THE EFFECTS OF DIETARY LIPID ON SPAWNING PERFORMANCE AND EGG QUALITY IN BLACK SEA BASS *Centropristis striata*

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ABSTRACT

The objectives of this study were to determine the effects of dietary lipid on spawning performance and egg quality in black sea bass (BSB) (*Centropristis striata*). The study was conducted over two consecutive spawning seasons. During year one, adult broodstock (N = 162) were held in six 1.8-m dia. Controlled environment tanks in sea water (35 g/L) and were fed three different dietary treatments: two commercially-prepared diets each with 45% protein and two different lipid levels (12% and 20%), and a natural diet of frozen fish, Atlantic silversides *Menidia menidia*. Broodstock were fed to satiation 6d/week beginning 3 mos. before the spawning season and were subjected to simulated photothermal conditions that mimicked natural variation until the spawning season (Apr.-Jul. 2005) when constant temperature (mean = 20.2) and photoperiod (13 L:11 D) were maintained. Mature females (mean oocyte diameter (MOD) $\geq$ 330 μm) were implanted with a luteinizing hormone releasing hormone analog (LHRHa) pellet at a nominal dose of 72 μg/kg body weight (BW) and then held with 5 running males for volitional spawning. Egg collectors were monitored daily, and non-viable (sinking) eggs and viable (floating) eggs were quantified. Buoyant eggs were transferred to 18-L incubators at 35 g/L and 19^{o}C where fertilization and hatching success were determined as indices of egg quality. A total of 6 induced spawning trials were conducted for fish fed a natural diet of silversides, 7 for fish fed a 20% lipid diet, and 6 for fish fed a 12% lipid diet. Spawning performance varied widely among individual females within each treatment; however, fish fed a diet of silversides (31.9% lipid) had a significantly ($P < 0.05$) higher fertilization success (22.4%) than the commercially prepared diets with 20% and 12% lipid (4.8 and 0.6%, respectively). The silverside diet treatment also produced

significantly more yolk sac larvae (YSL) per female (21.8 x 10^3) than the 12% lipid treatment (0.3 x 10^3). Hatching success of the fertilized eggs was similar in all diets (silverside diet: 51.3%; 20% lipid diet: 58.6%; and 12% lipid diet: 40.0%), but only two spawns from the 12 % lipid diet yielded viable yolk-sac larvae. Eggs from the silverside treatment contained a significantly greater proportion of *n*-3 series fatty acids with docosahexaenoic acid (DHA) (22:6*n*-3) as the largest fraction. The eggs from commercially prepared dietary treatments contained significantly more *n*-6 fatty acids. The proportions of egg *n*-3 and *n*-6 fatty acids were similar to those found in the diets and are likely associated with the higher fertilization success of fish fed a diet of silversides.

During year two, broodstock were fed three different dietary treatments: frozen Atlantic silversides, and two commercially-prepared diets with 45% protein and two different lipid levels (18% and 23%). Spawning protocols were the same as for the previous season, with the following exception: mean nominal dose of LHRHa was 50 µg/kg BW. A total of 8 spawning trials was conducted for fish in each dietary treatment. No significant ($P< 0.05$) differences were detected among dietary treatments for spawning performance or egg quality parameters. As in year 1, eggs from the silverside treatment contained significantly ($P < 0.05$) more *n*-3 fatty acids than eggs from the commercially prepared dietary treatments and significantly ($P < 0.05$) fewer *n*-6 fatty acids. Docosahexaenoic acid was the largest portion of fatty acids in eggs of all treatments, with eggs from the silverside treatment containing a significantly larger proportion.

The results suggested that dietary lipid has pronounced effects on spawning performance, egg fatty acid composition, and egg quality in BSB. For fish fed the

commercially prepared diets, a dietary lipid level of 12% was clearly inadequate for successful reproduction. The poor spawning performance from the fish fed the 12% lipid diet may have been related to higher levels of linoleic acid (18:2*n*-6) found in this diet. For fish fed silversides, spawning performance varied greatly between experiments, which may indicate a variation in the quality of this natural diet.

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DEDICATION

I would like to dedicate this thesis to my mother Linda Adamson, who has never failed in believing in me. Without her encouragement and support this would never have been possible. I would also like to dedicate this to me wife KJ, whose patience and support has played an instrumental role in my success through graduate school.

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INTRODUCTION

Black Sea Bass Ecology and Reproductive Biology

The black sea bass (*Centropristis striata*) is a member of the family Serranidae, found in continental shelf waters of the northeastern Gulf of Mexico and the entire US Atlantic coast (Hood et al. 1994). Within this range, two subspecies have been identified based on morphometric, meristic, and mtDNA differences (Bowen and Avise 1990). These two subspecies occur in the Gulf of Mexico and the Northwest Atlantic. There is evidence of further segregation within the Atlantic coast population into separate stocks north and south of Cape Hatteras, NC. Evidence of separate sub-stocks is based on meristic and morphometric variation; however, segregation is still in question due to the lack of genetic data (Shepherd 1991).

Black sea bass are protogynous hermaphrodites, beginning life as a female and later switching sex to a male (Lavenda 1949). Sexual succession can occur at any age or size class. However, fish older than age 4 and larger than 220 mm SL are most likely to be males (McGovern et al. 2002). In southeastern US waters, the female size at 50% maturity is 108 mm SL (McGovern et al. 2002). However, fecundity is relatively low in small fish and increases rapidly with increasing length, weight and age (Wenner et al. 1986). The peak spawning period off the southeastern US coast takes place between March and May, and tapers off in June (Wenner et al. 1986, McGovern et al. 2002). Females may spawn several times within a single spawning season, which is consistent

with a multiple clutch group-synchronous spawner (Wallace and Selman 1981, Watanabe et al. 2003).

Black Sea Bass Fishery

Black sea bass support an important commercial fishery in the southeastern US and are one of the major reef fish species targeted by recreational and charter boats in North Carolina (Mercer 1989, Manooch et al. 1981). Black sea bass commercial landings dramatically increased in the 1960's after the advent of a harvesting technique using baited wire traps (Mercer 1989). The current status of the black sea bass stock off the southeastern US coast was assessed by the South East Data, Assessment, and Review stock assessment workshop in 2003 (SEDAR 2 2003). An age-structured model was applied to existing data and indicated a heavily exploited stock. The instantaneous fishing mortality rate (F) for 2001 was estimated to be 6 times the fishing mortality rate for maximum sustainable yield, and the spawning-stock biomass for 2002 was estimated to be 22% of the spawning-stock biomass for maximum sustainable yield. The values of these parameters indicate that the black sea bass stock off the southeastern US is overfished and overfishing is occurring. With a reduction of F to 50-90%, stock projections estimated a stock rebuilding time of 10 to 25 years (SEDAR 2 2003).

Larviculture and Growout

The culture of black sea bass has improved substantially within the past 10 years. Tucker (1984) was the first to raise black sea bass beyond the yolksac stage, and determined some of the basic larval rearing requirements. Recently, much work has been done in controlled spawning (Watanabe et al. 2003, Berlinsky et al. 2005), rearing through the larval and juvenile stages (Berlinsky et al. 2000, 2004, Copeland and Watanabe 2006), and the growout of subadults to marketable stages (Copeland et al. 2002, 2003). These studies have shown black sea bass to be a promising candidate for commercial culture.

Captive Spawning of Black Sea Bass

Finfish held in captivity often become reproductively dysfunctional, due to a failure of eggs to undergo final oocyte maturation (Mylonas and Zohar 2001). Final oocyte maturation can often be achieved in many species through the manipulation of environmental cues or zeitgebers in the laboratory (Bromage et al. 1993). However, in some species it is necessary to intervene in the hypothalamus-pituitary-gonadal axis (HPG) with exogenous hormones in addition to the manipulation of zeitgebers (Harvey and Hoar 1979). This can be done at various levels of the HPG axis, from the use of releasing hormones to the use of gonadotropins and sex steroids (Lam 1985). By implementing these methods, culturists have been able reduce the reliance on wild populations for seed stock and develop new candidate species for future commercial

applications (Bromage 1995).

Captive black sea bass were originally spawned by injecting fish with human chorionic gonadotropins (hCG) (Hoff 1970). Tucker (1984) improved upon this method by determining that spawning success was achieved most consistently by selecting females for induced spawning with a minimum oocyte diameter of 0.4 mm. In addition, it was determined that multiple, rather than single, injections of hCG increased spawning success (Tucker 1984). More recently, synthetic analogs of both luteinizing hormone releasing hormone (LHRH-a) and gonadotropin releasing hormone (GnRH-a) have been used to successfully spawn captive black sea bass (Watanabe et al. 2003, White 2004, Berlinsky et al. 2005). These techniques have greatly improved both the quality and predictability of volitionally spawned black sea bass. However, there is still a considerable amount of variation in spawning success among individuals and spawning periods. For commercial-scale egg production of this species to be successful, much more work is needed to improve the quality of eggs and reliability of spawning.

Broodstock Nutrition

Broodstock nutrition has been shown to have a substantial effect on fecundity, gonadal development, and egg quality of cultured fishes (Watanabe 1985). Due to the importance of broodstock nutrition, there have been many studies conducted over the last twenty years examining the reproductive nutritional requirements of various species important to aquaculture. These studies have examined many nutrients including vitamin

E (Watanabe et al. 1991), ascorbic acid (Blom and Dabrowski 1995), and astaxanthin (Verakunpiriya et al. 1997; Watanabe et al. 1991).

Total lipids and fatty acids have also been determined to have a significant effect on the reproductive performance of broodstock. Duray et al. (1994) showed that increasing dietary lipid levels from 12% to 18% improved hatching success and fecundity in rabbitfish (*Siganus guttatus*). Cerda et al. (1995) compared spawning performance of the sea bass (*Dicentrarchus labrax*) fed two commercial diets with different lipid levels (8% and 11%) and broodstock fed a natural diet of raw fish (bogue, (*Boops boops*), 20% lipid). The broodstock fed the 8% lipid diet for 6 months failed to produce viable eggs, and broodstock fed the 11% lipid diet failed to produce viable eggs during their second spawning season. The failure to produce viable eggs after a long-term deficiency in dietary lipid was attributed to a reduced plasma concentration of the sex steroids 17β-estradiol (E2) and testosterone (T) prior to spawning. Histological samples of the ovaries were also taken during the second spawning season and a noticeably larger proportion of atretic oocytes were seen in samples collected during the vitellogenic period of the fish fed the commercial diets (Cerda et al. 1995). Atretic oocytes are non-steroidogenic, and their higher proportion in the ovaries of fish fed the commercial diets may have contributed to decreased steroid levels (Nagahama 1983). Navas et al. (1998) also examined the effects of dietary lipid on spawning performance in the sea bass (*D. labrax*). Their findings showed an elevated plasma concentration of E2 during the vitellogenic period and reduced spawning performance in fish fed commercial diets with 10% and 22% lipid compared to fish fed a natural diet of raw fish. It was suggested that

high levels of monounsaturated fatty acids in the commercial diets may have contributed to increased steroidogenesis in the follicle by inducing production of peroxisomes, which are organelles important in the metabolism of lipids, eicosanoids, and cholesterol (Navas et al. 1998). Although these findings appear contradictory with respect to the plasma E2 levels and spawning performance, both studies showed significantly reduced spawning performance in the fish fed commercial diets and demonstrate that dietary lipid levels can have a significant effect on the production of reproductive hormones responsible for successful spawning.

Another important role of lipids in spawning is to supply the metabolic energy required for gonadal development (Sargent 1995). In capelin (*Mallotus villosus*), a large portion of the lipids mobilized from the muscle tissue prior to spawning was not found in either the gonads or the liver. This suggested that the lipids were catabolized to provide energy for spawning, with a preference shown for the monounsaturates 20:1 and 22:1. The biosynthesis of vitellogenin was also determined to be responsible for a substantial portion of the energetic requirements prior to spawning in some species (Henderson et al. 1984). In Atlantic cod (*Gadus morhua*) and rainbow trout (*Salmo gairdneri*), a substantial decrease in fecundity was seen in broodstock fed a restricted ration of food (Knox et al. 1988, Lambert and Dutil 2000). In addition, the restricted ration in Atlantic cod increased the risk of brooder mortality following a spawn (Lambert and Dutil 2000).

It has been clearly demonstrated that maternal dietary lipid composition, specifically fatty acids, has a marked effect on the fatty acid profile of spawned eggs (Watanabe et al. 1984; Mourente and Ordizola 1990; Harel et al. 1994, Fernandez-

Palacios et al. 1995; Rodriguez et al. 1998; Mazorra et al. 2003). Watanabe et al. (1984) established that red sea bream (*Chrysophrys major*) broodstock fed a diet deficient in *n*-3 HUFA spawned eggs that contained a lower fraction of *n*-3 highly unsaturated fatty acids (HUFA) when compared to broodstock fed a diet high in *n*-3 HUFA.

It has also been shown that broodstock dietary lipid has a significant effect on egg and larval viability. Furuita et al. (2003) determined that in Japanese flounder (*Paralichthys olivaceus*) broodstock, dietary arachodonic acid (AA) level showed a considerable variation in larval quality. A dietary AA level of 0.6% produced larvae that had a lower percentage of abnormalities (ex. abnormal body curvature and tail loss), higher survival at 3d post hatch (DPH), and a higher SAI (survival activity index based on a starvation tolerance test) when compared to a dietary AA level of 0.1%. It was also found that a dietary AA level of 1.2% negatively affected larval quality and, larvae failed to survive beyond 3 DPH (Furuita et al. 2003).

Egg Quality

Egg quality can be simply defined as "the egg's potential to produce viable fry" (Kjorsvik et al. 1990). In a commercial aquaculture setting it is very important to have a quick and efficient method of separating the viable eggs from the non-viable eggs as early as possible to avoid wasting resources. There are several methods for doing so and there seems to be little agreement on a method that works for all marine species. Percentage of fertile eggs or fertility rate has been shown to be correlated with high

quality eggs in several marine species and blastomere morphology has also been used as a quality indicator (Kjorsvik et al. 1990, 2003; Shields et al. 1997). However, in many marine species fertilization rate has been shown to be a poor indicator of survival at later stages (Kjorsvik et al. 1990; Sargent 1995), and blastomere morphology may be difficult to use in a commercial setting. Many marine hatcheries implement a rapid method of using egg buoyancy to determine egg quality in which high quality eggs will float and poor quality eggs will sink (McEvoy 1984; Carrillo et al. 1989; Kjorsvik et al. 1990; Sargent 1995).

Lipids

The fatty acid (FA) composition of eggs has been shown to have a strong correlation with blastomere morphology and hatching success (Evans et al. 1996; Pickova et al. 1997). Of the FA found in marine finfish eggs, the polyunsaturated fatty acids (PUFA) are the most abundant, equaling as much as 50% of the total egg lipids of ripe ovaries (Tocher and Sargent 1984). The *n*-6 and *n*-3 groups of PUFA are termed essential fatty acids (EFA), due to the inability of de novo synthesis of these EFAs in most animals, and must be acquired from the diet (Tacon 1990). The *n*-3 PUFA are the most abundant in the marine ecosystem and are readily available to carnivorous fishes. For this reason, it is believed that marine teleosts have lost the ability to produce the enzymes Δ^5-desaturase and C_{18} and C_{20} fatty acid elongase. These enzymes are required for converting the *n*-3 precursor 18:3*n*-3 (linolenic acid) to 20:5*n*-3 (EPA) and 22:6*n*-3

(DHA). They are also required for the conversion of the *n*-6 precursor 18:2*n*-6 (LA) to 20:4*n*-6 (AA) (Sargent et al. 2002).

DHA, EPA and AA are important physiological components in fish. DHA has been shown to be a critical part of biological membranes in fish and particularly important in neural cell membranes (Bell and Dick 1991, Valentine and Valentine 2004). EPA and AA are precursors to the biologically important compounds eicosanoids, autocrines which function in localized physiological regulation. They are important in many functions ranging from reproduction to blood clotting (Sargent et al. 2002). For example, AA has been shown to have a significant regulatory effect on steroidogenesis in goldfish (Mercure and Kraak 1996). The ratio of EPA and AA in cellular membranes is also an important consideration. This is because AA produces more biologically active eicosanoids than EPA (Sargent et al. 2002), and EPA has been shown to be a strong inhibitor of the eicosanoids derived from AA (Bell et al. 1994). The appropriate ratio of these HUFA in the lipid reserves of eggs is critical for hatching success, development and growth of the embryo, and survival through the onset of exogenous feeding of larvae (Sargent 1995; Rainuzzo et al. 1997; Bruce et al. 1999). However, the appropriate amounts of HUFA required for embryo and larval development varies among species and must be determined for individual species (Sargent et al. 1999; Sargent et al. 2002).

Due to the importance of EFA in developing embryos, there have been many studies that have examined the EFA composition of eggs and its relation to egg quality. In the striped mullet (*Mugil cephalus*), high levels of AA were associated with a high percentage of fertility in spawned eggs (Tamaru et al. 1992; Ako et al. 1994). This was

also seen in Atlantic halibut (*Hippoglossus hippoglossus*), along with high levels of DHA (Evans et al. 1996). In both species, high fertility was also correlated with a high percentage of total lipids.

