both growth and storage during the summer but restored only lipid reserves during the winter. Similarly, Bull et al. (1996) demonstrated that wintering Atlantic salmon juveniles will adjust foraging and lipid levels differently depending on whether it is early or

late winter. These examples show that life history characteristics and expectations should be evaluated with consideration for fitness consequences within the environmental context (Ricklefs 1991; Metcalfe et al. 2002).

A major consequence of adaptive plasticity is that individuals are different. Large differences in resource allocation patterns may exist within the same population when alternative strategies have similar fitness (Gross 1982; Ware 1982; Jonsson and Jonsson 1997). Chance and a restricted range of sensory perception can produce a wide variety of environmental experiences in a population (Ware 1982). Further, differences in individual ability affect the amount of resources an animal may acquire. Patterns in resource acquisition and allocation strategy interact to produce correlations among life history traits. If variation in resource availability is large with respect to variation in allocation strategy, a positive correlation among traits should appear; conversely, variation in allocation strategy may diffuse or reverse the correlation (Van Noordwijk and DeJong 1986; Rowe and Houle 1996). Probable fitness benefits differ among individuals, so a uniform response should not be expected, even within a population where individuals experience similar conditions (Metcalfe and Monaghan 2001).

Is Fatter Fitter?

The fitness consequences of storage only recently have received much attention from evolutionary ecologists (Bonnet et al. 1998). Given the diversity of fish life histories, one may well ask under which circumstances it pays to be

fatter. Research on compensatory growth in fishes has demonstrated that growth is rarely at maximum (Metcalfe and Monaghan 2001). Because storage is generally related to growth (Calow 1994; Madsen and Shine 2002), we may expect that condition is also rarely at maximum.

Investigators studying birds have proposed that realized lipid content and body weight results from a tradeoff of costs versus benefits to produce an optimal body mass (OBM), which is usually less than maximal (Lima 1986; Rogers 1987; Witter and Cuthill 1993). This model predicts that the relative fitness value of body mass and storage should increase to some optimum and then decline (Figure 6.2, top panel). Environmental harshness and stochasticity tend to select for increased OBM, whereas predation risk and locomotive/maintenance costs tend to reduce OBM (Lima 1986; Rogers 1987; Rogers and Smith 1993). In essence, animals with greater relative mass are more resistant to starvation but are forced to forage more often to maintain that mass and so are at greater risk to predation (Grubb 1995). The interaction between starvation and predation risks is more important than either factor alone and may produce counterintuitive effects (e.g., deaths by starvation decreasing with resource scarcity) that are mediated via changes in behavior (McNamara and Houston 1987; Welton and Houston 2001). Actions of multiple factors, such as increased stochasticity coupled with decreased resource level, are likely to have multiplicative effects on OBM (McNamara and Houston 1990). The OBM is set according to local conditions and is actively regulated by programmed (innate) cycles and individual experience (Rogers and Smith 1993; Witter and Cuthill 1993). Although OBM

typically considers the non-breeding period (e.g., Rogers and Smith 1993), reproductive costs also act to determine OBM during breeding season (Witter and Cuthill 1993). The latter represents a relaxation of the assumption that survival is the fitness component being maximized.

The OBM model has important implications for condition indices. Each individual has an OBM it should seek to maintain; therefore, both positive and negative correlations between body mass and lipid content theoretically are possible within a population (McNamara and Houston 1990). Given that resources are commonly limited in natural environments, I believe that weights less than OBM will be more common in wild populations and therefore negative correlations to lipid content should be rare. When a positive correlation exists, condition indices have descriptive value (Witter and Cuthill 1993). However, it is plain that condition indices do not track fitness monotonically because index values result from a tradeoff (Saltz et al. 1995). Therefore, fatter is fitter only under certain circumstances that may change in time and space. These circumstances should be characterized by one or more of the following: decreased average energetic gain, increased variability in gain, increased average energetic expenditure, and increased variability in expenditures (Witter and Cuthill 1993).

The OBM model is well-established in avian ecology but its transferability to other vertebrate taxa is unclear. The concept that increased storage and body mass entail costs as well as benefits is intuitive but is most obvious in birds because of their high homeothermic and locomotive costs. The costs of storage

are not as severe in ectotherms as in endotherms (Bonnet et al. 1998). Given that the aquatic environment provides more body support in terms of locomotion, I propose that costs are even lower for fishes than reptiles. In general, it follows that OBM should be relatively higher in fishes than in other taxa, with reptiles and mammals being intermediate between birds and fishes (Figure 6.2, bottom panel).

Just because plumpness is more likely to be adaptive for fish than other taxa does not mean that plumpness is not associated with significant costs for fish. Increased plumpness may increase swimming costs and increase exposure to predators. Structural growth and reproductive opportunities may be foregone in order to increase plumpness. The magnitude of these costs will be situation-specific. Impact of ecological costs can change with size (Peters 1983); hence relative OBM will change within the population because fish populations typically consist of a wide range of sizes. Resource limitations may have the short-term effect of detracting from amount of energy available to be allocated or the long-term effect of changing allocation strategy, e.g., increasing growth (to become a better swimmer, predator, or predator-avoider) or increasing reproductive effort. Severe resource limitation can result in early maturity, slow growth, and reduced lifespan (Alm 1946; Jansen and Mackay 1991; Jansen 1996; Ylikarjula et al. 1999). However, in cases of abundant resources, high investment may be maintained in all compartments (Glazier 1999), resulting in positive correlations among growth, storage, and condition indices.

General Discussion of Use and Interpretation

The preceding theoretical considerations of storage strategy and body mass have several implications for interpretation and generality of condition indices. Fish are plastic in phenotype and can modify energy allocation to support a variety of life histories (subject to phylogenetic constraint). Life history theory deals explicitly with resource allocation. These considerations are intimately related to condition and its physiological expression. Condition indices should function best when: 1) environmental stresses do not greatly increase activity or maintenance costs, 2) reproduction is postponed (energy is invested in the parent), and 3) resources are variable relative to costs (storage is advantageous over growth). Realized condition index values result from a combination of life history decisions and performance.

Condition indices measure balance between resource acquisition and expenses. Somatic storage has been shown to be correlated to resource acquisition for a wide range of taxa (see Glazier 1999 and references therein). Ware (1982) demonstrated that it is the surplus (i.e., net resource gain) that is important. While fatter may not necessarily be fitter, barring any pathologies, a fatter animal has more stored resources. Studies of compensatory growth have found that condition index value is an indicator of prior nutritional stress and inversely related to strength of the subsequent compensatory response (Jobling et al. 1994). As such, the value of a condition index is as a general indicator of net nutritional balance relative to conspecifics with a similar life history strategy (and thus a similar OBM).

A strict compositional interpretation of condition indices will always be confounded for three reasons. In theory, storage and condition are thought of as a depot of usable resources within an animal's body, and further defined as lipid. In reality, the line between structural and storage tissues is blurred (Van der Meer and Piersma 1994). While energy is most efficiently stored as lipid, energy is not always a limiting factor (Ricklefs 1991). Animals may mobilize lipid, protein, carbohydrate, or minerals for use in bodily processes (Bonnet et al. 1998) and storage may take a number of forms. Diet composition and trophic position may also influence storage composition, depending whether dietary energy or protein is most limiting to growth (see Bowen et al. 1995). Secondly, the many factors that influence condition (e.g., Le Cren 1951) limit the strength of any single correlation to an index. As Huston (2002) mathematically demonstrated, interaction of multiple limiting factors produces a pattern in which variance increases as circumstances become more "favorable", but as one factor becomes extremely limited, the dependent variable drops to low level. Thus as condition index increases, the probability of a single, precise correlation to a constituent variable decreases. An exception occurs in the laboratory where other factors can be controlled. Lastly, suborganismal changes may not be on same cycle or respond at same time or size scale as whole-body weight. Time lags may confound interpretation because interpretation is usually done on an instantaneous basis. Size-wise, important components (e.g., lipids) are not necessarily on same scale as whole-body weight and can be overwhelmed by changes or inertia in other components (e.g., water). These issues militate

against precise interpretations. Whole-body measures change slowly and substantial losses of energy may occur in the interim (Brown and Murphy 2004), thus compositional interpretation will always be imprecise.

