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Lipid and Fatty Acid Composition of the Endogenous Energy Sources of Striped Bass (*Morone saxatilis*) Eggs

MAXWELL B. ELDRIDGE[a,*], **JEANNE D. JOSEPH**[b], **KAREN M. TABERSKI**[a] and **GLORIA T. SEABORN**[b], [a] *Southwest Fisheries Center, Tiburon Laboratory, National Marine Fisheries Service, 3150 Paradise Drive, Tiburon, CA 94920, and* [b] *Southeast Fisheries Center, Charleston Laboratory, National Marine Fisheries Service, PO Box 12607, Charleston, SC 29412*

ABSTRACT

The unique physiological flexibility of the early life stages of striped bass is attributed to the calorie-rich endogenous energy sources of the striped bass egg. Eggs of different aged striped bass from geographically separate populations were examined for lipid and fatty acid compositions and were found to be basically similar. Yolk components of the eggs contained significantly less total lipid than oil globules, were more diverse in lipid class composition and consisted mostly of polar lipids. Oil globules were entirely lipid material consisting predominantly of steryl/wax esters. Fatty acid compositions of yolk and oil globules differed according to their respective lipid compositions. The functional significance of these lipids is discussed in relation to the ecological context of the early life stages.
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INTRODUCTION

Studies on the feeding ecology and bioenergetics of striped bass embryos and larvae have indicated that early life stages are able to endure long periods of starvation and that they lack a critical period, defined as the sensitive and brief time when larvae begin active feeding and must convert from endogenous to exogenous energy sources (1,2). This physiological flexibility is largely attributed to the egg oil globule which persists for extended periods through the embryonic into the larval stage. At fertilization, the striped bass egg is characterized by a large single oil globule which comprises 55% of the total egg weight and 71% of the total caloric content. Proteinaceous yolk (5600 cal g^{-1}) and the oil globule (9500 cal g^{-1}) combine to an average 2.04 calories per egg. Lipid stores of the striped bass egg are of interest not only for their physiological and ecological functions, but also because they serve as reservoirs for lipophilic contaminants.

The following report presents information on the lipid and fatty acid compositions of the yolk and oil globule components of fully mature striped bass eggs. The object of our study was to further understanding of the physiology of the early life stages of striped bass by providing a detailed description of their initial lipid stores.

MATERIALS AND METHODS

Specimen Collection and Preparation

Five sexually mature striped bass were collected from the San Francisco Bay/Delta estuary and 2 females from the Coos River in Oregon. California fish were younger (5 and 6 yr) and smaller (62.4 cm mean standard length) than those from Oregon (8 and 14 yr; 78.5 cm standard length) such that fecundities also differed (0.6 vs 1.8 million eggs per female for California and Oregon, respectively). Subsamples of the left ovary (~150 g wet weight) were quick-frozen and stored at −40 C until analyzed. Eggs were removed from the center of each ovary, homogenized and centrifuged to separate yolk and oil globule components. The yolk and oil layers were aspirated into separate containers and lipids extracted from each fraction by the chloroform/methanol extraction procedure of Bligh and Dyer (3).

Prior to lipid analysis samples were stripped of solvent and ca. 50 mg of yolk or oil lipid was weighed and dissolved in 1 ml $CHCl_3$. This solution, or appropriate dilutions, was used for thin layer chromatography (TLC), quantitative analysis of lipid classes by TLC with flame ionization detection (FID) and gas liquid chromatography (GLC).

Thin Layer Chromatography/Flame Ionization Detection

Lipid classes present in the yolk and oil samples were separated and identified by comparison with a standard mixture on Silica Gel G plates (Supelco, Bellefonte, PA) developed in hexane/diethyl ether/acetic acid (80:20:1) and visualized with iodine and by spraying with 5% molybdophosphoric acid in 95% ethanol. A second plate, spotted and developed in the same manner, was sprayed with Liebermann-Burchard reagent after development, to confirm the presence of steryl esters.

Quantitation of individual lipid classes was achieved by TLC/FID. A comprehensive description of this relatively new technique is given by Ackman (4). An Iatroscan TH-10 analyzer was used with an Omniscribe B-5000 dual pen strip

*To whom correspondence should be addressed.

chart recorder. The FID was operated with a hydrogen flow rate of 160 ml/min and air flow rate of 2000 ml/min. Chromarods (type SII) were spotted with ca. 20 μg of lipid in $CHCl_3$ solution and developed in hexane/diethyl ether/formic acid (85:15:0.02). Following development, the rods were dried at 110 C for 4 min and scanned at a rate of 0.42 cm/sec. Peak areas were measured with a stepping pen integrator and were converted to weight percent lipid class by the use of correction factors (5,6) derived from authentic lipid standards. Steryl esters (SE) and wax esters (WE) could not be separated by the solvent system described above nor by any of the several other solvent mixtures attempted and are therefore reported as a sum.