Objectives

The objectives of this study are to determine the effect of dietary lipid level on the spawning performance, fatty acid profile, and egg quality of black sea bass.

Hypothesis

H_o: Dietary lipid level will have no significant effect on spawning performance, fatty acid profile or egg quality in black sea bass.

H_A: Dietary lipid level will have a significant effect on spawning performance, fatty acid profile or egg quality in black sea bass.

METHODS

Experimental Animals

Adult black sea bass ranging in size from 0.30 – 3.27 kg were used for this study. The oldest fish (2.3 ± 0.12 kg = $\bar{X}$ ± SE, N = 77) were collected in November 2000 by baited wire traps off Carolina Beach (Copeland et al. 2003; Watanabe et al. 2003). Younger fish (1.1 ± 0.9 kg, N = 25) were F1 progeny of wild-caught broodstock. In November 2004, additional fish averaging 0.30 ± .08 kg (N = 60) were collected.

Broodstock were used for spawning experiments over the course of two spawning

seasons during the period November 2004 to August 2006. Fish were fed a diet of Atlantic silversides (*Menidia menidia*) for at least two weeks before each trial and then randomly assigned to the dietary treatments described below.

Experimental System

An outdoor recirculating system was used to hold and spawn the black sea bass broodstock (Fig. 1). The system consisted of six 2,000-L tanks (dia. = 1.83 m, depth = 0.81 m), a 2,000-L sump, an external bead filter, a foam fractionator and an ultraviolet sterilizer. An exchange rate of approximately 25% per day was maintained with seawater (32-35 g/L) pumped from the Atlantic Intracoastal Waterway. A 3-hp heat pump was used to control the water temperature, and the photoperiod during the spawning season was controlled with timer-controlled fluorescent lights and light-proof covers.

Experimental Design

To compare the effects of dietary lipid on spawning performance, two isoprotein (45%) commercially prepared diets with different lipid levels (20% and 12%) and a natural diet of frozen fish, Atlantic silversides were fed to three separate groups of black sea bass broodstock (Table 1, Table 2, Table 3, Fig. 2). The diets were fed to each treatment group beginning in January 2005, three months before the spawning season. Fish were hand fed to apparent satiation 6 days a week. There was a total of 54 fish per dietary treatment (1.5 ± 0.8 kg). During the non-spawning period, fish in each treatment

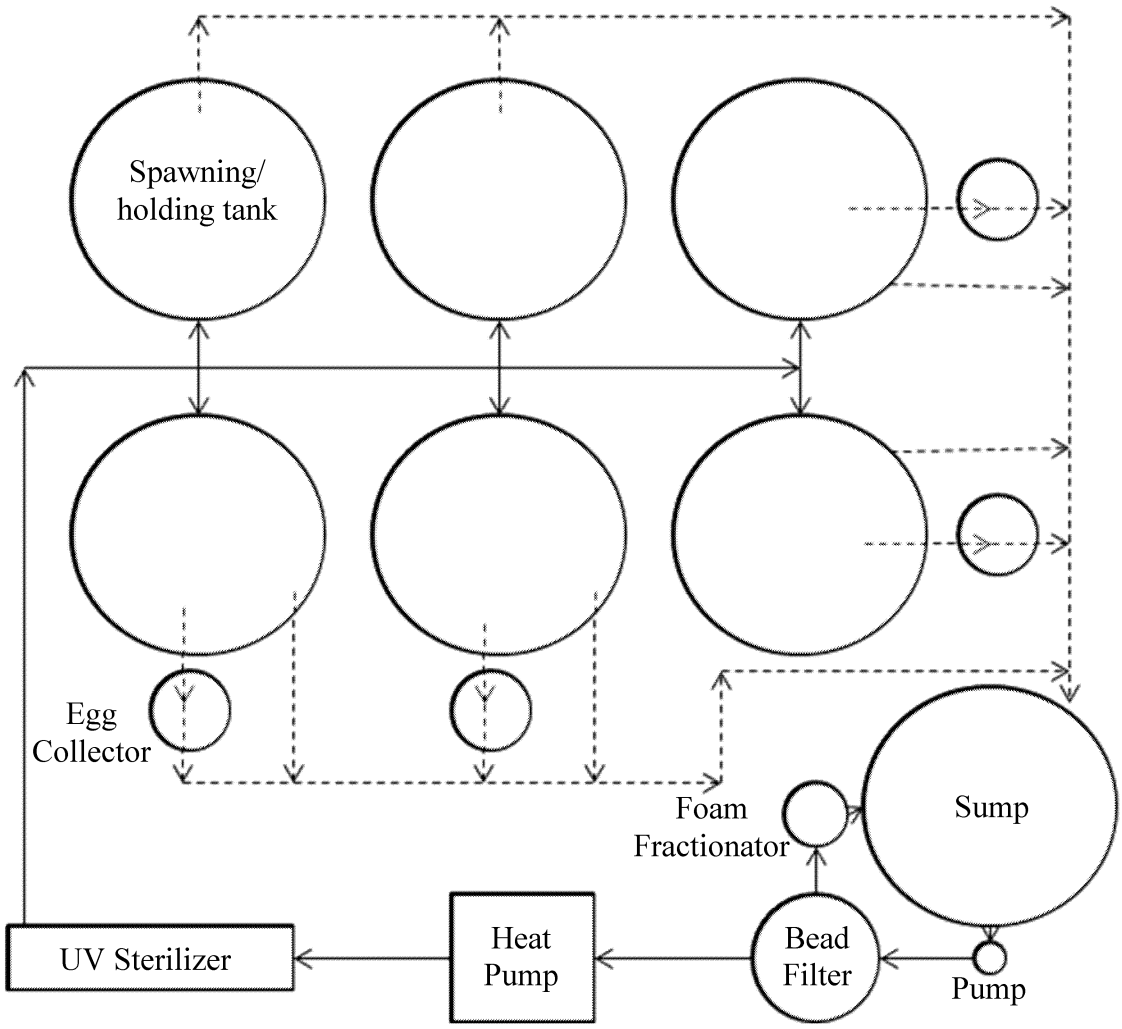

Figure 1. Line drawing of the spawning/holding tanks, egg collectors and filtration equipment used for broodstock nutrition experiments. Arrows represent the directional flow of water in the system.

were held in two tanks at a density of 27 fish per tank and a sex ratio of 2: 1 (female: male). The temperature was maintained at 17.1 ± 0.52^{o}C and fish were exposed to an ambient photoperiod by maintaining the tank covers ajar.

Based on the results of the 2005 spawning season, two commercially prepared diets with lipid levels of 23% and 18%, and a natural diet of frozen fish, Atlantic silversides were evaluated during a second spawning season (2006) (Table 4, Table 5, Table 6, Fig. 3). Fish were hand fed to apparent satiation (6 days a week) beginning five months before spawning season.

A total of 38-40 fish was used for each dietary treatment. Fish were held at a density of 17-21 fish per tank prior to spawning season, a sex ratio of 2: 1 (female: male), and under 17.7 ± 0.8^{o}C and ambient light.

Induced Spawning

During the 2005 spring-summer spawning season, fish from each treatment were combined into one tank at a density of 54 fish per tank. The remaining three tanks were used for spawning. Eligible females and males were transferred to a spawning tank at a ratio of 5 males to 1 female. During spawning season, the temperature and photoperiod were maintained at 20.1 ± 0.1^{o}C and 13 L: 11 D, respectively. Female candidates were selected based on ovarian biopsy, and fish with a minimum MOD of 0.330 mm were selected for induced spawning. Oocytes were preserved with 10% formalin in seawater (White 2004). MOD was determined by measuring 40 -100 oocytes of calibrated digital

Table 1. Commercial diet formulations given in percent as prepared by Integral Fish Foods Inc. (Grand Junction, CO) for the 2005 spawning season. All diets were stabilized with vitamin C at 400 mg/kg.

	Diet (% Lipid)	
Ingredient	20%	12%
Fishmeal [a]	32.0	32.0
Fish meal analog [b]	22.5	20.0
Soymeal [c]	7.5	7.0
Whole wheat	20.0	15.0
Wheat middlings	0.0	17.5
Fish oil [d]	11.7	2.0
Soy oil	2.0	2.0
Monosodium phosphate	0.8	0.8
Vitamin + mineral premix	3.0	3.0
Dl methionine	0.3	0.3
NaCl	0.5	0.5

[a] Menhaden (64 % protein)
[b] Contains some or all: 85% bloodmeal, hydrolysed feather meal, poultry byproducts meal, Dl methionine, lysine, monosodium phosphate
[c] 46 % protein
[d] Menhaden

Table 2. Proximate composition and calculated energetic values of the frozen Atlantic silversides (SS) and experimental diets used during the 2005 spawning season. Values are given in percent of dry weight and represent mean ± s.e. (N = 3).

	Diets		
	SS	20% Lipid	12% Lipid
Moisture	68.4 ± 1.6	8.4 ± 0.1	8.9 ± 0.1
Protein	58.8 ± 0.2	46.3 ± 0.1	46.6 ± 0.1
Lipid	31.9 ± 0.0	20.4 ± 0.7	11.6 ± 0.1
Fiber	nd [a]	1.1 ± 0.0	2.3 ± 0.1
Ash	10.2 ± 0.1	10.9 ± 0.0	12.1 ± 0.0
NFE [b]	nd	21.3 ± 0.6	27.4 ± 0.3
Energy [c]	6.39 ± 0.01	5.43 ± 0.04	4.86 ± 0.01

[a] Not detected
[b] Nitrogen free extract.
[c] Calculated based on energetic values for protein, lipid and NFE as 5.7, 9.5, 4 kcal/g respectively.

Table 3. Fatty acid profile of diets fed to black sea bass during the 2005 spawning season: Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. Values represent mean ± s.e. (N = 3).

Fatty acids		Diet	
	SS 2005	20% Lipid	12% Lipid
% lipid	31.9 ± 0.03	18.7 ± 0.64	10.6 ± 0.10
14:0	6.04 ± 0.01	7.78 ± 0.04	5.75 ± 0.02
15:0	1.49 ± 0.01	0.66 ± 0.01	0.55 ± 0.01
16:0	21.8 ± 0.05	22.7 ± 0.03	23.2 ± 0.09
16:1*n* – 7	14.4 ± 0.02	10.4 ± 0.04	7.56 ± 0.04
16:2	nd [b]	1.50 ± 0.01	0.82 ± 0.04
16:3*n* – 3	nd	1.93 ± 0.01	0.75 ± 0.03
16:4	nd	0.74 ± 0.00	nd
Unknown [a]	0.59 ± 0.02	nd	nd
Unknown [a]	1.95 ± 0.02	nd	nd
Unknown [a]	0.77 ± 0.00	nd	nd
18:0	4.35 ± 0.03	4.82 ± 0.02	5.81 ± 0.03
18:1*n* – 9	23.6 ± 0.05	13.8 ± 0.05	19.9 ± 0.11
18:1*n* – 7	4.85 ± 0.08	2.97 ± 0.01	2.27 ± 0.07
18:2*n* – 6	nd	9.66 ± 0.06	18.8 ± 0.06
18:4*n* – 3	0.82 ± 0.01	1.94 ± 0.01	1.14 ± 0.04
Unknown	0.74 ± 0.02	nd	nd
20:4*n* – 6	0.77 ± 0.01	1.00 ± 0.01	0.73 ± 0.01
20:5*n* – 3	3.31 ± 0.04	9.10 ± 0.06	5.78 ± 0.09
20:1	1.22 ± 0.00	1.07 ± 0.02	nd
20:1*n* – 9	0.77 ± 0.00	0.95 ± 0.01	0.85 ± 0.01
22:5*n* – 3	2.83 ± 0.04	1.95 ± 0.01	1.18 ± 0.02
22:6*n* – 3	9.30 ± 0.08	6.72 ± 0.04	4.92 ± 0.12
Saturates	34.6 ± 0.17	36.3 ± 0.13	35.3 ± 0.10
Monoenes	47.2 ± 0.12	29.2 ± 0.03	30.6 ± 0.16
PUFAS	17.1 ± 0.14	34.6 ± 0.11	34.1 ± 0.25
Σ *n* – 6	0.78 ± 0.01	10.7 ± 0.06	19.5 ± 0.07
Σ *n* – 3	16.4 ± 0.14	21.7 ± 0.13	13.8 ± 0.29
EPA/AA	4.30 ± 0.03	9.07 ± 0.03	7.89 ± 0.21
DHA/EPA	2.81 ± 0.04	0.74 ± 0.00	0.85 ± 0.01
Σ *n* – 3/Σ *n* – 6	21.1 ± 0.05	2.03 ± 0.02	0.71 ± 0.02

[a] 17-C FA with undetermined configuration.
[b] Not detected.

Table 4. Commercial diet formulations given in percent as prepared by Integral Fish Foods Inc. (Grand Junction, CO) for the 2006 spawning season. All diets were stabilized with vitamin C at 400 mg/kg.

	Diet (% Lipid)	
Ingredient	23%	18%
Fishmeal [a]	28.0	28.0
Fish meal analog [b]	26.0	23.5
Soymeal [c]	7.5	7.5
Whole wheat	10.0	10.0
Wheat middlings	3.0	6.5
Fish oil [d]	9.5	5.5
Soy oil	2.0	2.0
Monosodium phosphate	0.8	0.8
Vitamin + mineral premix	3.0	3.0
Dl methionine	0.3	0.3
NaCl	0.5	0.5

[a] Menhaden (64 % protein)
[b] Contains some or all: 85% bloodmeal, hydrolysed feather meal, poultry byproducts meal, Dl methionine, lysine, monosodium phosphate
[c] 46 % protein
[d] Menhaden

Table 5. Proximate composition and calculated energetic values of the frozen Atlantic silversides (SS) and experimental diets used during the 2006 spawning season. Values are given in percent of dry weight and represent means ± s.e. (N = 3).

		Diets	
	SS	23% Lipid	18% Lipid
Moisture	71.1 ± 0.4	6.3 ± 0.2	6.5 ± 0.1
Protein	57.0 ± 0.4	45.0 ± 0.2	43.7 ± 0.0
Lipid	35.2 ± 0.9	23.2 ± 0.5	17.9 ± 0.2
Fiber	nd	1.5 ± 0.0	1.8 ± 0.0
Ash	9.6 ± 0.1	11.0 ± 0.0	12.6 ± 0.0
NFE [a]	nd	19.3 ± 0.6	24.1 ± 0.1
Energy [b]	6.59 ± 0.06	5.54 ± 0.03	5.15 ± 0.01

[a] Nitrogen free extract.
[b] Calculated based on energetic values for protein, lipid and NFE as 5.7, 9.5, 4 kcal/g respectively.

Table 6. Fatty acid profile (% TFA) of diets fed to black sea bass during the 2006 spawning season: Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid. Values represent mean ± s.e. (N = 3).

Fatty acids		Diet	
	SS 2006	23% Lipid	18% Lipid
% lipid	35.2 ± 0.87	21.7 ± 0.52	16.7 ± 0.15
14:0	5.61 ± 0.25	3.93 ± 0.17	3.85 ± 0.01
15:0	2.10 ± 0.06	nd [b]	nd
16:0	22.5 ± 0.36	20.8 ± 0.93	20.2 ± 0.03
16:1*n* – 7	12.4 ± 0.12	6.29 ± 0.06	6.13 ± 0.08
16:2	nd	0.81 ± 0.15	0.66 ± 0.01
16:3*n* – 3	nd	0.75 ± 0.02	0.69 ± 0.07
16:4	nd	1.75 ± 0.12	1.77 ± 0.03
16:3*n* – 3	nd	0.75 ± 0.02	0.69 ± 0.07
Unknown [a]	0.73 ± 0.00	nd	nd
Unknown [a]	2.38 ± 0.05	nd	nd
Unknown [a]	0.99 ± 0.02	nd	nd
Unknown [a]	0.92 ± 0.09	nd	nd
18:0	3.92 ± 0.06	4.73 ± 0.24	4.57 ± 0.01
18:1*n* – 9	20.4 ± 1.10	20.8 ± 0.32	20.1 ± 0.04
18:1*n* – 7	4.52 ± 0.06	5.18 ± 1.03	4.13 ± 0.01
18:2*n* – 6	nd	10.6 ± 0.35	11.1 ± 0.02
18:4*n* – 3	1.16 ± 0.06	1.76 ± 0.12	1.86 ± 0.01
Unknown	1.95 ± 0.28	nd	nd
20:4*n* – 6	0.94 ± 0.06	0.56 ± 0.05	0.65 ± 0.01
20:5*n* – 3	4.49 ± 0.25	12.5 ± 1.08	13.4 ± 0.02
20:1	0.89 ± 0.00	0.67 ± 0.05	0.73 ± 0.00
20:1*n* – 9	0.61 ± 0.00	0.82 ± 0.06	0.87 ± 0.05
22:5*n* – 3	2.97 ± 0.20	1.48 ± 0.02	1.52 ± 0.01
22:6*n* – 3	10.6 ± 0.57	6.18 ± 0.61	7.10 ± 0.03
Unknown	nd	0.64 ± 0.05	0.66 ± 0.00
Saturates	36.1 ± 0.68	29.5 ± 1.33	28.6 ± 0.04
Monoenes	42.4 ± 1.15	33.8 ± 1.17	32.0 ± 0.03
PUFAS	20.8 ± 1.09	36.1 ± 2.31	38.8 ± 0.03
Σ *n* – 6	0.97 ± 0.06	11.1 ± 0.38	11.8 ± 0.02
Σ *n* – 3	19.8 ± 1.03	22.5 ± 2.07	24.5 ± 0.01
EPA/AA	4.78 ± 0.06	22.5 ± 0.17	20.7 ± 0.24
DHA/EPA	2.37 ± 0.01	0.49 ± 0.01	0.53 ± 0.00
Σ *n* – 3/Σ *n* – 6	20.5 ± 0.16	2.02 ± 0.15	2.09 ± 0.00

[a] 17-C FA with undetermined configuration.
[b] Not detected.

