

Effect of Water Temperature on Growth, Survival, and Biochemical Composition of Largemouth Bass *Micropterus salmoides*

JAMES H. TIDWELL, SHAWN D. COYLE, LEIGH ANNE BRIGHT,
AARON VANARNUM, AND DAVID YASHARIAN

Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky 40601 USA

Abstract

The effects of water temperature on growth rate, survival, and biochemical composition of juvenile largemouth bass *Micropterus salmoides* were evaluated under controlled conditions in tanks for 12 wk. Feed-trained juvenile largemouth (9.1 ± 1.9 g) were stocked into nine 3,610-L polyethylene tanks inside a greenhouse structure at 140 fish/m³ (500 fish/tank). Three treatment temperatures were evaluated (20, 26, and 32 C) with three replicates per treatment. Bass were fed to apparent satiation twice daily using a commercially available floating salmonid diet (45% crude protein and 16% lipid). After 97 d bass grown at 26 and 32 C had significantly higher ($P < 0.05$) average weights, SGR, condition factor (K), and production rates (kg/m³) than those in the 20 C treatment. Bass in the 26 C treatment had significantly lower ($P < 0.05$) FCR and higher percent protein deposit (PPD) than bass raised at 20 and 32 C, which were not significantly different ($P > 0.05$). There were no significant differences ($P > 0.05$) in survival among treatments which averaged 97%, overall. Bass raised at 32 C had higher ($P < 0.05$) lipid levels in the liver than other treatments. Whole body amino acid concentrations were not significantly impacted ($P > 0.05$) by culture temperature. Largemouth bass raised at 26 C had significantly lower ($P < 0.05$) levels of stearic acid (18:0) and significantly higher ($P < 0.05$) levels of arachidonic acid (20:4 n-6) than bass raised at 20 or 32 C. Bass raised at 32 C had significantly higher ($P < 0.05$) levels of linoleic acid (18:2 n-6), total saturates, and dienes than bass raised at 26 or 20 C. These data indicate that growth and feed conversion efficiency are reduced at 20 C. Growth is similar at 26 and 32 C, but feed and dietary protein are more efficiently utilized at 26 C.

The largemouth bass represents one of the most important freshwater fish in North America in terms of sportfishing activities and expenditures. In recent years interest in the production of largemouth of larger sizes for sales into both sportfish (> 50 g) and foodfish markets (> 500 g) has increased. Asian consumers appear to desire live largemouth bass above other freshwater fish, and demand for large largemouth bass has grown dramatically, and at times exceeds availability. These factors have resulted in increasing interest in them as an aquaculture species, not only in the U.S. but in Europe and Asia as well.

Temperature is the single-most pervasive environmental factor in poikilothermic animals (Stickney 1979). Temperature can affect fish growth directly by controlling feed consumption, nutrient requirements, and

food passage time (Smith 1989). Culture temperature may also affect the amount of lipid deposited, as well as its fatty acid profile, due to the role of unsaturated fatty acids in maintaining bio-membrane fluidity at different environmental temperatures (Sargeant et al. 1989). These changes could affect the nutrient demands of the fish, as well as their organoleptic attributes, when raised at different temperatures.

Previous work has demonstrated that largemouth bass may contain unusually high levels of the polyunsaturated fatty acid docosahexaenoic acid (DHA, 22:6 n-3) for a warmwater, freshwater fish (Tidwell et al. 1996). These concentrations are even higher than those found in coldwater fishes such as salmon. High concentrations of DHA may make largemouth desirable as a human food item as this is a fatty acid identified

as being advantageous for cardiovascular health in humans (Nettleton 1985). However, as a highly unsaturated fatty acid normally found in coldwater fishes, its high concentration in largemouth, which is considered a warmwater fish is unexplained. The biochemical analysis of largemouth reared at different temperatures could be advantageous to a better understanding of the role of polyunsaturated fatty acids in largemouth bass nutrition.

Properties of fish tissue proteins can be affected by the environmental temperature at which they are synthesized (Love 1980). If differences are of significant magnitude, nutritional requirements could potentially differ for fish cultured at different temperatures. Catfish diets for winter feeding normally contain less protein than those used at higher temperatures (Lovell 1989). Practical and profitable production of largemouth bass requires that efficient and economical feeds be formulated which support rapid growth. Since most bass are grown in ponds and actively feed at temperatures lower than better studied species such as catfish (Tidwell et al. 1996), it could be that adjustments in feed formulations for seasonally different culture temperatures may be justified for largemouth bass.

