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Effect of Dietary Lipid Level and Protein Energy Ratio on Growth and Body Composition of Largemouth Bass *Micropterus salmoides*

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Abstract—A feeding trial was conducted in aquaria with juvenile largemouth bass *Micropterus salmoides* to examine the effects of increasing dietary lipid levels on growth and body composition. Feed-trained largemouth bass fingerlings were graded to a similar size (16.3 ± 2.4 g) and randomly stocked into 15 113.6-L glass aquaria at 25 fish/aquarium. Fingerlings were fed twice daily to apparent satiation with one of five isonitrogenous extruded experimental diets based on practical ingredients. Diets contained approximately 40% crude protein and either 0, 5, 10, 15, or 20% added lipid. Due to background lipids in the ingredients, this equated to total lipid levels of 7, 10, 16, 20, and 23%, respectively. These diets had protein to energy ratios of 137, 120, 106, 95, and 86 mg/kcal, respectively. There were three replicate aquaria per dietary treatment. After 12 wk, there were no statistically significant differences ($\underline{P} > 0.05$) in average weight (g), specific growth rate (% body weight/d), survival (%), or protein efficiency ratio (PER, %) among fish fed the five diets, which averaged 79.3 ± 5.6, 1.9 ± 0.1, 99.5 ± 1.5, and 2.11 ± 0.19, respectively. Juvenile largemouth bass fed diets containing 15 and 20% added lipid had significantly lower ($P \leq 0.05$) feed conversion ratios (FCR) (1.1 ± 0.0 and 1.1 ± 0.1, respectively) than fish fed diets containing 0, 5, and 10% added lipid (1.4 ± 0.1, 1.3 ± 0.1, and 1.3 ± 0.2, respectively). Proximate analysis of whole body samples indicated a significantly higher ($P \leq 0.05$) lipid content in fish fed 15 and 20% added lipid compared to fish fed lower lipid levels. While FCR was lowest in fish fed the 15 and 20% added lipid diets, increased whole body lipid deposition may indicate that these levels are above optimal levels for juvenile largemouth bass. It appears that 7–16% total dietary lipid (P/E:137–106 mg/kcal) is sufficient to support efficient growth without impacting body composition in juvenile largemouth bass when fed a diet containing 40% crude protein.

Largemouth bass *Micropterus salmoides* (LMB) are recognized as one of the most commercially important freshwater sport fish in North America (Coyle et al. 2000). Traditionally, production of small juveniles for stock enhancement has been the focus of LMB production. Due to newly-emerging markets for larger fish (> 1 kg), such as for corrective pond stocking and as a live food fish in ethnic Asian markets, demand has increased in recent years. This demand has been identified to be in excess of 318,000 kg/yr at > $6.60 per kg (Tidwell et al. 2000).

Generally, feed costs constitute one of the largest components of production costs for most aquaculture operations, and protein is the most expensive dietary component in commercial feeds. Diets for carnivorous species such as largemouth bass usually contain not only high levels of crude protein (> 40% of diet); but also contain high levels of expensive fish meal (> 25% of diet). In some species, dietary protein levels can be decreased by increasing non-protein energy sources. By supplying sufficient non-protein energy, the catabolism of protein for energy is minimized (Lovell 1989). This is generally referred to as protein sparing. Although, dietary lipid can be used as a non-protein energy source, excessive dietary lipid levels can have a negative impact on body composition (Nematipour et al. 1992; Tidwell et al. 1996). Therefore, evaluating the effects of increasing non-protein energy through the supplementation of lipids is important.

Research has been conducted on protein with regards to dietary requirements (Anderson et al. 1981; Tidwell et al. 1996), essential amino acid and highly unsaturated fatty acids (HUFAs) (Coyle et al. 2000), as well as carbohydrate tolerance (Goodwin et al. 2000) of largemouth bass. However, the influence of varying dietary lipid and subsequent changes in the protein/energy ratios has not yet been evaluated. Coyle et al. (2000) suggested that further research is needed to determine these parameters for largemouth bass, as a precursor to the formulation of species-specific production diets. Consequently, the objective of this study was to evaluate the effect of increasing levels of added lipid, and the resulting range of protein/energy ratios, on the growth and body composition of juvenile largemouth bass.