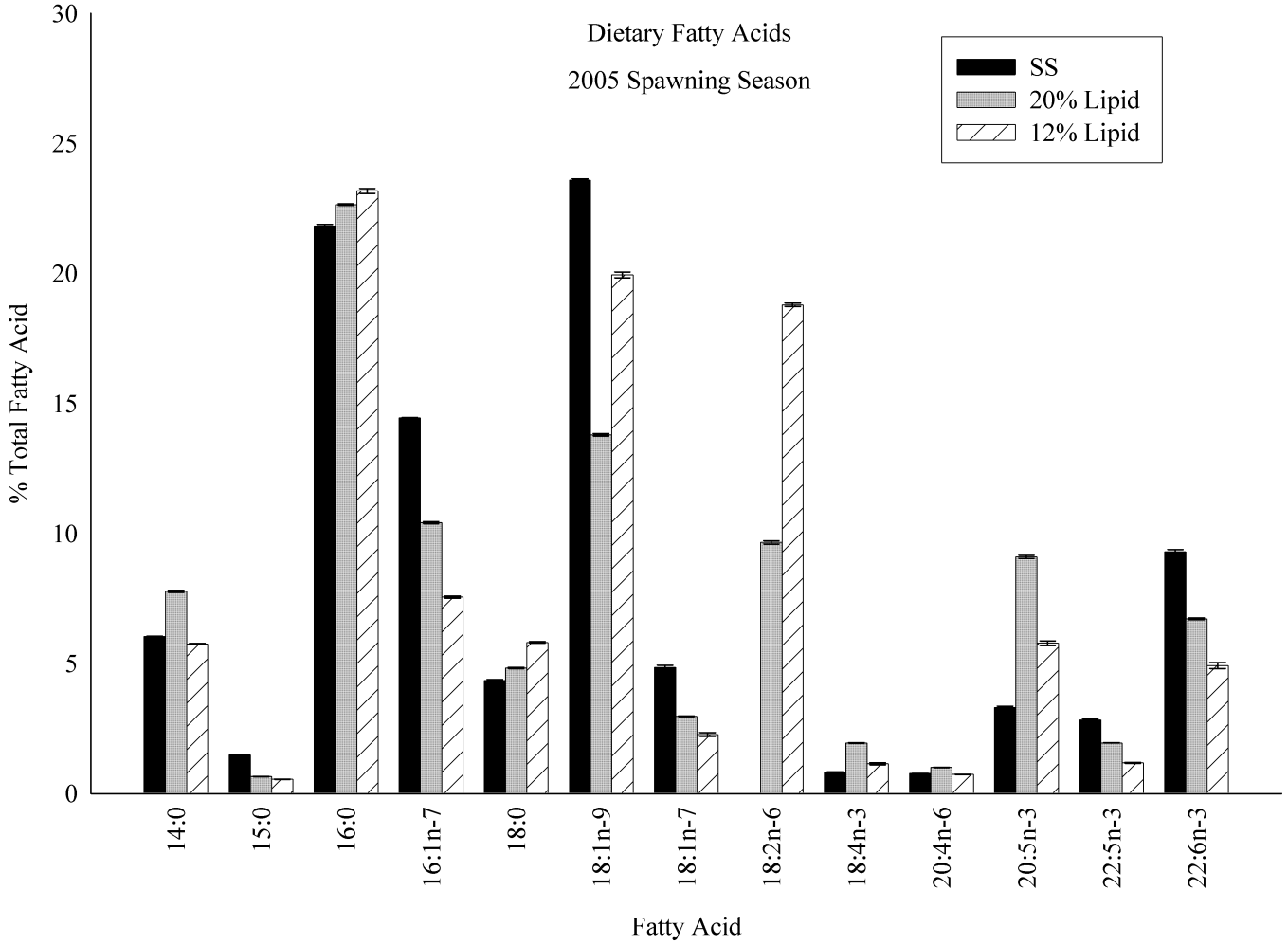

Figure 2. Major dietary fatty acids (% TFA) (mean ± s.e.) (N = 3) identified in the silversides (SS) and commercially prepared diets during the 2005 spawning season.

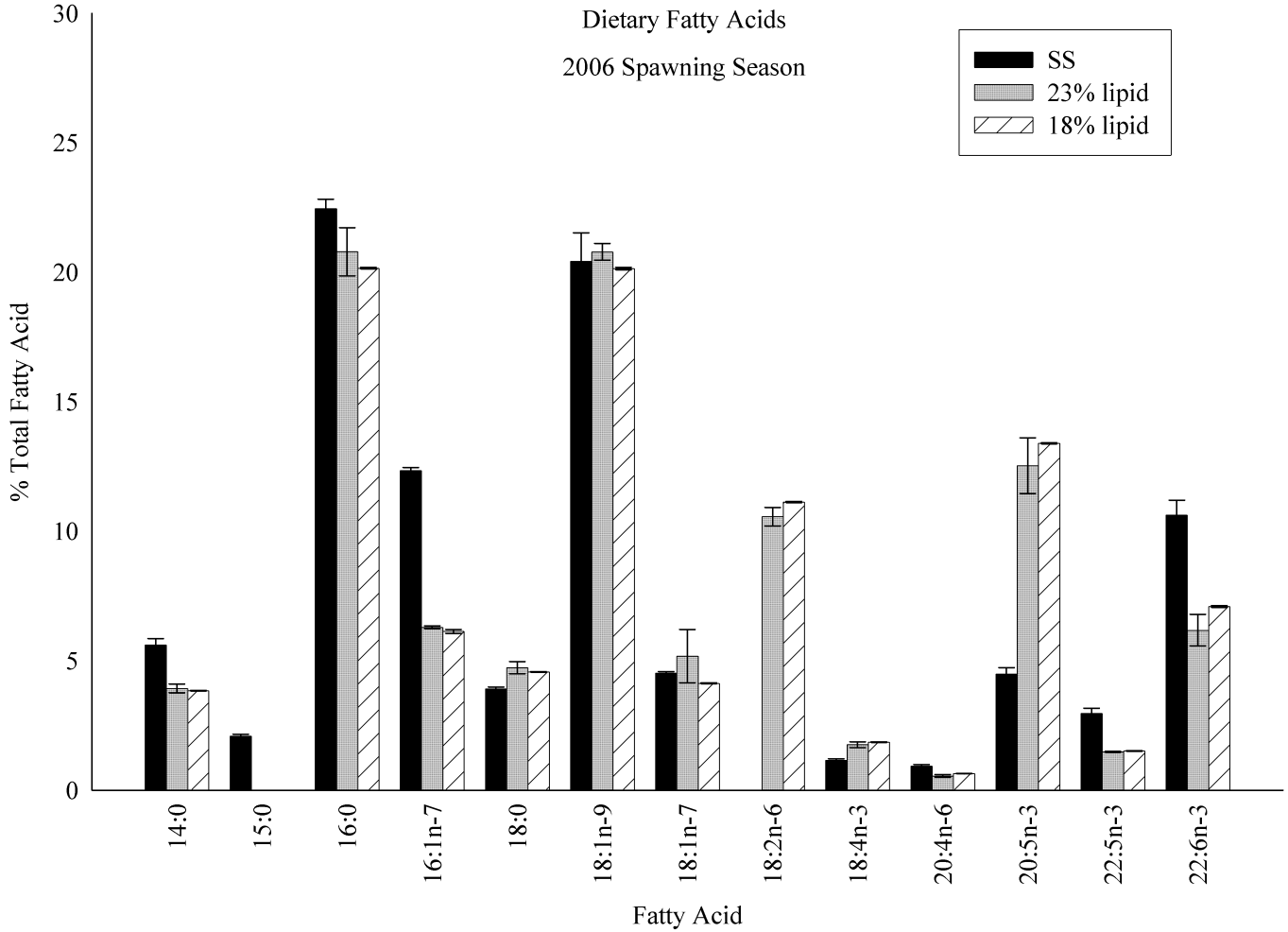

Figure 3. Major dietary fatty acids (% TFA) (mean ± s.e.) (N = 3) identified in the silversides (SS) and commercially prepared diets during the 2006 spawning season.

photomicrographs taken with a digital camera attached to a dissecting microscope. Oocytes were measured using image analysis software (ImageJ v1.37b).

To induce ovulation, the selected females were implanted in the dorsal musculature with a luteinizing hormone releasing hormone analog (LHRHa) pellet at a dose of 50-75 μg per kg body weight in a slow release matrix (95% cholesterol and 5% cellulose) (Sherwood et al. 1988). Males were periodically monitored for spermiation by applying slight abdominal pressure, and a relative score (1-4) was assigned based on the quantity of milt released. A small sample of sperm was placed on a microscope slide and activated with seawater to verify motility under a compound microscope. Only males with a spermiation score ≥ 3 and verified motility were used for spawning trials.

During the 2006 spawning season, temperature and photoperiod were maintained at 19.1 ± 0.1 C and 13 L: 11 D, respectively. Spawning protocols were the same as described above except that in one trial, females from each treatment were implanted with a gonadotropin-releasing hormone agonist in an ethylene-vinyl acetate implant (Mylonas and Zohar 2001). In one trial, 2 females from each treatment were implanted concurrently and held with five males (group spawning). If fish failed to spawn volitionally after 6 d post implant (p.i.) the female was removed from the tank and checked for ovulation by applying slight abdominal pressure. If hydrated oocytes were present, eggs were stripped manually from the female and fertilized in-vitro (Daniels and Watanabe 2002; Woolridge 2005).

Egg Collection

Cylindrocone egg collectors, which were plumbed to receive the tank effluent stream, were monitored daily for a maximum of 10 d p.i. for spawned eggs. A 250-µm mesh standpipe retained the eggs in the collector. If eggs were present, they were siphoned into a 50-µm mesh bag suspended in seawater.

Egg Quality

After collection, eggs were quantified volumetrically and then transferred to an acrylic 15-L hatching cone in 32 ppt seawater. The non-viable eggs were allowed to sink and then transferred to a graduated cylinder to estimate the number of eggs volumetrically (1,345 eggs/ml) (White 2004). The remaining viable eggs which were floating and neutrally buoyant, were concentrated using a 250 µm screen and then transferred to a graduated cylinder with 250-500 ml of sea water. The eggs were gently mixed, and the mean numbers of eggs contained in three 1-ml samples was used to estimate total number of eggs. During the 2006 spawning season, the salinity of the incubation water containing the neutrally buoyant viable eggs was raised until the eggs floated (not exceeding 42 ppt) and then transferred to a graduated cylinder to estimate number of eggs. A sample of at least 100 buoyant eggs from each spawn were examined under a dissecting microscope to determine fertilization rate. Hatching success was also monitored as an index of egg quality. Approximately 3,000 eggs from each spawn were incubated in duplicate 18-L black plastic aquaria with an exchange rate of 40% per hour

at 20.1 ± 0.1°C and at 36 g/L. Hatching success was monitored on d 3 post-fertilization using volumetric methods.

Fatty Acid Analysis

To determine the effects of dietary lipid on the fatty acid composition of the eggs, a sample of the viable floating eggs from each spawn was dried on a 50 μm screen and stored under nitrogen at -25°C until extraction. A modified Folch et al. (1957) method was used for lipid extraction with 1:1 chloroform: methanol (C: M) ratio. Egg samples were homogenized for 5 min. with a handheld glass homogenizer in C: M, sonicated for 2 min., then transferred to a round bottom flask for evaporation in a rotary evaporator. Samples were then filtered through a medium porosity glass fritted disk filter into a pre-weighed flask by transferring with 1 ml C:M three times. Samples were then placed on a rotary evaporator and transferred to a vacuum desiccator for 30 min. prior to gravimetric determination of lipid. Lipid was then transferred to a glass vial in 3 ml of 1:3 methanol: dichloromethane under nitrogen and stored at -35°C for later analysis.

FAME Preparation

For analysis of total fatty acids, approximately 3 mg of extracted lipid in 1 ml of solvent was transferred to a conical vial with magnetic stir bar and solvent was evaporated under nitrogen. For saponification of lipid, 1 ml of 0.5 M NaOH/MeOH was

added to sample and refluxed with stirring for 30 min. Fatty acids were then transesterified with 1.5 ml of 10-15% boron trifluoride-methanol (Morrison and Smith 1964) and refluxed with stirring for 30 min. The solution was then allowed to cool to room temperature and fatty acid methyl esters (FAME) were then purified. One ml of saturated NaCl and 1 ml of hexane was added to the vial and then the vial was shaken for ten seconds. The organic layer was transferred to a mini SiO_2 column for purification. Hexane (1 ml) was added again to the vial, shaken, and organic layer transferred to a mini-column. A solution of 20% ether hexane was then added to the vial, mixed, and organic layer transferred to the mini-column. To ensure FAMEs were eluted, 1 ml of 50% ether hexane was added to the column. FAME solution was evaporated under nitrogen and transferred to vial in chloroform at a concentration of approximately 5 µg/µl for GC analysis.

Gas Chromotography

FAMEs were analyzed with GC-FID (Hewlett Packard 6890 Series GC/FID with a HP 18596C autosampler and a 30 m x 0.25 mm HP-5 capillary column with a 0.25 µm film thickness) with split injector. One µl of sample was injected at a split ratio of 10:1 and a 2 ml/min. flow rate of He. The GC method for analysis of the egg FAMEs consisted of an initial temperature of 195 C held for 8 min., with a 15^{o}C /min. ramp to 255^{o}C. Commercially prepared diets were analyzed with the following method to provide greater resolution for the 18 carbon FAMEs: An initial temperature of 170^{o}C held for 8 min., 1.5^{o}C /min. ramp to 195^{o}C and held for 3 min., then 15^{o}C /min. ramp

to 255°C. A standard FAME mixture (GLC-84, Nu-Chek Prep) was run periodically for reference. FAMEs were identified with the addition of standards of oleic, vacenic, and linoleic methyl esters individually to sub samples. Representative samples were also analyzed with GC-MS (Varian CP-3800 GC with a 1079 injector and a 30 m X 0.25 mm WCOT fused silica column coupled to a Saturn 2200 MS) for verification and identification of FA where standards were unavailable. One µl of sample was injected at a split ratio of 20:1 and a 1.2 ml/min. flow rate of He. The GC method began with an initial temperature of 50°C with a 30°C /min. ramp to 200°C, then a 10°C /min. ramp to 250°C and hold for 10 min.

Diet Analysis

Diets were analyzed to determine their FA composition following the same procedures described for egg fatty acid determination, with the exception of the homogenization method. The diets were ground in a small food processor and then homogenized with a motor driven homogenizer in 1:1 chloroform: methanol for 2 min. The Atlantic silversides were freeze dried to determine percent moisture prior to lipid extraction. Percent moisture of commercially prepared diets was determined gravimetrically after grinding in a food processor and drying in an oven at 120°C for 2 h. Ash content was determined gravimetrically after exposure to 600°C for 6h in a muffle furnace. Percent protein for all diets and fiber for the commercially prepared diets were analyzed according to AOAC methods (NJ Feed Laboratory, Inc., Trenton, New Jersey).

Data Analysis

Fertilization success and survival of yolk sac larvae to first feeding were expressed as percentages. A weighted average was used to calculate fertilization and hatching success. Egg lipid was expressed as % wet weight and fatty acid composition was expressed as % of total fatty acids. Quantitative values were expressed as treatment means. All percentage data was arcsine transformed. When two treatments were compared, a Bartlett's test was performed to test for homogeneity of variances, and a t-test was used to compare means. If assumptions were violated, Wilcoxin's Rank Sums Test was used. When more than two treatment means were compared, an F-Max test was used to test for homogeneity of variance. Treatment means were compared by a one-way ANOVA, and a Tukey's test was used for multiple comparisons among means. When an assumption of ANOVA was violated, means were compared in pairs using the Wilcoxin's Rank Sums Test. All statistical analysis was performed with JMP 6.0 statistical software (SAS Institute Inc., Cary, NC.).

RESULTS

2005 Spawning Season

Spawning Results

A total of 30 females were implanted for induced spawning; 12 fish from the silverside treatment, 8 from the 20% lipid treatment, and 10 from the 12% lipid treatment

(Table 7). There was no significant correlation ($P < 0.05$) between female body weight, or condition index (female weight/(standard length)^3) and egg quality parameters. Mean female weight from 1.2 to 1.7 kg among treatments, with no significant differences (Table 7). The greatest percentage of fish responding to hormone treatment was observed in the 20% lipid treatment (88%), while the 12% lipid treatment was intermediate (60%), and the SS treatment showed the lowest percentage of fish responding (50%) among treatments (Table 7). The mean latency period for induced spawning ranged from 2.3 to 3.9 d pi, with no significant differences. The majority of females spawned on multiple days following hormone treatment, with the exception of two trials from the 12% lipid treatment in which only 1 spawning event was observed. The mean number of spawns per trial was 3.3 for the SS treatment, 4.3 for the 20% lipid treatment, and 2.5 for the 12% lipid treatment (Table 7). Spawning related mortalities were seen in all treatments following hormone implantation. Four mortalities were observed in both the SS and 12% lipid treatments, and 3 mortalities in the 20% lipid treatment (Table 7).