The objective of the study was to evaluate the effect of water temperature on growth, survival, and biochemical composition of largemouth bass raised at different temperatures.

Materials and Methods

The study was conducted in nine circular 3,610-L polyethylene tanks (Polytank Inc. Litchfield, Minnesota, USA) housed within a greenhouse located at the Aquaculture Research Center, Kentucky State University, Frankfort, Kentucky, USA. The greenhouse was covered with a shade cover to reduce ambient light levels by 60%. On 29 August 2000 each tank was stocked with 650 feed trained juvenile largemouth bass (average weight, 9.1 ± 1.9 g; total length, 8.9 ± 0.7 cm, $N = 150$). Fish were fed a

commercial salmonid diet (45% crude protein, 16% crude lipid, Nelson and Sons, Murrumbidgee, Utah, USA) to apparent satiation twice daily.

Each tank received a constant water flow of approximately 4 L/min. Dissolved oxygen levels were maintained by constant aeration. Water was recirculated through three heat pump units (AquaLogic, San Diego, California, USA) to maintain temperatures, with each heat pump supplying three replicate tanks. Each temperature treatment (three tanks) was constantly recirculated through its associated heat pump to maintain temperature. Approximately 25% of the total water volume of each three tank system was replaced daily by a constant flow of approximately 1 L/min of new water from a storage reservoir. After initial stocking, temperatures in all tanks were maintained at 24 C for a 7-d conditioning period. After the conditioning period, temperatures were gradually adjusted over a 5-d period to achieve treatment temperatures of 20, 26, and 32 C. Target temperatures were chosen to represent approximate overall summer temperatures in the northern to southern extremes of the potential culture regions.

Water temperature and dissolved oxygen concentrations were determined twice daily at approximately 0900 and 1500 h using a YSI Model 57 dissolved oxygen meter (YSI Instruments Co., Yellow Springs, Ohio, USA). Total ammonia-nitrogen (TAN) and nitrite-nitrogen were determined weekly, according to outlined procedures for a HACH DR/2000 spectrophotometer (Hach Co., Loveland, Colorado, USA), using water samples collected from each tank at approximately 1300 h. The pH was determined weekly at approximately 1300 h using an electronic pH meter (Hanna Instruments, Ltd., Mauritius). Un-ionized ammonia was calculated based on TAN, water temperature, and pH according to Boyd (1979). A sample of > 50 fish from each tank was collected every 3 wk, group

TABLE 1. Overall means (\pm SD) for total ammonia-nitrogen, un-ionized ammonia, nitrite-nitrogen, pH, and dissolved oxygen concentration in tanks in which largemouth bass were raised at three water temperatures. Means within a row followed by different letters are significantly different ($P < 0.05$).

Variable	Culture temperature (C)		
	20	26	32
Total ammonia-nitrogen (mg/L)	0.73 \pm 0.00 b	0.99 \pm 0.17 a	0.82 \pm 0.02 ab
Un-ionized ammonia (mg/L)	0.29 \pm 0.02 c	0.37 \pm 0.01 b	0.40 \pm 0.01 a
Nitrate (mg/L)	0.13 \pm 0.01 c	0.20 \pm 0.00 b	0.22 \pm 0.00 a
pH	8.86 \pm 0.02 a	8.75 \pm 0.03 b	8.74 \pm 0.02 b
Dissolved oxygen (mg/L)	7.6 \pm 0.1 a	5.9 \pm 0.2 b	5.1 \pm 0.1 b
Dissolved oxygen (% saturation)	85.7 \pm 1.1 a	73.0 \pm 2.0 b	68.3 \pm 1.5 c

weighed to the nearest gram, counted, and returned to their respective tank.

On 7 December 2000 all fish in each tank were captured, bulk weighed, and counted. From each tank 50 randomly selected individuals were also individually weighed and measured for total length. Twenty fish from each tank were randomly selected for chemical analysis. Ten fish from each tank were sacrificed for proximate analysis. Five whole fish and the livers of five fish from each tank were separately homogenized in a blender, and a pooled sample of each was frozen for subsequent proximate analyses by a commercial analytical laboratory (Woodson-Tenent Laboratories, Dayton, Ohio, USA). A separate composite sample of five fish from each tank was homogenized, frozen, and stored (-40 C) for subsequent amino acid analyses. Additionally, five fish from each tank were sacrificed and a composite sample of livers, and a composite sample of white muscle (removed from the fillet mid-section), were separately homogenized in a blender and immediately frozen in liquid nitrogen (-196 C) and stored (-40 C) for subsequent fatty acid analyses.

