Alternative Methods to Predict Fish Proximate Composition

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Abstract: We used a multiple linear regression approach to develop models predicting water, protein, and lipid content of bluegills *(Lepomis macrochirus)* under 4 measurement approaches varying in terms of time and money. Inputs were length, weight, relative weight, total body electrical conductivity, and water. Models predicting water and protein weights were very accurate (<5% mean error). No regression predicting lipid weight was accurate enough to be used as a predictor (>37% mean error). We then attempted to reduce inaccuracy by standardizing lipid weight 4 ways. No standardization substantially improved predictive accuracy (>30% mean error). However, our results suggest that increasing the range of values used to fit the regressions may increase precision and accuracy of prediction.

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The chief components of fish tissue include water, protein, and lipid. The amount or percentage of each within a fish's body is termed proximate composition. Proximate composition is often determined in studies of fish physiology, growth, and nutrition. Typically, the carcass is weighed, cut up, and homogenized. The homogenate is then dried to determine water content (wet weight – dry weight) and to prepare tissue for subsequent analyses. Samples of the homogenized tissue are subjected to ether extraction and the Kjeldahl process to determine lipid and protein content, respectively. Thus, direct determination of proximate components is expensive in terms of time and money and also requires the death of the fish and the destruction of its carcass.

Several methods have been used in fish physiology to estimate proximate composition. External physical measurements (e.g., length, weight, and condition index) were calibrated to body composition by McComish et al. (1971) for a laboratory-reared bluegill *(Lepomis macrochirus)* population. They developed precise equations (R^2>0.58) but did not verify accuracy. Total body electrical conductivity (TOBEC) has been calibrated to proximate composition for channel catfish (*Ictalurus punctatus,* Jaramillo et al. 1993) and red drum (*Sciaenops ocellatus,* Bai et al. 1994). Both studies found that regressions incorporating TOBEC in combination with body measurements gave a very precise fit to their data (R^2>0.85). Although destructive, determination of water content may allow estimation of the other components (i.e., protein and lipid) without further analysis. Water content has been correlated to lipid content in alewives (*Alosa pseudoharengus,* Flath and Diana 1985) and juvenile striped bass (*Morone saxatilis,* Sutton 1997). Flath and Diana merely noted that a strong inverse relationship existed between percent lipid and percent water. Sutton found the relationship between percent water and lipid as a percentage of the lipid-free dry weight to be very precise (R^2=0.98) but did not assess the accuracy of his equation.

For our research into bluegill physiology and condition, we need a quick, inexpensive, and sensitive method to estimate proximate composition. Because we are studying bluegills in natural settings, we are not able to use the equations of McComish et al. (1974). To estimate composition, we constructed predictive models of bluegill composition by regressing variables measured by the above procedures against proximate composition as determined by traditional laboratory methods. We considered 4 alternative approaches in order of increasing expense and effort (Table 1). External costs include little in addition to collection, non-destructive costs include the one-time capital expense of the TOBEC unit, destructive costs include the one-time capital expense of the drying oven, and integrative costs combine destructive and non-destructive costs. Our objective was to compare the accuracy of each alternative for estimating water, protein, and lipid.

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Table 1. Alternative approaches for estimation and variables included under each option. Approaches are listed in decreasing order of effort.

Approach	Variables measured
Integrative	Length, weight, Wr, water content, TOBEC
Destructive	Length, weight, Wr, water content
Non-destructive	Length, weight, Wr, TOBEC
Field measurement	Length, weight, Wr

Methods

We selected populations in 2 small impoundments, Lower Travis Lake and Smoots Pond, at Fort A. P. Hill in northeastern Virginia. Specimens were collected in August 1998 by daytime electrofishing (N=43 and N=41, respectively). Minimum size collected was 80 mm total length, the smallest length for which relative weight (W_r) is applicable for bluegills (Murphy et al. 1991). To ensure that all lengths in each population were represented in our sample, we defined 3 length strata based on percentiles of lengths collected previously and sampled evenly within each strata. These strata were 80–113 mm, 114–122 mm, and ≥123 mm for Lower Travis Lake and 80–153 mm, 154–187 mm, and ≥188 mm for Smoots Pond.