Individuals will differ in amount of investment in nutritional condition, as well as how that investment is expressed. Intermediate amounts of resources will favor individual variability because some animals inevitably will be better at acquiring resources than others (Basset and Glazier 1995; Jobling and Koskela 1996). At some point, some individuals will seek alternative strategies for allocating their diminishing resources to make the best of the situation. This will create variability in allocation which would militate against a clear correlation between storage and body mass. Abundant resources should favor congruity by relaxing limiting factors and reducing tradeoffs: if there is plenty for all, then alternative life histories may not be necessary. Because storage likely is either a fixed proportion or an increasing proportion of assimilated resources (Calow 1994), condition should be tied to surplus energy for growth and reproduction. The latter is a key interpretation and should hold even if the fitness strategy does not mandate a high level of somatic investment. Therefore, condition indices are rough measures of growth and proximate composition when resources are abundant because individual allocation strategies should converge within sex and maturity groups.

The concept of condition has great generality but little precision. Many taxa show strong positive relationships between measures of resource storage and acquisition (Glazier 1999). As a measure of resource surplus, size-specific

weight has descriptive value (Witter and Cuthill 1993). Given the imprecision of expression, inferences from significant results are conservative and lack of a difference is not informative (Rolff and Joop 2002). This is certainly true if one adheres to a strict compositional definition of condition as lipid storage. However, such a narrow view may ignore the true usefulness of a condition index as an indicator of somatic investment. This point needs direct experimental confirmation. Large differences or sudden changes will show relative well-being with higher values indicating better condition. Very low values should indicate extended periods of stress. Very high values are characteristic of individuals with ample reserves. Intermediate values can be exhibited by individuals in a variety of circumstances.

The utility of condition indices seems apparent in their widespread use. I define utility as the generation of biological and ecological insight. Valid use depends on purpose, performance criteria, and context (Rykiel 1996). The most rigorous way to obtain information is to directly measure the parameter in question, not to use an indirect index. Depending on the nature of the parameter and the circumstances of the investigation, direct measurement may not be feasible, efficient, or necessary. That is why indices are used, despite continuing disputes about their nature and calculation. Use of condition indices is warranted where mensurative resolution is not necessary or possible, e.g., due to study objectives and logistical constraints. Index assumptions relative to the intended use should be stated and verified insofar as is feasible (see Chapter 1). However, no single index is likely to capture a wide range of physiological

information (Brown and Murphy 2004); use of multiple indices may be useful in this regard. Interpretation proceeds from the assumptions, therefore all likely violations should be examined and their implications discussed. Even if fatter is not necessarily fitter, measures of relative plumpness can have utility. In particular, I see condition indices as useful in demographic and life history studies. Demographic monitoring for management purposes does not typically demand a high level of precision. Here condition indices should be useful indicators of the balance of foraging versus metabolic demand, the effects of reproduction, or the per capita abundance of resources. For life history studies, condition indices should be good general indicators of somatic investment versus emphasis on reproduction. Important considerations should be productivity and variability of the system on a per capita basis, population phenology, mortality and maturity schedules, and limiting factors in ecology and diet. These considerations should dictate the requisite ancillary data necessary to verify assumptions (Chapter 1) and thus clarify interpretation. Additional information will be needed as more precision is required. For the most precise and rigorous uses, all should be evaluated; under less rigorous circumstances, it may be enough to satisfy the first few. For example, to use a condition index as a measure of energetic balance, information on stress or consumption (not both) should be considered. To use as a predictor of body composition, the investigator needs to know or control important limiting factors and life history variations. Following these steps will enable investigators to make valid inferences about

relative well-being of comparable groups suitable for their particular circumstances.

In conclusion, condition can be viewed as a bridge between hierarchical levels of physiological and ecological organization. Condition provides gross information about fine-scale physiological processes in a form relevant to population dynamics and life history. These links have implications for evolutionary fitness and management. Condition indices have been, and will continue to be, useful generators of biological insight. Increased rigor will increase their usefulness.

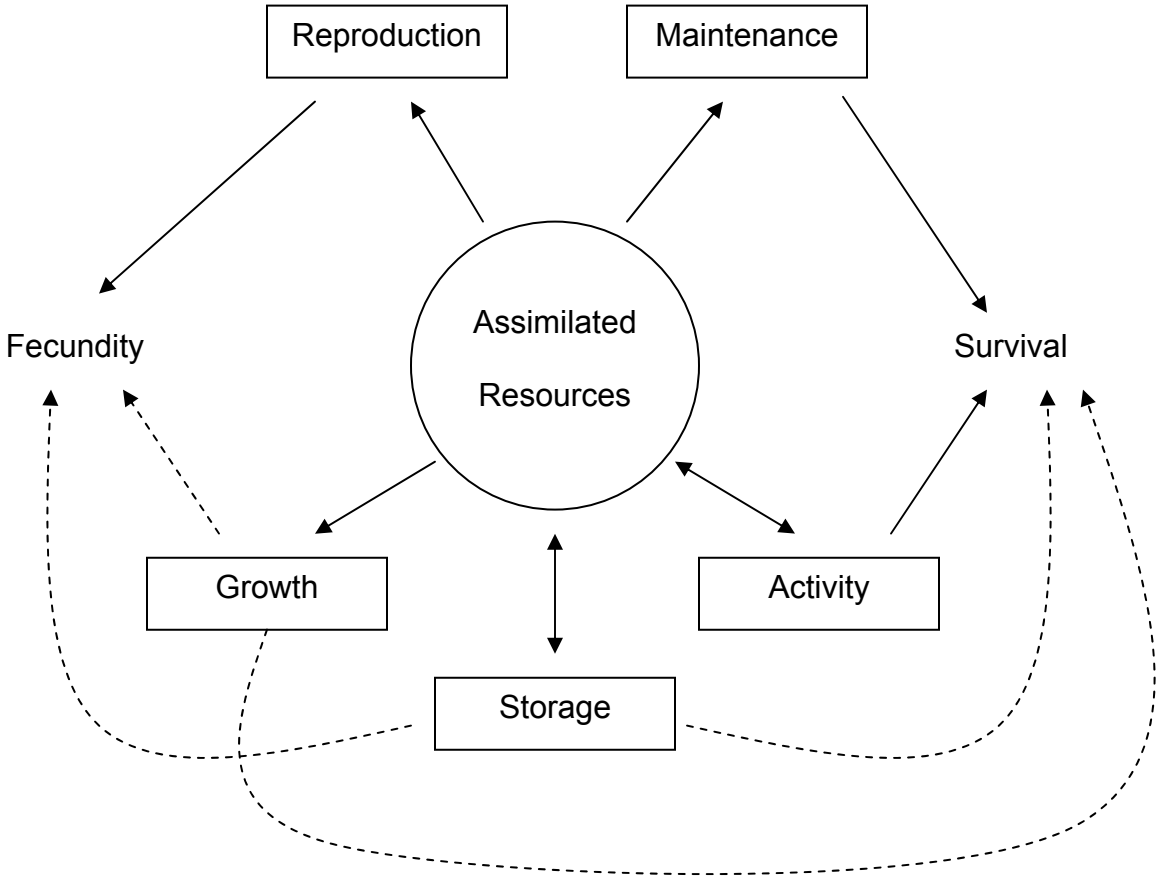

Figure 6.1. Relationship of reproduction, growth, storage, maintenance, and activity to assimilated resources and fitness components (fecundity and survival). Arrows indicate direction of interaction. Solid lines show direct effects, dashed lines show indirect effects.