Gas Liquid Chromatography

Methyl esters of fatty acids of all yolk and oil samples were prepared using 14% boron trifluoride in methanol, following saponification with 0.5 N potassium hydroxide. Esters were extracted with hexane and purified by TLC prior to GLC analysis. Fatty acid methyl esters were separated isothermally at 210 C on a Quadrex fused silica wall coated open tubular column (50 m × 0.21 mm id) coated with Carbowax 20-M operated in a Hewlett-Packard 5830 gas chromatograph equipped with an FID. Helium was used as the carrier gas at a flow of 1.5 ml/min (60 psig). Individual methyl ester peaks were provisionally identified using pure standards, secondary standards and equivalent chain length calculations. Relative composition was obtained by area normalization directly from the Hewlett-Packard 18850A terminal. Aliquots of the methyl esters were hydrogenated and rechromatographed to ensure the accuracy of identification of major components and to confirm that there was no significant loss of polyunsaturates during GLC.

RESULTS AND DISCUSSION

Lipid Composition

The high total lipid content of the egg was largely attributable to the oil globule. Yolk, which comprised an average 38% of the egg dry weight, contained 3.8% total lipids, while the oil globule was 100% extractable lipids. Eggs of other fishes, even those with distinct oil globules, have been found to have significantly less total lipid contents than eggs from striped bass (7-10). For example, a survey of eggs from 6 species by Kaitaranta and Ackman (10) showed total lipids to vary 2.4-9.2%.

Lipid classes differed sharply between egg yolk and oil globule components (Table 1), thus confirming that our technique successfully separated yolk and oil globule lipids. Only wax and steryl esters and triacylglycerols were present in the oil globules with steryl and wax esters averaging 90.4% of the total lipids. This is also the major lipid found in some marine zooplankton, particularly arctic upper-water crustacea (11,12). The presence of wax esters in fish is documented mostly in somatic tissues (muscle and liver) of deep-water forms (13-15). The occurrence of steryl/wax esters in eggs is ecologically more diverse (10), including marine and freshwater species: *Mugil cephalus* (16), *Merluccius capensis, Theragra chalcogramma,* and *Scomber japonicus* (17), *Trichogaster cosby* (18), *Perca fluviatilis, Lota lota* (19), and *Sciaenops ocellati* (7). We suspected wax esters were in striped bass eggs because Dergaleva and Shatunovskiy (20) had found declining concentrations of this lipid class in larvae and juveniles of striped bass from 8 to 40 days after hatching. An important morphological feature common to eggs of all these species is the presence of discrete oil globules.

Although yolk lipids comprised a small portion of the yolk weight, it was more diverse in its composition than oil globule lipids. Phospholipids were an average 78.9% of yolk total lipids. This is not unusual since phospholipids are documented as the major lipid of egg yolks in a variety of fishes (10,19,21-23). Yolk is the endogenous energy source first consumed by the embryo and larvae (1), and is totally absorbed within 5 days after hatching (at 18 C), a period characterized by extensive tissue differentiation and growth. Since phospholipids are the major lipid constituents of biomembranes, it seems logical they were preferentially transferred

TABLE 1

Lipid Class Composition (Percent) of Oil Globules and Yolk Lipids of Striped Bass Eggs[a]

Egg component	Steryl and wax esters	Triacylglycerols	Free fatty acids	Sterols	Phospholipids
Yolk	5.3 ± 1.7	6.5 ± 1.9	6.6 ± 1.8	2.8 ± 0.9	78.9 ± 2.9
Oil globule	90.4 ± 2.8	9.6 ± 2.8	---	---	---

[a]Values presented as means ± standard errors for all fish combined, 4 determinations per fish.

from the yolk during this period. Free fatty acids and sterols were also present in small quantities and, like phospholipids, were present only in the yolk. The varying amounts of free fatty acids were probably due to hydrolysis of the samples during storage or laboratory work-up. Sterols were most likely cholesterol, which is common to most animal tissues.

