Ratio of 20:3(n-9) to 20:5(n-3) in Phospholipids as an Indicator of Dietary Essential Fatty Acid Sufficiency in Striped Bass, *Morone saxatilis*, and Palmetto Bass, *M. saxatilis* × *M. chrysops*

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ABSTRACT. The effects of feeding different sources of brine shrimp nauplii with different fatty acid compositions on growth, survival, and fatty acid composition of striped bass, *Morone saxatilis* and palmetto bass (*M. saxatilis* × *M. chrysops*) were determined. The sources of brine shrimp were Chinese (CH), with a high percentage of 20:5(n-3), eicosapentaenoic acid (EPA), and Colombian (COL), San Francisco Bay (SFB), and Great Salt Lake (GSL), with low percentages of EPA but high percentages of 18:3(n-3), linolenic acid. None of the brine shrimp sources contained a measurable amount of 22:6(n-3), docosahexaenoic acid (DHA). After enrichment with menhaden oil to increase the content of EPA and DHA, the GSL brine shrimp nauplii were also fed to hybrid striped bass. Growth and survival of fish larvae fed brine shrimp nauplii with high

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percentages of EPA and DHA (CH and GSLE) were higher ($P < 0.05$) than those of fish fed brine shrimp with a low percentage of EPA (COL, SFB, and GSL). The ratio of 20:3(n-9), eicosatrienoic acid (ETA), to DHA in polar lipids (phospholipids) of fish, traditionally used as an indicator of essential fatty acid (EFA) sufficiency of the diet, was not a reliable indicator of essential fatty acid sufficiency of diets for larval striped bass and hybrid striped bass. However, the ratio of ETA to EPA appears to be an appropriate indicator. An ETA-to-EPA ratio in phospholipids of less than 0.10 is consistent with an EFA sufficient diet.

INTRODUCTION

Striped bass, *Morone saxatilis*, and palmetto bass (striped bass × white bass, *M. chrysops*) are important freshwater food and sport fish in North America. Because most inland waters do not provide essential spawning requirements, larvae and juveniles must be supplied by hatcheries (Stevens 1966). Striped bass larvae often receive live foods during initial feeding and do not assimilate prepared diets well (Braid and Shell 1981).

The importance of essential fatty acid nutrition on growth and development of fishes has been established with several species (Cowey et al. 1976; Ackman 1980; Watanabe et al. 1983; Satoh et al. 1989). An adequate dietary source of n-3 highly unsaturated fatty acids (HUFA) is necessary for normal growth and development of striped bass (Webster and Lovell 1990a, 1990b; Tuncer and Harrell 1992) and palmetto bass larvae (Clawson and Lovell 1992). Webster and Lovell (1990a) showed that striped bass larvae fed brine shrimp nauplii high in 18:3(n-3) but low in n-3 HUFA did not grow and develop normally, while larvae fed nauplii high in 20:5(n-3), eicosapentaenoic acid (EPA), showed normal growth. Clawson and Lovell (1992) reported similar results for palmetto bass.

Polar (or phospholipid) fatty acid ratios in liver and other tissues have been used as subclinical indicators of dietary fatty acid sufficiency in several animals. A low ratio of 20:3(n-9), eicosatrienoic acid (ETA), to 20:4(n-6), arachidonic acid, in phospholipids indicates a dietary essential fatty acid (EFA) deficiency in mammals (Mohrhauer and Holman 1963; Ahluwalia et al. 1967). Castell et al.

(1972) proposed that the phospholipid ratio of ETA to 22:6(n-3), docosahexaenoic acid (DHA), be used in salmonids and that a ratio < 0.40 indicated EFA sufficiency. The purpose of the present study was to feed striped bass and palmetto bass brine shrimp nauplii with varying compositions of fatty acids and relate fish growth and survival to various fatty acid ratios in neutral and polar lipids in the fish, which may serve as an indicator of EFA sufficiency of the diet.

MATERIALS AND METHODS

Two feeding studies were conducted, one with striped bass larvae and another with palmetto bass larvae. The studies were conducted at the Alabama State Fish Hatchery, Marion, Alabama.