After collection, fish were put on ice and transported to the laboratory. Individuals were measured for total length (TL, to the nearest 1.0 mm) and weight (W, to the nearest 0.1 g). We calculated relative weight $W_r = W/W_s \times 100$, where W_s is the 75th percentile weight at length from the equation developed by Hillman (1982). To measure TOBEC, we used an Em-Scan Model SA-3000 base unit paired with a SA-3114 detection chamber (Em-Scan, Inc., Springfield, Ill.). This unit detects fluctuations in a magnetic field set up in the detection chamber that are related to electrical conductivity. The mean of 3 readings was used. If coefficient of variation (CV) exceeded 5%, we would continue to take readings until the best 3-reading CV was ≤5%. After beings canned, fish were individually bagged and frozen. Frozen individuals were diced, dried at 60 C for a minimum of 24 hours, ground with a mortar and pestle, and dried again until weight stabilized. Water weight (WATER) was determined by subtraction. The dried and homogenized carcass was bagged and stored in a dessicator. Lipid weight (LIPID) and protein weight (PROTEIN) were determined from duplicate samples of each dried fish via ether extraction and the micro-Kjeldahl process (Assoc. Official Analytic Chemists 1984) by personnel of the Virginia Tech Forage Testing Laboratory.

We used multiple linear regression (MLR) to predict proximate composition following the recommendations of Neter et al. (1990) for building and validating models. A scatterplot matrix of all variables was examined to determine the appropriateness of various transformations and if inclusion of interaction terms was warranted. We used natural logarithm transformations of water and protein weight ($X' = \ln[X+1]$) because of increased variation in the residuals at higher predicted values. To linearize relationships of the predictors to these response variables, we also log-transformed weight and TOBEC values. Residuals of lipid weight increased and then decreased as predicted values rose. No transformation adequately corrected this pattern so we used the untransformed measurements. We pooled all fish, then split the data evenly into 2 sets (N=42 each), a training set to build models, and a validation set to test them. We stratified the data set into thirds based on length, and within strata randomly assigned each individual to a data set. For each alternative scenario and response variable, we constructed all possible regressions using the RSQUARE option in SAS (SAS Inst. 1996). The significance of the regression was evaluated by analysis of variance and that of the individual coefficients by partial F-tests. The

"best" model for each scenario was chosen for validation based on relative bias and precision. To make this choice, we examine adjusted R^2, model mean square error (MSE), the Collin Mallow statistic (C_p), and prediction error sum of squares (PRESS). We looked for the model with a high adjusted R^2, low MSE and PRESS, and C_p near the number of parameters in the model. These 4 criteria usually agreed; if not, we emphasized PRESS and C_p.

Best models were validated by several means (Neter et al. 1990) to see if they usefully described the data. Coefficients were inspected to see if each variable contributed to the relationship in a logical and statistically significant manner. We constructed an identical model (i.e. using the same variables) with the validation data to see if the coefficients were stable and precision similar. Finally we input the validation data into the training set models and examined mean square prediction error (MSPR) and tested the predicted versus observed values with a paired *t*-test as per Bai et al. (1994) and Jaramillo et al. (1994).

After having chosen a best model for each approach, we then examined them in terms of accuracy, i.e., how well the training set model predicted the validation set values. For our research, we wanted models that would predict within ± 5% of the true value, thus our main criteria was mean error rate <5%. We also wanted to assess the effects of measurement precision. Because the scale we used to measure weight was accurate to the nearest 0.1 g, we determined the number of errors generated that could not be attributable to rounding error (>0.1 g and also >5%). We then chose the least expensive alternative (in terms of time and effort) with an acceptable level of accuracy.

After our initial efforts, we found that lipid variability caused all models to be too inaccurate. To reduce this variability, we standardized lipid content by several measures of body size. Standardization by weight produces lipid content as a percentage of wet weight (WETLIP), standardization by dry weight expresses lipid content as a percentage of dry weight (DRYLIP), standardization by lipid-free dry weight produces a lipid index (LI, Johnson et al. 1985), and standardization by standard weight (W_s) produces a relative lipid weight (L_r, see Anderson and Gutreuter 1983). We then repeated the modeling process using these standardized lipid contents as response variables and including the percentage of water (%WATER) as a predictive variable. We did not compute the number of large errors (as previously defined) for this part of the study because the results were not expressed in grams.

Results

Lengths of bluegills ranged from 75 to 205 mm and W_r ranged from 54 to 102 (N=84). Water content was 72.4%–78.9%. Protein and lipid, as percentage of dry weight, ranged from 58.2% to 75.3% and 1.9% to 14.2%, respectively. Correlations between length and proximate composition were relatively precise for water and protein, but lipid content was much more variable (Fig. 1). Most fish had lipid weights of <1.0 g.

