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Cold Tolerance and Fatty Acid Composition of Striped Bass, White Bass, and Their Hybrids

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Abstract.—Cold tolerance of striped bass *Morone saxatilis*, white bass *M. chrysops*, palmetto bass (female striped bass × male white bass), and sunshine bass (female white bass × male striped bass) were compared under controlled laboratory conditions. Two groups of each taxon were acclimated at 20°C in a recirculating-water system housed in an environmental chamber and were fed either a natural or prepared diet for 84 d. The fatty acid composition of the natural diet was 13% more unsaturated than that of the prepared diet. Fish fed the natural diet subsequently had unsaturated : saturated fatty acid ratios 10–25% higher than fish fed the prepared diet. After being subjected to identical simulated cold fronts (10°C drop in surface water temperature, as if the fish were confined in cages or pens), all groups of fish fed the prepared diet suffered high mortality (50–90%) whereas there was zero mortality among the groups receiving the natural diet. White bass and sunshine bass fed the prepared diet had higher survival rates (50% and 40%, respectively) compared with their striped bass and palmetto bass counterparts (10% and 20%, respectively). The lower incipient lethal temperature was higher for fish fed the prepared diet (5.9, 4.8, 2.5, and 1.9°C for striped bass, palmetto bass, sunshine bass and white bass, respectively) than for those fed the natural diet (near 0.0°C, but 1.8°C for sunshine bass). Both studies reflect a maternal affect on cold tolerance, with white bass being most tolerant. We demonstrated that diet-induced muscle fatty acid composition directly affects cold tolerance of striped bass, white bass, and their hybrids.

Striped bass *Morone saxatilis* have been cultured and propagated for more than 100 years in the United States. Subsequent research revealed that hybrids of the striped bass and white bass *Morone chrysops* were better suited for culture (Bishop 1968; Logan 1968; Williams 1971, 1976; Ware 1975; Bonn et al. 1976; Kerby and Joseph 1979), yet retained the appealing taste of the striped bass (Lareau 1987). Production of hybrid striped bass is currently one of the fastest growing segments of commercial aquaculture in the United States.

There have been relatively few studies of the culture of striped bass or hybrid striped bass in cages. Harrell et al. (1988) obtained nearly 100% survival of striped bass overwintered in cages in freshwater ponds on the Eastern Shore of Maryland. However, striped bass reared in cages in a brackish-water environment in New York had unacceptably high mortality rates that were attributed to low water temperatures during the fall and winter (Valenti et al. 1976). Recently, Hogans (1994) demonstrated that striped bass could be successfully overwintered in cages in marine water. Wil-

liams et al. (1981) and Woods et al. (1983) demonstrated that hybrid striped bass could be successfully grown in cages in estuarine waters in South Carolina and North Carolina, respectively.

Culture of striped bass or hybrid striped bass in the Midwest has not been as extensively established as in the coastal regions of the United States. When raising hybrid striped bass in cages, several producers have reported sudden losses of hybrids when the water temperature rapidly decreased by several degrees in a relatively short period of time (Valenti 1989; A. M. Kelly and C. C. Kohler, personal observation). The rapid onset of cold temperatures has been reported as the cause of death in several species of fish (Verrill 1901; Storey 1937; Galloway 1941; Gunther 1941; Ash et al. 1974; Coutant 1977; Mitchell 1990). It is believed that the lipid composition in the fish muscle plays a vital role in the ability of fish to adapt from one temperature to another (Hazel 1984; Greene and Selivonchick 1987; Henderson and Tocher 1987). Phospholipids are the class of lipids in which the most obvious changes occur. As environmental temperatures decrease, the invariable response is an increase in fatty acid unsaturation (Johnston and Roots 1964; Caldwell and Vernberg 1970; Hazel 1979; Cossins and Prosser 1982). Conversely, as ambient temperatures increase, phospholipid saturation must also increase to avoid excess fluidity.

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The dynamics of lipid composition of cells occurs in order to maintain a constant fluid matrix for enzymes associated with membranes (Greene and Selivonchick 1990). Different species of fish differ in their patterns of fat deposition and mobilization, which in turn affects the temperature range in which the species can grow and survive. For example, the Nile tilapia *Oreochromis niloticus* does not store excess lipids in the musculature but rather relies on visceral deposits that it is incapable of mobilizing at low temperatures, which results in high mortalities between 8°C and 6.5°C (Sato et al. 1984). Viola et al. (1988) demonstrated that the common carp *Cyprinus carpio*, which is capable of mobilizing lipids from muscular and visceral deposits, is able to survive to 4.5°C under the same conditions.