Specific growth rate (SGR, % body wt/d) was calculated as $SGR = [(\ln W_f - \ln W_i)] \times 100$, where W_f = mean weight at the end of the period, W_i = mean weight at the beginning of the period, and t = time in days of the period (Ricker 1975). Feed conversion ratio (FCR) was calculated as $FCR = \text{weight of feed fed (g)/live weight}$

gain (g). Percentage protein deposited (PPD) was calculated as $[(\text{final body protein} - \text{initial body protein}) \times 100] / \text{total protein fed}$. Condition factor (K) was calculated from $K = 100 \times W \cdot L^{-3}$, where W = weight (g) and L = total length (cm) (Weatherly and Gill 1987).

Data were analyzed by one-way analysis of variance (ANOVA) using Statistix version 4.1 (Statistix Analytical Software 1994) to determine the effects of temperature on growth, condition factor, feed conversion, body composition, and survival. If ANOVA indicated significant differences, means were separated using Fisher's Least Significant Difference (LSD) test (Steel and Torrie 1980). All percentage and ratio data were transformed to arc sin values prior to analysis (Zar 1984). Results are presented in their untransformed form to facilitate interpretation.

Results and Discussion

Measured temperatures were maintained very near target treatment temperatures, averaging (\pm SD) 20.5 ± 0.0 , 26.5 ± 0.5 , and 31.3 ± 0.1 C over the study period. Dissolved oxygen concentrations averaged over the duration of the study were significantly different ($P < 0.05$) between all three treatments (Table 1). This was primarily due to the decreased solubility of oxygen at increasing water temperatures (Boyd 1979). However, when oxygen concentrations were compared as percentage of saturation, values still differed significantly

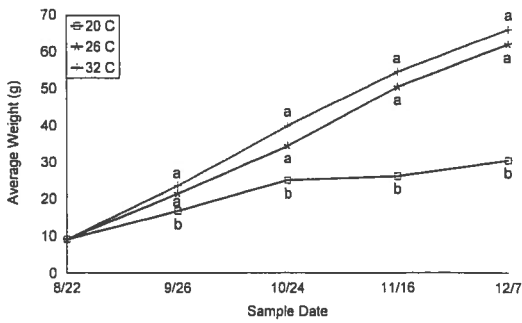


FIGURE 1. Relationship between largemouth bass body weight and sample date when raised at three culture temperature (20, 26, and 32 C). Each symbol represents the mean of three replicate tanks. Sample means with different letters are significantly different ($P < 0.05$).

between culture temperatures ($P < 0.05$) in an inverse relationship (Table 1). These decreases in oxygen concentrations beyond those accounted for by the direct effect of temperature on water's ability to dissolve oxygen, are likely due to demands created by increased metabolism of fish at higher temperatures. Increasing water temperature increases both maintenance demand and activity levels of fish (Weatherly and Gill 1987). Oxygen consumption is considered the best measure of increased metabolism in fish and represents the summation of all other metabolic processes (Coutant 1975).

Total ammonia-nitrogen (TAN) concentrations were significantly higher ($P <$

0.05) in the 26 C treatment tanks than in the 20 C treatment. There was no significant difference in TAN in the 20 C and 32 C treatments ($P > 0.05$) (Table 1). The concentration of un-ionized ammonia was significantly higher ($P < 0.05$) in the 32 C treatment than in the 26 and 20 C treatment (Table 1). This was primarily due to the increasing amount of TAN shifted to the un-ionized form at increasing temperatures (Boyd 1979). Nitrite concentrations were also significantly higher ($P < 0.05$) at the higher culture temperatures (Table 1), although levels were not likely sufficient to cause health problems as largemouth bass are known to be very tolerant of nitrite (Palachek and Tomasso 1984). pH was significantly higher ($P < 0.05$) in the 20 C treatment tanks than in the 26 or 32 C tanks, which were not different ($P > 0.05$) from each other (Table 1).

The growth pattern of bass raised at different culture temperatures is shown in Fig. 1. Consistent differences were established by the first sample date. After 97 d, the average weight of bass cultured at 20 C was significantly lower ($P < 0.05$) than fish in the 26 and 32 C treatments, which were not significantly different from each other ($P > 0.05$) (Table 2). Bass grown at 26 and 32 C had significantly higher ($P < 0.05$) weight gain (%), SGR (%), condition factors (K),

TABLE 2. Final average weights, production, feed conversion, survival, weight gains, specific growth rates, and protein efficiency ratio, condition factor (K), and hepatosomatic index (HSI) for largemouth bass raised at three temperatures for 97 d. Values are mean \pm SE of three replicates. Means within a row followed by different letters are significantly different ($P < 0.05$) by ANOVA.