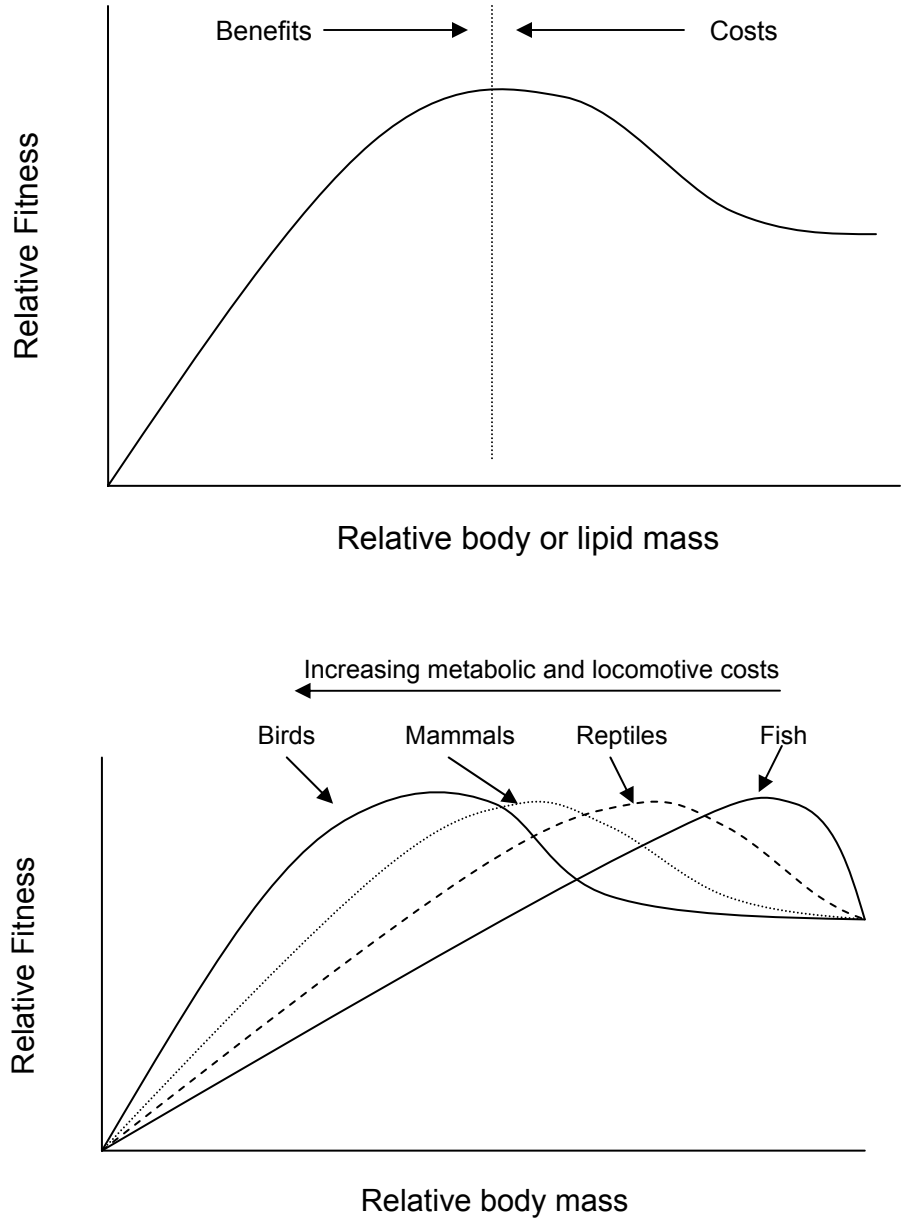

Figure 6.2. Relationship of relative body or lipid mass to fitness with reference to ecological and physiological costs and benefits of increased mass (adapted from Grubb 1995, top panel) and the effect of increasing metabolic and locomotive costs on relationship of relative body mass to fitness among vertebrate taxa (bottom panel). The arrow indicates how costs increase among taxa.

References

Adams, S. M., R. B. McLean, and J. A. Parrotta. 1982. Energy partitioning in largemouth bass under conditions of seasonally fluctuating prey availability. Transactions of the American Fisheries Society 111:549-558.

Adams, S. M., and R. B. McLean. 1985. Estimation of largemouth bass, *Micropterus salmoides* Lacepede, growth using the liver somatic index and physiological variables. Journal of Fish Biology 26:111-126.

Adams, S. M., and D. L. DeAngelis. 1987. Indirect effects of early bass-shad interactions on predator population structure and food web dynamics. Pages 103-117 *in* W. C. Kerfoot and A. Sih, editors. Predation: direct and indirect impacts on aquatic communities. University Press of New England, Hanover, New Hampshire.

Aday, D. D., D. A. Allen, and W. E. Kelso. 2000. Field and laboratory determinations of hypoxic effects on RNA-DNA ratios of bluegill. American Midland Naturalist 143:433-442.

Aday, D. D., C. M. Kush, D. H. Wahl, and D. P. Philipp. 2002. The influence of stunted body size on the reproductive ecology of bluegill *Lepomis macrochirus*. Ecology of Freshwater Fish 11:190-195.

Alasalvar, C., K. D. A. Taylor, E. Zubcov, F. Shahidi, and M. Alexis. 2002. Differentiation of cultured and wild sea bass (*Dicentrarchus labrax*): total lipid content, fatty acid and trace mineral composition. Food Chemistry 79:145-150.

Alm, G. 1946. Reasons for the occurrence of stunted fish populations. Institute of Freshwater Fishery Research (Drottningholm) Report 25:1-146.

Anderson, R. O. 1959. The influence of season and temperature on growth of the bluegill, *Lepomis macrochirus* Rafinesque. Doctoral dissertation. University of Michigan, Ann Arbor.

Anderson, R. O. 1980. Proportional stock density (PSD) and relative weight (W_r): interpretive indices for fish populations and communities. Pages 27-33 *in* S. Gloss and B. Shupp, editors. Practical fisheries management: more with less in the 1980's. Proceedings of the 1st Annual Workshop of the New York Chapter, American Fisheries Society. New York Cooperative Fishery Research Unit, Ithaca.

Anderson, R. O. and S. J. Gutreuter. 1983. Length, weight, and associated structural indices. Pages 283-300 in L.A. Nielsen and D.L. Johnson, editors. Fisheries Techniques. American Fisheries Society, Bethesda, Maryland.

Anderson, R. O., and R. M. Neumann. 1996. Length, weight, and associated structural indices. Pages 447-482 *in* B. R. Murphy and D. W. Willis, editors. Fisheries Techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

AOAC (Association of Official Analytical Chemists). 1990. Official methods of analysis, 15th edition. AOAC, Washington, DC.

Arndt, S. K. A., T. J. Benfey, and R. A. Cunjak. 1996. Effect of temporary reductions in feeding on protein synthesis and energy storage of juvenile Atlantic salmon. Journal of Fish Biology 49:257-276.

Arrington, D. A., K. O. Winemiller, W. F. Loftus, and S. Akin. 2002. How often do fishes “run on empty”? Ecology 83:2145-2151.

Basset, A., and D. S. Glazier. 1995. Resource limitation and intraspecific patterns of weight x length variation among spring detritivores. Hydrobiologia 316:727-737.

Beamish, F. W. H. 1964. Influence of starvation on standard and routine oxygen consumption. Transactions of the American Fisheries Society 93:103-107.

Beckman, B. R., D. A. Larsen, C. Sharpe, B. Lee-Pawlak, C. B. Schreck, and W. W. Dickhoff. 2000. Physiological status of naturally reared juvenile spring Chinook salmon in the Yakima River: seasonal dynamics and changes associated with smolting. Transactions of the American Fisheries Society 129:727-753.

Belk, M. C., and L. S. Hales. 1993. Predation-induced differences in growth and reproduction of bluegills (*Lepomis macrochirus*). Copeia 1993:1034-1044.

Bertschy, K. A., and M. G. Fox. 1999. The influence of age-specific survivorship on pumpkinseed sunfish life histories. Ecology 80: 2299-2313.

Biro, P. A., J. R. Post, and E. A. Parkinson. 2003. From individuals to populations: prey fish risk-taking mediates mortality in whole-system experiments. Ecology 84:2419-2431.

Blackwell, B. G., M. L. Brown, and D. W. Willis. 2000. Relative weight (W_r) status and current use in fisheries assessment and management. Reviews in Fisheries Science 8:1-44.