Diets influence the fatty acid composition in several species of fish (Henderson and Tocher 1987; Lovell 1989; Seo et al. 1994), and the ability of a fish to alter its lipid composition when placed in colder water is one factor that determines survival. For example, summer harvest syndrome is an anomaly seen in goldfish *Carassius auratus* when they are harvested in the summer and placed in tanks containing water that is colder than the pond water (Mitchell 1990). The death of these fish is thought to be a result of the fat that the goldfish consume or produce (Mitchell 1990). Goldfish with high concentrations of saturated body fat are less tolerant of temperature change than fish with high concentrations of unsaturated body fat. Similarly, rainbow trout *Oncorhynchus mykiss* that have been fed diets high in saturated fats stiffen and die when placed in cold water (Mitchell 1990). In these fish, the fat apparently hardens in the colder water, causing the fat-impregnated muscles to stiffen and the fish to become exhausted and lose movement.

Although it has been hypothesized that temperature is closely linked to membrane composition, relatively few studies have been conducted to determine if a correlation exists between lipid composition and cold tolerance. This study was designed to determine the effect of a sudden temperature change (a simulated cold front) on striped bass, white bass, and their hybrids fed either a natural or prepared diet, as well as to determine their lower incipient lethal temperature. The association of fatty acid composition and unsaturated: saturated fatty acid ratios in these fish were examined with respect to their tolerance to cold.

Methods

Several hundred similar-size (~150 g) striped bass, white bass, palmetto bass (female striped

bass × male white bass) and sunshine bass (female white bass × male striped bass) were maintained at 20°C. Fish were held in 209-L tanks within a recirculating-water system equipped with a biofilter. Hybrids were not half-sibling relatives. Before being subjected to cold treatments, two groups of each taxon were fed for a minimum of 84 d either a natural diet of fathead minnows *Pimephales promelas* or a prepared commercial diet (40% crude protein and 10% crude fat).

Cold front studies.—The cold front studies were conducted in 10 aquaria (38-L) connected to two limestone biofilters (209-L). The system was equipped with a 1.0-hp (746-W) cooling unit (Frigid Units, Toledo, Ohio) and was inside a temperature-controlled environmental chamber. A control group was maintained at 20°C in a separate environmental chamber for each run.

For each run, 10 randomly selected individuals (mean weight = 250 ± 10 g) from one taxon-treatment combination were stocked into 10 aquaria. Fish were maintained at 20°C and fed their respective diets for an additional 5 d before the start of the experiment. Any fish showing signs of stress or disease was replaced during the 5-d holding period. Following acclimation, the chilling unit and environmental chamber were adjusted to produce an even 10°C decrease in temperature over 24 h (~1.0°C/2.4 h). The fish were maintained at that temperature (10.0 ± 0.5°C) for an additional 24 h. The protocol was chosen to simulate the change in surface water temperature that occurred when significant losses of hybrid striped bass in cages were experienced in a previous pond study conducted in southern Illinois (Kelly and Kohler 1996).

Fish were observed every hour to record deaths and signs of stress during the chill down study. Temperature, dissolved oxygen, pH, nitrite, and total ammonia were measured every 4 h with a Hach test kit.

Cold tolerance studies.—The cold tolerance studies were conducted in two 1,500-L raceways divided into two compartments. The raceways were part of a recirculating-water system equipped with a limestone biofilter. Each raceway was equipped with a 1.0-hp (746-W) cooling unit (Frigid Units). Separate batteries of runs were made for each feed treatment group. Ten randomly selected individuals (mean weight = 250 ± 10 g) of each of the four taxa from the same feed treatment were stocked by group into randomly selected raceway compartments. The temperature of the cold tolerance system was maintained at 20 ± 1°C. Fish

were fed their respective diets and allowed to acclimate to the system for 14 d before the initiation of the chill-down process. Temperature was decreased from acclimation temperature to 3°C at a rate of 1°C/d. This temperature regime is reported to allow for temporal physiological adaptation (no temperature shock) of test fish and to provide good estimates of lower incipient lethal temperature limits (Shaffland and Pestrak 1982). The number of dead fish at each subsequent temperature stanza was recorded for each group. The study was triplicated in separate runs for each feed treatment. Probit analysis was employed to determine the lower incipient lethal temperature for each taxon.

