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Preliminary investigations on the effects of dietary lipid on the spawning performance and egg quality of black sea bass *Centropristis striata* L

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Abstract

Adult black sea bass *Centropristis striata* broodstock ($N = 162$) were fed three different dietary treatments: two commercially prepared diets with 45% protein and two different lipid levels (12% and 20%) (diets 1 and 2), and a diet of frozen Atlantic silversides *Menidia menidia* (SS, diet 3). Broodstock were held under controlled photothermal conditions and induced to spawn with an LHRHa pellet (72 μg kg^{-1} bw). Dietary lipid had pronounced effects on spawning performance and egg quality. Diet 3 (SS) produced a significantly ($P < 0.05$) higher fertilization success (22.4%) than diets 1 (0.6%) and 2 (4.8%). The hatching success of fertilized eggs was similar in all diets (range = 40–58.6%), but only two spawns from diet 1 (12% lipid) yielded viable yolk-sac larvae (YSL). Diet 3 (SS) also produced significantly more YSL per female (21.8×10^3) than the diet 1 (0.3×10^3). Eggs from diet 3 (SS) contained a significantly greater proportion of n-3 series fatty acids, with docosahexaenoic acid (DHA) as the largest fraction. Eggs from commercially prepared dietary treatments contained significantly more n-6 fatty acids. The poor spawning performance of fish fed diet 1 (12% lipid) may be related to higher levels of linoleic acid and lower levels of DHA in the diet.

Keywords: *Centropristsi striata*, reproduction, lipid, broodstock, egg fatty acids

Introduction

The black sea bass (BSB) *Centropristis striata* L. 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, Godcharles & Barco 1994). 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 (Manooch, Abbas & Ross 1981; Mercer 1989).

Recently, much work has been carried out in controlled spawning (Watanabe, Smith, Berlinsky, Woolridge, Stuart, Copeland & Denson 2003; Berlinsky, King & Smith 2005; Denson, Jenkins, Berlinsky & Smith 2007), rearing larvae through juvenile stages (Berlinsky, Watson, Nardi, & Bradley 2000; Atwood, Young, Tomasso & Smith 2004; Berlinsky, Taylor, Howell, & Bradley 2004; Copeland & Watanabe 2006) and growout of subadults to marketable stages (Copeland, Watanabe & Carroll 2002; Copeland, Watanabe, Carroll & Wheatly 2003). These studies have shown BSB to be a promising candidate for commercial culture. Induced spawning of BSB has considerably improved both the quality and the predictability of spawning. However, there is considerable variation in spawning success among individuals and among spawning clutches from the same individual. For commercial-scale egg production, more work is needed to improve the quality of eggs and the reliability of spawning.

Total lipids and fatty acids (FA) in broodstock diets were determined to have a significant effect on the reproductive performance in several commercially important species (Duray, Kohno & Pascual 1994; Cerdá, Zanuy, Carillo, Ramos & Serrano 1995; Navas, Mañanós, Thrush, Ramos, Zanuay, Carillo, Zohar & Bromage 1998). Broodstock dietary lipid has a significant effect on egg and larval viability in Japanese flounder

Paralichthys olivaceus (Temminck and Schlegel) (Furuita, Yamamoto, Shima, Suzuki & Takeuchi 2003), and broodstock dietary lipid composition, specifically FA, has a marked effect on the FA profile of spawned eggs in many species (Watanabe, Ohhashi, Itoh, Kitajima & Fujita 1984; Mourente & Odriozola 1990; Harel *et al.* 1994; Fernandez-Palacios *et al.* 1995; Rodriguez, Cejas, Martin, Badia, Samper & Lorenzo 1998; Mazorra *et al.* 2003).

The FA composition of eggs was shown to have a strong correlation with blastomere morphology and hatching success in both Baltic cod *Gadus morhua* L. (Pickova, Dutta, Larsson & Kiessling 1997) and Atlantic halibut *Hippoglossus hippoglossus* L. (Evans, Parrish, Brown & Davis 1996). Polyunsaturated fatty acids (PUFA) are the most abundant FA found in the eggs of marine finfish, equalling as much as 50% of the total egg lipids of ripe ovaries (Tocher & 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 fish. For this reason, it is believed that marine teleosts have lost the ability to produce the enzymes Δ5-desaturase and C18 and C20 FA elongase. These enzymes are required for converting the n-3 precursor 18:3n-3 (linolenic acid) to 20:5n-3 eicosapentaenoic acid(EPA) and 22:6n-3 docosahexaenoic acid (DHA). They are also required for the conversion of the n-6 precursor 18:2n-6 linoleic acid (LA) to 20:4n-6 (AA) (Sargent, Tocher & Bell 2002).