Boggs, C. L. 1994. The role of resource allocation in understanding reproductive patterns. Pages 25-33 *in* S. R. Leather, A. D. Watt, N. J. Mills, and K. F. A. Walters, editors. Individuals, populations, and patterns in ecology. Intercept, Andover, England.

Bohlin, T., C. Dellefors, and U. Faremo. 1994. Probability of first sexual maturation of male parr in wild sea-run brown trout (*Salmo trutta*) depends on condition factor 1 year in advance. Canadian Journal of Fisheries and Aquatic Sciences 51:1920-1926.

Bolger, T., and P. L. Connolly. 1989. The selection of suitable indices for the measurement and analysis of fish condition. Journal of Fish Biology 34:171-182.

Bonnet, X., D. Bradshaw, and R. Shine. 1998. Capital versus income breeding: an ectothermic perspective. Oikos 83:333-342.

Booth, D. J., and J. A. Keast. 1986. Growth energy partitioning by juvenile bluegill sunfish, *Lepomis macrochirus* Rafinesque. Journal of Fish Biology 28:37-45.

Bowen, S. H., E. V. Lutz, and M. O. Ahlgren. 1995. Dietary protein and energy as determinants of food quality: trophic strategies compared. Ecology 76:899-907.

Bradley, M. C., N. Perrin, and P. Calow. 1991. Energy allocation in the cladoceran, *Daphnia magna* Straus, under starvation and refeeding. Oecologia 86:414-418.

Brawn, V. M. 1969. Buoyancy of Atlantic and Pacific herring. Journal of the Fisheries Research Board of Canada 26:2077-2091.

Breck, J. E. 1996. Mechanisms of recruitment failure in bluegill ponds. Michigan Department of Natural Resources, Fisheries Division, Research Report 2024, Lansing.

Breck, J. E. 1998. Development of a warmwater fish community model. Michigan Department of Natural Resources, Fisheries Division, Research Report 2033, Lansing.

Brenden, T. O., B. R. Murphy, and T. Copeland. Unpublished manuscript. Standard weight equation variability and the consequences of extrapolating the length-weight relationship: establishing new guidelines for the regression-line-percentile technique. Submitted to North American Journal of Fisheries Management.

Broekhuizen, N., W. S. C. Gurney, A. Jones, and A. D. Bryant. 1994. Modelling compensatory growth. Functional Ecology 8:770-782.

Brown, J. H., P. A. Marquet, and M. L. Taper. 1993. Evolution of body size: consequences of an energetic definition of fitness. American Naturalist 142:573-584.

Brown, M. L., and B. R. Murphy. 1991. Relationship of relative weight (W_r) to proximate composition of juvenile striped bass and hybrid striped bass. Transactions of the American Fisheries Society 120:509-518.

Brown, M. L., and B. R. Murphy. 2004. Seasonal dynamics of direct and indirect condition indices in relation to energy allocation in largemouth bass (*Micropterus salmoides* Lacepede). Ecology of Freshwater Fish 13:23-36.

Buijse, A. D., and R. P. Houthuijzen. 1992. Piscivory, growth, and size-selective mortality of age 0 pikeperch (*Stizostedion lucioperca*). Canadian Journal of Fisheries and Aquatic Sciences 49:894-902.

Bull, C. D., N. B. Metcalfe, and M. Mangel. 1996. Seasonal matching of foraging to anticipated energy requirements in anorexic juvenile salmon. Proceedings of the Royal Society of London B 263:13-18.

Bulow, F. J., C. B. Coburn, and C. S. Cobb. 1978. Comparison of two bluegill populations by means of the RNA – DNA ratio and liver-somatic index. Transactions of the American Fisheries Society 107:799-803.

Busacker, G. P., I. R. Adelman, and E. M. Goolish. 1990. Growth. Pages 363-388 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Cade, B. S., J. W. Terrell, and R. L. Schroeder. 1999. Estimating effects of limiting factors with regression quantiles. Ecology 80:311-323.

Calow, P. 1994. From physiological ecology to population and evolutionary ecology with speculation on the importance of storage processes. Pages 349-358 *in* S. R. Leather, A. D. Watt, N. J. Mills, and K. F. A. Walters,

editors. Individuals, populations, and patterns in ecology. Intercept, Andover, England.

Carlander, K. D. 1977. Handbook of freshwater fishery biology, volume 2. Iowa State University Press, Ames.

Caulton, M. S., and E. Bursell. 1977. The relationship between changes in condition and body composition in young *Tilapia rendalli* Boulenger. Journal of Fish Biology 11:143-150.

Charnov, E. L., G. H. Orians, and K. Hyatt. 1976. Ecological implications of resource depression. American Naturalist 110:247-259.

Coble, D. W. 1988. Effects of angling on bluegill populations: management implications. North American Journal of Fisheries Management 8:277-283.

Cone, R. S. 1989. The need to reconsider the use of condition indices in fisheries science. Transactions of the American Fisheries Society 118:510-514.

Cook, S. B. 1994. Effects of food particle size on short-term food intake and growth of bluegill (*Lepomis macrochirus*). Doctoral dissertation. Southern Illinois University, Carbondale.

Copeland, T., and R. F. Carline. 2004. Relationship of lipid content to size and condition in walleye fingerlings from natural and aquacultural environments. North American Journal of Aquaculture 66:237-242.

Copeland, T., J. J. Ney, and B. R. Murphy. 1999. Alternative methods to predict fish proximate composition. Proceedings of the Annual Conference of Southeastern Fish and Wildlife Agencies Volume 53:110-118.

Corti, M., R. S. Thorpe, L. Sola, V. Sbordoni, and S. Cataudella. 1988. Multivariate morphometrics in aquaculture: a case study of six stocks of the common carp (*Cyprinus carpio*) in Italy. Canadian Journal of Fisheries and Aquatic Sciences 45:1548-1554.

Craig, J. F. 1977. The body composition of adult perch, *Perca fluviatilis*, in Windermere, with reference to seasonal changes and reproduction. Journal of Animal Ecology 46:617-632.

Craig, S. R., D. S. MacKenzie, G. Jones, and D. M. Gatlin. 2000. Seasonal changes in the reproductive condition and body composition of free-ranging red drum, *Sciaenops ocellatus*. Aquaculture 190:89-102.

Cunjak, R. A., and G. Power. 1987. The feeding and energetics of stream-resident trout in winter. Journal of Fish Biology 31:493-511.

Deacon, L. I., and J. A. Keast. 1987. Patterns of reproduction in two populations of pumpkinseed sunfish, *Lepomis gibbosus*, with differing food resources. Environmental Biology of Fishes 19:281-296.

Delahunty, G., and V. L. DeVlaming. 1980. Seasonal relationships of ovary weight, liver weight, and fat stores with body weight in the goldfish, *Carassius auratus* (L.). Journal of Fish Biology 16:5-13.

DeVlaming, V., G. Grossman, and F. Chapman. 1982. On the use of the gonosomatic index. Comparative Biochemistry and Physiology 73A:31-39.

DiCenzo, V. J., M. J. Maceina, and W. C. Reeves. 1995. Factors related to growth and condition of the Alabama subspecies of spotted bass in reservoirs. North American Journal of Fisheries Management 15:794-798.

Dodson, P. 1978. On the use of ratios in growth studies. Systematic Zoology 27:62-67.

Drake, M. T., J. E. Claussen, D. P. Philipp, and D. L. Pereira. 1997. A comparison of bluegill reproductive strategies and growth among lakes with different fishing intensities. North American Journal of Fisheries Management 17:496-507.

Dygart, P. H. 1990. Seasonal changes in energy content and proximate composition associated with somatic growth and reproduction in a representative age class of female English sole. Transactions of the American Fisheries Society 119:791-801.

Ehlinger, T. J., and D. S. Wilson. 1988. Complex foraging polymorphism in bluegill sunfish. Proceedings of the National Academy of Sciences of the United States of America 85:1878-1882.