Egg Production

There were no significant differences ($P < 0.05$) for mean total fecundity (eggs/female) and relative fecundity (eggs/kg BW) among treatments (Table 8). Mean total and relative fecundity for the SS treatment were 242.6 ± 87.8 and 192.5 ± 54.7 x 10^3 eggs, respectively. The 20% lipid treatment had a mean total fecundity of 155.1 ± 36.6 x 10^3 eggs and a mean relative fecundity of 128.2 ± 29.4 x 10^3 eggs, while the 12% lipid treatment had a mean total fecundity of 173.2 ± 31.9 x 10^3 eggs and relative

fecundity of 116.2 ± 21.7 x 10^3 eggs (Table 8).

The mean percentage of buoyant eggs was significantly ($P < 0.05$) higher in the SS treatment (23.3 ± 7.7%) when compared to the commercially prepared diets, while there was no significant difference between the 20% (6.1 ± 2.6%) and 12% (1.0 ± 0.6%) lipid treatments (Table 8). Mean fertilization success of the buoyant eggs was 96.8 ± 1.8% for the SS treatment, 84.2 ± 7.3% for the 20% lipid treatment, and 61.6 ± 23.6% for the 12% lipid treatment, with no significant differences (Table 8). The mean number of fertilized eggs produced per female in the SS treatment (69.5 ± 29.2 x 10^3) was significantly ($P < 0.05$) higher than in the 12% lipid treatment (1.3 ± 0.9 x 10^3). The 20% lipid treatment produced an intermediate number of fertilized eggs (11.8 ± 7.4 x 10^3) (Table 8), which was not significantly different from the SS or 12% lipid treatments. Fertilization success (%) was also significantly ($P < 0.05$) higher in the SS treatment (22.4 ± 7.3) compared to the 20% (4.8 ± 2.3) and 12% (0.6 ± 0.4) lipid treatments (Fig. 4, Table 8).

Hatching success (%) was not significantly different between the SS (51.3 ± 13.5) and 20% (58.6 ± 8.8) lipid treatments. Hatching success for the 12% lipid treatment (which represented only 1 spawning trial) was 40% (Table 8, Fig. 4).

Total and relative yolk-sac larvae (YSL) production (no./female and no./kg body weight) in the SS treatment (26.6 ± 12.0 and 21.8 ± 10.3 x 10^3, respectively) were significantly ($P < 0.05$) higher than in the 12% lipid treatment (0.3 ± 0.3 and 0.3 ± 0.3 x 10^3, respectively). The 20% lipid treatment was intermediate for both total and relative YSL produced (5.1 ± 2.9 and 4.0 ± 2.1 x 10^3, respectively) (Table 8, Fig. 5).

Table 7. Spawning results (2005 spawning season) for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. Values represent means ± s.e. (range).

	Dietary Treatment		
	SS	20% Lipid	12% Lipid
No. of females implanted	12	8	10
LHRHa (50 ug/Kg)	3	2	0
LHRHa (75 ug/Kg)	9	6	10
Fish body wt. (kg)	1.2 ± 0.2	1.4 ± 0.2	1.7 ± 0.4
(range)	(0.8 – 1.7)	(0.8 – 2.3)	(0.8 – 3.2)
Fish responding (No., %)	(6, 50)	(7, 88)	(6, 60)
Latency period (d pi)	3.5 ± 0.7	3.9 ± 1.2	2.3 ± 0.2
(range)	(2 – 6)	(2 –10)	(2 – 3)
No. spawns per trial (d)	3.3 ± 0.6	4.3 ± 0.7	2.5 ± 0.8
(range)	(2 – 6)	(2 – 8)	(1 – 6)
Mortalities (No., %)	(4, 33)	(3, 38)	(4, 40)

Table 8. Egg production and egg quality (2005 spawning season) for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. Data represents mean ± s.e. (range). Means not sharing a common letter are significantly different ($P < 0.05$).

Parameter	SS	20%	12%
Eggs/ female (x 10^3)	242.6 ± 87.8 (64.5 – 616.5) n = 6	155.1 ± 36.6 (35.0 – 351.3) n = 7	173.2 ± 31.9 (80.7 – 277.1) n = 6
Eggs/ kg body wt. (x 10^3)	192.5 ± 54.7 (48.7 – 353.9) n = 6	128.2 ± 29.4 (15.3 – 240.1) n = 7	116.2 ± 21.7 (35.7 – 173.0) n = 6
Buoyant eggs (%)	23.3 ± 7.7 a (0.7 – 48.1) n = 6	6.1 ± 2.6 b (0.0 – 15.6) n = 7	1.0 ± 0.6 b (0.0 – 3.1) n = 6
Fertilization success (% buoyant eggs)	96.8 ± 1.8 (88.3 – 100.0) n = 6	84.2 ± 7.3 (67.2 – 97.0) n = 6	61.6 ± 23.6 (38.0 – 85.2) n = 2
Fertilized eggs per female (x 10^3)	69.5 ± 29.2 a (0.6 – 158.2) n = 6	11.8 ± 7.4 ab (0.0 – 53.3) n = 7	1.3 ± 0.9 b (0.0 – 5.1) n = 6
Fertilization success (% overall)	22.4 ± 7.3 a (0.7 – 48.1) n = 6	4.8 ± 2.3 b (0.0 – 15.2) n = 7	0.6 ± 0.4 b (0.0 – 2.6) n = 6
Hatching success (%)	51.3 ± 13.5 (16.4 – 83.4) n = 5	58.6 ± 8.8 (41.8 – 71.2) n = 3	40.0 nd (40.0 – 40.0) n = 1
YSL per female (x 10^3)	26.6 ± 12.0 a (0.0 – 71.5) n = 6	5.1 ± 2.9 ab (0.0 – 19.4) n = 7	0.3 ± 0.3 b (0.0 – 1.9) n = 6
YSL/ kg body wt. (x 10^3)	21.8 ± 10.3 a (0.0 – 63.1) n = 6	4.0 ± 2.1 ab (0.0 – 13.3) n = 7	0.3 ± 0.3 b (0.0 – 1.7) n = 6

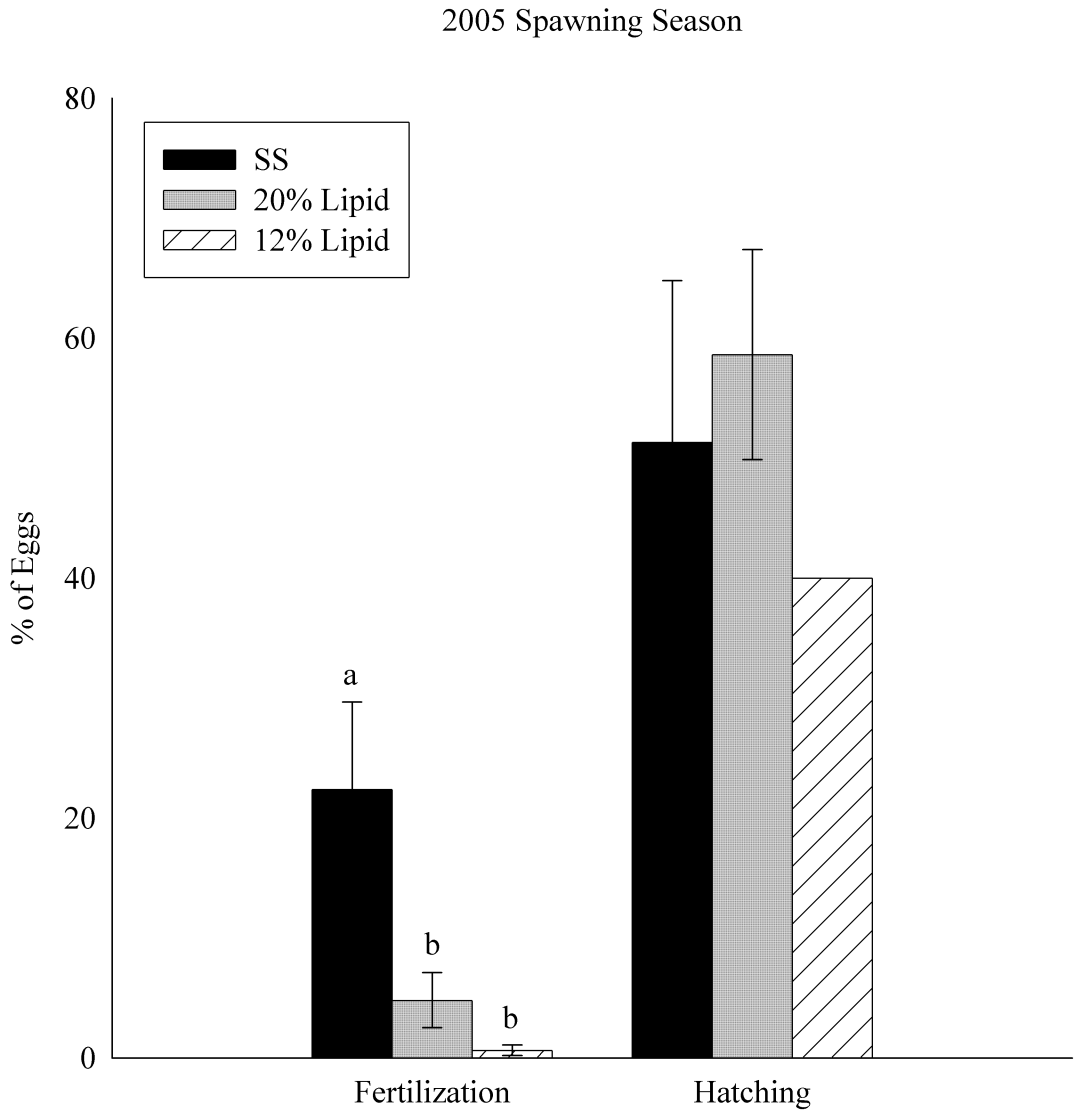

Figure 4. Fertilization and hatching success (mean ± s.e.) (N = 1 – 6) of eggs during the 2005 spawning season for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. For each parameter, means not sharing a common letter are significantly different ($P < 0.05$).

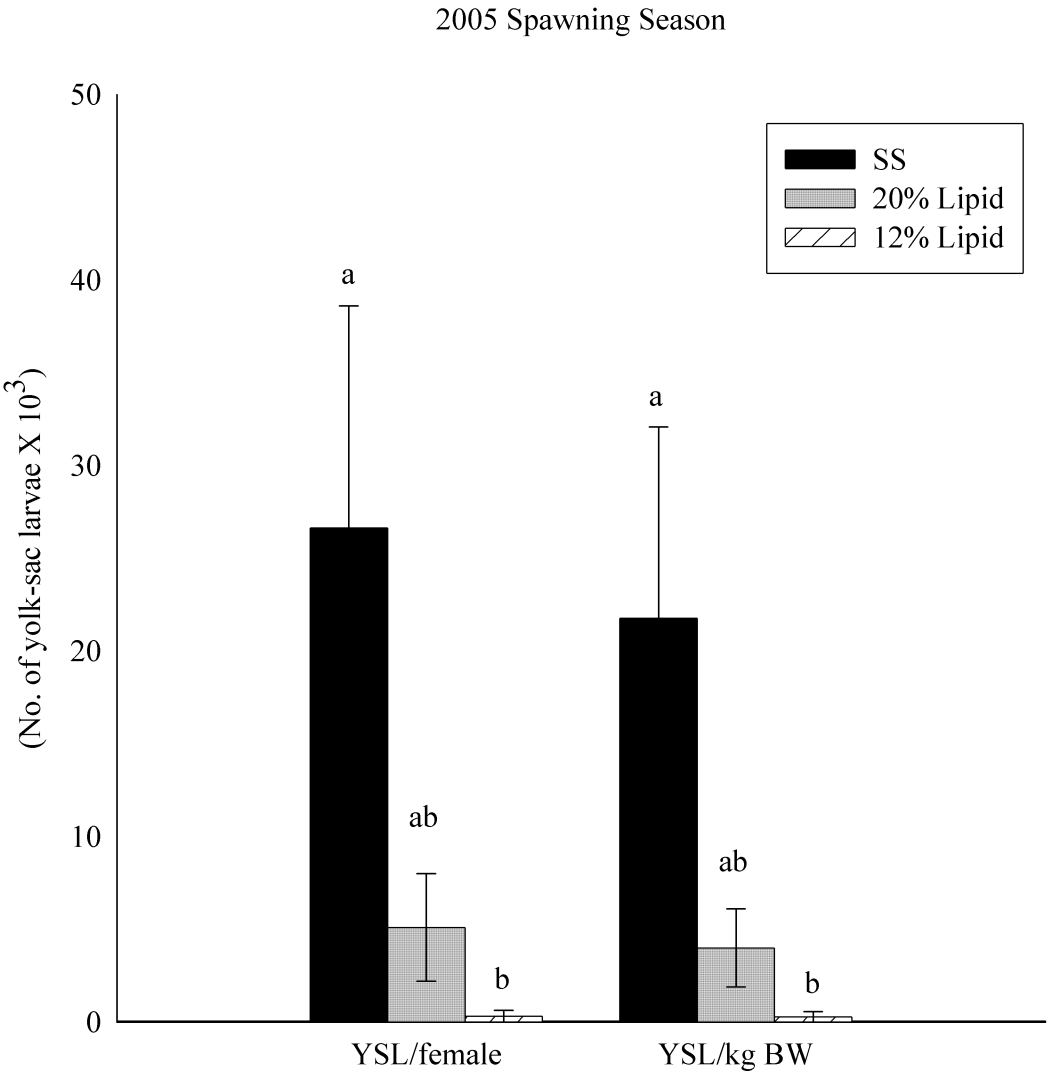

Figure 5. Total and relative yolk-sac larvae ($x10^3$) (mean ± s.e.) (N = 6 – 7) produced during the 2005 spawning season for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. For each parameter, means not sharing a common letter are significantly different ($P < 0.05$).

Egg Fatty Acids

There were no significant differences in the percent total lipid of eggs between the SS (4.79 ± 0.19%) and 20% lipid treatment (4.71 ± 0.73%). Percent total lipid of the 12% lipid treatment (4.95% lipid) was also similar, however was not included in any statistical comparisons since this represented a single sample (Table 9).

A total of 16 FA were identified in the egg samples, with a considerable amount of variation in FA profile among treatments. The major FA found in the eggs were palmitic acid (16:0), oleic acid (18:1*n*-9), and DHA (22:6*n*-3) with DHA comprising the largest percentage in the SS treatment (28.6 ± 1.46% total fatty acids (TFA)) and palmitic acid as the largest percentage in both the 20% and 12% lipid treatments (20.1 ± 0.56 and 20.0% TFA respectively). (Table 9, Fig. 6).

Significant ($P < 0.05$) differences between the SS and 20% lipid treatment were found in 11 FA (Table 9 and Fig. 6). The 20% lipid treatment had a significantly ($P < 0.05$) higher amount of 14:0 (2.42 ± 0.18% TFA) than the SS treatment (1.44 ± 0.09% TFA). There was a significantly ($P < 0.05$) higher amount of 15:0 in the SS treatment (0.65 ± 0.01% TFA) than in the 20% lipid treatment which contained < 0.50% TFA. Palmitoleic (16:1*n*-7) and palmitic (16:0) acids were significantly ($P < 0.05$) higher in the 20% lipid treatment (8.52 ± 0.32 and 20.1 ± 0.56% TFA, respectively) than in the SS treatment (7.06 ± 0.44 and 17.5 ± 0.61% TFA, respectively). The SS treatment contained a higher amount of 17:0 (1.31 ± 0.12% TFA) than the eggs from the 20% lipid treatment (< 0.50% TFA). LA (18:2*n*-6) was significantly ($P < 0.05$) higher in the 20% lipid treatment (9.46 ± 0.38% TFA) than in the SS treatment (1.59 ± 0.43% TFA). The FA

19:0 was detected in the SS treatment (0.67 ± 0.09% TFA) but not in the 20% or 12% lipid treatments. The 20% lipid treatment eggs contained significantly ($P < 0.05$) more EPA (20:5*n*-3) and 20:? (9.19 ± 0.11% and 0.89 ± 0.03% TFA, respectively) than the SS treatment (6.96 ± 0.16% and 0.55 ± 0.10% TFA, respectively), while the SS treatment contained significantly ($P < 0.05$) more DHA (22:6*n*-3) and DPA (22:5*n*-3) (28.6 ± 1.46% and 4.64 ± 0.19% TFA, respectively) than the 20% lipid treatment eggs (17.5 ± 0.56% and 2.67 ± 0.13% TFA, respectively) (Table 9, Fig. 6).