Fatty acid determination.—Thirty fish from each group were euthanatized, weighed, and filleted. Minced homogenous samples were prepared from the pool of fillets of 10 fish from each group. Portions were taken from each of the combined samples for moisture and lipid analyses.

Moisture was determined following standards of the Association of Official Analytical Chemists (AOAC 1990). Lipids were extracted by the method outlined by Bligh and Dyer (1959). The lipid residue was weighed for total lipid determination, and a subsample was removed for fatty acid analyses. Lipids were separated into nonpolar lipids (triglycerides, cholesterol) and polar lipids (phospholipids) according to the method outlined by Juaneda and Rocquelin (1985). Phospholipids were saponified with 0.5 N methanolic sodium hydroxide and esterified with boron trifluoride in methanol (AOAC 1990).

Methyl esters of the fatty acids of phospholipids were separated with a gas chromatograph (Hewlett-Packard 5890A, Hewlett-Packard, Inc., Avondale, Pennsylvania) fitted with a permanently bonded polyethylene glycol and fused silica capillary column (Restek Stabilwax; 30 m × 0.53-mm inner diameter, 1.0-μm film thickness; Restek Corp, Bellefonte, Pennsylvania). A program rate of 195°C (9 min hold) to 230°C at 7°C/min and 230°C (3 min hold) to 245°C at 3°C/min was employed. Peak areas were integrated with a Hewlett-Packard 3390A integrator, and concentrations of each methyl ester were quantified based on percent of total identified fatty acid concentrations of each lipid. Quantitation of fatty acid methyl ester was achieved by comparison of retention times of unknowns to authentic standards obtained from NuChek Prep (Elysian, Minnesota) run on the same Restek column. Response factors were calculated with methyl eicosanoate (21:0) as an internal standard. Peak areas of fatty acid methyl

TABLE 1.—Percent mortality of striped bass, white bass, palmetto bass, and sunshine bass fed either a natural or a prepared diet after being subjected to cold front temperatures (10°C decrease over 24-h period). Control groups were maintained at 20°C throughout the study. An asterisk denotes a value that is significantly different from the control ($P \leq 0.05$).

Taxon	Natural diet		Prepared diet	
	Treatment	Control	Treatment	Control
Striped bass	0	0	90*	0
White bass	0	0	50*	0
Palmetto bass	0	0	80*	0
Sunshine bass	0	0	60*	0

esters were expressed as percentages of total peak area.

An analysis of variance (ANOVA) was used to analyze fatty acid methyl ester data and unsaturated: saturated ratios, and mean differences were submitted to Duncan's multiple-range test to determine significant ($P \leq 0.05$) differences. Statistical differences ($P \leq 0.05$) between moisture and lipid content for striped bass, white bass, and their hybrids fed either a natural or prepared diet were determined with univariate analyses (SAS Institute 1991).

Results

Only fish fed the prepared diet died when subjected to the simulated cold front (Table 1). Mortality rates were higher for striped bass and palmetto bass than for white bass and sunshine bass. Fish fed the prepared diet also died at higher temperatures than fish fed the natural diet (Figure 1). Striped bass fed the prepared diet had the highest lower incipient lethal temperature (LILT; 5.9°C), followed by palmetto bass (4.8°C), sunshine bass (2.5°C), and white bass (1.9°C). Fish fed the natural diet had considerably lower LILT, with all taxa being near 0.0°C, except palmetto bass, which had a LILT of 1.8°C. Palmetto bass was the only taxon in which disease became a problem at lower temperatures. Three of the 10 fish were diagnosed with saprolegniasis during the course of the study.

The unsaturated: saturated (U : S) fatty acid ratio was significantly higher for the natural diet than for the prepared diet (2.19 and 1.78, respectively; Table 2). The phospholipid fatty acid composition for the muscle lipids for all four taxa were reflective of the diets they received (i.e., the natural diet had a higher percentage of unsaturated fatty acids, as did the muscle tissue of the fish that consumed the natural diet; Table 2). The U : S fatty acid ratio was significantly higher for all taxa fed the pre-