Variable	Culture temperature (C)		
	20	26	32
Average weight (g)	30.5 \pm 0.8 b	61.9 \pm 0.6 a	66.1 \pm 3.6 a
Production (kg/m ³)	2.8 \pm 0.02 b	7.12 \pm 0.04 a	6.63 \pm 0.61 a
Feed conversion ratio	1.2 \pm 0.0 a	1.0 \pm 0.0 b	1.1 \pm 0.1 a
Survival (%)	96.8 \pm 1.9 a	97.7 \pm 1.3 a	86.5 \pm 9.8 a
Weight gain (%)	325.1 \pm 1.8 b	666.2 \pm 2.9 a	627.4 \pm 48.9 a
Specific growth rate (%/d)	1.3 \pm 0.0 b	2.0 \pm 0.0 a	1.9 \pm 0.1 a
Percent protein deposited	33.4 \pm 1.0	39.9 \pm 1.6 a	32.5 \pm 3.5 b
K factor	1.3 \pm 0.1 b	1.4 \pm 0.0 a	1.4 \pm 0.1 a
HSI	2.6 \pm 0.4 a	2.3 \pm 0.2 a	2.0 \pm 0.34 a

TABLE 3. Proximate composition of whole body, white muscle tissue, and livers (% of wet weight) of largemouth bass raised at three temperatures for 97 d. Values are means \pm SE of three replicates. Means within a row followed by different letters are significantly different ($P < 0.05$) by ANOVA.

Variable	Culture temperature (C)		
	20	26	32
Whole body			
Moisture (%)	69.9 \pm 0.9 b	71.7 \pm 0.2 a	72.3 \pm 1.1 a
Protein (%)	17.4 \pm 0.3 a	17.3 \pm 0.5 a	17.0 \pm 0.1 a
Lipid (%)	6.0 \pm 0.4 a	5.7 \pm 0.4 a	4.5 \pm 0.8 a
Ash (%)	4.0 \pm 0.4 a	3.9 \pm 0.3 a	3.7 \pm 0.1 a
White muscle			
Moisture (%)	76.6 \pm 0.9 b	76.4 \pm 0.3 b	78.1 \pm 0.5 a
Protein (%)	19.9 \pm 1.1 a	20.8 \pm 0.5 a	21.0 \pm 0.8 a
Lipid (%)	0.3 \pm 0.1 a	0.4 \pm 0.1 a	0.3 \pm 0.2 a
Ash (%)	1.3 \pm 0.3 a	1.5 \pm 0.1 a	1.5 \pm 0.0 a
Liver			
Moisture (%)	68.9 \pm 2.1 a	68.4 \pm 6.0 a	71.3 \pm 3.2 a
Protein (%)	15.2 \pm 1.3 a	14.4 \pm 0.8 a	14.1 \pm 1.0 a
Lipid (%)	2.2 \pm 0.1 b	3.0 \pm 1.0 b	5.9 \pm 1.7 a
Ash (%)	1.1 \pm 0.0 a	1.0 \pm 0.0 b	1.1 \pm 0.1 a

and production (kg/m^3) than those in the 20 C treatment. There was no significant difference ($P > 0.05$) in survival among treatments, which averaged 96.5% overall. However, fish in the 32 C treatment were diagnosed with *Aeromonas sp.* on day 80 of the 97-d study and these tanks were fed Terramycin (TM50) medicated feed for 10 d during the study. This epizootic in the high temperature treatment may indicate that 32 C is high enough to represent stressful conditions for largemouth.

Bass in the 26 C treatment had significantly lower ($P < 0.05$) FCR and higher ($P < 0.05$) percentage protein deposited (PPD) than bass raised at 20 and 32 C, which were not significantly different ($P > 0.05$) (Table 2). These data indicate that largemouth bass gain weight and convert feed and dietary protein more efficiently at 26 C than at 20 or 32 C. Largemouth bass will feed at 20 C and convert feed efficiently, though appetite is reduced compared to higher temperatures. As culture temperature is increased to 26 and 32 C, appetite and growth both increase. At the higher end of that range, feed and protein efficiencies decrease. At 32 C food intake did not increase

further, while energy demands for maintenance and activity increase (Weatherly and Gill 1987) as evidenced by the reduced oxygen concentrations in 32 C tanks being reduced below levels accounted for by direct effects on the water's ability to dissolve oxygen. These factors could result in decreased feed conversion efficiency. Other possible explanations may be shifts of enzyme activities to different isozymes, and stress reactions due to increased occurrence of disease (i.e., *Aeromonas sp.*) (Keembiyehetty and Wilson 1998).