Ehlinger, T. J., M. R. Gross, and D. P. Philipp. 1997. Morphological and growth rate differences between bluegill males of alternative reproductive life histories. North American Journal of Fisheries Management 17:533-542.

Encina, L., and C. Granado-Lorencio. 1997, Seasonal variations in the physiological status and energy content of somatic and reproductive tissues of chub. Journal of Fish Biology 50:511-522.

Ersbak, K., and B. L. Haase. 1983. Nutritional deprivation after stocking as a possible mechanism leading to mortality in stream-stocked brook trout. North American Journal of Fisheries Management 3:142-151.

Filbert, R. B., and C. P. Hawkins. 1995. Variation in condition of rainbow trout in relation to food, temperature, and individual length in the Green River, Utah. Transactions of the American Fisheries Society 124:824-835.

Flath, L. E., and J. S. Diana. 1985. Seasonal energy dynamics of the alewife in southeastern Lake Michigan. Transactions of the American Fisheries Society 114:328-337.

Fischer, R. U., J. D. Congdon, and M. Brock. 1996. Total body electrical conductivity (TOBEC): a tool to estimate lean mass and nonpolar lipids of an aquatic organism? Copeia 1996:459-462.

Fischer, R. U., J. D. Congdon, C. D. DiFonzo, and J. M. Aho. 1998. Lipid and reproductive cycles of bluegills (*Lepomis macrochirus*) exposed to 35 years of elevated environmental temperatures. Journal of Thermal Biology 23:359-367.

Fox, M. G. 1994. Growth, density, and interspecific influences on pumpkinseed sunfish life histories. Ecology 75:1157-1171.

Frischknecht, M. 1993. Breeding colouration of male three-spined sticklebacks as an indicator of energy investment in vigour. Evolutionary Ecology 7:439-450.

Fullerton, A. H., J. E. Garvey, R. A. Wright, and R. A. Stein. 2000. Overwinter growth and survival of largemouth bass: interactions among size, food, origin, and winter severity. Transactions of the American Fisheries Society 129:1-12.

Gabelhouse, D. W. 1987. Responses of largemouth bass and bluegills to removal of surplus largemouth bass from a Kansas pond. North American Journal of Fisheries Management 7:81-90.

Gabelhouse, D. W. 1991. Seasonal changes in body condition of white crappies and relations to length and growth in Melvern Reservoir, Kansas. North American Journal of Fisheries Management 11:50-56.

Gaylord, T. G., and D. M. Gatlin. 2000. Assessment of compensatory growth in channel catfish *Ictalurus punctatus* R. and associated changes in body condition indices. Journal of the World Aquaculture Society 31:326-336.

Gerking, S. D. 1952. The protein metabolism of sunfishes of different ages. Physiological Zoology 25:358-372.

Gerking, S. D. 1955. Influence of rate of feeding on body composition and protein metabolism of bluegill sunfish. Physiological Zoology 28:267-282.

Gerking, S. D. 1962. Production and food utilization in a population of bluegill sunfish. Ecological Monographs 32:31-78.

Gerking, S. D. 1966. Annual growth cycle, growth potential, and growth compensation in the bluegill sunfish in northern Indiana lakes. Journal of the Fisheries Research Board of Canada 23:1923-1956.

Gershanovich, A. D., N. M. Markevich, and Z. T. Dargaleva. 1984. Using the condition factor in ichthyological research. Journal of Ichthyology 24:78-90.

Glazier, D. S. 1999. Trade-offs between reproductive and somatic (storage) investment in animals: a comparative test of the Van Noordwijk and DeJong model. Evolutionary Ecology 13:539-555.

Glazier, D. S. 2000. Is fatter fitter? Body storage and reproduction in ten populations of the freshwater amphipod *Gammarus minus*. Oecologia 122:335-345.

Glebe, B. D., and W. C. Leggett. 1981. Latitudinal differences in energy allocation and use during the freshwater migrations of American shad (*Alosa sapidissima*) and their life history consequences. Canadian Journal of Fisheries and Aquatic Sciences 38:806-820.

Goede, R. W., and B. A. Barton. 1990. Organismic indices and an autopsy-based assessment as indicators of health and condition of fish. Pages 93-108 *in* S. M. Adams, editor. Biological indicators of aquatic ecosystem stress. American Fisheries Society Symposium 8, Bethesda, Maryland.

Grigorakis, K., M. A. Alexis, K. D. A. Taylor, and M. Hole. 2002. Comparison of wild and cultured gilthead sea bream (*Sparus aurata*); composition, appearance and seasonal variations. International Journal of Food Science and Technology 37:477-484.

Gross, M.R. 1982. Sneakers, satellites, and parentals: polymorphic mating strategies in North American sunfishes. Zeitschrift fur Tierpsychologie 60:1-26.

Gross, M. R., and E. L. Charnov. 1980. Alternative male life histories in bluegill sunfish. Proceedings of the National Academy of Sciences (USA) 77:6937-6940.

Grubb, T. C. 1995. On induced anabolism, induced caching, and induced construction as unambiguous indices of nutritional condition. Proceedings of the Western Foundation of Vertebrate Zoology 6:258-263.

Gutreuter, S., and W.M. Childress. 1990. Evaluation of condition indices for estimation of growth of largemouth bass and white crappie. North American Journal of Fisheries Management 10:434-441.

Harder, J. D., and R. L. Kirkpatrick. 1996. Physiological methods in wildlife research. Pages 275-306 *in* T. A. Bookhout, editor. Research and management techniques for wildlife and habitats. The Wildlife Society, Bethesda, Maryland.

Hartman, K. J., and S. B. Brandt. 1995. Estimating energy density of fish. Transactions of the American Fisheries Society 124:347-355.

Hayes, J. P., and J. S. Shonkwiler. 2001. Morphometric indicators of body condition: worthwhile or wishful thinking? Pages 8- 38 *in* J.R. Speakman, editor. Body composition analysis of animals: a handbook of non-destructive methods. Cambridge University Press, New York.

Hayward, R. S., D. B. Nolte, and N. Wang. 1997. Use of compensatory growth to double hybrid sunfish growth rates. Transactions of the American Fisheries Society 126:316-322.

Hayward, R. S., and N. Wang. 2001. Failure to induce overcompensation of growth in maturing yellow perch. Journal of Fish Biology 59:126-140.

Heath, D., and D. A. Roff. 1987. Test of genetic differentiation in growth of stunted and nonstunted populations of yellow perch and pumpkinseed. Transactions of the American Fisheries Society 116:98-102.

Heidinger, R. C., and S. D. Crawford. 1977. Effect of temperature and feeding rate on the liver-somatic index of the largemouth bass, *Micropterus salmoides*. Journal of the Fisheries Research Board of Canada 34:633-638.

Henson, J. C. 1991. Quantitative description and development of a species-specific growth form for largemouth bass, with application to the relative weight index. Master's thesis. Texas A & M University, College Station.

Hillman, W. P. 1982. Structure and dynamics of unique bluegill populations. Master's thesis. University of Missouri, Columbia.

Htun-Han, M. 1978. The reproductive biology of the dab, *Limanda limanda* (L.), in the North Sea: gonosomatic index, hepatosomatic index and condition factor. Journal of Fish Biology 13:369-378.

Hubert, W. A., R. D. Gipson, and R. A. Whaley. 1994. Interpreting relative weight values of lake trout stocks. North American Journal of Fisheries Management 14:212-215.

Huston, M. A. 2002. Introductory essay: critical issues for improving predictions. Pages 7-21 *in* J. M. Scott, P. J. Heglund, M. L. Morrison, J. B. Haufler, M. G. Raphael, W. A. Wall, and F. B. Samson, editors. Predicting species

occurrences: issues of accuracy and scale. Island Press, Washington, D.C.

Iles, T. D. 1984. Allocation of resources to gonad and soma in Atlantic herring *Clupea harengus* L. Pages 331-347 *in* G. W. Potts and R. J. Wootton, editors. Fish reproduction: strategies and tactics. Academic Press, London.

Iles, T. D., and R. J. Wood. 1965. The fat/water relationship in North Sea herring (*Clupea harengus*), and its possible significance. Journal of the Marine Biological Association of the United Kingdom 45:353-366.