The sum of saturated fatty acids (SFA) was significantly ($P < 0.05$) greater in the 20% lipid treatment (31.8 ± 0.61% TFA) than in the SS treatment (29.1 ± 0.77% TFA). Total SFA in the 12% lipid treatment (31.2% TFA) was similar to the 20% lipid treatment. The sums of *n*-6 and *n*-3 series FA were also significantly different between the SS and 20% lipid treatments, with the SS treatment having a higher sum of *n*-3 FA (40.1 ± 1.70% TFA) and a lower sum of *n*-6 FA (3.55 ± 0.41% TFA) than the 20% lipid treatment (29.4 ± 0.72 and 11.1 ± 0.36% TFA, respectively). The sums of the *n*-6 and *n*-3 series FA in the in the 12% lipid treatment were 14.7 and 24.7% TFA, respectively (Table 9, Fig. 7).

The ratios of EFAs and *n*-3 to *n*-6 series FA were also significantly different between the SS and 20% lipid treatments (Table 9, Fig. 8). Eggs from the SS treatment contained a significantly ($P < 0.05$) higher ratio of DHA/EPA (4.10 ± 0.20) and *n*-3/*n*-6 (12.0 ± 1.52) than the 20% lipid treatment (1.91 0.06 and 2.65 ± 0.11 respectively). The 20% lipid treatment had a higher EPA/AA ratio (5.51 ± 0.14) than the SS treatment (3.60 ± 0.22). The ratios of EPA/AA, DHA/EPA, and *n*-3/*n*-6 for the 12% lipid treatment

(5.58, 2.29, and 1.68 respectively) were similar to the eggs from the 20% lipid treatment (Table 9, Fig. 8).

2006 Spawning Season

Spawning Results

During the 2006 spawning season, 9 females were implanted for induced spawning in each treatment. Mean female weight ranged from 1.4 – 1.7 kg with no significant differences among treatment means (Table 10). There was no significant correlation ($P < 0.05$) between female body weight, or condition index (female weight/(standard length)^3) and egg quality parameters. A total of eight spawning trials were conducted for each dietary treatment, including 7 individual spawning trials and 1 group spawning consisting of 2 females. One strip spawning was conducted in the SS treatment after the female failed to spawn volitionally by 6 d pi. Percentage of females responding to hormone treatment during the 2006 spawning season was high (89%), with only 1 fish from each treatment failing to respond to hormone implantation (Table 10). Mean latency period ranged from 2.3 to 3.1 d pi among treatments (Table 10). The mean number of spawns per trial varied greatly and ranged from 3.3 to 5.7 d, with no significant differences among treatment means (Table 10). Mortalities of implanted females were observed in all treatments, including 1 from the SS treatment, and 2 from the 23 and 18% lipid treatments (Table 10).

Table 9. Egg fatty acid profile (% TFA) (2005 spawning season) for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. Values represent mean ± s.e. and letters identify significant differences (P < 0.05).

		Diet treatment	
Fatty acids	SS	20% Lipid	12% Lipid
n	5	4	1
% lipid	4.79 ± 0.19	4.71 ± 0.73	4.95
14:0	1.44 ± 0.09 b	2.42 ± 0.18 a	1.99
15:0	0.65 ± 0.01 a	tr [a] b	tr
16:0	17.5 ± 0.61 b	20.1 ± 0.56 a	20.0
16:1*n* – 7	7.06 ± 0.44 b	8.52 ± 0.32 a	7.93
17:0	1.31 ± 0.12 a	tr b	tr
18:0	8.83 ± 0.53	9.01 ± 0.59	9.25
18:1*n* – 9	13.5 ± 0.58	13.9 ± 0.33	16.7
18:1*n* – 7	3.31 ± 0.25	2.90 ± 0.08	2.79
18:2*n* – 6	1.59 ± 0.43 b	9.46 ± 0.38 a	13.5
19:0	0.67 ± 0.09 a	nd [b] b	nd
20:?[c]	0.55 ± 0.10 b	0.89 ± 0.03 a	0.83
20:1	tr	0.51 ± 0.18	0.55
20:4*n* – 6	1.96 ± 0.11	1.67 ± 0.06	1.23
20:5*n* – 3	6.96 ± 0.16 b	9.19 ± 0.11 a	6.84
Unknown	tr	tr	0.62
22:5*n* – 3	4.64 ± 0.19 a	2.67 ± 0.13 b	2.26
22:6*n* – 3	28.6 ± 1.46 a	17.5 ± 0.56 b	15.6
Totals			
Σ Saturates	29.1 ± 0.77 b	31.8 ± 0.61 a	31.2
Σ Monoenes	23.9 ± 0.90	25.4 ± 0.51	27.4
Σ PUFAS	43.7 ± 1.68	40.6 ± 0.74	39.4
Σ *n* – 6	3.55 ± 0.41 b	11.1 ± 0.36 a	14.7
Σ *n* – 3	40.1 ± 1.70 a	29.4 ± 0.72 b	24.7
EPA/AA	3.60 ± 0.22 b	5.51 ± 0.14 a	5.58
DHA/EPA	4.10 ± 0.20 a	1.91 ± 0.06 b	2.29
Σ *n* – 3/ Σ *n* – 6	12.0 ± 1.52 a	2.65 ± 0.11 b	1.68

[a] Trace amounts (< 0.5 %) detected
[b] Not detected
[c] Polyunsaturated with number of double bonds unidentified

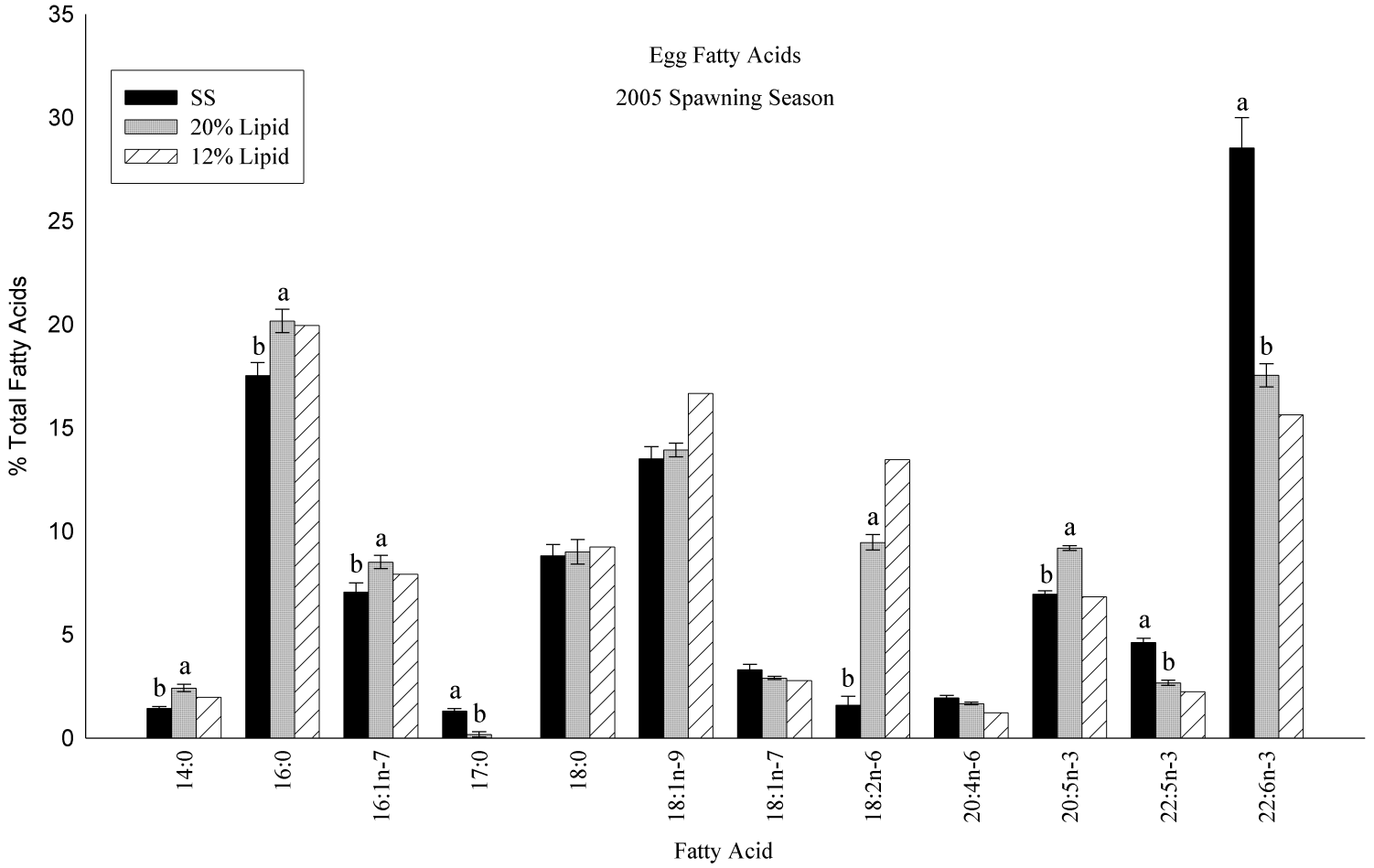

Figure 6. Fatty acid profile (% TFA) (mean ± s.e.) (N = 1 – 5) of eggs spawned during the 2005 spawning season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. For each FA, means not sharing a common letter are significantly different ($P < 0.05$).

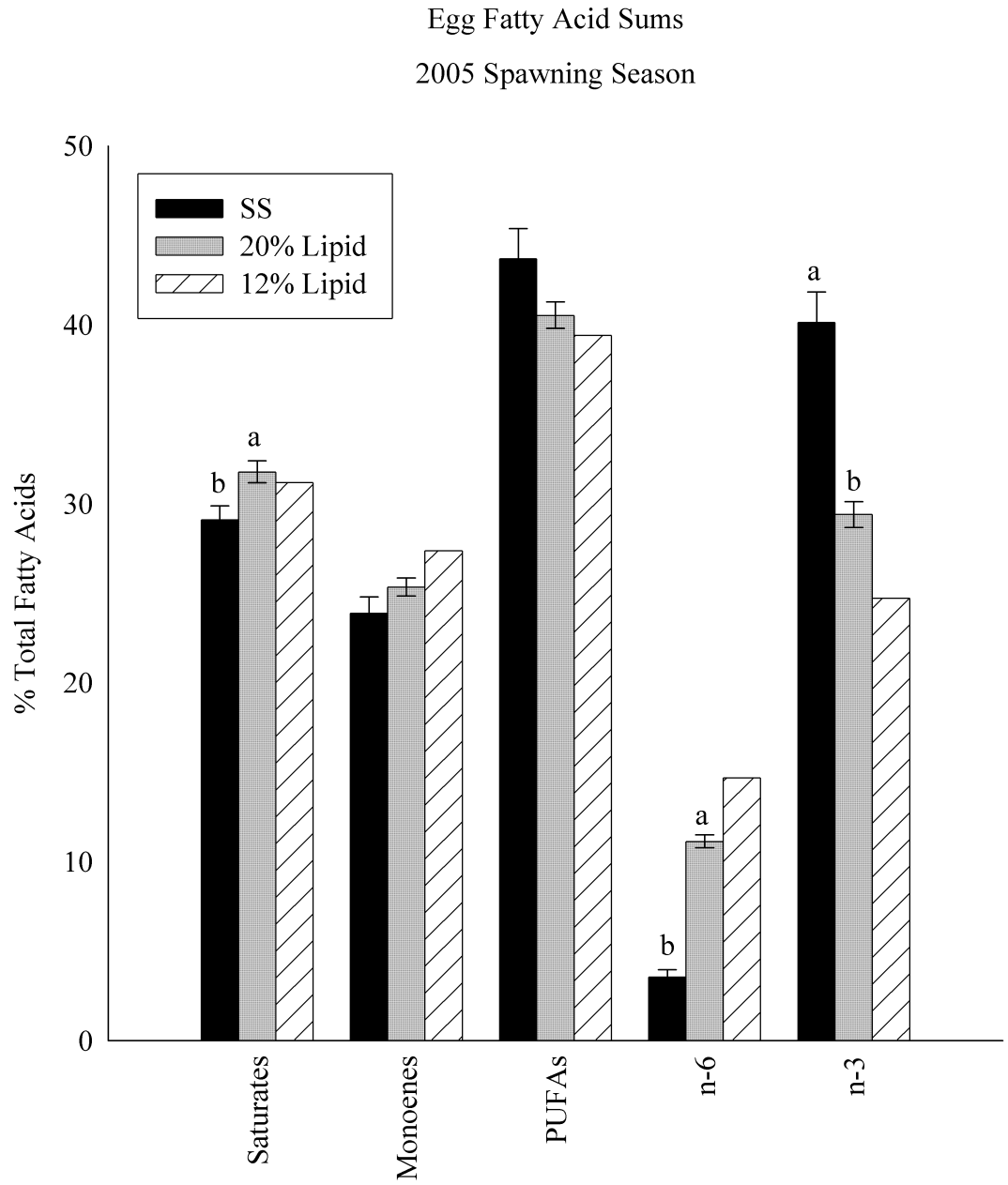

Figure 7. Sums of fatty acid classes and *n*-6 and *n*-3 series FA (% TFA) (mean ± s.e.) (N = 1 – 5) found in eggs spawned during the 2005 spawning season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. For each FA, means not sharing a common letter are significantly different ($P < 0.05$).

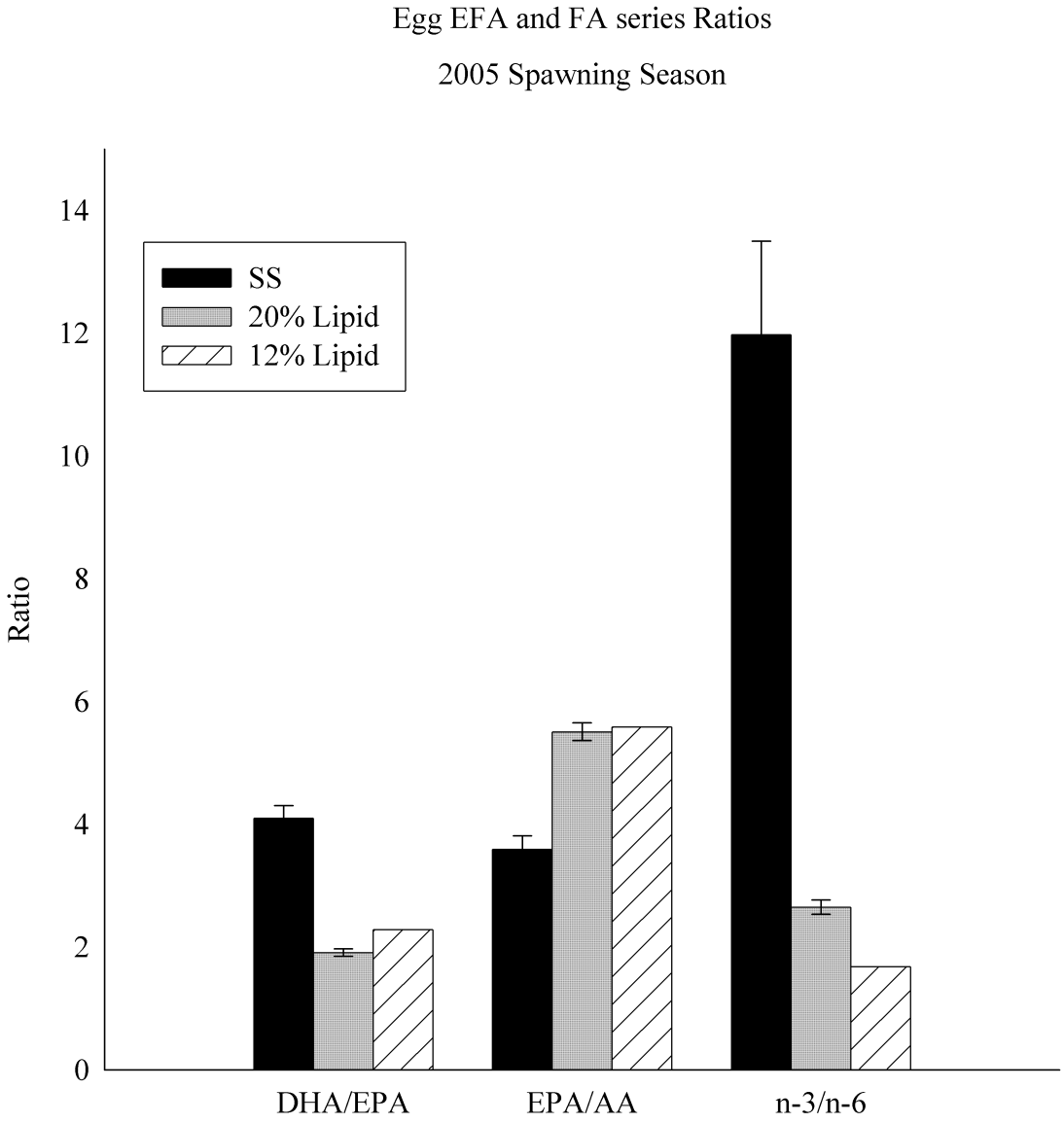

Figure 8. Essential fatty acid (EFA) ratios and *n*-3/*n*-6 ratios (mean ± s.e.) (N = 1 – 5) from eggs spawned during the 2005 season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid. For each FA, means not sharing a common letter are significantly different ($P < 0.05$).