Experiment 1

Hatching Brine Shrimp Nauplii

Brine shrimp cysts from three geographical sources were obtained from commercial suppliers: Chinese (Cans 686532-686536; Biomarine, Inc.,[1] Hawthorne, California), Colombian (lot #120; Aquarium Products, Glen Burnie, Maryland), and San Francisco Bay (lot #1106; San Francisco Bay Brands, Inc., Newark, California). Previous research had indicated that these sources of brine shrimp had greatly different compositions of n-3 fatty acids (Webster and Lovell 1990a). The cysts were hatched in water with salt (NaCl) added to give a specific gravity of 1.02, in 56-L conical hatching containers. Water was continually aerated and continual illumination provided by fluorescent ceiling lights. After 30 hours of incubation at 28°C, aeration was turned off, and the hatched nauplii settled to the bottom of the container and were collected by opening a valve fitted on the bottom of the container into a nylon net. The nauplii were rinsed with fresh well water and fed to the striped bass larvae.

1. Use of trade or manufacturer's name does not imply endorsement.

Feeding Striped Bass Larvae

The various sources of brine shrimp were fed as the only food to newly-hatched striped bass larvae in 112.5-L flowing-water aquaria at the Alabama Striped Bass Hatchery, Marion, Alabama. Aeration was provided to all aquaria. The larvae were obtained from adult striped bass collected from the wild and induced to spawn by injection with human chorionic gonadotropin (Bonn et al. 1976). Larvae were transferred from the hatching tanks to the aquaria after 4 days post-hatch. Well water (19 ± 1°C) was supplied to the aquaria at a rate of 13.5 L/minutes. Ceiling fluorescent lights provided continual illumination. Aquaria were treated every 8 hours with furacin (Hess and Clark, Inc., Ashland, Ohio) to prevent fungal infection, and uneaten brine shrimp were siphoned from aquaria every 8 hours.

The larvae were fed one source of brine shrimp nauplii every 3 hours at a density of 10-20 nauplii/mL aquarium water for 17 days. Nauplii density was determined by counting three 1-mL samples taken 10 minutes after adding nauplii to the aquarium. At the conclusion of the experiment, striped bass larvae were flash-frozen with liquid CO_2 and stored under nitrogen for subsequent lipid extraction. Larvae used for analysis were not fed 12 hours prior to sampling.

Growth rate of the fish was measured at 3-day intervals. Fifty larvae were randomly collected and preserved in 10% formalin for subsequent measurement of standard length with dial calipers (Webster and Lovell 1990a). Survival rate was determined by adding the number of larvae taken at each sampling period to the number of fish surviving at the conclusion of the experiment.

Lipid Analysis

Total lipids were extracted from the larvae and brine shrimp nauplii with chloroform-methanol (2:1; v/v) with 0.5% water by the method of Bligh and Dyer (1959), except the larvae were not homogenized due to their small size. Total lipids were separated into triacylglycerol (TG), phospholipid (PL), and free fatty acid (FFA) classes by thin-layer chromatography (TLC) by methods described in Kates (1986). Glass plates (20 × 20 cm) precoated with silica gel

60 (E. Merck, Darmstadt, Germany) were developed in hexane-diethyl ether-acetic acid (79:20:4; v/v) (Weete et al. 1983).

Lipids were visualized on the plates with iodine vapor and identified by comparison of Rf values with a standard containing triacylglycerol, free fatty acid, sterol and wax ester, and phospholipid classes (Applied Science Laboratories, Deerfield, Illinois), which was spotted alongside the lipid samples. Silica gel was scraped from the plates, washed once with methanol-chloroform (2:1; v/v) and two times with methanol-chloroform (1:1; v/v) to obtain the lipid. The solvent was evaporated under nitrogen.

Triacylglycerol (TG) and phospholipid (PL) classes were converted to their fatty acid methyl esters by transesterification using sodium methoxide in methanol (Applied Science Laboratories). Screwcap test tubes (Teflon lined) were used, with 2 mL sodium methoxide-methanol and 1 mL of benzene as cosolvent. After heating for 20 minutes at 80°C and cooling, 3 mL diethyl ether and 3 mL water was added. The esters were recovered in the diethyl ether phase and dried over anhydrous sodium sulfate. Free fatty acids (FFA) were methylated using BF_3/methanol (Morrison and Smith 1964). Fatty acid methyl ester analysis was conducted using a Hewlett-Packard (Avondale, Pennsylvania) gas chromatograph 5710A equipped with a flame-ionization detector and a 30-m capillary column DB-225 (J & W Scientific, Folsom, California). Carrier gas was nitrogen at a column pressure of 1.06 kg/cm^2. Injector and detector temperatures were both 250°C, and the oven temperature was programmed from 180 to 210°C at 1°C/minute. Fatty acids were recorded and quantitated on a Hewlett-Packard 3380A integrator-recorder and were identified by comparison of their retention times with those of standards (Nu-Chek Prep, Elysian, Minnesota).