Jakob, E. M., S. D. Marshall, and G. W. Uetz. 1996. Estimating fitness: a comparison of body condition indices. Oikos 77:61-67.

Jansen, W. A., and W. C. Mackay. 1991. Body composition and reproductive investment of stunted yellow perch. Verhandlungen der Internationalen Vereinigung fur theoretische und angwandte Limnologie 24:2356-2361.

Jansen, W. A. 1996. Plasticity in maturity and fecundity of yellow perch, Perca flavescens (Mitchill): comparisons of stunted and normal-growing populations. Annales Zoologici Fennici 33:403-415.

Jenkins, R. E., and N. M. Burkhead. 1993. Freshwater fishes of Virginia. American Fisheries Society, Bethesda, Maryland.

Jennings, M. J., and D. P. Philipp. 1992. Reproductive investment and somatic growth rates in longear sunfish. Environmental Biology of Fishes 35:257-271.

Jensen, A. J. 1980. The 'gut index', a new parameter to measure the gross nutritional state of arctic char, *Salvelinus alpinus* (L.) and brown trout, *Salmo trutta* L. Journal of Fish Biology 17:741-747.

Jobling, M. 1980. Effects of starvation on proximate chemical composition and energy utilization of plaice *Pleuronectes platessa* L. Journal of Fish Biology 17:325-334.

Jobling, M., and S. J. S. Johansen. 1999. The lipostat, hyperphagia and catch-up growth. Aquaculture Research 30:473-478.

Jobling, M., and J. Koskela. 1996. Interindividual variations in feeding and growth in rainbow trout during restricted feeding and in a subsequent period of compensatory growth. Journal of Fish Biology 49:658-667.

Jobling, M., O. H. Meloy, J. Dos Santos, and B. Christiansen. 1994. Compensatory growth response of the Atlantic cod: effects of nutritional history. Aquaculture International 2:75-90.

Johansen, S. J. S., M. Ekli, B. Stangnes, and M. Jobling. 2001. Weight gain and lipid deposition in Atlantic salmon, *Salmo salar*, during compensatory growth: evidence for lipostatic regulation? Aquaculture Research 32:963-974.

Johnson, B. L., and D. B. Noltie. 1997. Demography, growth, and reproductive allocation in stream-spawning longnose gar. Transactions of the American Fisheries Society 126:438-466.

Johnson, D. H., G. L. Krapu, K. J. Reinecke, and D. G. Jorde. 1985. An evaluation of condition indices for birds. Journal of Wildlife Management 49:569-575.

Jonas, J. L., C. E. Kraft, and T. L.Margenau. 1996. Assessment of seasonal changes in energy density and condition in age-0 and age-1 muskellunge. Transactions of the American Fisheries Society 125:203-210.

Jonsson, N., and B. Jonsson. 1997. Energy allocation in polymorphic brown trout. Functional Ecology 11:310-317.

Justus, J. A., and M. G. Fox. 1994. The cost of early maturation on growth, body condition and somatic lipid content in a lake pumpkinseed (*Lepomis gibbosus*) population. Ecology of Freshwater Fish 3:9-17.

Keast, A. 1985. The piscivore feeding guild of fishes in small freshwater ecosystems. Environmental Biology of Fishes 12:119-129.

Kirkpatrick, R. L. 1975. Applicability of basic physiology and nutrition research to practical wildlife management. Proceedings of the Southeastern Association of Game and Fish Commissions 30:476-480.

Layzer, J. B., and M. D. Clady. 1987. Phenotypic variation of young-of-year bluegills (*Lepomis macrochirus*) among microhabitats. Copeia 1987:702-707.

Le Cren, E. D. 1951. The length-weight relationship and seasonal cycle in gonad weight and condition in the perch (*Perca fluviatilis*). Journal of Animal Ecology 20:201-219.

Legler, R. E. 1977. New indices of well-being for bluegills. Master's thesis. University of Missouri, Columbia.

Lemke, A. E. 1977. Optimum temperature for growth of juvenile bluegills. Progressive Fish-Culturist 39:55-57.

Lemly, A. D., and J. F. Dimmick. 1982. Growth of young-of-the-year and yearling centrachids in relation to zooplankton in the littoral zone of lakes. Copeia: 1982:305-321.

Liao, H., C. L. Pierce, D. H. Wahl, J. B. Rasmussen, and W. C. Leggett. 1995. Relative weight (W_r) as a field assessment tool: relationships with growth, prey biomass, and environmental conditions. Transactions of the American Fisheries Society 124:387-400.

Lima, S. L. 1986. Predation risk and unpredictable feeding conditions: determinants of body mass in birds. Ecology 67:377-385.

Loy, A., E. Ciccotti, L. Ferrucci, and S. Cataudella. 1996. An application of automated feature extraction and geometric morphometrics: temperature-related changes in body form of *Cyprinus carpio* juveniles. Aquacultural Engineering 15:301-311.

Maceina, M. J., and B. R. Murphy. 1988. Variation in the weight-to-length relationship among the Florida and northern largemouth bass and their intraspecific F_1 hybrid. Transactions of the American Fisheries Society 117:232-237.

Madon, S. P., and D. A. Culver. 1993. Bioenergetics model for larval and juvenile walleyes: an *in situ* approach with experimental ponds. Transactions of the American Fisheries Society 122:797-813.

Madsen, T. and R. Shine. 2002. Short and chubby or long and slim? Food intake, growth, and body condition in free-ranging pythons. Austral Ecology 27:672-680.

Mann, R. H. K. 1973. Observations on the age, growth, reproduction, and food of the roach *Rutilus rutilus* (L.) in two rivers in southern England. Journal of Fish Biology 5:707-736.

Mann, R. H. K. 1976. Observations on the age, growth, reproduction, and food of the chub *Squalius cephalus* (L.) in the River Stour, Dorset. Journal of Fish Biology 8:265-288.

Marwitz, T. D., and W. A. Hubert. 1997. Trends in relative weight of walleye stocks in Wyoming reservoirs. North American Journal of Fisheries Management 17:44-53.

McComish, T. S. 1971. Laboratory experiments on growth and food conversion by the bluegill. Doctoral dissertation. University of Missouri, Columbia.

McComish, T. S., R. O. Anderson, and F. G. Goff. 1974. Estimation of bluegill (*Lepomis macrochirus*) proximate composition with regression models. Journal of the Fisheries Research Board of Canada 31:1250-1254.

McDonald, D. G., C. L. Milligan, W. J. McFarlane, S. Croke, S. Currie, B. Hooke, R. B. Angus, B. L. Tufts, and K. Davidson. 1998. Condition and performance of juvenile Atlantic salmon (*Salmo salar*): effects of rearing

practices on hatchery fish and comparison with wild fish. Canadian Journal of Fisheries and Aquatic Sciences 55:1208-1219.

McNamara, J. M., and A. I. Houston. 1987.Starvation and predation as factors limiting population size. Ecology 68:1515-1519.

McNamara, J. M., and A. I. Houston. 1990. Value of fat reserves and the tradeoff between starvation and predation. Acta Biotheoretica 38:37-61.

Medford, B. A., and W. C. Mackay. 1978. Protein and lipid content of gonads, liver, and muscle of northern pike (*Esox lucius*) in relation to gonad growth. Journal of the Fisheries Research Board of Canada 35:213-219.

Metcalfe, N. B., and P. Monaghan. 2001. Compensation for a bad start: grow now, pay later? Trends in Ecology and Evolution 16:254-260.

Metcalfe, N. B., C. D. Bull, and M. Mangel. 2002. Seasonal variation in catch-up growth reveals state-dependent somatic allocations in salmon. Evolutionary Ecology Research 4:871-881.

Miglavs, I., and M. Jobling. 1989. The effects of feeding regime on proximate body composition and patterns of energy deposition in juvenile Arctic charr, *Salvelinus alpinus*. Journal of Fish Biology 35:1-11.