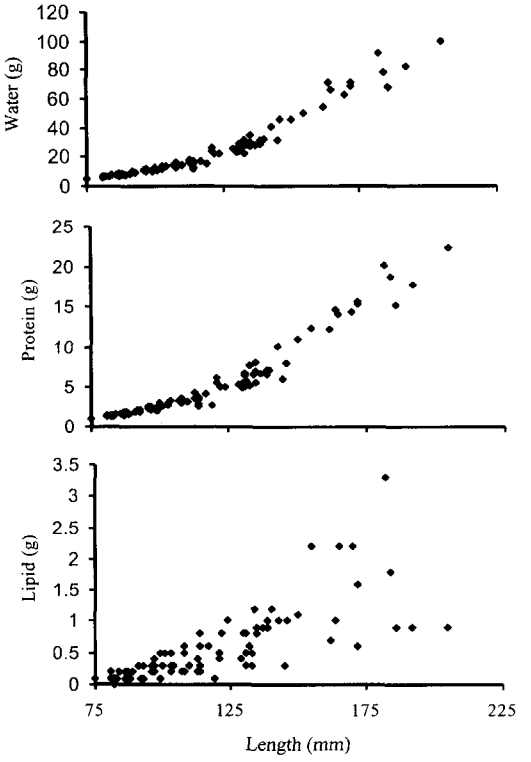

Figure 1. Relationships of proximate components to length in bluegills captured in Lower Travis Lake and Smoots Pond in August 1998.

There were 10 possible best models to be considered for validation, 2 for water weight and 4 each for protein and lipid weight (Table 2). For water weight, a model incorporating log weight and W_r was best when either the integrative approach was used or when only external measurements were considered for predictors. For lipid weight, a model that included only weight was best when external measurements were considered for predictors or destructive measurement was used. When predicting lnWATER and LIPID, 2 of the approaches yielded identical models; therefore we validated 8 regression models. With 1 exception, all appeared to be valid models. The exception was the model predicting log protein from length, log weight, W_r, and log water weight. The coefficient for length was not statistically significant (partial F-test, $P=0.07$). This model passed other validation methods so we decided to also test this model for accuracy.

Model Accuracy

We examined only 1 model that predicted water weight. This model had a mean error rate of 0.05% and did not generate any large errors. It is acceptable for use and has the form

Table 2. Variables included in "best" models from each of the 4 approaches for estimation. The asterisk indicates models that were not validated. Abbreviations are given in the text.

Dependent variable	Approach			
	Integrative	Destructive	Non-destructive	Field
lnWATER	lnW, Wr	n/a	n/a	lnW, Wr
lnPROTEIN	lnW, LnTOBEC Wr, LnWater	lnW, LnTOBEC	TL, lnW Wr, LnWater	lnW, Wr
LIPID	W, TOBEC Wr, WATER	W	W, WATER	W
DRYLIP	TOBEC, %Water	TL, %WATER	TL, TOBEC*	TL, W*
LI	TOBEC, %Water	TL, %WATER	TL, TOBEC*	TL, W*
WETLIP	TOBEC, %Water	TL, %WATER	TL, TOBEC*	TL, W*
L_r	Wr, TOBEC %WATER	W, Wr, %WATER	W, TOBEC*	W*

$$\text{lnWATER} = -0.25765 + 0.974784\ \text{lnW} + 0.000822\ W_r,\ R^2 = 0.9997.$$

We examined 4 models that predicted protein weight (Table 3). Three had acceptable mean error rates. The model incorporating only physical measurements had the lowest mean error rate and also the lowest number of large errors. Because this model would also be the cheapest in terms of time and money, we chose this model for use. It has the form

$$\text{lnPROTEIN} = -1.281465 + 0.842539\ \text{lnW} + 0.002454\ W_r,\ R^2 = 0.9926.$$

Table 3. Accuracy of validated models.