Statistical Analysis

Length and survival data were analyzed using SAS ANOVA procedure (Statistical Analysis Systems 1985) and means compared using Duncan's multiple-range test. Analysis of variance (ANOVA) was computed on percentage of TG, PL, and FFA classes in the total lipid and the percentage of fatty acids found in each class. Percentages were arcsine-transformed prior to analysis (Zar 1984). Duncan's multiple range test was used to determine where differ-

ences existed among means. Untransformed data are reported to facilitate comparison with results from other related studies.

Experiment 2

The palmetto bass were produced from a striped bass female collected from Smith Lake, Alabama and a white bass male collected from the Coosa River, Alabama. The fish were spawned in a hatchery as described by Bonn et al. (1976). Five days after hatching, 10,000 larvae were counted into each of twelve 112-L aquaria supplied with a continual flow of well water. The 12 aquaria of larvae were randomly assigned one of the three groups of brine shrimp nauplii. Feeding and management of the fish were similar to those described for experiment 1. The fish were fed for 18 days.

Two sources of brine shrimp were purchased from a commercial supplier (Biomarine, Inc., Hawthorne, California). One strain was from the Great Salt Lake (GSL), Utah (lot #667246), and the other strain was from China (CH) (lot #501222). Brine shrimp from these sources had previously been shown to contain low (GSL) and high (CH) quantities of n-3 HUFA (Webster and Lovell 1990a). The cysts were hatched as described in experiment 1. One-half of the hatched nauplii from the GSL strain were transferred to an n-3 HUFA enrichment medium prior to feeding to the larvae. The enrichment medium was a suspension of menhaden oil in saline water. The menhaden oil came from the Gulf of Mexico (Zapata Haney, Inc., Hammond, Louisiana) and was fortified with 125 mg of ethoxyquin/kg to retard oxidation. The emulsifying agent was gum xanthum (Sigma Chemicals, St. Louis, Missouri). The emulsion containing 0.5% gum xanthum, 5% menhaden oil, and 94.5% saline (1.025 specific gravity) water was prepared as described by Clawson (1991). The nauplii were held in the emulsion for 3 to 9 hours prior to feeding. The fish were fed as described in experiment 1. Samples (2 g) of brine shrimp nauplii and larval fish were collected and frozen for fatty acid analysis as described in experiment 1. Length and survival measurements, as well as statistical analysis procedures were the same as those described in experiment 1.

RESULTS

Table 1 shows percentages of selected fatty acids in the total lipids from 5-day-old unfed striped bass and hybrid striped bass, menhaden oil, and brine shrimp fed in experiments 1 and 2. There were major differences in fatty acid composition among the various sources of brine shrimp. Most notable were the proportions of 16:1(n-7), palmitoleic acid; 18:3(n-3), linolenic acid; and 20:5(n-3), EPA. Both lots of Chinese (CH) brine shrimp had relatively high percentages of palmitoleic acid and EPA and relatively low percentages of linolenic acid. This is in contrast to San Francisco Bay (SFB), Colombian (COL), and Great Salt Lake (GSL) sources which contained relatively low percentages of palmitoleic acid and EPA but high percentages of linolenic acid. Enrichment of GSL nauplii (GSLE) reduced linolenic acid, increased EPA, and added a significant amount of DHA. None of nonenriched nauplii contained significant amounts of DHA.

Percentage length increase and percentage survival for the larvae in the two experiments are presented in Table 2. In experiment 1, the fish fed the CH brine shrimp had significantly larger size increase, 120%, and percentage survival, 41.0%, than fish fed the COL or SFB brine shrimp, which had 92 and 100% length increases and 7.8 and 7.8% percentage survival, respectively. In experiment 2, length increase and percentage survival were significantly greater for the fish fed the CH brine shrimp, 143% and 23%, respectively, and the GSLE brine shrimp, 147% and 22%, respectively, than for those fed the GSL brine shrimp, 67% and 16%, respectively. There was no significant difference in size increase or survival between the fish fed the CH and the enriched GSL brine shrimp.

Phospholipids comprised 17 to 29% of the total lipids in striped bass larvae (Table 3). There was no significant difference in PL percentage among the three sources of brine shrimp evaluated in experiment 1. Triglycerols comprised 63 to 77% and free fatty acids comprised 5.6 to 7.9%. There was no significant difference among brine shrimp in these components.