Egg Production

During the 2006 spawning season, no significant differences among treatments were detected for total fecundity (199.8 – 246.2 x 10^3) or relative fecundity (119.4 – 184.4 x 10^3/kg) (Table 11). There were no significant differences among treatments in the percentage of buoyant eggs (4.7 – 9.6) or fertilization success (%) of buoyant eggs (70.7 – 82.3). The mean number of fertilized eggs per female (5.8 – 21.7 x 10^3) was also not significantly different among treatments (Table 11). Overall fertilization success (%) ranged from 3.8 – 8.5, with no significant differences among treatments (Fig. 9, Table 11). Hatching success (%) was similar in all three treatments, (34.3 – 45.2), with no significant differences (Table 11, Fig. 9). No significant differences were detected among treatments for total YSL per female (1.3 – 8.2 x 10^3) and relative YSL production (0.8 – 6.8 x 10^3/kg) (Table 11, Fig. 10).

Egg Fatty Acids

For the 2006 season, there were no significant differences in egg percent lipid composition for the SS, 23%, and 18% lipid treatments (4.21 - 5.17% lipid) (Table 12). A total of 16 FA were identified in the eggs from the 2006 spawning season. The predominant FA were similar to those observed in the eggs from the 2005 spawning season, with DHA (22:6*n*–3) representing the largest percentage in the silverside treatment (28.6 ± 0.53% TFA), and palmitic acid (16:0) as the largest percentage in the

Table 10. Spawning results (2006 spawning season) for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid. Values represent means ± s.e. (range).

	Dietary Treatment		
	SS	23% Lipid	18% Lipid
Fish implanted (total)	9	9	9
LHRHa (50 ug/Kg)	8	8	8
EVAC (75 ug/Kg)	1	1	1
Fish body wt. (kg)	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.1
(range)	(1.5 – 2.1)	(1.0 – 1.8)	(0.9 – 1.7)
Fish responding (No., %)	(8, 89)	(8, 89)	(8, 89)
Latency period (d pi)	2.7 ± 0.6	3.1 ± 1.0	2.3 ± 0.2
(range)	(1 – 6)	(2 – 9)	(2 – 3)
No. spawns per trial (d)	3.3 ± 0.7	5.1 ± 0.9	5.7 ± 1.7
(range)	(1 – 6)	(1 – 8)	(1 – 14)
Strip spawns	1	0	0
Group spawns	1	1	1
Mortalities (No., %)	(1, 11)	(2, 22)	(2, 22)

Table 11. Mean egg production and egg quality (2006 spawning season) for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid. Data represents means ± s.e. (range).

Parameter	SS	23%	18%
Eggs/ female (x 10^3)	199.8 ± 36.0 (41.7 – 336.3) n = 7	246.2 ± 52.6 (16.1 – 402.2) n = 7	218.6 ± 94.3 (16.1 – 686.0) n = 7
Eggs/ kg body wt. (x 10^3)	119.4 ± 22.7 (27.0 – 208.6) n = 7	156.4 ± 34.7 (16.9 – 286.2) n = 7	184.4 ± 101.5 (18.2 – 765.6) n = 7
Buoyant eggs (%)	9.6 ± 4.5 (0.0 – 32.3) n = 7	4.7 ± 2.2 (0.0 – 16.7) n = 7	8.7 ± 2.9 (0.0 – 18.6) n = 7
Fertilization success (% buoyant eggs)	82.3 ± 7.7 (54.0 – 98.1) n = 5	70.7 ± 11.5 (21.0 – 97.5) n = 6	80.4 ± 7.4 (48.0 – 99.5) n = 6
Fertilized eggs per female (x 10^3)	10.0 ± 4.2 (0.0 – 30.3) n = 7	5.8 ± 2.6 (0.0 – 20.0) n = 7	21.7 ± 10.7 (0.0 – 68.4) n = 7
Fertilization success (% overall)	8.5 ± 3.9 (0.0 – 27.4) n = 7	3.8 ± 1.8 (0.0 – 13.8) n = 7	7.7 ± 2.7 (0.0 – 17.6) n = 7
Hatching success (%)	39.9 ± 11.5 (25.0 – 62.4) n = 3	34.3 ± 6.5 (22.7 – 51.2) n = 4	45.2 ± 3.1 (39.0 – 51.3) n = 4
YSL per female (x 10^3)	3.6 ± 2.6 (0.0 – 18.9) n = 7	1.3 ± 0.6 (0.0 – 4.4) n = 7	8.2 ± 4.2 (0.0 – 26.3) n = 7
YSL/ kg body wt. (x 10^3)	2.0 ± 1.5 (0.0 – 10.8) n = 7	0.8 ± 0.4 (0.0 – 2.9) n = 7	6.8 ± 4.1 (0.0 – 29.4) n = 7

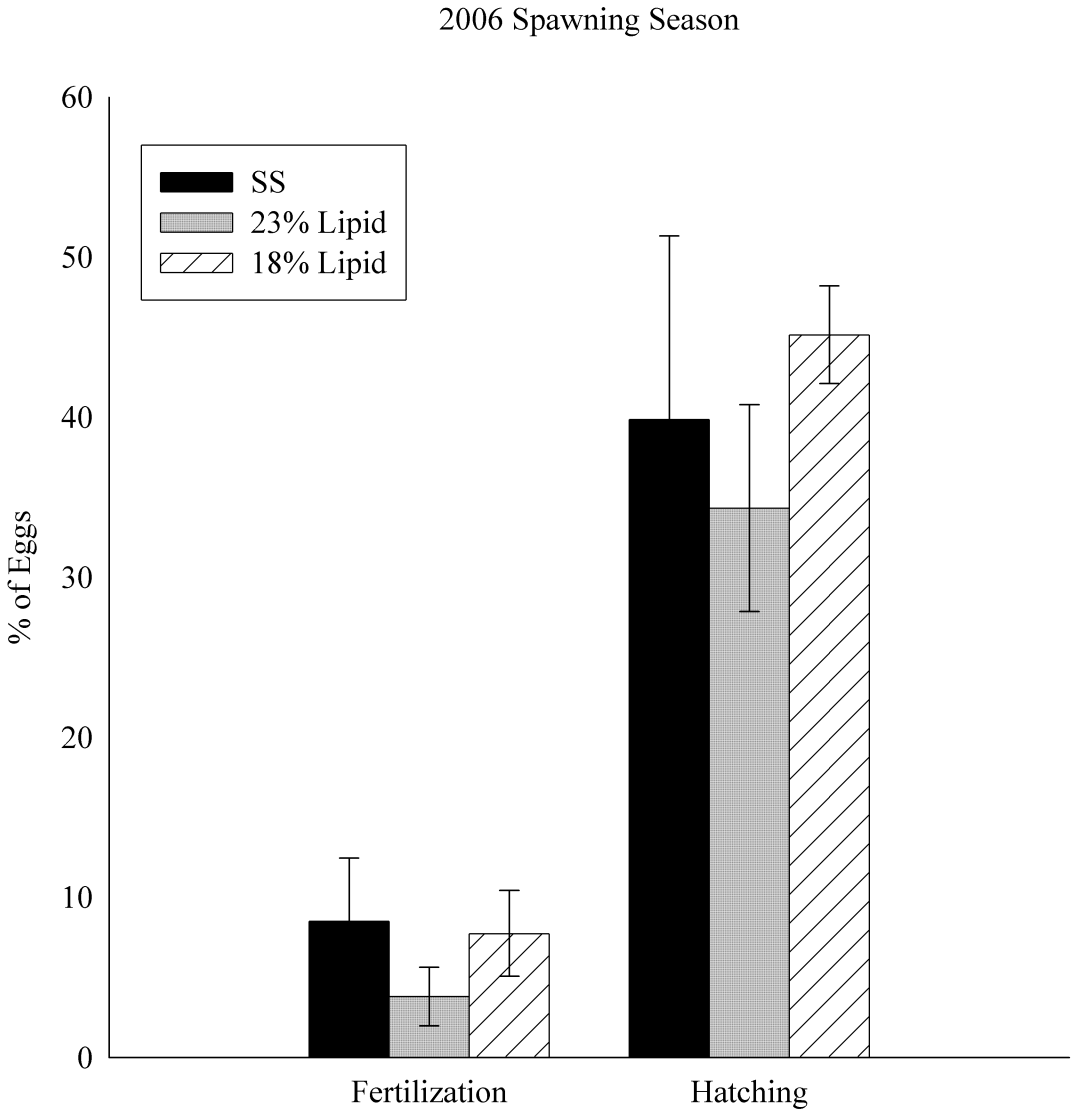

Figure 9. Fertilization and hatching success (%) (mean ± s.e.) (N = 7) of eggs during the 2006 spawning season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid.

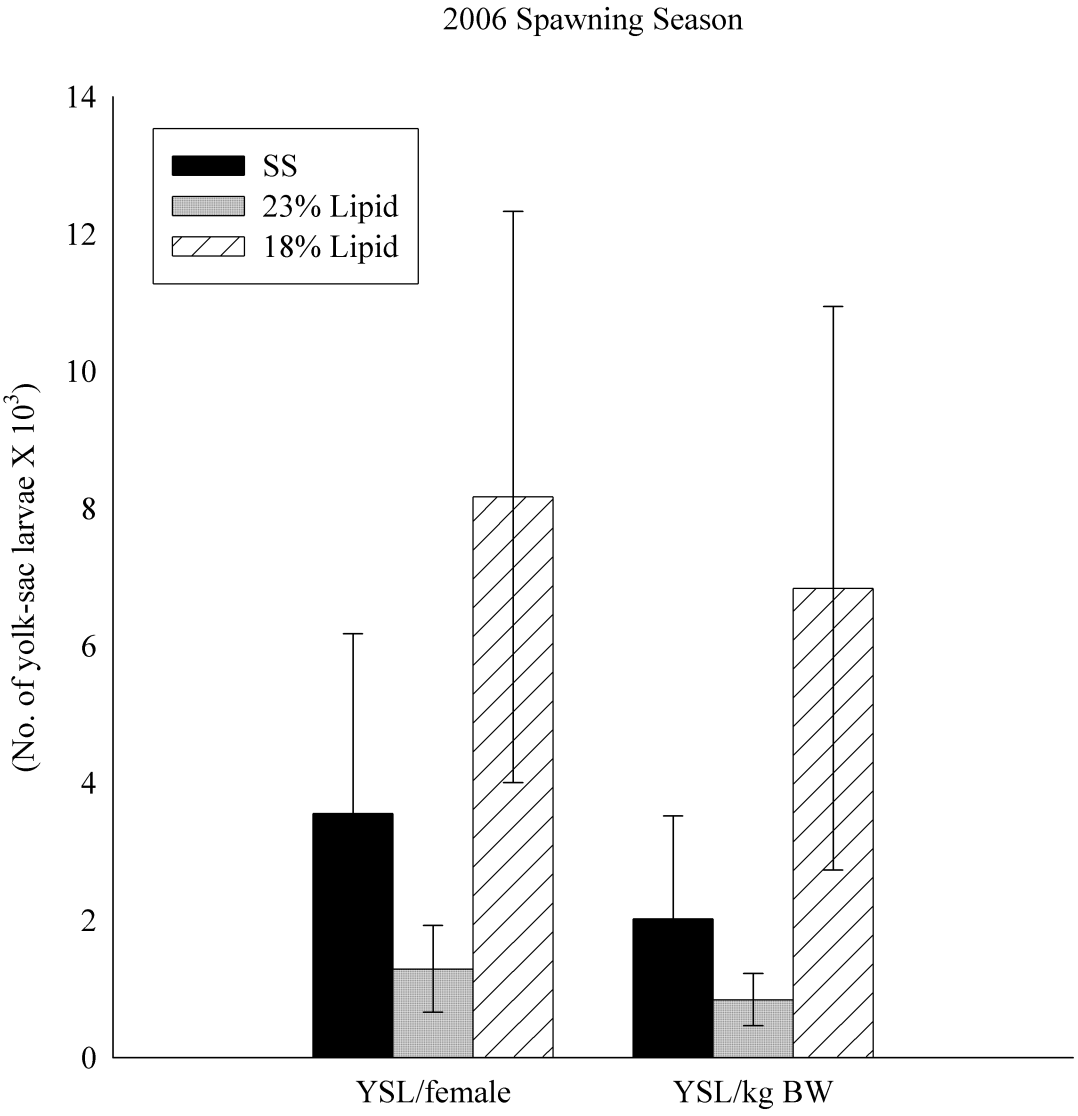

Figure 10. Total and relative yolk-sac larvae (x10^3) (mean ± s.e.) (N = 7) produced for the 2006 spawning season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid.

23% (19.7 ± 0.76% TFA) and 18% (18.7 ± 0.49% TFA) lipid treatments (Table 12, Fig. 11).

Six of these FA showed significant differences between the SS and commercial diets. The FA 17:0 was detected in the SS treatment eggs (1.28 ± 0.11% TFA), while only trace amounts (< 0.50% TFA) were detected in the 18% lipid treatment with none detected in the 23% lipid treatment. The eggs from the 23% and 18% lipid treatments contained a significantly ($P < 0.05$) higher percentage of linoleic (18:2*n*-6) FA (8.81 ± 0.30% and 8.07 ± 0.39% TFA, respectively) than the SS treatment (1.36 ± 0.22% TFA). Oleic acid (18:1*n*-9) and stearic acid (18:0) were significantly ($P < 0.05$) higher in the eggs from the 18% lipid treatment (16.9 ± 0.68% and 11.6 ± 0.42% TFA, respectively) than in the SS treatment (14.2 ± 0.55% and 8.75 ± 0.42% TFA, respectively). AA (20:4*n*-6) was significantly ($P < 0.05$) higher in the SS treatment (2.21 ± 0.10% TFA) than in the 23% and 18% lipid treatments (1.04 ± 0.03% and 1.15 ± 0.07% TFA). The 23% and 18% lipid treatments contained significantly ($P < 0.05$) higher percentages of EPA (20:5*n*-3) (11.8 ± 0.45% and 11.3 ± 0.49% TFA) than the SS treatment (7.71 ± 0.18% TFA). Both DHA (22:6*n*-3) and DPA (22:5*n*-3) were significantly ($P < 0.05$) higher in the SS treatment (28.6 ± 0.53% and 4.92 ± 0.18% TFA, respectively) than in the 23% (15.0 ± 0.61% and 2.78 ± 0.09% TFA, respectively) and 18% (17.5 ± 1.10% and 2.91 ± 0.20% TFA, respectively) lipid treatments (Table 12, Fig. 11).

The eggs produced in the 23% lipid treatment contained a significantly ($P < 0.05$) higher amount of SFA (31.9 ± 1.02% TFA) than eggs from the SS treatment (28.6 ±

0.72% TFA), while eggs from the 18% lipid treatment (31.4 ± 0.68% TFA) did not differ significantly from the other treatments. There were no significant differences in the sums of monoenes (23.8 – 27.0% TFA) and PUFA (39.3- 44.7% TFA) among treatments; however, the sum of *n*-6 series FA was significantly ($P < 0.05$) lower in the SS treatment (3.56 ± 0.27% TFA) than in the 23% (9.84 ± 0.31% TFA) and 18% (9.23 ± 0.37% TFA) lipid treatments, while the sum of *n*-3 series FA in the SS treatment was significantly lower (41.1 ± 0.68% TFA) than the 23% (29.5 ± 1.00% TFA) and 18% (31.7 ± 1.53% TFA) lipid treatments (Table 12, Fig. 12).

Significant differences ($P < 0.05$) were detected among all three treatments in the ratios of EPA/AA and DHA/EPA. The 23% lipid treatment had the highest EPA/AA ratio (11.4 ± 0.13), the 18% lipid treatment had an intermediate ratio (9.86 ± 0.31) while the SS treatment had the lowest EPA/AA ratio (3.51 ± 0.11). The highest ratio of DHA/EPA was found in the SS treatment (3.71 ± 0.04), with the 18% lipid treatment (1.55 ± 0.09) intermediate, and the lowest ratio in the 23% lipid treatment (1.27 ± 0.03). The ratio of *n*-3/*n*-6 series FA was significantly ($P < 0.05$) higher in the SS treatment (11.7 ± 0.69) than in the 23% (3.01 ± 0.14) and 18% (3.45 ± 0.21) lipid treatments (Table 12, Fig. 13).

Correlation of Egg Fatty Acids and Egg Quality Parameters

There was no significant correlation between egg fatty acids (LA, AA, EPA, DHA, EPA/AA, and DHA/EPA) and overall fertilization success (%) or hatching success

Table 12. Egg fatty acid profile (% TFA) (2006 spawning season) for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid. Values represent mean ± s.e. and letters identify significant differences ($P < 0.05$).

