

Development of a Morphological Index of the Nutritional Status of Juvenile Largemouth Bass

CHRIS D. SMITH

*Wildlife and Fisheries Management Institute, Mailstop 2125,
Texas Tech University, Lubbock, Texas 79409-2125, USA*

CHRISTOPHER L. HIGGINS*

*Department of Biological Sciences, Texas Tech University,
Lubbock, Texas 79409-3131, USA*

GENE R. WILDE

*Wildlife and Fisheries Management Institute, Mailstop 2125,
Texas Tech University, Lubbock, Texas 79409-2125, USA*

RICHARD E. STRAUSS

*Department of Biological Sciences, Texas Tech University,
Lubbock, Texas 79409-3131, USA*

Abstract.—We describe the morphological changes associated with starvation in larval largemouth bass *Micropterus salmoides* and develop bivariate and multivariate morphological indices of nutritional status. We obtained hatchery-reared largemouth bass, raised them until completion of fin development, and divided them into two randomized experimental groups of fed and unfed fishes. Fed fishes were provided with newly hatched brine shrimp twice daily. We quantified morphological changes in body shape using 23 morphometric characters. After only 3 d of food deprivation, we were able to detect statistically significant differences in morphology between the fed and unfed fish using multivariate analysis. The magnitude of the mean difference increased over time. An unexpected result suggested that a simple, bivariate ratio of standard length to body depth at the anus was almost as efficient and robust at classifying fed and unfed largemouth bass from an independent data set as a multivariate index based on all 23 morphometric characters. The relative ease of use, combined with a small level of misclassification error rate and a firm foundation in whole-body changes, makes these ratio indices effective tools for detecting food deprivation in larval largemouth bass.

High and variable mortality rates among larval fishes are a major cause of interannual variation in recruitment. Various explanations have been proposed to account for this mortality, including cannibalism (Baras and Jobling 2002), predation (Elliot and Leggett 1998), disease (Munday and Nakai 1997), environmental variation (Frank and Leggett 1982), and food deprivation (Jonas and Wahl 1998). Currently, much of the evidence points to food deprivation as being the primary factor responsible for poor survival among larval fishes (Hjort 1914; Cushing 1978; Sinclair 1988). Without adequate food at, or shortly following, the time of hatching, the survival of larval fishes may decrease directly as a result of starvation or indirectly from decreased growth rates, which pro-

long the period during which larvae are most vulnerable to predation.

A variety of techniques have been developed for diagnosing the nutritional status of larval fishes. These include a number of biochemical, histological, and morphological indices. Biochemical and histological indices reflect changes at the cellular and tissular levels of organization, respectively, whereas morphological indices reflect changes at the organismal level (Ferron and Leggett 1994). Although the effects of starvation may take longer to appear at the organismal level than at the cellular level, morphological indices provide a direct and integrative approach to assess whole-body changes resulting from food deprivation. Morphological indices have been used for years to detect changes in shape resulting from suboptimal feeding (Theilacker 1978) under the basic premise that certain body parts (e.g., abdomen) respond differently to starvation than other, less sensitive parts

* Corresponding author: chris.higgins@ttu.edu

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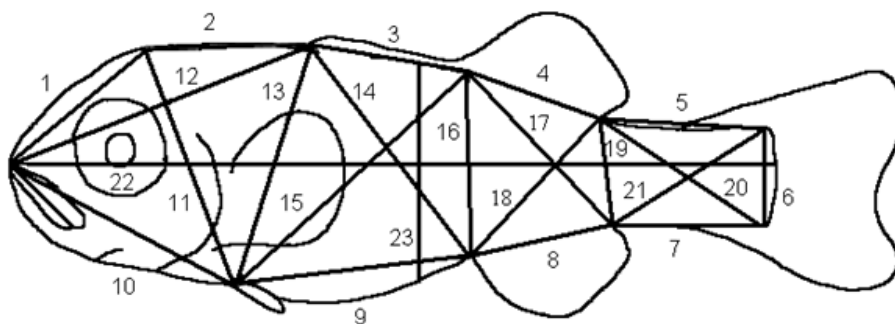


FIGURE 1.—Diagram of the truss network and two supplemental measurements used for morphological analyses and development of indices of nutritional status of juvenile largemouth bass.