Materials and Methods

Experimental Diets

Five practical diets were formulated to be iso-nitrogenous and to contain approximately 40% crude protein. Lipid in the form of cod liver oil was supplemented at either 0, 5, 10, 15, or 20% of the total formulation. Due to background lipids in the ingredients, this equated to total lipid levels of 7, 10, 16, 20, and 23%, respectively (Table 1). This produced protein to energy ratios of approximately 137, 120, 106, 95, and 86 mg/kcal, respectively. All diets contained fixed amounts of fish meal, soybean meal, and wheat. Levels of digestible carbohydrate were held constant among all diets by decreasing the inclusion of non-nutritive filler (Celufil; USB Corp., Cleveland, Ohio, USA).

Diet ingredients were homogenized in a mixer for even distribution of water and lipid. In the experimental diets that received added lipid, 2% of the total formulation (by weight) was added into the ingredient mixture and homogenized. If the experimental diet formulation required greater than 2% added lipid, additional amounts were top-coated onto finished pellets. After thorough mixing, moist diet ingredients were extruded through a 3-mm diameter die using a Wenger X85 single-screw cooker-extruder (Wenger Manufacturing, Inc., Sabetha, Kansas, USA) and were cut to a length of 3 mm. Moist pellets were air dried for 24 h, then transferred to an oven and dried at 150 C until pellets contained < 10% moisture to prevent mold growth. Pellets were top-coated with additional fish oil (if required). Diets were stored at -20 C until fed. Two samples of each diet were analyzed for proximate analysis (Table 1) by a commercial laboratory (Woodson-Tenant Laboratories, Dayton, Ohio, USA). Protein/energy ratios for experimental diets were calculated based on ingredient composition tables (Table 1).

Fish and Experimental System

Feed-trained largemouth bass fingerlings were purchased from a commercial producer (Mayer's Fish Farm, Bardstown, Kentucky, USA), graded to a similar size (16.3 ± 2.4 g), and randomly stocked into 15 113.6-L glass aquaria at 25 fish/aquarium. There were three replicate tanks per experimental diet. Five fish were taken prior to stocking for baseline whole body proximate analyses. For a 1-wk conditioning period, fish were allowed to acclimate to aquaria and were fed a commercial trout diet (Nelson's Silver Cup Fish Food, Murray, Utah, USA) once daily. For the next 12 wk, fish were fed twice daily (0800 and 1600 h) to apparent satiation over 30 min, with one of the five experimental diets. The amount of diet fed was recorded at the end of each day.

Water temperature was maintained at 26.1 ± 0.97 C by ambient temperature control. Overhead fluorescent lights (Philips, New York, New York, USA) with a manual control switch (TORK, Mount Vernon, New York, USA) were used to maintain a photoperiod schedule 12 h light/12 h dark. Water quality was maintained by recirculation through a common biofilter (Bubble Bead Filtration Systems Inc., Model BBF-10, Marion, Texas, USA) so that all aquaria shared similar water chemistry. Dissolved oxygen (DO) and temperature were monitored twice daily using a YSI 85 dissolved oxygen meter (YSI Company, Inc., Yellow Springs, Colorado, USA). Levels of total ammonia-nitrogen (TAN), un-ionized ammonia, nitrite-nitrogen, and pH were monitored three times/week with a spectrophotometer (HACH Odyssey DR 2500 spectrophotometer, HACH Company, Loveland, Colorado, USA). Alkalinity was monitored three times/wk by titration (HACH digital titrator, HACH Company, Loveland, Colorado, USA). Salinity was maintained at 0.3 ppt.

Harvest Procedures

After 12 wk, fish from each individual aquarium were harvested, bulk weighed, then individually weighed and measured (total length) and counted. Nine fish from each tank were randomly selected, anesthetized with quinaldine sulfonate (Tranquil, Sure-Life Laboratories, Seguin, Texas, USA), and processed for body composition analyses. Three whole fish samples, three white muscle

TABLE 1. *The formulated (% as-is) and analyzed composition of the five diets formulated to be isonitrogenous with different levels of lipid.*