While it is well known that changes in culture temperature can influence the body composition of fish, reactions among species vary, increasing the need to evaluate impacts on individual species (Cui and Wootton 1988). In this study, there was no significant difference ($P > 0.05$) in the protein, lipid, or ash content of whole body or white muscle of largemouth bass raised at the three treatment temperatures (Table 3). This differs from channel catfish *Ictalurus punctatus* in which lipid deposition has been shown to increase as culture temperature increases (Andrews and Stickney 1972). Moisture content of bass tissues was

TABLE 4. Amino acid composition (% of total amino acids) of largemouth bass raised at three water temperatures. Values are mean \pm SE of three replicates. There were no significant differences ($P > 0.05$) within amino acids by temperature as evaluated by ANOVA.

Amino acid	Culture temperature (C)		
	20	26	32
Arginine	6.47 \pm 0.06	6.48 \pm 0.08	6.63 \pm 0.29
Cystine	0.83 \pm 0.01	0.84 \pm 0.02	0.80 \pm 0.09
Histidine	2.38 \pm 0.02	2.23 \pm 0.07	2.16 \pm 0.18
Isoleucine	4.37 \pm 0.03	4.31 \pm 0.13	4.16 \pm 0.23
Leucine	7.63 \pm 0.09	7.48 \pm 0.26	7.31 \pm 0.33
Lysine	8.07 \pm 0.04	8.15 \pm 0.13	7.98 \pm 0.33
Methionine	3.04 \pm 0.05	3.03 \pm 0.19	3.12 \pm 0.40
Phenylalanine	4.29 \pm 0.06	4.12 \pm 0.12	4.07 \pm 0.10
Threonine	4.43 \pm 0.03	4.47 \pm 0.04	4.41 \pm 0.14
Tryptophan	0.94 \pm 0.03	0.94 \pm 0.02	0.99 \pm 0.09
Tyrosine	3.13 \pm 0.06	3.09 \pm 0.10	2.99 \pm 0.23
Valine	4.85 \pm 0.02	4.85 \pm 0.07	4.75 \pm 0.19

effected by temperature, producing a significant decrease ($P < 0.05$) in moisture content of whole body samples of fish grown at 20 C compared to 26 and 32 C, and decreased moisture content ($P < 0.05$) of white muscle for fish reared at 20 and 26 C compared to 32 C (Table 3). These findings agree with those of Niimi and Beamish (1974) who reported that moisture content of largemouth bass tissue was lowest at the lowest water temperature they tested. However, the actual magnitude of changes in moisture levels in the present study were quite small and are not likely biologically significant.

Hepatosomatic Index (HSI) consistently decreased as culture temperatures increased (Table 2), though differences were not statistically significant ($P > 0.05$). This agrees with Heidinger and Crawford (1977) who reported increased temperature lowered HSI in largemouth bass. Keembiyehetty and Wilson (1998) reported that in sunshine bass liver metabolism was altered between the two study temperatures (26.7 and 32.2 C) with increased temperatures shifting lipid from the liver to visceral storage. They reported that this may have indicated stress at the higher temperature. In this study, there appeared to be the opposite trend in largemouth bass. Lipid concentrations in

the liver were significantly increased ($P < 0.05$) at 32 C (Table 3) while whole body lipid concentrations showed a decreasing trend with temperature. Since lipid concentrations in white muscle were low ($< 0.05\%$), and did not change with temperature, this indirectly indicates decreased visceral storage. In largemouth bass visceral fat stores are tightly bound to pyloric caeca and accurate direct measurements are extremely difficult.

Properties of fish tissue proteins are thought to be affected by the environmental temperature at which they are synthesized (Love 1980). If differences were of sufficient magnitude, specific amino acid requirements could potentially differ for fish cultured at different temperatures. However, in this study there was no significant difference ($P > 0.05$) in whole body concentrations of amino acids (% of total amino acids) among bass raised at the different temperatures (Table 4). Based on the data, adjustments in protein quality or amino acid composition do not appear justified within the temperature range evaluated.