(e.g., eye). The greatest advantage of morphological indices is attributable to the short processing time, lost cost, and ease of attainability (Theilacker 1978; Martin and Wright 1987). However, few studies have accounted for allometric growth when formulating nutritional indices, an omission that could lead to erroneous conclusions about the nutritional status of larval fishes.

In this study, we (1) describe morphological changes in larval largemouth bass *Micropterus salmoides* as a consequence of food deprivation using a multivariate aspect to account explicitly for allometry and variation across the entire body, (2) develop multivariate and bivariate (ratio) indices of nutritional condition based on whole-body changes, and (3) test the utility of these indices using an independent sample of largemouth bass of known nutritional history. We use largemouth bass because of its ecological importance as a keystone species and its societal importance as a highly sought recreational fish.

Methods

We obtained naturally occurring larvae of largemouth bass in May 1996 from Starr Lake near College Station, Texas, and hatchery-reared larvae in May 1997 from the A. E. Wood State Fish Hatchery, Texas Parks and Wildlife Department, San Marcos. Fish obtained in 1996 (Florida largemouth bass *M. salmoides floridanus*) were used in an initial study to assess necessary sample sizes; fish obtained in 1997 (northern largemouth bass) were used to develop the index of nutritional status. We used the same experimental design and methods for fish obtained in both years. On arrival, we transferred the fish to two 80-L aquaria maintained at room temperature (20–22°C) until completion of fin development. During this holding period, all fish were fed to satiation twice daily on

newly hatched brine shrimp *Artemia* spp. We then divided them into two experimental groups using a design similar to that of Powell and Chester (1985) and Zamal and Ollevier (1995): larvae were divided into “fed” (control) and “unfed” (treatment) groups, with three replicates per group and approximately 125 fish per replicate. Each replicate was maintained in a separate 45-L aquarium maintained at 17–21°C. We supplied the fed groups with brine shrimp twice daily and allowed them to feed to satiation, whereas we did not supply any food to the unfed groups. We harvested 8 fish/d from each of the replicates, euthanatized them with a lethal dose of MS-222, fixed them in 10% neutral buffered formalin for 24 h, and subsequently transferred them to a 70% ethanol solution.

We used a computerized imaging system with input from an Olympus SZH10 dissecting scope and a Sony CCD/RGB color video camera to capture images of the left side of each fish, with the dorsal fins on the same geometric plane as the anal fin. Because the head of the fish is generally broader than the tail, we raised the tail of each fish to lie within the same plane as the head and paid careful attention to consistent positioning to obtain unbiased measurements (Strauss and Bond 1990). With each fish correctly positioned, we digitized 13 anatomical landmarks and converted them to 23 interlandmark distances (Figure 1) using a program written in Matlab (Mathworks 1997). We used 10 of the landmarks to form a truss network of 21 morphometric characters. A truss network is a systematically arranged set of distances among a preselected set of anatomical landmarks that are assumed to be homologous, or at least analogous, among specimens (Strauss and Bookstein 1982). We used a truss network because trusses generally ensure regular coverage of the body in area and orientation, and allow measurement error to be

partitioned statistically from the measurements (Strauss and Bond 1990). The remaining three landmarks were used to provide two supplemental morphometric characters that typically are used by fishery biologists—standard length and body depth at the anus—to augment the truss measurements.

We performed discriminant function analysis (DFA) on log-transformed measurements from largemouth bass to determine which morphometric characters best separated fed and unfed fish and to estimate classification functions to predict group membership. In addition, we conducted multivariate analyses of variance (MANOVAs) to test for morphological differences among replicates of fed fish, among replicates of unfed fish, and between fed and unfed largemouth bass. There were no significant differences among replicates within either the fed or unfed group, so individuals within replicates were pooled within groups for the remaining statistical analyses. There was, however, a significant difference between fed and unfed fish. Given that a significant difference occurred and that the loadings from the DFA corresponded to size allometry factors (i.e., all loadings on the first discriminant function were positive and of similar magnitude), we estimated a size-independent canonical variable by (1) finding the pooled within-group principal components (PCs); (2) regressing the first PC, which characterized within-group size variation, from each character independently; and (3) using the regression residuals in a canonical discriminant analysis (Strauss 1995). We performed a “size-free” discriminant function analysis (SF-DFA) to determine the degree to which fed and unfed fish could be optimally distinguished using the 23 morphometric characters, independent of allometric size variation.