Miller, P. J. 1979. A concept of fish phenology. Pages 1-28 *in* P. J. Miller, editor. Fish phenology: anabolic adaptiveness in teleosts. Zoological Society of London Symposium 44, London.

Montgomery, D. C., and E. A. Peck. 1992. Introduction to linear regression analysis, 2nd edition. John Wiley and Sons, New York.

Mosher, T. D. 1984. Responses of white crappie and black crappie to threadfin shad introductions to a lake containing gizzard shad. North American Journal of Fisheries Management 4:365-370.

Murphy, B. R., M. L. Brown, and T. A. Springer. 1990. Evaluation of the relative weight (W_r) index, with new applications to walleye. North American Journal of Fisheries Management 10:85-97.

Murphy, B. R., D. W. Willis, and T. A. Springer. 1991. The relative weight index in fisheries management: status and needs. Fisheries (Bethesda) 16(2):30-38.

Murphy, B. R., and D. W. Willis. 1991. Application of relative weight (W_r) to western warmwater fisheries. U.S. Forest Service General Technical Report RM-207:243-248.

Murphy, B. R., and D. W. Willis, editors. 1996. Fisheries techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

Murphy, D. D., and B. D. Noon. 1991. Coping with uncertainty in wildlife biology. Journal of Wildlife Management 55:773-782.

Murphy, M. E., and J. R. King. 1991. Ptilochronology: a critical evaluation of assumptions and utility. Auk 108:695-704.

Neter, J., M. H. Kutner, C. J. Nachtsheim, and W. Wasserman. 1996. Applied linear statistical models, 4th edition. Irwin, Chicago, Illinois.

Neumann, R. M., and B. R. Murphy. 1991. Evaluation of the relative weight (W_r) index for assessment of white crappie and black crappie populations. North American Journal of Fisheries Management 11:543-555.

Neumann, R. M., and B.R. Murphy. 1992. Seasonal relationships of relative weight to body composition in white crappie, *Pomoxis annularis* Rafinesque. Aquaculture and Fisheries Management 23:243-251.

Neumann, R. M., and M. K. Flammang. 1997. Relative weight as a body condition index for chain pickerel. Journal of Freshwater Ecology 12:19-26.

Ney, J. J. 1993. Bioenergetics modeling today: growing pains on the cutting edge. Transactions of the American Fisheries Society 122:736-748.

Nibbelink, N. P., and S. R. Carpenter. 1998. Interlake variation in growth and size structure of bluegill (*Lepomis macrochirus*): inverse analysis of an individual-based model. Canadian Journal of Fisheries and Aquatic Sciences 55:387-396.

Nicieza, A. G., and N. B. Metcalfe. 1997. Growth compensation in juvenile Atlantic salmon: responses to depressed temperature and food availability. Ecology 78:2385-2400.

Niimi, A. J. 1972. Changes in the proximate body composition of largemouth bass (*Micropterus salmoides*) with starvation. Canadian Journal of Zoology 50:815-819.

Novinger, G. D. 1973. The effect of food quantity on ovary development and condition of female bluegill. Master's thesis. University of Missouri, Columbia.

Olson, M. H. 1996. Ontogenetic niche shifts in largemouth bass: variability and consequences for first-year growth. Ecology 77:179-190.

Orban, E., G. DiLena, T. Nevigato, I. Casinin, G. Santaroni, A. Marzetti, and R. Caproni. 2002. Quality characteristics of sea bass intensively reared and from lagoon as affected by growth conditions and the aquatic environment. Journal of Food Science 67:542-546.

Ormerod, S. J., and S. J. Tyler. 1990. Assessments of body condition in dippers *Cinclus cinclus*: potential pitfalls in the derivation and use of condition indices based on body proportions. Ringing and Migration 11:31-41.

Osenberg, C. W., E. E. Werner, G. G. Mittleback, and D. J. Hall. 1988. Growth patterns in bluegill (*Lepomis macrochirus*) and pumpkinseed (L. gibbosus) sunfish: environmental variation and the importance of ontogenetic niche shifts. Canadian Journal of Fisheries and Aquatic Sciences 45:17-26.

Owen, M., and W. A. Cook. 1977. Variations in body weight, wing length, and condition of mallard *Anas platyrynchos platyrynchos* and their relationship to environmental changes. Journal of Zoology (London) 183:377-395.

Packard, G. C., and T. J. Boardman. 1988. The misuse of ratios, indices, and percentages in ecophysiological research. Physiological Zoology 61:1-9.

Partridge, L., and P. H. Harvey. 1988. Ecological context of life history evolution. Science 241:1449-1455.

Pearse, A. S. 1925. The chemical composition of certain fresh-water fishes. Ecology 6:7-16.

Perrin, N., M. C. Bradley, and P. Calow. 1990. Plasticity of storage allocation in *Daphnia magna*. Oikos 59:70-74.

Perrin, N., and R. M. Sibly. 1993. Dynamic models of energy allocation and investment. Annual Review of Ecology and Systematics 24:379-410.

Peters, R. H. 1983. Ecological implications of body size. Cambridge University Press, New York.

Piersma, T., and A. Lindstrom. 1997. Rapid reversible changes in organ size as a component of adaptive behaviour. Trends in Ecology and Evolution 12:134-138.

Pope, K. L., and D. W. Willis. 1996. Seasonal influences on freshwater fisheries sampling data. Reviews in Fisheries Science 4:57-73.

Porath, M. T., and E. J. Peters. 1997. Use of relative weights (W_r) to assess prey availability. North American Journal of Fisheries Management 17:628-637.

Post, J. R., and E. A. Parkinson. 2001. Energy allocation strategy in young fish: allometry and survival. Ecology 82:1040-1051.

Prairie, Y. T. 1996. Evaluating the predictive power of regression models. Canadian Journal of Fisheries and Aquatic Sciences 53:490-492.

Rencher, A. C. 1995. Methods of multivariate analysis. John Wiley and Sons, New York.

Reznick, D. N., and B. Braun. 1987. Fat cycling in the mosquitofish (*Gambusia affinis*): fat storage as a reproductive adaptation. Oecologia 73:401-413.

Rice, J. A., J. E. Breck, S. M. Bartell, and J. F. Kitchell. 1983. Evaluating the constraints of temperature, activity, and consumption on the growth of largemouth bass. Environmental Biology of Fishes 9:263-275.

Ricklefs, R. E. 1991. Structures and transformations of life histories. Functional Ecology 5:174-183.

Rieman, B. E., and J. B. Dunham. 2000. Metapopulations and salmonids: a synthesis of life history patterns and empirical observations. Ecology of Freshwater Fish 9:51-64.

Rikardsen, A. H., and M. Johansen. 2003. A morphometric method for estimation of total lipid level in live Arctic charr: a case study of its application on wild fish. Journal of Fish Biology 62:724-734.

Roff, D. A. 1984. The evolution of life history parameters in teleosts. Canadian Journal of Fisheries and Aquatic Sciences 41:989-1000.

Roff, D. A. 1992. The evolution of life histories: theory and analysis. Chapman and Hall, New York.

Rogers, C. M. 1987. Predation risk and fasting capacity: do wintering birds maintain optimal body mass? Ecology 68:1051-1061.

Rogers, C. M., and J. N. Smith. 1993. Life-history theory in the nonbreeding period: tradeoffs in avian fat reserves? Ecology 74:419-426.

Rolff, J., and G. Joop. 2002. Estimating condition: pitfalls of using weight as a fitness correlate. Evolutionary Ecology Research 4:931-935.

Rose, C. J. 1989. Relationship between relative weight (W_r) and body composition in immature walleye. Masters thesis. Texas A & M University, College Station.

Rowe, L., and D. Houle. 1996. The lek paradox and the capture of genetic variance by condition dependent traits. Proceedings of the Royal Society of London B 263:1415-1421.

Rykiel, E. J. 1996. Testing ecological models: the meaning of validation. Ecological Modelling 90:229-244.

Salam, A., and P. M. C. Davies. 1994. Body composition of northern pike (*Esox lucius* L.) in relation to body size and condition factor. Fisheries Research 19:193-204.