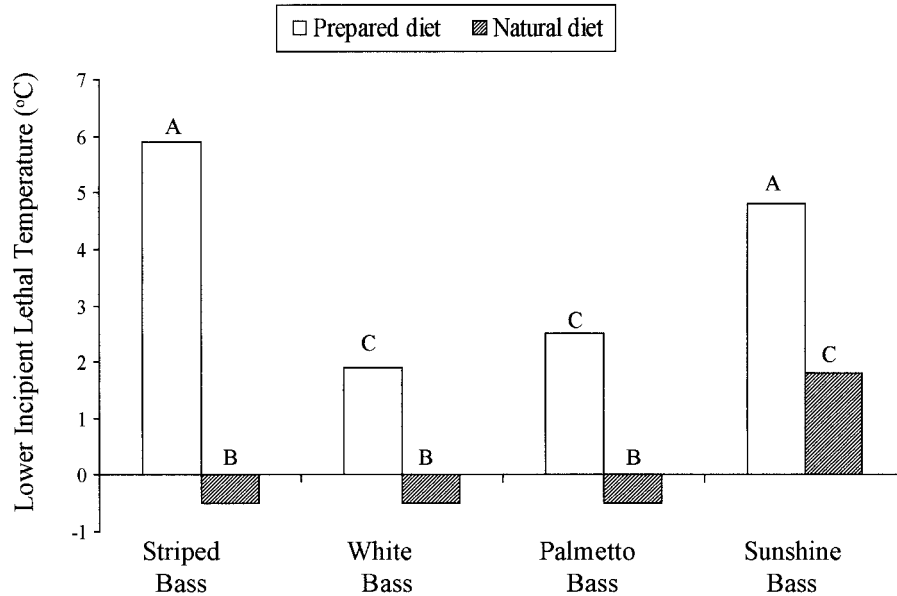


FIGURE 1.—Lower incipient lethal temperatures (LILT) for striped bass, white bass, palmetto bass, and sunshine bass fed either a prepared or natural diet. Among all taxa, bars labeled with the same letter are not significantly different ($P > 0.5$). Negative LILT values are reported as near 0°C because fish would not survive in ice.

pared diet (Figure 2). No significant differences existed in the percent moisture or percent lipid between the various parental and hybrid species fed either the natural or prepared diet (Table 3).

Water quality varied little during the trials. Temperature in the treatment tanks were maintained as prescribed, pH was constant at 7.2, total ammonia ranged from 0.0 to 0.1 mg/L, and nitrites were less than 0.01 mg/L.

Discussion

We demonstrated that diet-induced muscle fatty acid composition directly affects cold tolerance of striped bass, white bass, and their hybrids. Fish fed fathead minnows had U : S fatty acid ratios 10–25% higher than fish fed a prepared diet. When subjected to a simulated cold front, all groups of fish fed the prepared diet suffered high mortality (50–90%) whereas the groups fed the natural diet experienced zero mortalities. The LILT was also higher for fish fed the prepared diet.

The amount of unsaturated fatty acids in the muscle is believed to affect a fish's ability to tolerate lower temperatures (Hoar and Dorchester 1949; Hoar and Cottle 1952a, 1952b). In general, the tissue temperature of fish is within 1°C of the ambient water temperature (Carey et al. 1971; Reynolds et al. 1976). Physiologically, fish are affected by variations in water temperature in two

ways (Hochachka and Somero 1984). First, temperature determines the rate of chemical reactions, and secondly, temperature dictates the point of equilibrium between the formation and disruption of the macromolecular structures in biological membranes. Structural flexibility, therefore, is a requirement for integrity of biological membranes (Hazel 1993). Cold temperatures constrain this flexibility and, as a result, stabilize less active conformations. The rate of increase in the ability of fish to tolerate higher temperatures usually requires less than 24 h at temperatures above 20°C, whereas the gain in resistance to lower temperatures is a much slower process, requiring up to 20 d in some species (Doudoroff 1942; Brett 1944). The rate of resistance to lower temperatures is governed in part by the rate of metabolism, which is depressed at lower environmental temperatures. The simulated cold front in this study resulted in higher mortalities in the striped bass and the palmetto bass when compared with the white bass and the sunshine bass. Although no studies have been conducted to determine the amount of time required to gain resistance to lower temperatures in striped bass, white bass, or their hybrids, this study suggests that when these fish receive a natural diet they are well suited to sudden changes in water temperature. However, when these fish are fed a prepared diet, this ability to adapt to sudden

TABLE 2.—Fatty acid composition (%) of the phospholipids in striped bass (SB), white bass (WB), palmetto bass (PB), and sunshine bass (SunB) that were fed either a prepared or natural diet, and the fatty acid composition (%) of the prepared diet and natural diet (fathead minnows) used in this study. An asterisk denotes an unsaturated : saturated (U : S) fatty acid ratio for the natural diet that is significantly higher than its paired value for the prepared diet ($P \leq 0.05$); ND is not detected.