Fatty acid composition of the non-polar lipids (triaclylglycerols and free fatty acids) and polar lipids (phospholipids) of the fed larvae are shown in Table 4. There were higher concentrations of

TABLE 1. Percentages of selected fatty acids (area %) in the total lipids from 5-d old unfed striped bass (STB) and Palmetto bass (PSTB), menhaden fish oil, and various sources of brine shrimp nauplii. Brine shrimp sources were: Chinese (CH), Colombian (COL), San Francisco Bay (SFB), Great Salt Lake (GSL), and Great Salt Lake enriched with menhaden oil (GSLE).

				Experiment 1			Experiment 2		
Fatty acid	STB	PSTB	Menhaden oil	CH	COL	SFB	CH	GSL	GSLE
16:1(n-7)	21.4	14.2	11.1	19.8	5.6	5.4	15.5	4.1	5.6
18:3(n-3)	5.7	8.7	2.0	3.6	24.2	25.2	7.5	34.2	18.2
20:3(n-9)				0.1	0.2	0.3	0.2	0.3	0.3
20:5(n-3)	5.0	4.0	14.0	10.4	3.0	1.2	10.5	1.2	11.3
22:6(n-3)	9.0	5.5	8.1	0.0	0.0	0.0	< 0.1	< 0.1	4.1
Other[1]	58.9	67.6	62.7	66.2	67.2	68.2	66.5	60.5	60.8

[1]Other fatty acids measured were 12:0, 14:0, 14:1(n-5), 15:0, 16:0, 16:1(n-9), 16:2(n-4), 16:3(n-4), 16:4(n-3), 17:0, 18:0, cis-18:1(n-9), trans-18:1(n-9), 18:1(n-7), 18:2(n-6), 18:4(n-3), 20:0, 20:1(n-9), 20:2(n-6), 20:3(n-9), 20:4(n-6), 20:5(n-6), 22:0, 22:1(n-9), 22:2(n-6), 22:3(n-9), 22:4(n-6), 22:5(n-6), and 22:5(n-3).

TABLE 2. Length increase and survival of striped bass larvae (experiment 1) and palmetto bass larvae (experiment 2) fed different sources of brine shrimp nauplii. Values are means ± SE of four replications per treatment. Means in a column with different letters were significantly different ($P < 0.05$). Sources of brine shrimp were Chinese (CH), Colombian (COL), San Francisco Bay (SFB), Great Salt Lake (GSL), and Great Salt Lake enriched with menhaden oil (GSLE).

Source	Length increase (%)	Survival (%)
	Experiment 1	
CH	120.3 ± 4.4a	41.0 ± 3.4a
COL	91.5 ± 5.6b	7.8 ± 0.1b
SFB	100.0 ± 4.9b	7.8 ± 0.3b
	Experiment 2	
CH	143.3 ± 8.5a	23.2 ± 2.0a
GSL	67.4 ± 9.8b	15.9 ± 1.4b
GSLE	147.2 ± 8.7a	22.1 ± 1.1a

n-3 HUFA in the phospholipid fraction. The striped bass larvae fed the CH nauplii had much higher concentration of EPA than those fed COL or SFB. All striped bass larvae contained DHA. The palmetto bass fed the CH and the GSLE brine shrimp contained high concentrations of EPA while those fed the GSL nauplii had only a trace amount. The palmetto bass fed the GSLE brine shrimp contained significantly more DHA than the larvae fed CH or GSL brine shrimp. Prior to feeding, the larvae of striped bass and palmetto bass each had higher percentages of DHA than EPA in their lipids. After feeding, the percentage of DHA decreased and the percentage of EPA increased in the polar lipids.

The 20:3(n-9), eicosatrienoic acid (ETA), to EPA ratio and the ETA-to-DHA ratio for nonpolar lipids were not accurate indicators for growth. Striped bass larvae fed CH brine shrimp had an ETA-to-DHA ratio of 7.0, while larvae fed COL and SFB brine shrimp had

TABLE 4. Percentages of selected fatty acids in nonpolar (triacylglycerols and free fatty acids) and polar (phospholipids) lipids in striped bass (experiment 1) and palmetto bass (experiment 2) larvae fed different sources of brine shrimp nauplii. Values are means ± SE of 3 replications. Fatty acid means in a row for the same species with different letters were significantly different ($P < 0.05$). Sources of brine shrimp were Chinese (CH), Columbian (COL), San Francisco Bay (SFB), Great Salt Lake (GSL), and Great Salt Lake enriched with menhaden oil (GSLE).