Leslie and Buckley (1976) demonstrated that fish can adjust their pattern of biosynthesis of fatty acids rapidly according to the prevailing temperature. In the present study several significant differences in fatty acid

TABLE 5. Fatty acid composition (% of total fatty acids) of white muscle samples of largemouth bass raised at three water temperatures. Values are mean \pm SE of three replicates. Means within a row followed by different letters are significantly different ($P < 0.05$) by ANOVA. Diet fatty acid composition is included for comparative purposes.

Fatty acid	Water temperature (C)			Diet
	20	26	32	
14:0	5.57 \pm 1.97 a	7.16 \pm 1.18 a	6.58 \pm 1.94 a	8.18 \pm 0.18
16:0	22.62 \pm 1.59 a	21.28 \pm 0.28 a	27.29 \pm 5.29 a	25.03 \pm 0.33
16:1 n-7	11.15 \pm 2.39 a	11.76 \pm 1.78 a	10.00 \pm 2.50 a	10.53 \pm 0.15
18:0	5.92 \pm 0.61 a	3.96 \pm 0.82 b	6.19 \pm 0.84 a	5.30 \pm 0.02
18:1 n-9	19.13 \pm 2.19 a	18.28 \pm 1.55 a	18.65 \pm 3.19 a	16.92 \pm 0.20
18:2 n-6	6.28 \pm 0.2 b	6.20 \pm 0.33 b	9.02 \pm 2.00 a	6.40 \pm 0.11
18:3 n-3	0.70 \pm 1.22 a	2.03 \pm 0.47 a	0.00 \pm 0.00 b	2.27 \pm 0.08
20:4 n-6	0.70 \pm 1.22 b	2.89 \pm 0.35 a	0.60 \pm 1.03 b	2.06 \pm 0.35
20:5 n-3	6.63 \pm 0.67 a	6.63 \pm 0.24 a	3.75 \pm 3.26 a	10.37 \pm 0.16
22:5 n-6	5.92 \pm 0.61 a	4.92 \pm 0.12 a	3.75 \pm 3.26 a	2.62 \pm 0.01
22:6 n-3	14.66 \pm 3.7 a	13.39 \pm 3.55 a	14.17 \pm 4.75 a	7.89 \pm 0.10
Saturates	34.12 \pm 3.14 b	32.40 \pm 0.45 b	40.06 \pm 3.81 a	38.27 \pm 0.17
Monenes	30.64 \pm 3.78 a	30.36 \pm 2.77 a	28.65 \pm 5.10 a	28.65 \pm 0.28
Diene	6.28 \pm 0.2 b	6.20 \pm 0.33 b	9.02 \pm 2.00 a	6.36 \pm 0.06
PUFA	28.96 \pm 6.87 a	31.03 \pm 3.40 a	22.26 \pm 10.57 a	26.72 \pm 0.03
n-3	22.33 \pm 6.21 a	23.22 \pm 3.44 a	17.92 \pm 6.95 a	22.0 \pm 0.36
n-6	12.90 \pm 0.78 a	14.01 \pm 0.47 a	13.37 \pm 1.97 a	11.03 \pm 0.28
n-3/n-6	1.72 \pm 0.38 a	1.66 \pm 0.30 a	1.32 \pm 0.39 a	2.00 \pm 0.08
U.I. ¹	187	186	161	157

¹ U.I. = Unsaturation Index, the summed products of weight percentage and the number of double bonds.

composition were identified (Table 5). Stearic acid (18:0) was significantly lower ($P < 0.05$) in fish raised at 26 C than for fish raised at 20 and 32 C, which were not significantly different ($P > 0.05$) (Table 5). Linoleic acid (18:2 n-6) was significantly higher ($P \leq 0.05$) and linolenic acid (18:3 n-3) was significantly lower ($P \leq 0.05$) in the white muscle of largemouth bass raised at 32 C compared to those raised at 20 or 26 C. Arachidonic acid (20:4 n-6) was significantly higher ($P < 0.05$) in bass cultured at 26 C than for bass cultured at 20 or 32 C. For summary categories, saturated fatty acids showed a significant response to culture temperature, being significantly higher ($P < 0.05$) in bass cultured at 32 C than in bass cultured at 20 or 26 C (Table 5). Body lipids of fish from warm waters are usually more saturated than those of fish from cooler waters (Gopakumar and Nair 1972). Dienes were also significantly higher ($P < 0.05$) in fish raised at 32 C than at 20 or 26 C. Concentrations of PUFA, n-3 fatty acids,

n-6 fatty acids, and n-3/n-6 ratios were not significantly different ($P > 0.05$) among fish raised at the three temperatures.