To create a simplified index of nutritional status, we reduced the number of measurements to those that are most useful in detecting food deprivation in larval largemouth bass. We chose two morphometric characters that were highly correlated with the first size-free discriminant function and that described differences in opposite directions (Figure 2). Morphometric character x_{13} had the highest correlation in the positive direction and x_{10} had the highest correlation in the negative direction. Because the two additional measurements (standard length [x_{22}] and body depth at anus [x_{23}]) typically are used by fishery biologists and are highly correlated with the discriminant function, we chose also to analyze the ratio of these variables to compare its utility. Both ratios ($x_{13} : x_{10}$

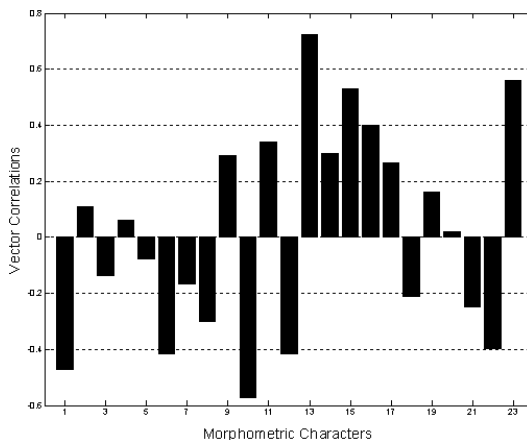


FIGURE 2.—Vector correlations between morphometric characters and the first size-free discriminant function. The numbers for each morphometric character correspond to those in Figure 1.

and $x_{23} : x_{22}$) as well as a multivariate index based on all 23 characters, were subjected to a classification algorithm that uses a jackknife approach to classify each known observation as either a fed or unfed fish. Finally, we tested the robustness and predictability of the three nutritional indices using an independent sample ($n = 125$) of Florida largemouth bass as a validation data set.

Results

Fed and unfed largemouth bass ranged in length from 8.0 to 15.4 mm and differed significantly in overall morphology (MANOVA; Wilk's lambda = 0.42; $F = 15.89$; $df = 23, 260$; $P < 0.001$). There was no difference in morphology of fed and unfed largemouth bass during days 0–3, but after the third day morphology of the two groups differed significantly (Wilk's lambda = 0.08; $F = 5.85$; $df = 23, 12$; $P = 0.001$). The degree to which fed and unfed fish differed continued to increase over time, but the patterns of change remained the same (Figure 3). That is, both fed and unfed fish grew in length with relatively little change along the horizontal axis. Most of the morphological differences between fed and unfed largemouth bass occurred along the vertical axis or in body depth, especially in the abdominal region.

The multivariate framework was used to describe whole-body changes in larval fish resulting from starvation and to develop a multivariate nutritional index. The bivariate ratios were used in an attempt to simplify the methodology for more standard applications. The bivariate ratios of $x_{13} : x_{10}$ and $x_{23} : x_{22}$ indicated there was a significant

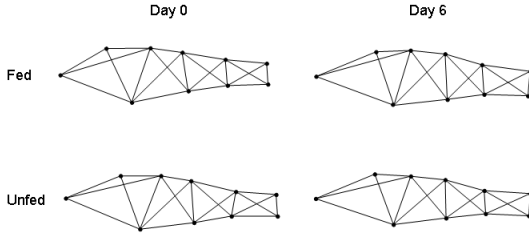


FIGURE 3.—Truss diagrams for average fed and unfed fish at day 0 and day 6 of the experiment. This diagram depicts the extent of morphological changes associated with starvation.

difference ($t = -3.1556, P = 0.003$; and $t = -3.3592, P = 0.002$, respectively) in the morphology of fed and unfed fish after just 1 d of food deprivation, which is slightly more powerful than the multivariate index. These ratios also reflect the fact that the difference between fed and unfed fish increased over time (Figure 4). Both morphometric ratios were highly correlated with size-free discriminant scores, although the ratio $x23 : x22$ had a slightly higher magnitude of correlation ($r =$