	Striped bass			Palmetto bass		
Fatty acid	CH	COL	SFB	CH	GSL	GSLE
			Nonpolar lipids			
16:1(n-7)	16.3 ± 1.3a	4.4 ± 2.0b	5.2 ± 1.1b	8.9 ± 1.4c	6.5 ± 1.0a	12.0 ± 1.1b
18:3(n-3)	3.1 ± 0.8b	17.3 ± 2.4a	14.5 ± 3.7a	7.6 ± 1.6c	24.0 ± 1.2a	18.0 ± 1.4b
20:3(n-9)	0.7 ± 0.6	0.6 ± 0.3	1.4 ± 0.5	0.8 ± 0.1	0.7 ± 0.1	1.0 ± 0.1
20:5(n-3)	6.7 ± 2.6a	2.9 ± 2.7b	0.9 ± 0.7b	1.3 ± 0.2c	<0.1a	2.0 ± 0.6b
22:6(n-3)	0.1 ± 0.1b	2.0 ± 1.5ab	2.4 ± 0.3a	<0.1	<0.1	<0.1
Other[1]	73.1	72.8	75.6	81.4	68.8	67.0
ETA:EPA[2]	0.10	0.21	1.56	0.62	>7.0	0.50
ETA:DHA[3]	7.00	0.30	0.58	>8.0	>7.0	>10.0

	Polar lipids					
16:1(n-7)	8.2 ± 0.7a	2.6 ± 1.0b	6.1 ± 1.7a	4.0 ± 0.8c	2.5 ± 0.4a	8.5 ± 1.2b
18:3(n-3)	3.1 ± 0.4b	17.1 ± 1.9a	14.1 ± 2.7a	5.6 ± 1.9b	18.5 ± 1.1a	3.5 ± 1.2b
20:3(n-9)	0.3 ± 0.3	1.5 ± 0.4	0.9 ± 0.6	1.1 ± 0.1	0.8 ± 0.1	1.0 ± 0.1
20:5(n-3)	20.6 ± 1.2a	5.4 ± 1.9b	5.6 ± 2.2b	17.0 ± 1.9c	1.0 ± 0.1a	22.0 ± 1.0b
22:6(n-3)	1.6 ± 0.4b	2.3 ± 1.2ab	4.4 ± 1.0a	1.3 ± 1.1c	<0.1a	4.0 ± 1.1b
Other[1]	66.2	71.1	68.9	71.0	77.2	61.0
ETA:EPA[2]	0.015	0.278	0.161	0.065	0.800	0.045
ETA:DHA[3]	0.19	0.65	0.20	0.85	>8.0	0.25

[1] Other fatty acids measured were 12:0, 14:0, 14:1(n-5), 15:0, 16:0, 16:1(n-9), 16:2(n-4), 16:3(n-4), 16:4(n-3), 17:0, 18:0, cis-18:1(n-9), trans-18:1(n-9), 18:1(n-7), 18:2(n-6), 18:4(n-3), 20:0, 20:1(n-9), 20:2(n-6), 20:4(n-6), 20:4(n-3), 20:5(n-6), 22:0, 22:1(n-9), 22:2(n-6), 22:3(n-3), 22:4(n-6), 22:5(n-6), and 22:5(n-3).

[2]ETA:EPA = 20:3(n-9) to 20:5(n-3) ratio.

[3]ETA:DHA = 20:3(n-9) to 22:6(n-3) ratio.

(Webster and Lovell 1990a; Clawson and Lovell 1992), use of the ETA-to-EPA ratio may be more appropriate for determining dietary EFA sufficiency than the ETA-to-DHA ratio.

Use of the ETA-to-EPA ratio in non-polar lipids had inconsistent results. In experiment 1, striped bass fed the CH brine shrimp had the lowest ETA-to-EPA ratio (0.10) and the highest growth and survival. However, palmetto bass fed CH and GSLE brine shrimp had the highest growth and percentage survival but had higher (0.62 and 0.50, respectively) ETA-to-EPA ratios. These ratios were lower than the value for fish fed GSL brine shrimp (>7.0), which had lower growth and survival. The ETA-to-EPA ratio in phospholipids was consistently indicative of growth and survival rates. Fish with an ETA-to-EPA ratio of less than 0.10 had high growth rate and percentage survival. Thus, an ETA-to-EPA ratio in phospholipids of less than 0.10 may be indicative of EFA sufficiency of the diet for larval striped and palmetto bass.

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