		Diet treatment	
Fatty acids	SS	23% Lipid	18% Lipid
n	4	4	5
% lipid	4.36 ± 0.18	5.17 ± 0.74	4.21 ± 0.16
14:0	0.95 ± 0.18	1.58 ± 0.11	1.48 ± 0.17
15:0	tr	tr	tr
16:0	17.6 ± 0.55	19.7 ± 0.76	18.7 ± 0.49
16:1*n* – 7	6.25 ± 0.34	6.27 ± 0.57	6.30 ± 0.56
17:0	1.28 ± 0.11 a	nd b	tr b
18:0	8.75 ± 0.42 b	10.6 ± 0.97 ab	11.6 ± 0.42 a
18:1*n* – 9	14.2 ± 0.55 b	16.9 ± 0.68 ab	16.9 ± 0.79 a
18:1*n* – 7	3.34 ± 0.05	3.84 ± 0.31	3.15 ± 0.12
18:2*n* – 6	1.36 ± 0.22 b	8.81 ± 0.30 a	8.07 ± 0.39 a
19:0	0.66 ± 0.11	nd	tr
20:?[c]	0.68 ± 0.02	0.72 ± 0.02	0.73 ± 0.02
20:1	0.65 ± 0.04	0.86 ± 0.03	0.66 ± 0.10
20:4*n* – 6	2.21 ± 0.10 a	1.04 ± 0.03 b	1.15 ± 0.07 b
20:5*n* – 3	7.71 ± 0.18 b	11.8 ± 0.45 a	11.3 ± 0.49 a
Unknown	nd	tr	nd
22:5*n* – 3	4.92 ± 0.18 a	2.78 ± 0.09 b	2.91 ± 0.20 b
22:6*n* – 3	28.6 ± 0.53 a	15.0 ± 0.61 b	17.5 ± 1.10 b
Totals			
Σ Saturates	28.6 ± 0.72 b	31.9 ± 1.02 a	31.4 ± 0.68 ab
Σ Monoenes	23.8 ± 0.76	27.0 ± 0.95	26.4 ± 1.17
Σ PUFAS	44.7 ± 0.91	39.3 ± 1.05	40.9 ± 1.67
Σ *n* – 6	3.56 ± 0.27 b	9.84 ± 0.31 a	9.23 ± 0.37 a
Σ *n* – 3	41.1 ± 0.68 a	29.5 ± 1.00 b	31.7 ± 1.53 b
EPA/AA	3.51 ± 0.11 c	11.4 ± 0.13 a	9.86 ± 0.31 b
DHA/EPA	3.71 ± 0.04 a	1.27 ± 0.03 c	1.55 ± 0.09 b
Σ *n* – 3/ Σ *n* – 6	11.7 ± 0.69 a	3.01 ± 0.14 b	3.45 ± 0.21 b

[a] Trace amounts (< 0.5 %) detected
[b] Not detected; [c] Polyunsaturated with number of double bonds unidentified

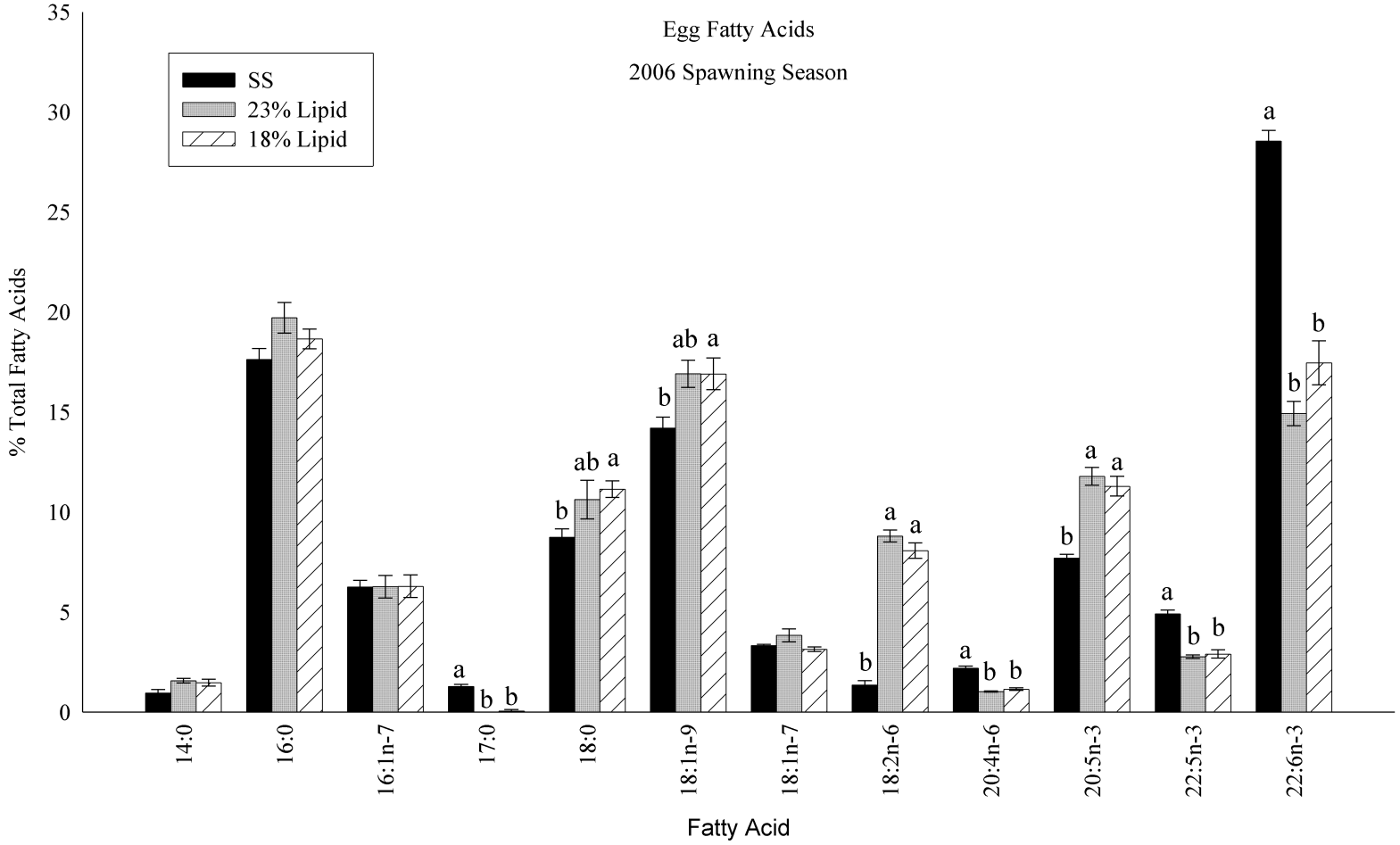

Figure 11. Fatty acid profile (% TFA) (mean ± s.e.) (N = 4 – 5) of eggs spawned during the 2006 spawning season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid. For each FA, means not sharing a common letter are significant different ($P < 0.05$).

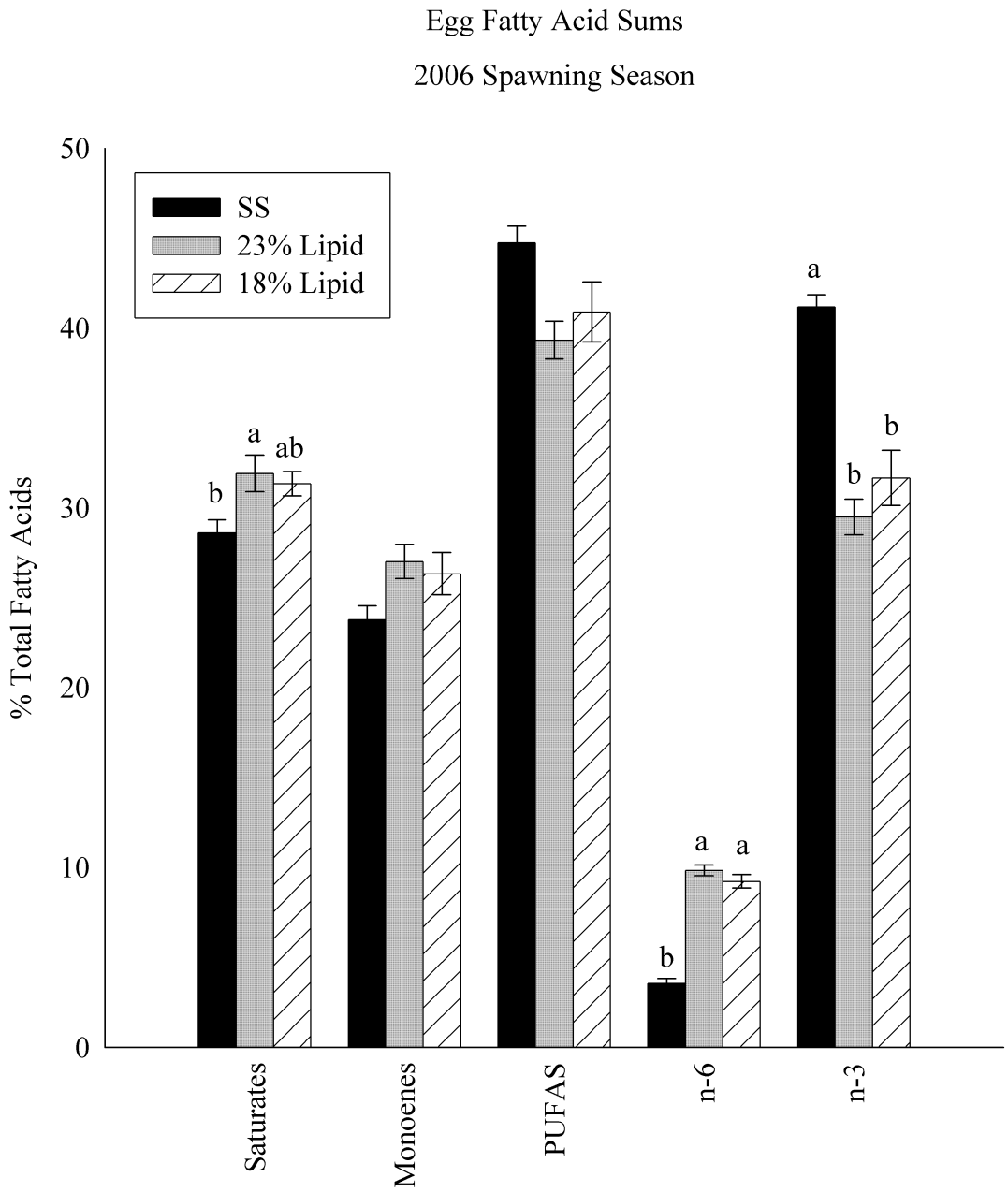

Figure 12. Sums of fatty acid classes and *n*-6 and *n*-3 series FA (% TFA) (mean ± s.e.) (N = 4 – 5) found in eggs spawned during the 2006 spawning season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid. For each FA, means not sharing a common letter are significantly different ($P < 0.05$).

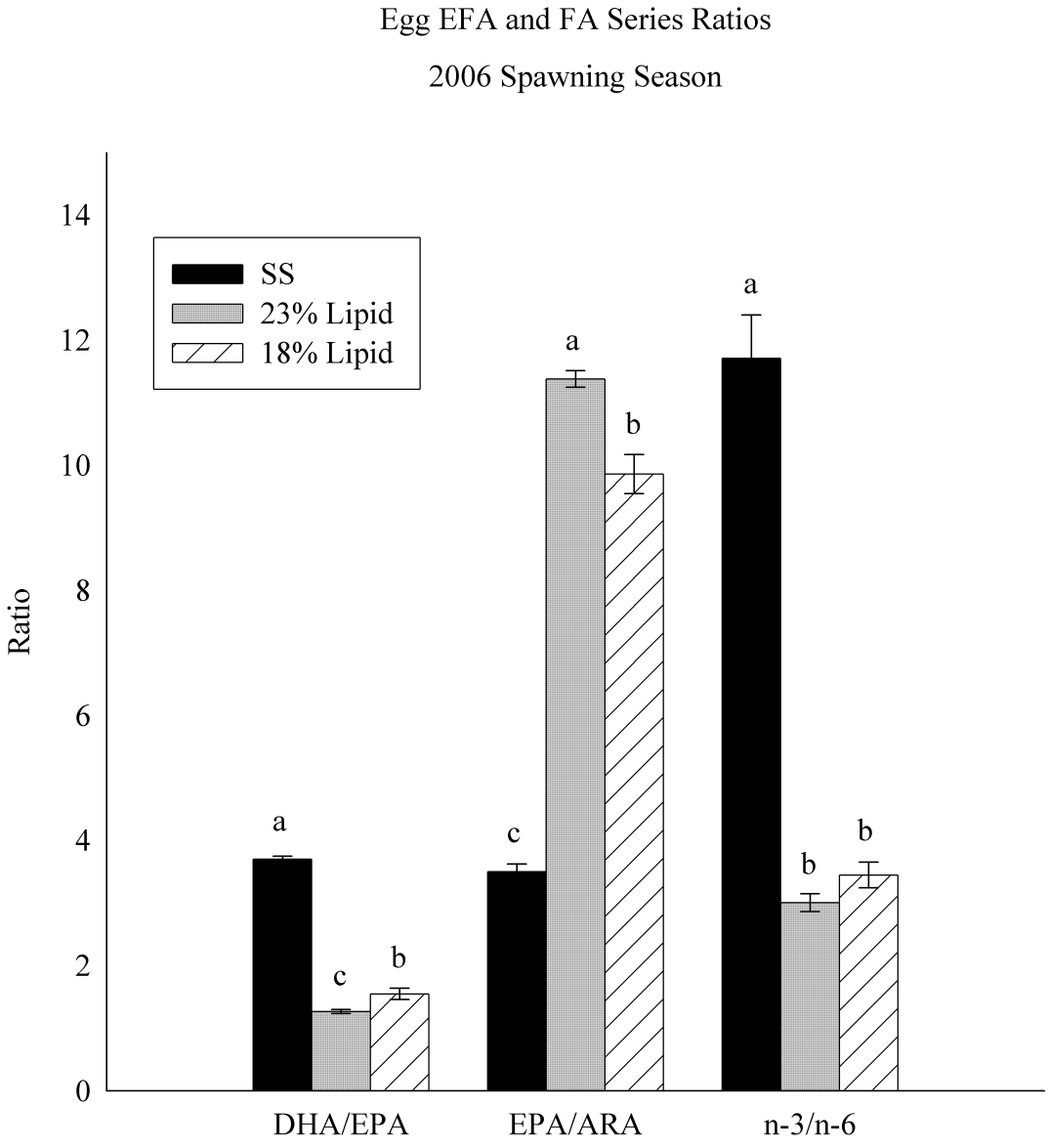

Figure 13. Essential fatty acid (EFA) ratios and *n*-3/*n*-6 ratios (mean ± s.e.) (N = 4 – 5) from eggs spawned during the 2005 season for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 23 or 18% lipid. For each FA, means not sharing a common letter are significantly different ($P < 0.05$).

(%) for the 2005 spawning season (Table 13). In the 2006 season, there was a negative correlation ($P < 0.5$) between egg EPA content and fertilization success. Where data for the 2005 and 2006 were combined, there was a significant positive correlation ($P < 0.05$) between egg AA, DHA, and DHA/EPA content with fertilization success. In addition there was a negative correlation ($P < 0.05$) of egg LA, EPA, and EPA/AA content with fertilization success (Table 13).

DISCUSSION

Female Response

According to available data, the timing of ovulation of BSB following LHRHa implantation is variable and difficult to predict (Watanabe et al. 2003, White 2004, Berlinsky et al. 2005). In this study, BSB females implanted with a slow release pellet containing LHRHa (50 ug/kg BW) had mean latency periods ranging from 2.3 to 3.9 d among dietary treatments, although individual females showed latency periods of up to 10 d pi. This is similar to the latency period (2 – 3 d pi) previously reported for BSB implanted with a slow release pellet containing 50 ug/kg LHRHa for volitional spawning (Watanabe et al. 2003, White 2004), but with significant variability among females. White (2004) reported a latency period of 2 – 6 d pi in BSB females implanted with LHRHa pellets (0 – 100 ug/kg), while Watanabe et al. (2003) reported latency periods exceeding 5 d pi with a range of 2 – 9 d pi in fish implanted with 50 ug/kg LHRHa. The

Table 13. Correlation between egg fatty acids and egg quality parameters for the 2005, 2006 and combined (2005, 2006) seasons. Significant correlation is indicated by (*), ($P < 0.05$).