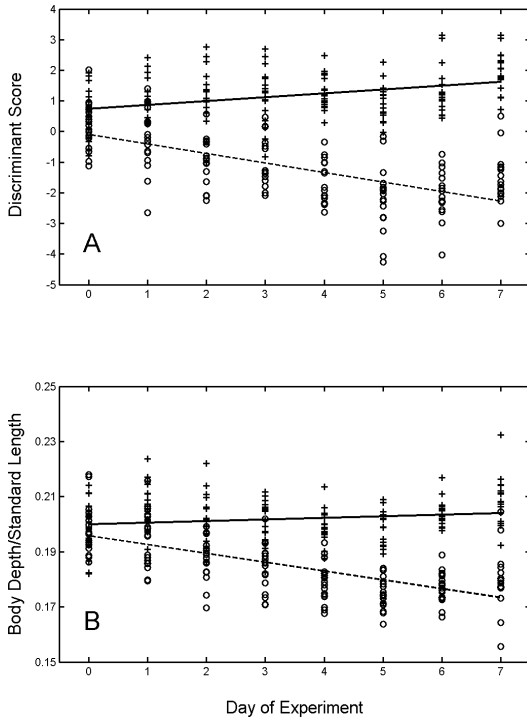


FIGURE 4.—(A) Discriminant scores and (B) ratios of body depth at anus to standard length for fed (plus signs) and unfed largemouth bass (circles) grouped by day of experiment. The solid and dashed lines are regression lines for fed and unfed fish, respectively.

TABLE 1.—Percentages of northern largemouth bass correctly classified as fed or unfed by multivariate and bivariate morphological indices of nutritional status decomposed into days of experiment and treatment groups. See text for additional details on the indices.

Day	Size-free DFA ^a		Ratio $x13 : x10$		Ratio $x23 : x22$	
	Fed	Unfed	Fed	Unfed	Fed	Unfed
0	44.4	55.6	55.6	44.4	5.6	11.1
1	50.0	61.1	72.2	55.6	77.8	66.7
2	72.2	70.6	83.3	76.5	66.7	82.4
3	88.9	77.8	88.9	66.7	88.9	83.3
4	94.4	88.9	94.4	100.0	94.4	88.9
5	81.3	100.0	87.5	94.4	93.8	100.0
6	94.4	100.0	77.8	66.7	100.0	100.0
7	100.0	100.0	88.9	76.5	94.4	88.2
Overall	91.5	78.2	82.4	67.7	83.1	78.9

^a DFA = discriminant function analysis.

$-0.84, P < 0.001$) than the ratio $x13 : x10$ ($r = -0.73, P < 0.001$).

Overall, the ratios $x23 : x22$ and $x13 : x10$ both correctly classified fed fish (83.1% and 82.4%, respectively) with a greater success rate than they did unfed fish (78.9% and 69.7%, respectively). Among these two indices, the ratio of $x23 : x22$ was more accurate at classifying largemouth bass into fed and unfed fish than ratio $x13 : x10$ (Table 1). In fact, the simple ratio of $x23 : x22$ was as efficient at classifying unfed largemouth bass as the multivariate index that incorporated all 23 morphometric characters (78.9% and 78.2%, respectively), and it was only slightly less efficient at classifying fed fish (83.1% and 91.5%, respectively) compared with the multivariate index. When used to classify the 125 Florida largemouth bass from the preliminary study, the ratio $x23 : x22$ consistently outperformed the other two indices at classifying unfed fish but was slightly less efficient at classifying fed fish (Table 2).

TABLE 2.—Percentages of Florida largemouth bass correctly classified as fed or unfed by multivariate and bivariate morphological indices of nutritional status decomposed into days of experiment and treatment groups. See text for additional details on the indices.

Day	Size-free DFA ^a		Ratio $x13 : x10$		Ratio $x23 : x22$	
	Fed	Unfed	Fed	Unfed	Fed	Unfed
0	20.0	16.7	100.0	0.0	30.0	33.3
1	87.0	0.0	100.0	0.0	52.2	47.4
2	75.0	4.8	100.0	0.0	75.0	57.1
3	58.3	50.0	100.0	0.0	95.8	85.7
4	50.0	50.0	100.0	0.0	100.0	50.0
Overall	98.9	0.0	100.0	0.0	85.4	43.2

^a DFA = discriminant function analysis.