	Lipid Supplement (%)				
Ingredient	0	5	10	15	20
Fish meal	30	30	30	30	30
Soybean meal	37	37	37	37	37
Wheat	15.6	15.6	15.6	15.6	15.6
Cod liver oil	0	5.0	10.0	15.0	20.0
Choline chloride	0.5	0.5	0.5	0.5	0.5
Mineral mix	0.3	0.3	0.3	0.3	0.3
Vitamin C	0.2	0.2	0.2	0.2	0.2
Vitamin mix	0.4	0.4	0.4	0.4	0.4
Di-Cal-P	1	1	1	1	1
Celufil	15.0	10.0	5.0	2.5	0.0
Analyzed Composition (as fed)					
Moisture (%)	11.5 ± 0.1	9.8 ± 0.1	7.9 ± 0.2	7.4 ± 0.1	5.3 ± 0.0
Protein (%)	38.4 ± 0.8	39.2 ± 0.3	40.0 ± 0.4	41.1 ± 0.7	40.7 ± 1.5
Ash (%)	8.4 ± 0.1	9.6 ± 0.1	8.5 ± 0.2	8.6 ± 0.0	8.5 ± 0.1
Lipid (%)	7.3 ± 0.2	10.1 ± 0.5	16.4 ± 0.3	19.7 ± 0.2	22.8 ± 0.4
NFE (%)	24.6 ± 0.4	25.8 ± 1.8	24.6 ± 0.8	22.5 ± 0.0	21.2 ± 1.8
Energy[a]	3.04	3.49	3.94	4.39	4.84
P/E (mg/kcal)[b]	137	120	106	95	86

[a]*Gross energy values of 4.0, 4.0, and 9.0 kcal/g for carbohydrate, protein, and lipid, respectively (Garling and Wilson 1976; Nematipour et al. 1992; Webster et al. 1995).*
[b]*P/E = protein to energy ratio in mg protein/kcal.*

samples from fillets, and three liver samples from individual fish in each aquarium were obtained, individually homogenized, and submitted for proximate analysis (Woodson-Tenant Laboratories, Dayton, Ohio, USA).

Statistical Analysis

Growth performance parameters were calculated as follows: Specific growth rate (SGR, % body wt/d) was calculated from SGR = [(ln W_f - ln W_i/t] X 100, where W_f = final weight (g), W_i = initial weight (g), and t = time in days. Protein efficiency ratio was calculated as PER = weight gain (g)/total protein fed (g). Feed conversion ratio was calculated as FCR = dry feed intake (g)/total wet weight gain (g). Hepatosomatic index was calculated on individuals as HSI = weight of liver (g)/weight of whole body (g). Data were analyzed by analysis of variance (ANOVA) using Statistix version 7.0 (Analytical Software, Tallahassee, Florida, USA) to determine treatment effects of the different diets on growth performance. If ANOVA indicated significant treatment effects ($P \leq 0.05$), the Least Significant Difference test (LSD) was used to determine differences among treatment means (Steel and Torrie 1980). All percentage and ratio data were transformed to arc sin values prior to analysis (Zar 1984). Data are presented in the untransformed form to facilitate interpretation.

Results

Over the 12-wk period, temperature, dissolved oxygen, total ammonia-nitrogen, un-ionized ammonia-nitrogen, nitrite-nitrogen, pH, and alkalinity averaged: 27.3 C, 5.5 mg/L, 0.40 mg/L, 0.02 mg/L, 0.15 mg/L, 7.91, and 87.03 mg/L, respectively.

After 12 wk, there were no significant differences ($P > 0.05$) in average harvest weight, SGR, PER, or survival rates among fish fed the five diets, which averaged 79.2 g, 1.9 %/d, 2.11, and 99.5%, respectively. Juvenile largemouth bass fed diets containing 20 and 23% total lipid (15 and 20% added lipid) had significantly lower ($P \leq 0.05$) feed conversion ratios (1.10 and 1.12, respectively) than

TABLE 2. *Means (± SEM) of average harvest weight, specific growth rate (%), survival, protein efficiency ratio (PER), feed conversion ratio (FCR), and hepatosomatic indices (HSI) of juvenile largemouth bass (initial weight 16.3 ± 2.4 g) fed five extruded isonitrogenous diets containing different levels of added lipid (0, 5, 10, 15, 20%). Values are means of three replications per diet. Significant differences (*P *< 0.05) are indicated by different letters following values within a row.*