Saltz, D., G. C. White, and R. M. Bartmann. 1995. Assessing animal condition, nutrition, and stress from urine in snow: a critical view. Wildlife Society Bulletin 23:694-698.

Savitz, J. 1971. Effects of starvation on body protein utilization of bluegill sunfish (*Lepomis macrochirus* Rafinesque) with a calculation of caloric requirements. Transactions of the American Fisheries Society 100:18-21.

Schaffer, W. M., and P. F. Elson. 1975. Adaptive significance of variations in life history among local populations of Atlantic salmon in North America. Ecology 56:577-590.

Schneider, J. C. 1999. Dynamics of quality bluegill populations in two Michigan lakes with dense vegetation. North American Journal of Fisheries Management 19:97-109.

Seaburg, K. G., and J. B. Moyle. 1964. Feeding habits, digestive rates, and growth of some Minnesota warmwater fishes. Transactions of the American Fisheries Society 93:269-285.

Secor, S. M., and J. M. Diamond. 2000. Evolution of regulatory responses to feeding in snakes. Physiological and Biochemical Zoology 73:123-141.

Shearer, K. D. 1994. Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture 119:63-88.

Shul'man, G. E. 1974. Life cycles of fish:physiology and biochemistry. Translated from Russian by N. Kaner. John Wiley and Sons, New York.

Sibly, R. M. 1991. The life history approach to physiological ecology. Functional Ecology 5:184-191.

Simpkins, D. G., W. A. Hubert, C. Martinez Del Rio, and D. C. Rule. 2003. Physiological responses of juvenile rainbow trout to fasting and swimming activity: effects on body composition and condition indices. Transactions of the American Fisheries 132:576-589.

Sinervo, B., and E. Svensson. 1998. Mechanistic and selective causes of life history trade-offs and plasticity. Oikos 83:432442.

Snee, R. D. 1977. Validation of regression models: methods and examples. Technometrics 19:415-428.

Springer, T. A., B. R. Murphy, S. Gutreuter, R. O. Anderson, L. E. Miranda, D. C. Jackson, and R. S. Cone. 1990. Properties of relative weight and other condition indices. Transactions of the American Fisheries Society 119:1048-1058.

Strange, R. J., and J. C. Pelton. 1987. Nutrient content of clupeid forage fishes. Transactions of the American Fisheries Society 116:60-66.

Sutton, S. G., T. P. Bult, and R. L. Haedrich. 2000. Relationships among fat weight, body weight, water weight, and condition factors in wild Atlantic salmon parr. Transactions of the American Fisheries Society 129:527-538.

Tyler, A. V., and R. S. Dunn. 1976. Ration, growth, and measures of somatic and organ condition in relation to meal frequency in winter flounder, *Pseudopleuronectes americanus*, with hypotheses regarding population homeostasis. Journal of the Fisheries Research Board of Canada 33:63-75.

Van der Meer, J., and T. Piersma. 1994. Physiologically inspired regression models for estimating and predicting nutrient stores and their composition in birds. Physiological Zoology 67:305-329.

Van Horne, B. 2002. Approaches to habitat modeling: the tensions between pattern and process and between specificity and generality. Pages 63-72 *in* J. M. Scott, P. J. Heglund, M. L. Morrison, J. B. Haufler, M. G. Raphael, W. A. Wall, and F. B. Samson, editors. Predicting species occurrences: issues of accuracy and scale. Island Press, Washington, D. C.

Van Noordwijk, A. J., and G. DeJong. 1986. Acquisition and allocation of resources: their influence on variation in life history tactics. American Naturalist 128:137-142.

Van Winkle, W., B. J. Shuter, B. D. Holcomb, H. I. Jager, J. A. Tyler, and S. Y. Whitaker. 1997. Regulation of energy acquisition and allocation to respiration, growth, and reproduction: simulation model and example using rainbow trout. Pages 103-137 *in* R. C. Chambers and E. A. Trippel,

editors. Early life history and recruitment in fish populations. Chapman and Hall, London.

VDEQ (Virginia Department of Environmental Quality). 1990. Water quality monitoring. Virginia Department of Environmental Quality. Available: www.deq.va.state.us/webapp/wqm_station.station_detail?p_sta_id=9-XBL000.20. (June 2003).

Wang, N., R. S. Hayward, and D. B. Noltie. 1998. Variation in food consumption, growth, and growth efficiency in juvenile hybrid sunfish held individually. Aquaculture 167:43-52.

Ware, D. M. 1982. Power and evolutionary fitness of teleosts. Canadian Journal of Fisheries and Aquatic Sciences 39:3-13.

Weatherley, A. H., and H. S. Gill. 1981. Recovery growth following periods of restricted rations and starvation in rainbow trout *Salmo gairdneri* Richardson. Journal of Fish Biology 18:195-208.

Weatherley, A. H., and H. S. Gill. 1983. Protein, lipid, water and caloric contents of immature rainbow trout, *Salmo gairdneri* Richardson, growing at different rates. Journal of Fish Biology 23:653-673.

Weatherley, A. H., and H. S. Gill. 1987. The biology of fish growth. Academic Press, New York.

Weber, L. P., P. S. Higgins, R. I. Carlson, and D. M. Janz. 2003. Development and validation of methods for measuring multiple biochemical indices of condition in juvenile fishes. Journal of Fish Biology 63:637-658.

Wedemayer, G. A., B. A. Barton, and D. J. McLeay. 1990. Stress and acclimation. Pages 451-490 *in* C. B. Schreck and P. B. Moyle, editors. Methods for fish biology. American Fisheries Society, Bethesda, Maryland.

Wege, G. J. and R. O. Anderson. 1978. Relative weight (W_r): a new index of condition for largemouth bass. Pages 79-91 *in* G. D. Novinger and J. G. Dillard, editors. New approaches to the management of small impoundments. American Fisheries Society, North Central Division, Special Publication 5. Bethesda, Maryland.

Welton, N. J., and A. I. Houston. 2001. A theoretical investigation into direct and indirect effects of state on the risk of predation. Journal of Theoretical Biology 213:275-297.

Werner, E. E., and D. J. Hall. 1988. Ontogenetic habitat shifts in bluegills: the foraging rate - predation risk tradeoff. Ecology 69:1352-1366.

Werner, E. E., J. F. Gilliam, D. J. Hall, and G. G. Mittelbach. 1983. An experimental test of the effects of predation risk on habitat use in fish. Ecology 64:1540-1548.

Wicker, A. M., and W. E. Johnson. 1987. Relationships among fat content, condition factor, and first-year survival of Florida largemouth bass. Transactions of the American Fisheries Society 116:264-271.

Willis, D. W., and C. G. Scalet. 1989. Relations between proportional stock density and growth and condition of northern pike populations. North American Journal of Fisheries Management 9:488-492.

Willis, D. W., C. S. Guy, and B. R. Murphy. 1991. Development and evaluation of a standard weight (W_s) equation for yellow perch. North American Journal of Fisheries Management 11:374-380.

Windell, J. T. 1966. Rates of digestion in bluegill sunfish. Investigations of Indiana Lakes and Streams 7:185-214.

Witter, M. S., and I. C. Cuthill. 1993. The ecological costs of avian fat storage. Philosophical Transactions of the Royal Society of London B 340:73-92.

Witter, M. S., and J. P. Swaddle. 1997. Mass regulation in juvenile starlings: response to change in food availability depends on initial body mass. Functional Ecology 11:11-15.

Wootton, R. J. 1977. Effect of food limitation during the breeding season on the size, body components, and egg production of female sticklebacks (*Gasterosteus aculeatus*). Journal of Animal Ecology 46:823-834.

Ylikarjula, J., M. Heino, and U. Dieckmann. 1999. Ecology and adaptation of stunted growth in fish. Evolutionary Ecology 13:433-453.

Zar, J. H. 1996. Biostatistical analysis, 3rd edition. Prentice-Hall, Inc. Saddle River, New Jersey.

Zera, A. J., and L. G. Harshman. 2001. The physiology of life history trade-offs in animals. Annual Reviews in Ecology and Systematics 32:95-126.