Olsen and Henderson (1997) compared the unsaturation index (UI) of arctic char tissue to the UI of the diet they were being fed. They indicated that a decrease from an UI of 287 in the feed to 170 in the charr tissues likely indicated that their essential fatty acid requirements were being met and excess unsaturated fatty acids were being oxidized. In this study the UI of the feed was 157 and the UI of LMB raised at 20 and 26 were 186 and 187, respectively (Table 5). This may indicate that for fish raised at these temperatures (20 and 26 C) the diets may need to be more highly unsaturated. The similarity of UI between the diet (157) and fish raised at 32 C (161) may indicate a reduced demand for unsaturated fatty acids and that level of dietary unsaturation is sufficient for fish raised at this higher temperature.

Another possible indication of a need for

more highly unsaturated fatty acids in the diet is based on levels of docosapentaenoic acid (22:5 n-6, DPA) in the fish. Levels of DPA in the largemouth bass white muscle were 43–126% greater than in the diet. This fatty acid is an end product of elongation and desaturation within the n-6 family (Olsen and Henderson 1987) and can be an indicator that insufficient DHA within the n-3 family is available (Neuringer and Conner 1989). To try to meet DHA needs when insufficient n-3 precursors are available, the closest analog may be produced. Levels of DPA were significantly negatively correlated ($P < 0.05$) with culture temperature, with concentrations increasing as culture temperatures decreased. This would support an increasing demand for unsaturated fatty acids at lower temperatures. Olsen and Henderson (1997) reported that in most freshwater fishes there is a clear preference of 18:3 n-3 over 18:2 n-6 for elongation and desaturation. They also reported that the very low levels of the delta-4 desaturation product 22:5 n-6 (DPA) in comparison with 22:6 n-3 (DHA) supported the view that this desaturation step is highly specific to n-3 PUFA in charr *Salvelinus alpinus*. However, these current data would appear to indicate that the delta-4 desaturation step in the n-6 pathway may be relatively active in largemouth bass.

While most individual fatty acids closely reflected concentrations in the diet as expected (Worthington and Lovell 1973), two fatty acids recognized as nutritionally essential showed relatively large deviations from dietary concentrations. The concentration of eicosapentaenoic acid (EPA, 20:5 n-3) was 36–64% lower in bass white muscle than in the diet. Its lowest concentration was in fish raised at 32 C. This may indicate it is being used more as an energy source at the higher temperature. Concentrations of docosahexaenoic acid (DHA, 22:6 n-3) in the white muscle of the bass were 70–85% greater than concentrations in the diet. This agrees with Tidwell et al. (1996) who reported high DHA concentrations in

largemouth bass and hypothesized that this may indicate their high relative importance to the species. When Coyle et al. (2000) fed largemouth juveniles diets containing high DHA levels, under controlled conditions, growth was not improved but whole body lipid concentrations were decreased while protein concentrations were increased. Further investigations into the roles of EPA and DHA in largemouth bass nutrition and metabolism appear justified.

In summary, at 26 C growth, survival, feed conversion, and protein retention appear near optimal. At 20 C, feed conversion and survival remain good but growth rate is reduced approximately 35%. At 32 C, growth is similar to that at 26 C but feed efficiency and protein retention is decreased and fish may be under chronic stress conditions and more susceptible to disease outbreaks. For largemouth bass raised at cooler temperatures a shift to more unsaturated fatty acids in the diet may be advantageous.

Acknowledgments

We thank Karla Hochstrasser for typing the manuscript and Randy Kendall for keeping the heat pump system operating. This research was partially funded by a grant from Illinois-Indiana Sea Grant Program, through support of Kentucky's Regional University Excellence Trust Fund to the Aquaculture Program as Kentucky State University's Program of Distinction, and by USDA/CSREES grant to Kentucky State University under agreement KYX-80-91-04A.