	Lipid Supplement (%)				
	0	5	10	15	20
Harvest weight (g)	1.38 ± 2.30a	84.81 ± 2.00a	77.07 ± 4.98a	74.67 ± 3.08a	77.87 ± 3.15a
Total diet fed (kg)	2.19 ± 0.02a	2.10 ± 0.09a	1.89 ± 0.05ab	1.56 ± 0.08b	1.68 ± 0.03b
SGR (%/d)	1.90 ± 0.03a	1.94 ± 0.03a	1.83 ± 0.08a	1.79 ± 0.0a5	1.84 ± 0.05a
Survival (%)	98.67 ± 1.33a	98.67 ± 1.33a	100 ± 0.00a	100 ± 0.00a	100 ± 0.00a
PER	1.94 ± 0.06a	2.08 ± 0.06a	2.02 ± 0.15a	2.28 ± 0.01a	2.25 ± 0.10a
FCR	1.40 ± 0.04a	1.28 ± 0.03a	1.30 ± 0.09a	1.10 ± 0.01a	1.12 ± 0.05a
HSI	1.52 ± 0.08b	1.66 ± 0.09b	2.30 ± 0.07a	2.40 ± 0.16a	2.58 ± 0.09a

TABLE 3. *Means of whole-body, white muscle, and liver percentage moisture, fat, protein, and ash (wet-weight basis) of largemouth bass fed five extruded isonitrogenous diets containing different levels of added lipid (0, 5, 10, 15, 20%). Values are means of three replications per diet. Significant differences (*P *< 0.05) are indicated by different letters following values within a row.*

	Lipid Supplement (%)				
Analysis	0	5	10	15	20
Whole Body					
Moisture	68.3 ± 0.5a	68.0 ± 1.1a	67.2 ± 1.2ab	65.4 ± 0.7c	65.9 ± 0.9bc
Fat	7.5 ± 0.6c	8.1 ± 0.7bc	9.8 ± 0.4b	12.3 ± 2.1a	12.0 ± 0.8a
Protein	18.6 ± 0.1a	17.5 ± 0.5a	17.1 ± 0.5a	17.5 ± 1.8a	17.0 ± 1.1a
Ash	4.9 ±0.2a	5.0 ± 0.5a	4.0 ± 0.5b	4.4 ± 0.1ab	3.9 ± 0.6b
White Muscle					
Moisture	76.0 ± 0.4a	76.1 ± 1.0a	76.0 ± 0.7a	76.8 ± 0.8a	75.5 ± 0.8a
Fat	1.6 ± 0.4b	1.9 ± 0.4b	2.8 ± 0.4a	3.0 ± 0.6a	2.9 ± 0.5a
Protein	19.7 ± 1.0a	20.7 ± 1.1a	19.3 ± 0.4a	20.2 ± 0.8a	19.4 ± 1.4a
Ash	1.3 ± 0.0a	1.3 ± 0.1a	1.3 ± 0.1a	1.3 ± 0.1a	1.3 ± 0.1a
Liver					
Moisture	68.3 ± 0.4a	68.6 ± 0.8a	67.9 ± 0.6a	70.1 ± 1.5a	68.4 ± 0.9a
Fat	2.7 ± 1.3b	2.3 ± 0.5b	1.3 ± 0.7b	4.0 ± 0.3a	2.2 ± 0.6b
Protein	10.8 ± 2.2a	13.2 ± 1.9a	10.8 ± 0.6a	11.4 ± 0.6a	10.9 ± 1.2a
Ash	0.7 ± 0.3a	0.8 ± 0.2a	0.8 ± 0.1a	0.8 ± 0.2a	0.8 ± 0.1a

fish fed diets containing 7% (1.40), 10% (1.28), and 16% (1.30) total lipid (Table 2). Fish fed diets with 7 and 10% total lipid consumed significantly ($P \leq 0.05$) more diet (2.2 kg and 2.1 kg, respectively) than fish fed 16% total lipid (1.9 kg), which was significantly higher ($P \leq 0.05$) than fish fed 20% (1.6 kg) and 23% (1.7 kg) total lipid (Table 2). Proximate analysis of whole fish indicated that fish fed diets with 7% total lipid had significantly lower ($P \leq 0.05$) whole body lipid levels than fish fed 16, 20 and 23% total lipid diets (Table 3). Fish fed 10% total lipid had significantly lower whole body lipid levels ($P \leq 0.05$) than those fed 20 and 23% total lipid, but were not significantly different ($P > 0.05$) from fish fed either 7 or 16% total lipid (Table 3). Whole body proximate analysis also indicated that fish fed the 7% total lipid diet had a significantly higher ($P \leq 0.05$) moisture content than fish fed 20 and 23% total lipid, while fish fed 16% total lipid were significantly higher ($P \leq 0.05$) in moisture content than those fed 20% total lipid (Table 3). There were no significant differences (*P*

> 0.05) in protein efficiency ratio (PER) between treatments which averaged 2.11%, overall.