Fatty Acid Composition

Fish lipids are generally characterized by high contents of long-chain polyunsaturated fatty acids. Such was the case with both striped bass yolk and oil globule lipids (Table 2). The dominant isomers of the C_{18} to C_{20} monoethylenic fatty acids were the 18:1ω9 and 20:1ω9 reported elsewhere for fish lipids (24). The respective fatty acid composition of yolk and oil globule lipids basically reflected differences in their lipid classes. The fatty acid composition of the yolk lipids was typical of marine phospholipids being dominated by palmitic (16:0), eicosapentaenoic (20:5ω3) and docosahexaenoic (22:6ω3) acids. Oil globule lipids were made up of fatty acids very similar to those found in somatic tissues of meso- and bathypelagic fishes, animals known to often have large amounts of wax esters (14,25). The principal acids were oleic (18:1ω9), palmitoleic (16:1ω7) and docosahexaenoic (22:6ω3). Egg lipids of striped bass from this study had a mixed fatty acid composition which is indicative of overlapping freshwater and marine environments (26). Total C_{16} and C_{18} fatty acids were high; palmitic acid was high in the yolk and the tetraenoic acids low. The lack of a clear pattern agrees with the life history of the species, namely, that it inhabits the estuarine zone.

The fatty acid composition was also consistent among individual females regardless of location, age, condition or pollutant burden. Lasker and Theilacker (27) also found remarkably similar fatty acid patterns among sardine eggs of different females at different stages of maturation.

The functional roles of high lipid contents and steryl/wax esters in fish embryos and larvae have not been determined, but it may be proposed that they generally serve in marine organisms for metabolic energy or buoyancy (28). Their most clearly identified purpose is in providing energy, especially in situations of food deprivation (15,25,29), emergencies of delayed hatch (30), or when large reserves of energy are needed for rapid embryonic development (7). In the context of the early life stage ecology of striped bass, the unique lipid characteristics serve to provide: (a) abundant energy for rapid embryonic development (incubation period = 48 hr at 18 C); (b) necessary flotation of pelagic eggs and early larvae until formation of functional swim bladders; and (c) long-term energy for feeding larvae because of uncertainty of food availability (e.g., dietary zooplankton patchiness).

TABLE 2

Fatty Acid Composition of Yolk and Oil Globule Components of Striped Bass Eggs[a]

Fish fatty acids	Yolk	Oil globule
Saturates		
14:0	1.2±0.1	1.6±0.4
16:0	17.5±1.9	6.2±0.6
18:0	9.9±0.9	0.7±0.2
Monoenes		
16:1ω9	1.3±0.2	2.2±0.4
16:1ω7	4.6±0.5	13.8±1.6
18:1ω9	15.5±1.8	35.7±2.7
18:1ω7	4.2±0.4	5.4±0.6
20:1ω11	trace	0.3±0.4
20:1ω9	1.2±0.3	1.1±0.2
20:1ω7	0.1±0.0	0.1±0.1
Dienes		
18:2ω6	0.8±0.3	1.8±0.6
20:2ω6	0.2±0.1	0.2±0.0
Trienes		
18:3ω3	0.2±0.1	0.7±0.2
20:3ω6	0.1±0.0	0.1±0.0
Tetraenes		
18:4ω3	0.3±0.1	0.8±0.3
20:4ω3	0.2±0.1	0.6±0.1
20:4ω6	3.3±0.4	1.3±0.3
22:4ω6	0.3±0.3	trace
Pentaenes		
20:5ω3	8.6±3.8	6.4±1.6
22:5ω6	0.2±0.1	0.3±0.1
22:5ω3	2.4±0.7	2.9±0.7
Hexaenes		
22:6ω3	19.9±2.2	10.0±1.5

[a]Percent composition by weight presented as means ± standard error.

The occurrence of discrete oil globules in fish eggs and larvae is more common than one might think. In a worldwide review of fish egg surveys, Ahlstrom and Moser (31) found that most pelagic marine fish eggs have discrete oil globules. As the oil globules of these and inshore and estuarine species are analyzed, we believe that steryl/wax esters will be found to be the principal form of endogenous energy in the eggs.

Recent findings show that striped bass eggs from geographically distant populations contained high levels of petrochemicals, pesticides and chlorinated hydrocarbons (32) which correlated with reduced reproductive capacity and lowered early life stage survival. The advantages of lipid to the embryo and larva are then offset by the facility with which lipophilic and toxic chemicals are accumulated from the environment.

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