Discussion

The morphology of larval largemouth bass changes with starvation, most modifications occurring in the vertical direction, especially in the abdominal region. These changes are best captured with a multivariate perspective, but our results suggest that a simple bivariate morphological index can provide useful information on the nutritional status of juvenile largemouth bass. Based on our results, we suggest that a ratio of depth to length of 0.195 can provisionally be used to classify larval largemouth bass as either being fed (≥ 0.195) or unfed (< 0.195), but see Figure 4 for a depiction of the variability of the ratio within and among days. The ratio of body depth at the anus to standard length correctly classified fish from the original data set (northern largemouth bass) as accurately as the multivariate metric based on 23 morphological characters and correctly classified Florida largemouth bass more accurately than the multivariate metric. Thus, the simple morphological index of nutritional status for larval largemouth bass was as efficient and more robust than the multivariate index. This finding, coupled with the fact that standard length and body depth can be quickly measured, suggest that the simple ratio of depth to length may be a useful tool for biologists and fisheries managers alike. However, it is important to note that this index was derived from a multivariate perspective describing whole-body changes that resulted from starvation, which is a more objective approach than a priori choosing two morphological variables to use as an index of nutritional status.

There are two limitations to the morphological method developed here. The first involves the conditions under which the indices were developed. Rarely will food be completely unavailable to larval largemouth bass in the wild. In studies involving the feeding habits of postlarval largemouth bass (> 20 mm), very few researchers have reported finding fish with completely empty stomachs during the peak feeding hours (Miller and Kramer 1971; Elliot 1976; Wilde and Paulson 1988). However, the extreme condition of food unavailability was used to determine whether the truss network and ratios could detect morphological changes resulting from starvation. A second limitation is in the applicability of the index to wild-caught fish. There may be morphological differences in wild-caught and laboratory-reared fish that prevent accurate calibration of the index (Blaxter 1971; Martin and Wright 1987). For ex-

ample, Blaxter (1971) noted that racial differences in morphology occurred between larvae of different stocks of herring *Clupea* spp. The same may be true for larval largemouth bass; however, the utility of our index for both subspecies and for fish collected from a private lake suggest our indices are robust to these differences. If morphological differences occur between different year-classes or populations of largemouth bass, application of the index developed here may require refinement from year to year and from stock to stock (Martin and Wright 1987). Despite these limitations, we advocate the use of a simple, morphological index of the nutritional status of larval fish (e.g., depth to length) because of the simplicity and inexpensive nature of conducting morphometric studies. Our technique is shown to work for largemouth bass and suggests that it will work for other species, but a multivariate grounding describing whole-body changes is necessary to alleviate known statistical problems associated with ratios when extending this procedure to other species.

Theilacker (1978), McGurk (1985), and Powell and Chester (1985) selected morphological measurements that they believed might be affected by food deprivation. To avoid prior expectations, we used an overall coverage of the body with the truss network, supplementing those measurements with measurements of body depth at the anus and standard length to ensure that our indices reflected whole-body changes. The complete coverage, combined with the multivariate statistical techniques, allowed the systematic elimination of unnecessary measurements and aided in selecting those measurements that were most informative in detecting the effects of food deprivation in largemouth bass. Ratios of body depth to standard length were effective in detecting food deprivation, regardless of fish size. The high correlation between the ratio $x_{23} : x_{22}$ and size-free canonical discriminant scores suggests that two variables, among the 23 originally measured, were adequate for a nutritional index. McGurk (1985) reported that ratios were not effective as nutritional indices because of their high correlation with fish size. Because our indices were applied to a small range in length of largemouth bass, they can be effective as an indicator of condition in larval fish. Theilacker (1978) successfully classified 94% of food-deprived larvae tested using a ratio of body depth at the pectoral fin to standard length. The ease of use, combined with a small level of misclassification error, makes ratio indices an effective tool for detecting food deprivation in larval largemouth

bass. The difficulty in distinguishing between fed largemouth bass and those held without food for only 1 d can be overcome by assessing the condition of largemouth bass over time (Martin and Wright 1987). Negative trends in the ratios of body depth to standard length over several days indicated that largemouth bass were deteriorating in condition.

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