Literature Cited

- Andrews, J. W. and R. R. Stickney. 1972. Interactions of feeding rates and environmental temperature on growth, food conversion, and body composition of channel catfish. *Transactions of the American Fisheries Society* 101:94–99.
- Boyd, C. E. 1979. Water quality in warmwater fish ponds. Auburn University, Agricultural Experiment Station, Auburn, Alabama, USA.
- Coutant, C. C. 1975. Responses of bass to natural and artificial temperature regimes. Pages 272–285 in R. H. Stroud and H. Clepper, editors. *Black bass*

- biology and management. Sport Fishing Institute, Washington, D.C., USA.
- 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.
- Cui, Y. and R. J. Wootton.** 1988. Effects of ration, temperature and body size on the body composition and energy content of the minnow, *Phoxinus phoxinus* (L.). *Journal of Fish Biology* 32:749–764.
- Gopakumar, K. and M. R. Nair.** 1972. Fatty acid composition of eight species of Indian marine fish. *Journal of Science Food Agriculture* 23:493–496.
- 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.
- Keembiyehetty, C. N. and R. P. Wilson.** 1998. Effect of water temperature on growth and nutrient utilization of sunshine bass (*Morone chrysops* × *Morone saxatilis*) fed diets containing different energy/protein ratios. *Aquaculture* 166:151–162.
- Leslie, J. M. and J. T. Buckley.** 1976. Phospholipids composition of goldfish (*Carassius auratus* L.) liver and brain and temperature-dependence of phosphatidyl cholinesynthesis. *Comparative Biochemistry and Physiology* 53B:335–337.
- Love, R. M.** 1980. The chemical biology of fishes, volume 2. *Advances 1968–1978*. Academic Press, New York, New York, USA.
- Lovell, R. T.** 1989. Nutrition and feeding of fish. Van Nostrand Reinhold, New York, New York, USA.
- Nettleton, J.** 1985. Seafood nutrition: facts, issues, and marketing of nutrition in fish and shellfish. Osprey Books, Huntington, New York, USA.
- Neuringer, M. and W. E. Conner.** 1989. Omega-3 fatty acids in the retina. Pages 177–190 in C. Galli and A. P. Simopoulos, editors. *Dietary ω3 and ω6 fatty acids, biological effects and nutritional essentiality*. Plenum Press, New York, New York, USA.
- Niimi, A. J. and F. W. H. Beamish.** 1974. Bioenergetics and growth of largemouth bass (*Micropterus salmoides*) in relation to body weight and temperature. *Canadian Journal of Zoology* 52:447–456.
- Olsen, R. E. and R. J. Henderson.** 1997. Muscle fatty acid composition and oxidative stress indices of arctic charr, *Salvelinus alpinus* (L.), in relation to dietary polyunsaturated fatty acid levels and temperature. *Aquaculture Nutrition* 3:227–238.
- Palachek, R. M. and J. R. Tomasso.** 1984. Toxicity of nitrite to channel catfish (*Ictalurus punctatus*), tilapia (*Tilapia aurea*), and largemouth bass (*Micropterus salmoides*): evidence for a nitrite exclusion mechanism. *Canadian Journal of Fisheries and Aquatic Sciences* 41:1739–1744.
- Ricker, W. E.** 1975. Computation and interpretation of biological statistics of fish populations. *Canadian Bulletin of Fisheries and Aquatic Sciences*, No. 191, Department of Fisheries and Oceans, Pacific Biological Station, Nanaimo, B.C.
- Sargent, J., R. J. Henderson, and D. R. Tocher.** 1989. The lipids. Pages 153–218 in J. E. Halver, editor. *Fish nutrition*. Academic Press, New York, New York, USA.
- Smith, L. S.** 1989. Digestive functions in teleost fishes. Pages 331–421 in J. E. Halver, editor. *Fish nutrition*. Academic Press, New York, New York, USA.
- Statistix Analytical Software.** 1994. *Statistix user's manual, version 4.1*. Analytical Software, Tallahassee, Florida, USA.
- Steel, R. G. and J. H. Torrie.** 1980. *Principals and procedures of statistics*. McGraw-Hill Book Co., New York, USA.
- Stickney, R. R.** 1979. *Principles of warmwater aquaculture*. John Wiley & Sons, New York, New York, 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.
- Weatherly, A. H. and H. S. Gill.** 1987. *The biology of fish growth*. Academic Press, New York, USA.
- Worthington, R. E. and R. T. Lovell.** 1973. Fatty acids of channel catfish (*Ictalurus punctatus*): variance components related to diet, replications within diets, and variability among fish. *Journal of the Fisheries Research Board of Canada* 30:1604–1608.
- Zar, J. H.** 1984. *Biostatistical analysis*, 2nd edition. Prentice-Hall, Englewood Hills, New Jersey, USA.