2005 Season	Fertility (%)		Hatching Success (%)	
	N=10		N=8	
Fatty Acid	r	P	r	P
LA	-0.450	0.19	0.227	0.58
AA	0.618	0.05	-0.570	0.13
EPA	-0.197	0.58	0.407	0.31
DHA	0.513	0.12	-0.305	0.46
EPA/AA	-0.508	0.13	0.567	0.14
DHA/EPA	0.422	0.22	-0.369	0.36
2006 Season				
	N=13		N=10	
LA	-0.439	0.13	0.095	0.79
AA	0.460	0.11	-0.083	0.81
EPA	-0.557*	0.04	0.108	0.76
DHA	0.488	0.09	0.064	0.86
EPA/AA	-0.526	0.06	0.046	0.89
DHA/EPA	0.512	0.07	-0.026	0.94
2005 and 2006 Seasons				
	N=23		N=18	
LA	-0.434*	0.03	0.177	0.48
AA	0.469*	0.02	-0.155	0.53
EPA	-0.379	0.07	0.0258	0.91
DHA	0.501*	0.01	-0.093	0.71
EPA/AA	-0.413*	0.04	-0.014	0.95
DHA/EPA	0.472*	0.02	-0.115	0.64

latency period after hormone administration can be affected by the species sensitivity to handling stress, water temperature, the gonadal stage of development during hormone administration, and dose (Mylonas and Zohar 2001). The high variability in the latency periods observed in BSB in general may indicate that environmental factors (ie. handling stress, social stress) or initial stage of gonadal development was not uniform among studies. Cerda et al. (1997) determined that BSB oocytes incubated *in vitro* showed a variable response to hCG depending on both the level of atresia in the ovaries and the developmental state of follicles in the ovaries. It is possible that the stage of gonadal development may not have been uniform among spawning candidates for induced spawning in this study. Although the MOD of fish spawned in this study were within the range of MOD (305-448 um) used successfully for induced spawning of BSB in earlier studies (Watanabe et al. 2003), the actual developmental state of the ovaries may not be apparent from this data. BSB are multiple clutch group-synchronous spawners and may have several clutches of oocytes at various stages of development simultaneously (Wallace and Selman 1981, Watanabe et al. 2003). The MOD and maximum oocyte diameter of a BSB ovarian sample does not account for atresia within the ovary, proportion of pre-vitellogenic oocytes, or the oocyte diameters of the dominant clutch, all of which may affect the responsiveness of the oocytes to GtH (Cerda et al. 1997).

Berlinsky et al. (2005) described the use of a calculated developmental index (number of oocytes > 400 um/total number of oocytes measured) for candidate selection to determine the developmental stage of the dominant clutch of oocytes. However, there were no significant differences in fecundity, fertilization success, or number of spawns

among fish grouped by developmental index. Since BSB are protogynous hermaphrodites, it was suggested that the proximity to sex inversion may also be a factor that affects the oocyte responsiveness to LHRHa (Berlinsky et al. 2005). The variability of female response to hormone treatment in this study can also be seen in the wide range of the number of spawns per female within each trial. During the 2005 and 2006 seasons, the number of spawns per female implanted with LHRHa ranged from 2 – 8 and from 1 – 14, respectively. Further studies are needed to develop selection criteria of female candidates for induced spawning that can be used to produce a predictable spawning response.

Spawning results

In this study, there were no significant effects of dietary lipid treatments on total egg production, which is contrary to what has been reported in many other studies. Duray et al. (1994) found that in rabbitfish (*Siganus guttatus*), a broodstock dietary lipid level of 18% yielded a higher relative fecundity (1,487 eggs/g BW) compared to a diet of 12% lipid (1,210 eggs/g BW). Cerda et al. (1995) also found reduced fecundity in *D. labrax* fed a commercially prepared diet (20% and 11% lipid) when compared to a diet of frozen fish. In this study, the total egg production for all dietary treatments during both the 2005 and 2006 spawning seasons was similar to results previously obtained for volitional spawning of BSB fed commercially prepared diets (44 – 54% protein, 9 – 15% lipid) (Watanabe et al. 2003) (149 X 10^3 eggs/female) or a mixed diet of commercially prepared pellets (50% protein, 12% lipid) of Atlantic silversides, smelt, squid and

krill (197 X 10^3 eggs/female), using comparable hormone dose (50 µg/kg BW LHRHa) (White 2004). This suggested that the fecundity of BSB was less affected by diet than other species of marine finfish.

A commonly used indicator of egg quality in marine finfish is the percentage of buoyant eggs from a spawn (McEvoy 1984; Carrillo et al. 1989; Kjorsvik et al. 1990; Sargent 1995). The quality of spawned eggs in this study, as measured by egg buoyancy, varied within and among dietary treatments. Other studies have reported high variability in the percent buoyancy of BSB eggs. Watanabe et al. (2003) found the percentage of buoyant eggs in BSB implanted with 50 µg/kg BW LHRHa ranged from 0 – 100% in successive daily spawns from the same female. Berlinsky et al. (2005) observed buoyancy rates between 0 – 100% for eggs strip-spawned from BSB induced with 31.0 – 61.5 µg/kg BW LHRHa. Despite the high variability in percentage of buoyant eggs observed in this study, the SS treatment during the 2005 spawning season produced a significantly higher percentage of buoyant eggs (23.3%) than the 20 and 12% lipid treatments (6.1% and 1% respectively). This was likely due to the higher percentage of EFA found in the SS (Table 5. Fig. 2). Watanabe et al. (1984) found that in red sea bream (*Pagrus major*), broodstock fed a diet deficient in EFA produced similar quantities of eggs as fish fed a diet containing EFA; however the percentage of buoyant eggs was considerably lower in the EFA deficient diet.

Fertilization success, another measure of egg quality, was also highly variable (0 – 48%) in this study, which is consistent with the findings of earlier studies with BSB. White (2004) reported fertilization success ranging from 0 – 100% in BSB fed a mixed

diet of raw fish and commercially prepared pellets and induced to spawn with LHRHa (5, 50 and 100 μg/kg BW). Watanabe et al. (2003) also reported a high variability in overall fertilization success (10% - 100%) of BSB fed a commercially prepared diet (44-54% protein, 9-15% lipid) and induced to spawn with LHRHa (50 μg/kg BW). Similar variability (0 – 98% fertilization success) was obtained following strip spawning of BSB implanted with LHRHa at various dose rates (6.3 – 50 μg/kg BW) (Berlinsky et al. 2005). High variability in fertilization success of marine teleosts during induced spawning is common and is likely a result of many interacting factors including stress, egg overripening, sperm quality, suboptimum spawning protocols, and hormone dose (Bromage et al. 1994, Coward et al. 2002). Mate selectivity and genetic compatibility of brooders are also likely to play a major role in fertilization success (Nordeide 2007).

In this study, mean fertilization success during the 2005 spawning season was significantly higher in the SS treatment (22.4%) than in the commercially prepared diets (20% lipid diet = 4.8% and 12% lipid diet = 0.6%). This was likely due to elevated dietary levels of DHA (22:6*n*-3) found in the SS (9.30% TFA) when compared to the commercially prepared diets (20% lipid diet = 6.72% TFA and 12% lipid diet = 4.92% TFA). During the 2006 spawning season, however, the silverside treatment and the commercial diets showed no significant difference in fertilization success, and fertilization success was considerably lower (8.5%) than the 2005 silverside treatment (22.4%). The poor spawning performance from the 2006 silverside treatment may have been due to poor diet quality. The silversides from the 2006 season were packaged in poorly sealed plastic bags which were less protective to oxidative degradation compared

to the packaging used during the 2005 season. Oxidation of the silversides from 2006 was evidenced by "freezer burn". Many compounds in marine fish are highly susceptible to oxidative degradation especially ascorbic acid (Passi et al. 2005). Dietary ascorbic acid is known to play an important role in steroidogenesis by affecting the aromatization of testosterone to estradiol (Waagbo et al. 1989). Aromatase activity is important in the reproductive physiology of teleosts and is also important in sexual succession in hermaphroditic species (Zohar 1989, Frisch 2004). Dietary ascorbic acid has also been shown to have a positive effect on male fertilization success. In male rainbow trout (*Oncorrhynchus mykiss*) broodstock, fish fed a diet deficient in ascorbic acid showed a significant reduction in motility and sperm concentration, both critical factors in fertilization success (Ciereszko and Dabrowski 1995). It is possible that excessive oxidation in the 2006 silverside diet following long term freezer storage led to a deficiency of ascorbic acid in male broodstock, which may have had a negative effect on fertilization rates. These results emphasize an important disadvantage of using frozen feeds.

Hatching success observed in this study (mean = 45%, range = 16.4 – 83.4%) was higher than reported in previous studies with BSB treated with LHRHa for induced spawning. White (2004) reported a mean hatch rate of 15.6% (range = 0 – 40.2%) with volitionally spawned BSB implanted with 50 μg/kg LHRHa, and Watanabe et al. (2003) reported a mean hatch rate of 27.2% (range = 4.3 – 83%) for BSB spawned with the same hormone dose. Berlinsky et al. (2005) reported hatch rates of 53 – 76% for BSB eggs strip spawned from multiple females implanted with LHRHa (7.1 – 23.0 μg/kg BW),

similar to the hatch rates found in this study.

Hatching success was similar during both spawning seasons (34.3 – 58.6%), with no significant differences among treatments. The lack of a dietary lipid effect on hatching success, especially between the SS diet and the commercially prepared diets, is surprising and contradictory to the finding that dietary lipid and FA composition have a significant effect on hatching success in many marine finfish species. Cerda et al. (1995) found that *D. labrax* fed a 7% lipid diet produced eggs with no hatching success, while broodstock fed a natural diet of fish produced the highest hatch rate (34 – 50%). These workers suggested that the poor hatching rates of fish fed commercially prepared diets were related to suboptimal DHA/EPA ratios (0.92 – 0.96) in these diets. Navas et al. (2001) also found that *D. labrax* broodstock fed a natural diet produced eggs with a higher hatching success than fish fed artificial diets with total lipid levels of 10 and 17%. The poor hatching success was also attributed to low DHA/EPA ratios in the diets (1.1 – 1.2) and in the eggs (2.4 – 3.2) compared to the ratios found in the natural diet (3.3) and eggs (4.9 – 6.4) from the fish fed a natural diet. In this study, BSB broodstock fed diets with a DHA/EPA ratio as low as 0.49 produced viable eggs, and an egg DHA/EPA ratio of 1.27 was sufficient for embryo viability. These findings suggest that the optimal fatty acid composition of marine fish eggs can vary greatly among species.

A striking result of this study was the low egg viability seen in the low lipid (12%) treatment. Only 2 of the 6 fish in this treatment that spawned produced fertilized eggs, and only 1 fish produced enough fertilized eggs to yield data on hatching success and biochemical composition. The overall percentage of fertilized eggs from the low

lipid treatment (1.1 and 2.6%) was considerably lower than the majority of spawns from the 20% lipid treatment (mean = 4.8%, range = 0 – 15.2%). The percentage of fertilized eggs in the 20% lipid treatment showed substantial variation (range = 0 – 15.2%) which precluded statistical resolution between the 12% and 20% lipid treatments. Furthermore, hatching percentage and fatty acid composition in the 12% lipid treatment represented only one trial, preventing statistical comparisons for these parameters. The evidence clearly indicated that the 12% lipid treatment resulted in poor egg production and viability, whereas the 20% lipid treatment produced a moderate amount of viable eggs and yolk-sac larvae.

Dietary and Egg Fatty Acids

In this study, the proportions of *n*-3 and *n*-6 dietary FA may have played a major role in the spawning performance of BSB broodstock. There was a considerable difference in the sum of *n*-6 series FA between the commercially prepared diets and the SS diets during both the 2005 and 2006 seasons with substantially less *n*-6 FA in the SS diet. The 12% lipid diet also had the lowest amount of *n*-3 FA of all the diets. The resulting *n*-3/*n*-6 ratio was much higher for the 2005 and 2006 SS diet (21.1 and 20.5 respectively) than in the 12% lipid diet (0.71). This may have resulted in higher fertilization success in the 2005 SS treatment and the low fertilization success in the 12% lipid treatment. It is well documented that, compared to terrestrial animals, marine finfish have a higher dietary requirement for the *n*-3 series FA than the *n*-6 series FA.

However, dietary intake of the *n*-6 series FA is also important and must be considered along with the ratio of *n*-3/*n*-6 FA when examining dietary FA (Sargent et al. 1995). The importance of obtaining the appropriate balance of *n*-3 and *n*-6 FA is related to the biological actions of the two series of FA. The *n*-6 series FA, specifically AA, are precursors to eicosanoids and the *n*-3 series FA are important components of biological membranes and are critical for maintaining proper membrane fluidity. The *n*-3 FA are also capable of inhibiting the bioconversion of *n*-6 FA to PGE_2 thus reducing eicosanoid activity (Lands 1991; Valentine and Valentine 2004). The ratio of EPA/AA is also commonly examined in biological systems due to their interactions in the production of eicosanoids; however the appropriate ratio is often species dependent (Bell et al. 1994; Sargent et al. 2002). In this study, the eggs from the silverside treatments from the 2005 and 2006 spawning seasons contained the lowest EPA/AA ratio of all of the dietary treatments (3.60 and 3.51 respectively) with the eggs from the commercial diet treatments containing much higher ratios (5.51 – 11.4). In European sea bass, an EPA/AA ratio of 3.7 has been shown to be a suitable ratio for good hatching and larval survival when compared to eggs with a ratio of 9.7 (Bruce et al. 1999). It is not clear at this point what the appropriate ratio is for BSB eggs, but if BSB have a similar requirement to European sea bass, than the silverside eggs may have been higher quality eggs.

In this study, a high proportion of LA (18:2*n*-6) in the 12% lipid diet may have contributed to the poor spawning performance and egg quality observed in this treatment. LA was the most noticeable FA contributing to the elevated *n*-6 FA in the 12% lipid diet, which was almost twice the percentage of the 20% lipid diet and not detected in the SS

diet. LA was also transferred proportionately to the eggs of fish fed the commercially prepared diets. The elevated percentage of LA in the 12% lipid diet was due to soy oil which was used in all the commercial diets and which represented a large proportion of the lipid in the low lipid diet. Lane and Kohler (2006) found that white bass (*Morone chrysops*) broodstock fed a diet high in LA produced eggs containing elevated LA resulting in poor hatching success. Vassallo-Agius et al. (2001) compared the spawning performance and egg FA composition of striped jack (*Pseudocaranx dentex*) broodstock fed either raw fish or pelleted feed containing soybean and corn meal. Fish fed a raw fish diet yielded higher egg production and fertilization success. The authors attributed the superior egg quality of the broodstock fed raw fish to a higher proportion of DHA and a lower proportion of LA in both the raw fish diet and the eggs. In most vertebrates, LA is an EFA that can be converted to AA, however marine finfish are not capable of effectively utilizing this pathway (Sargent et al. 2002). The functions that LA plays in marine finfish are not completely understood, however it is possible that it may have a negative effect on egg quality by interfering with the functions of AA, DHA and EPA (Sargent et al. 1995).

Increased dietary DHA may have contributed to the higher fertilization success observed in the 2005 SS treatment when compared to the commercially prepared diets. The SS diet from the 2005 and 2006 spawning seasons contained a considerably larger proportion of DHA than the commercially prepared diets, with the resulting eggs exhibiting a similar trend. DHA has been demonstrated to be an important FA in reproduction and early development in marine finfish. Rodriguez et al. (1998) compared

gilthead seabream (*Sparus aurata*) broodstock fed a diet deficient in *n*-3 HUFA with broodstock fed a control diet containing *n*-3 HUFA. The results demonstrated a significant reduction in fecundity, fertilization success, and hatching success in the *n*-3 HUFA deficient diet. The poor spawning success was primarily attributed to low levels of DHA and EPA found in female gonads and eggs. It was also suggested that the *n*-3 HUFA deficient diet may have also had a negative affect on sperm quality (Rodriguez et al. 1998). In human sperm, a reduced proportion of DHA was associated with male infertility. The results indicated the importance of DHA in maintaining proper membrane fluidity of spermatozoa and that DHA may also have other physiological roles required for successful fertilization (Zalata et al. 1998). In this study, it is likely that the elevated dietary DHA in the 2005 SS treatment was responsible for the significantly higher fertilization success. Increased dietary DHA may have also had a positive effect on sperm quality of the male broodstock from the 2005 SS treatment.

CONCLUSION

In conclusion, we have determined that dietary lipid in a broodstock diet can have a strong influence on egg fatty acid profile and spawning performance in black sea bass when fed 3 months prior to spawning, particularly with respect to percent of DHA in eggs, which reinforces the importance of broodstock lipid nutrition on egg quality and spawning performance. There was a considerable amount of variation in the latency period of black sea bass females induced to spawn with LHRHa, which is consistent with

other studies on induced spawning in BSB. It is not likely that broodstock diet was the cause of this unpredictable response. To improve the predictability of induced BSB spawning, more work is needed to improve the selection criteria for female candidates used for induced spawning, including developmental stage composition of intra-ovarian oocytes.

In this study, commercially prepared diets formulated with menhaden oil and soy oil significantly reduced the percentage of fertilized eggs produced when compared to fish fed a natural diet of Atlantic silversides. It is not clear if the lower fertility in fish fed diets containing menhaden oil was a result of crude lipid levels or specific dietary fatty acids; however, the low dietary DHA levels and the high *n*-6 series FA in the commercial diets may have had a negative effect on spawning performance. It was also not clear if a specific dietary lipid level between 18 – 23% was advantageous for reproductive performance in a black sea bass broodstock diet; however, a dietary lipid level of 12% was inadequate for use as a black sea bass broodstock feed. The poor performance of this diet may have been due to a small amount of soy oil used in the diet formulations which had a greater influence on the fatty acid profile in the 12% lipid formulation. The inclusion of soy oil as a large portion of the lipid source in the 12% lipid diet reduced the proportion of *n*-3 FA and increased the proportion of LA when compared to the 20% lipid diet. Further investigation into black sea bass broodstock nutrition is required with emphasis on the lipid sources as well as lipid levels used in the prepared diets. Particular importance should be placed on the inclusion of adequate levels of DHA, and low levels of LA, when formulating BSB broodstock diets. The use of fish oil similar in

composition to Atlantic silversides may improve the spawning performance of black sea bass broodstock fed a prepared diet.

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