FAME ^a	Fed prepared diet				Fed natural diet				Diet composition	
	SB	WB	PB	SunB	SB	WB	PB	SunB	Prepared	Natural
14:0	4.98	4.26	4.56	3.37	2.61	2.99	2.64	2.69	3.46	1.78
14:1(n-5)	0.63	0.12	0.16	0.18	0.67	0.38	0.14	0.17	0.28	0.27
15:0	0.54	0.42	0.34	0.43	0.37	0.42	0.40	0.27	0.38	0.47
16:0	24.10	22.87	24.86	23.71	22.22	19.80	23.46	20.14	21.54	22.23
16:1(n-7)	11.21	8.26	11.32	9.07	7.73	10.97	8.19	9.38	4.49	5.33
16:2(n-4)	1.44	0.76	0.56	0.68	0.65	0.99	0.64	0.33	0.58	0.34
16:3(n-4)	1.46	0.92	0.76	0.98	0.82	1.08	0.85	0.58	0.58	0.69
16:4(n-1)	0.13	0.11	0.13	0.18	0.09	0.07	0.09	0.07	0.20	0.09
18:0	3.05	4.28	3.42	4.11	3.77	2.84	3.91	4.25	9.93	6.27
18:1(n-9)	24.83	27.31	27.75	29.01	35.22	33.43	36.84	36.30	28.65	31.63
18:2(n-6)	8.26	12.01	11.83	7.22	3.90	3.60	4.59	3.53	18.30	14.16
18:3(n-6)	0.37	0.02	0.15	0.17	0.35	0.31	0.25	0.41	ND	0.33
18:3(n-3)	1.52	0.99	1.76	0.95	1.14	3.26	1.05	1.27	1.44	1.19
18:4(n-3)	0.65	0.52	0.71	0.82	0.45	1.42	0.45	0.33	0.57	0.17
20:0	0.12	0.20	0.30	0.22	0.20	0.12	0.19	0.15	0.33	0.24
20:1(n-9)	2.12	2.99	2.04	2.53	1.22	1.63	2.92	2.13	0.88	1.60
20:2(n-6)	0.74	0.74	0.61	0.55	0.80	0.93	0.80	0.85	0.18	1.05
20:3(n-6)	0.26	0.42	0.33	0.20	0.21	0.28	0.46	0.23	0.47	1.07
20:4(n-6)	1.09	0.63	0.81	0.96	4.88	4.12	1.67	5.94	0.61	2.89
20:4(n-3)	0.61	0.54	0.44	0.65	0.18	0.29	0.47	0.23	0.30	0.45
20:5(n-3)	4.69	4.08	2.58	6.34	3.12	4.18	3.50	4.33	2.80	2.24
22:0	0.21	0.32	0.12	0.17	0.22	0.31	0.32	0.11	0.28	0.38
22:4(n-6)	0.33	0.41	0.15	0.26	0.20	0.46	0.41	0.18	0.21	0.59
22:5(n-6)	1.66	1.42	0.83	1.56	2.17	1.85	1.28	0.72	0.68	0.90
22:6(n-3)	4.00	5.22	3.48	5.68	6.81	4.27	4.48	5.41	2.86	3.64
U : S	2.00	2.09	1.97	2.12	2.40*	2.78*	2.23*	2.62*	1.78	2.19*

^a FAME = fatty acid methyl esters. Ratio is number of carbon atoms to number of double bonds, and n designates the position of the first double bond from the methyl end.

decreases in water temperature is less apparent. This suggests that diet influences the fish's ability to adapt to sudden water changes.

Hoar and Dorchester (1949) attempted to correlate the melting points of different dietary fats with the ability of the goldfish to resist high and low temperatures. Their study was unable to demonstrate any correlation between the degree of unsaturation of the feed and the fish's ability to withstand high temperatures, possibly because fatty acid composition of the fish was not examined. Hoar and Cottle (1952a) examined the effect of diet on lower incipient lethal temperatures in goldfish and demonstrated that those fish fed herring oil resist low temperatures best; however, again no correlation between melting points and degree of unsaturation of the diet could be made. These studies also examined the ability of the fish to withstand sudden changes in temperature, which adds other physiological responses that may complicate the outcome of the study. To reduce the effect of other physiological responses, the present study eliminated the temperature shock by acclimating

the fish at 1°C/d to determine the possible role of various levels of saturated and unsaturated fatty acids on cold tolerance. Fish were fed either the natural or prepared diet throughout the cold tolerance study to maintain the type of dietary fatty acids that the fish consumed.