Dependent variable	Independent variables	Mean error (%)	*N* Large errors	Validation R^2
lnWATER	lnW, Wr	0.1	0	1.00
lnPROTEIN	lnW, lnTOBEC, Wr, lnWATER	4.4	11	0.99
	lnW, lnTOBEC	4.4	10	0.99
	TL, lnW, lnWATER, Wr	5.2	10	0.99
	lnW, Wr	4.3	7	0.99
LIPID	W, TOBEC, Wr, WATER	37.7	15	0.91
	W, WATER	41.9	16	0.87
	W	52.5	20	0.79
DRYLIP	TOBEC, %WATER	35.5		0.58
	TL, %WATER	31.2		0.50
LI	TOBEC, %WATER	38.3		0.54
	TL, %WATER	33.6		0.45
WETLIP	TOBEC, %WATER	35.6		0.60
	TL, %WATER	30.2		0.51
L_r	TOBEC, Wr, %WATER	35.3		0.59
	W, Wr, %WATER	34.8		0.51

We examined 3 models that predicted lipid weight (Table 3). Mean error rates were too high for all models and many large errors were generated. The model with the lowest error rate and lowest number of large errors has the form

$$\text{LIPID} = -0.346172 + 0.358551\ \text{W} - 0.035583\ \text{TOBEC} + 0.009958\ \text{W}_r - 0.442292\ \text{WATER};\ R^2 = 0.9093.$$

Standardized Lipid Models

There were 16 "best" models predicting standardized lipid content that we considered for validation (Table 2). Of these, there were 8 that were not validated. All invalid models did not explain significant amounts of variation when fit to the validation data (ANOVA, $P > 0.15$).

We examined the 8 valid models for accuracy (Table 3). Mean error rates were too high for all models (>30%). In some cases, estimates were more accurate than for lipid weight but any gains were modest.

Discussion

We were able to construct regression models predicting water and protein content with acceptable levels of accuracy (mean error rate <5%). Water content can be estimated very accuratedly using only external variables. Weight and W_r explained enough variability such that addition of TL or TOBEC did not significantly improve the fit of the model. Similarly, weight and W_r also were the best predictors of protein weight. Although not as accurate as the final model predicting water content, this model was accurate enough for our applications.

None of the models we constructed to predict lipid weight met our standard for accuracy. All generated high percentages of large errors and the mean error rate was much higher than our criterion for acceptance. Even standardizing lipid content did not provide enough precision and accuracy. Estimating lipid content with <30% error evidently requires more precision than our methods provided.

Standardization improved accuracy only slightly. However, validation R^2s of the standardized regressions were much less than the unstandardized models. This suggests that improvements may be possible. The range of lipid content in this study (0.0–3.3 g) was less than that found by Jaramillo et al. (1994, 4.1–12.9 g), or Bai et al. (1994, 0.46–6.82 g). Fitting regressions with a wider range of values may improve precision and accuracy. Incorporating data from other seasons and populations may provide this range.

Our methods differed from those of McComish et al. (1974). For our work, we focused on prediction of weight of proximate components rather than percentages. While percentages are often used to remove the effects of body size on the parameter of interest, errors in both the numerator and denominator can sometimes confound the results (Packard and Boardman 1988). We resorted to standardization only when we were not able to get an accurate predictor. Therefore, our results for prediction of water and protein content are not directly comparable to those of McComish et al.

Qualitatively, none of our validated regressions incorporated the exact same combination of variables that they found to be significant. Obviously, there were some physiological differences between their laboratory population and our wild populations. Their experimental fish were kept individually in indoor aquaria under a constant temperature and hand-fed chironomids (McComish 1971). Wild fish experience alternations of stress and feeding; members of the population may be similar in length, yet one may be starving while the other has just fed. These varying individual histories should be important to proximate composition, especially lipid content. We feel our models should hold for small impoundments in the Virginia piedmont and coastal plain during the summer. Such systems should have similar stresses and energy flow patterns.

Unlike Bai et al. (1994) and Jaramillo et al. (1994), we did not find TOBEC to be a useful addition to the predictive models, even in several of the "best" nondestructive models where it was explicitly considered. We considered the accuracy of several models for each dependent variable except water content. A model including TOBEC was the most accurate only when predicting lipid weight (LIPID). Given the limited nature of our data, we do not reject TOBEC as a useful indicator of proximate composition. It may add resolution when dealing with more extensive datasets.

In summary, we can accurately predict water and protein weight for our bluegill populations at the time of our collections, but not lipid content. A greater range of lipid values needs to be examined. We plan to continue to evaluate the robustness of our models with data collected during different seasons and from different populations. These additional data should provide for a greater range of values and perhaps improve the performance of lipid regressions. We will also further evaluate the utility of TOBEC in these assessments.

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