Proximate analysis of white muscle tissue indicated a significantly higher ($P \leq 0.05$) lipid content among fish fed 16, 20, and 23% added lipid compared to fish fed lower lipid inclusion levels (Table 3). Proximate analysis of liver tissue indicated that fish fed the diet with 20% added lipid had significantly higher ($P \leq 0.05$) fat content than fish fed diets with other lipid inclusion levels (Table 3). The HSI for fish fed diets containing 16% (2.3), 20% (2.4), and 23% (2.6) total lipid were significantly higher ($P \leq 0.05$) than in fish fed the 7% (1.5) and 10% (1.7) total lipid diets (Table 2).

Discussion

Commercial diets currently used in largemouth bass production typically contain 10–15% lipid with protein to energy (P/E) ratios of approximately 110 mg/kcal (personal communication, Chris Nelson, Nelson's Silver Cup Fish food, Murray, Utah, USA). Based on this study, the range of suitable protein to energy ratios for juvenile largemouth bass appears to be wide, with no improvements in growth among fish fed experimental diets ranging from 86 mg/kcal to 137 mg/kcal. Lipid deposition increased in fish fed diets with ≥ 20% total lipid, which may indicate that lower lipid levels of 7–10% total lipid (P/E of 137–120) may be advantageous to minimize lipid deposition.

In this study, fish fed lipid inclusions of 16–23% of diet resulted in increased lipid deposition in white muscle tissue. However, in whole body samples, increased lipid deposition was apparent only in fish fed diets containing 20 and 23% total lipid. Lovell (1989) stated that growth may include increased fat deposition in fish flesh that should not be confused with "true growth," which should reflect an increase in structural tissues such as muscle, bone, and organs. In food fish, excess body fat may be undesirable (NRC 1993), and excessive lipid reserves in tissues may ultimately lead to reduced growth due to appetite suppression (Watanabe et al. 2001; Jobling et al. 2002). However, for fish that are to be stocked into natural waters, higher lipid levels may represent reserves needed for increased survival (Lovell 1989). Therefore, appropriate lipid levels in diets of largemouth bass may depend on their ultimate use.

Fish fed diets with 20 and 23% total lipid resulted in significantly ($P < 0.05$) lower feed conversion ratios. Because fish eat to satisfy their energy requirement (NRC 1993), these lower feed conversion ratios may be explained by the fish consuming less diet at the higher lipid concentrations. Fish fed 20 and 23% total lipid diets consumed 1.6 and 1.7 kg of diet, which was significantly less than fish fed 7, 10, and 16% lipid diets (2.2, 2.1, and 1.9 kg, respectively). However, diets with higher lipid inclusions appear to exceed the energy demands of the fish, as evidenced by increased fat deposition. Gaylord and Gatlin (2000) state that relative changes in growth and feed utilization as well as lipid deposition in the body should give a reasonable indication of when dietary energy is adequate or excessive. Based on this study, it appears that lipid inclusions equal to or greater than 16% will result in increased lipid stores that may not be beneficial for juvenile largemouth bass.

Some similarities can be observed between the nutrient requirements of largemouth bass and hybrid striped bass *(Morone saxatilis* X *M. chrysops)*. Based on this study, a P/E ratio of 106–137 may be advantageous for largemouth bass food fish production. This range is similar to the optimal P/E reported for hybrid striped bass (112 mg/kcal) (NRC 1993). However, P/E is higher than the reported optimum for other commonly cultured finfish such as: channel catfish *Ictalurus punctatus* (Garling and Wilson 1976), 81–97 mg/kcal; rainbow trout *Onchorynchus mykiss* (Cho and Woodward 1989), 92–105 mg/kcal; red drum *Sciaenops ocellatus* (Daniels and Robinson 1986), 98 mg/kcal; nile tilapia *Oreochromis nilotica* (El-Sayed 1987), 103 mg/kcal; and common carp *Cyprinus carpio*, 108 mg/kcal (Takeuchi et al. 1979). Gaylord and Gatlin (2000) reported that hybrid striped bass fed lipid levels > 15% resulted in increased lipid deposition in whole body and liver tissues, as well as the peritoneal cavity. The authors state that an inclusion of 10% dietary lipid should be used in diets for hybrid striped bass for the most rapid weight gain, which is similar to the results of this study with largemouth bass.