Examination of the phospholipid contents of the striped bass, white bass, and their hybrids demonstrates that the fatty acid content appears to be maternally inherited. The fatty acid composition in the phospholipid fractions of the palmetto bass is similar to those of the striped bass, whereas the fatty acids of the sunshine bass are similar to those of the white bass. If the degree of unsaturation plays a role in the cold tolerance of a species, then it would be expected that the cold tolerance of the palmetto bass would be similar to the striped bass and the sunshine bass would be similar to the white bass. That is the pattern that emerged in this study. Although it is accepted that cold tolerance is a function of acclimation temperature, the median lethal temperature achieved in this study should

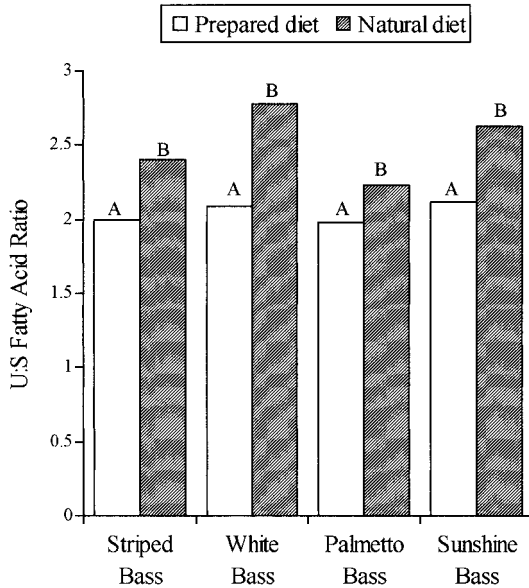


FIGURE 2.—Unsaturated : saturated (U : S) fatty acid ratios for striped bass, white bass, palmetto bass, and sunshine bass fed either a prepared or natural diet. Within each taxon, value bars labeled with the same letter are not significantly different ($P > 0.5$).

hold for striped bass, white bass, and their hybrids acclimated to 20°C.

A possible explanation for the higher LILTs of the fish fed the prepared diets may be explained by the variation in the fatty acid composition of menhaden oil in the prepared trout diets (used in the present study). Ackman et al. (1981) summarized the published information on fatty acid composition of menhaden oils. They showed that oils originating from menhaden caught from colder Atlantic waters were more unsaturated than the oils from menhaden obtained in the warmer waters of the Gulf of Mexico. Joseph (1985) demonstrated that there is little seasonal variation in the fatty acids of menhaden obtained from the Atlantic; however, those obtained from the Gulf of Mexico had fatty acids that differed significantly not only seasonally and geographically but also annually. Accordingly, the presence of menhaden oil in a feed does not ensure low levels of saturated fats, and the feed may, in fact, vary by batch.

The 20°C acclimation temperature maintained in the present study is similar to the preferred summer temperature of subadult striped bass (Coutant 1977, 1978; Coutant and Carroll 1980; Schaich and Coutant 1980; Waddle et al. 1980). It is also similar to the temperature at which the fish would be held in early autumn if they were cage reared

TABLE 3.—Muscle tissue moisture and lipid composition of striped bass, white bass, and their hybrids fed either a prepared or natural diet.

Taxon	Prepared diet		Natural diet	
	Moisture (%)	Lipid (%)	Moisture (%)	Lipid (%)
Striped bass	74.2	4.8	73.9	4.0
White bass	74.8	4.4	72.7	3.9
Palmetto bass	74.1	4.2	74.8	4.3
Sunshine bass	73.8	4.4	73.8	4.0

in ponds in the Midwest. When the phospholipids are more saturated and consequently less fluid, sudden drops in temperature result in more ridged membranes and subsequently more stress to the fish.

Fish deaths due to cold temperatures have frequently been reported. It is generally believed that deaths arise from the rapidity of dropping temperatures whereby the fish are unable to acclimate to the lower temperature despite being within their biokinetic range. It is consequently critical especially in autumn to feed fish of the genus *Morone*, and possibly other genera, a diet that is relatively low in saturated fats when they are confined to surface waters in cages or pens.

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