In summary, it appears that 7–16% total lipid (P/E of 137–106 mg/kcal) is sufficient to support good growth in juvenile largemouth bass when fed a diet containing 40% crude protein. Growth was similar and FCR was lower in fish fed diets containing 20 and 23% total lipid, but increased lipid deposition in tissues indicate that these levels are possibly above optimal levels for juvenile largemouth bass.

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Literature Cited

Anderson, R. J., E. W. Kleinholz, and S. A. Flickinger. 1981. Protein requirements of smallmouth bass and largemouth bass. Journal of Nutrition 111:1085–1097.

Cho, C.Y. and B. Woodward. 1989. Studies on the protein-to-energy ratio in diets for rainbow trout (*Salmo gairdneri*). European Association for Animal Production Publication 43. Wageningen, Netherlands, UK.

Coyle, S. D., J. H. Tidwell, and C. D. Webster. 2000. Response of largemouth bass *Micropterus salmoides* to dietary supplementation of lysine, methionine, and highly unsaturated fatty acids. Journal of the World Aquaculture Society 31:89–95.

Daniels, W. H. and E. H. Robinson. 1986. Protein and energy requirements of red drum. Aquaculture 53:232–243.

El-Sayed, A. M. 1987. Protein and energy requirments of *Tilapia zillii*. Doctoral dissertation. Michigan State University, East Lansing, Michigan, USA.

Garling, D. L. and R. P.Wilson. 1976. Optimum dietary protein to energy ratio for channel catfish fingerlings, *Ictalurus punctatus*. Journal of Nutrition 106:1368–1375.

Gaylord, T. G. and D. M. Gatlin III. 2000. Dietary lipid level but not L-carnitine affects growth performance of hybrid striped bass (*Morone chrysops* X *M. saxatilis*). Aquaculture 190:237–246.

Goodwin, A. E., D. M. Tieman, R. T. Lochmann, and A. J. Mitchell. 2000. Massive hepatic necrosis and nodular regeneration in largemouth bass fed feeds high in available carbohydrate. Page 16 *in* R. C. Cipriano, editor. 25th Annual Eastern Fish Health Workshop, Plymouth, Massachusetts, USA.

Jobling, M., A. V. Larsen, B. Andreassen, and R. L. Olsen. 2001. Adiposity and growth of post-smolt Atlantic salmon (*Salmo salar*). Aquaculture Research 33:533–541.

Lovell, R. T. 1989. Nutrition and feeding of fish. Van Nostrand Reinhold, New York, New York, USA.

National Research Council (NRC). 1993. Nutrient requirements of fish. National Academy Press, Washington, D.C., USA.

Nematipour, G. R., M. L. Brown, and D. M. Gatlin III. 1992. Effects of dietary energy:protein ratio on growth characteristics and body composition of hybrid striped bass, *Morone chrysops* X *M. saxatilis*. Aquaculture 107:359–368.

Steel, R. G. D. and J. H. Torrie. 1980. Principles and procedures of statistics, 2nd edition. McGraw-Hill, New York, New York, USA.

Takeuchi, T., T. Wantanabe, and C. Ogino. 1979. Optimum ratio of dietary energy to protein for carp. Bulletin of the Japanese Society of Scientific Fisheries 45:983–987.

Tidwell, J. H., S. D. Coyle, and T. A. Woods. 2000. Species profile largemouth bass. SRAC Publication 722, Stoneville, Mississippi, USA.

Tidwell, J. H., C. D. Webster, and S. D. Coyle. 1996. Effects of dietary protein level on second year growth and water quality for largemouth bass (*Micropterus salmoides*) raised in ponds. Aquaculture 145:213–223.

Watanabe, W. O., S. C. Ellis, and J. Chaves. 2001. Effects of dietary lipid and energy to protein ratio on growth and feed utilization of juvenile mutton snapper *Lutjanus analis* fed isonitrogenous diets at two temperatures. Journal of the World Aquaculture Society 32(1):30–40.

Webster, C. D., L. G. Tiu, J. H. Tidwell, P. Van Wyke, and R. D. Howerton. 1995. Effects of dietary protein and lipid levels on growth and body composition of sunshine bass (*Morone chrysops* X *M. saxatilis*) reared in cages. Aquaculture 131:291–301.

Zar, J. H. 1984. Biostatistical analysis, 2nd edition. Prentice-Hall, Englewood Cliffs, New Jersey, USA.