Docosahexaenoic acid has been shown to be a critical part of biological membranes in fish and is particularly important in neural cell membranes (Bell & Dick 1991; Valentine & Valentine 2004). Eicosapentaenoic acid and AA are precursors to the biologically important compounds eicosanoids, autocrines that 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, *Carassius auratus* L. (Mercure & Van Der Kraak 1996). The ratio of EPA to AA in cellular membranes is also an important consideration (Sargent *et al.* 2002). The appropriate ratio of these highly unsaturated fatty acids (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, Bell, Bell, Henderson & Tocher 1995; Rainuzzo, Reitan & Olsen 1997; Bruce, Oyen, Bell, Asturiano, Farndale, Carillo, Zanuy, Ramos & Bromage 1999). However, the appropriate amounts of HUFA required for embryo and larval development vary among species and must be determined for individual species (Sargent, McEvoy, Estevez, Bell, Bell, Henderson & Tocher 1999; Sargent *et al.* 2002). The objectives of this study were to determine the effects of dietary lipid level on the spawning performance and egg quality, including the FA profile, of BSB.

Methods

Experimental animals

Adult BSB ranging in size from 0.30 to 3.27 kg were used for this study. The oldest fish (2.3 ± 0.12 kg ± 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 F_1 progeny of wild-caught broodstock.

Experimental system

An outdoor recirculating system was used to hold and spawn the BSB broodstock. The system consisted of six 2000 L tanks (diameter = 1.83 m, depth = 0.81 m), a 2000 L sump, an external bead filter, a foam fractionator and an ultraviolet sterilizer. An exchange rate of approximately 25% day^{-1} was maintained with seawater (32–35 g L^{-1}) 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 lightproof 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 *Menidia menidia* L., were fed to three separate groups of BSB broodstock (Tables 1 and 2). Age classes of broodstock were evenly distributed among treatments. The diets were fed to each treatment group beginning in January 2005, 3 months before the spawning season. Fish were hand fed to apparent satiation 6 days a week. There were a total of 54 fish

Table 1 Commercial diet formulations (Integral Fish Foods, Grand Junction, CO, USA) and proximate composition of the frozen Atlantic silversides (SS) and experimental diets given in per cent of dry weight

	Diets		
	SS	20% lipid	12% lipid
Menhaden meal	–	32.0	32.0
Fish meal analog*	–	22.5	20.0
Soybean meal	–	7.5	7.0
Whole wheat	–	20.0	15.0
Wheat middlings	–	0.0	17.5
Menhaden oil	–	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
Dry matter	31.6	91.6	91.1
Crude protein	58.8	42.4	42.5
Total lipid	31.9	18.7	10.6
Fibre	–	1	2.1
Ash	10.2	10	11
Nitrogen free extract	–	21.3	27.4
Energy (calculated)‡	6.39	5.43	4.86

*Contains some or all: 85% bloodmeal, hydrolysed feather meal, poultry byproducts meal, DL methionine, lysine, monosodium phosphate.

†Proprietary commercial blend.

‡Based on energetic values for protein, lipid and NFE as 5.7, 9.5, 4 kcal g^{-1} respectively.

All diets were stabilized with vitamin C at 400 mg kg^{-1}.

Table 2 Fatty acid profile of diets fed to black sea bass: Atlantic silversides (SS) or pelleted diets containing 20% or 12% lipid

	Diet		
Fatty acids	SS	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
18:0	4.35 ± 0.03	4.82 ± 0.02	5.81 ± 0.03
16:1n-7	14.4 ± 0.02	10.4 ± 0.04	7.56 ± 0.04
18:1n-7	4.85 ± 0.08	2.97 ± 0.01	2.27 ± 0.07
18:1n-9	23.6 ± 0.05	13.8 ± 0.05	19.9 ± 0.11
20:1n-9	0.77 ± 0.00	0.95 ± 0.01	0.85 ± 0.01
20:1	1.22 ± 0.00	1.07 ± 0.02	ND
16:2	ND	1.50 ± 0.01	0.82 ± 0.04
18:2n-6	ND	9.66 ± 0.06	18.8 ± 0.06
16:3n-3	ND	1.93 ± 0.01	0.75 ± 0.03
16:4	ND*	0.74 ± 0.00	ND
18:4n-3	0.82 ± 0.01	1.94 ± 0.01	1.14 ± 0.04
20:4n-6	0.77 ± 0.01	1.00 ± 0.01	0.73 ± 0.01
20:5n-3	3.31 ± 0.04	9.10 ± 0.06	5.78 ± 0.09
22:5n-3	2.83 ± 0.04	1.95 ± 0.01	1.18 ± 0.02
22:6n-3	9.30 ± 0.08	6.72 ± 0.04	4.92 ± 0.12
Unknown†	3.31 ± 0.02	ND	ND
Unknown	0.74 ± 0.02	ND	ND
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
Σn-6	0.78 ± 0.01	10.7 ± 0.06	19.5 ± 0.07
Σn-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
Σn-3/Σn-6	21.1 ± 0.05	2.03 ± 0.02	0.71 ± 0.02

Values represent mean ± SE ($n = 3$).

*Not detected.

†17-C FA with undetermined configuration.

PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

(BW = 1.5 ± 0.8 kg) per dietary treatment. During the non-spawning period, fish in each treatment 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 °C, and fish were exposed to an ambient photoperiod by maintaining the tank covers ajar.

Induced spawning

During the 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 five males to one female. During the spawning season, the temperature and photoperiod were maintained at 20.1 ± 0.1 °C and 13 L:11 D respectively (Watanabe *et al.* 2003). Female candidates were selected based on ovarian biopsy, and fish with a minimum mean oocyte diameter (MOD) of 0.330 mm were selected for induced spawning (Watanabe *et al.* 2003). Oocytes were preserved with 10% formalin in seawater (White 2004). The mean oocyte diameter was determined by measuring 40–100 oocytes from digital photomicrographs taken using a dissecting microscope. Oocytes were measured using image analysis software (IMAGEJ v1.37b National Institute of Health, USA).

To induce ovulation, the selected females were implanted in the dorsal musculature with a luteinizing hormone releasing hormone analogue (LHRHa) pellet at a dose of 50–75 μg kg^{-1} body weight in a slow-release matrix (95% cholesterol and 5% cellulose) (Sherwood *et al.* 1988; Watanabe *et al.* 2003). 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.

Egg collection

Cylindrocone egg collectors, which were plumbed to receive the tank effluent stream, were monitored daily for a maximum of 10 dpi 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 32–35 g L^{-1} seawater. The non-viable eggs were allowed to sink and then transferred to a graduated cylinder to estimate the number of eggs volumetrically (1345 eggs mL^{-1}) (White 2004). The remaining viable eggs that were floating and neutrally buoyant were concentrated using a 250 μm screen and then transferred to a graduated cylinder with 250–500 mL of 32–35 g L^{-1} seawater. The eggs were gently mixed, and the mean numbers of eggs contained in three 1 mL samples were used to estimate the total number of eggs. At least 100 buoyant eggs from each spawn were examined under a dissecting microscope to determine the fertilization rate. Hatching success was also monitored as an index of egg quality. Approximately 3000 eggs from each spawn were incubated in duplicate 18 L black plastic aquaria with an exchange rate of 40% h^{-1} at 20.1 ± 0.1 °C and at 32–35 g L^{-1}. Hatching success was monitored on day 3 post fertilization using volumetric methods.

Fatty acid analysis

To determine the effects of dietary lipid on the FA 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, Lees and Stanley (1957) method was used for lipid extraction with a 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 and then transferred to a roundbottom flask for evaporation in a rotary evaporator. Samples were then filtered through a medium-porosity glass-fritted disk filter into a preweighed 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 before 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.

For analysis of total FAs, approximately 3 mg of extracted lipid in 1 mL of solvent was transferred to a conical vial with a magnetic stir bar and solvent was evaporated under nitrogen. For saponification of lipid, 1 mL of 0.5 M NaOH in MeOH was added to the sample and refluxed with stirring for 30 min. Fatty acids were then transesterified with 1.5 mL of 10–15% boron trifluoride–methanol (Morrison & 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 millilitre of saturated NaCl and 1 mL of hexane were added to the vial and then the vial was shaken for 10 s. The organic layer was transferred to a mini SiO_2 column for purification. Hexane (1 mL) was added again to the vial, shaken and the organic layer was transferred to a mini-column. A solution of 20% ether hexane was then added to the vial, mixed and the organic layer was transferred to the mini-column. To ensure that FAMEs were eluted, 1 mL of 50% ether hexane was added to the column. Fatty acid methyl esters solution was evaporated under nitrogen and transferred to vial in chloroform at a concentration of approximately 5 mg mL^{-1} for GC analysis.

Fatty acid methyl esters were analysed with GC-FID (Hewlett Packard 6890 Series GC/FID (Palo Alto, CA, USA) using an HP 18596C autosampler and a 30 m × 0.25 mm HP-5 capillary column with a 0.25 μm film thickness) with a split injector. One microlitre of the sample was injected at a split ratio of 10:1 and a 2 mL min^{-1} 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 °C min^{-1} ramp to 255 °C. Commercially prepared diets were analysed using the following method to provide greater resolution for the 18 carbon FAMEs: an initial temperature of 170 °C held for 8 min, 1.5 °C min^{-1} ramp to 195 °C and held for 3 min, and then 15 °C min^{-1} ramp to 255 °C. A standard FAME

mixture (GLC-84, Nu-Chek Prep, Elysian, MN, USA) was run periodically for reference. Fatty acid methyl esters were identified with the addition of standards of oleic, vacenic and linoleic methyl esters individually to subsamples. Representative samples were also analysed with GC-MS (Varian CP-3800 GC, Palo Alto, CA, USA with a 1079 injector and a 30 m × 0.25 mm WCOT-fused silica column coupled to a Saturn 2200 MS) for verification and identification of FA where standards were unavailable. One microlitre of sample was injected at a split ratio of 20:1 and a 1.2 mL min^{-1} flow rate of He. The GC method began with an initial temperature of 50 °C with a 30 °C min^{-1} ramp to 200 °C, then a 10 °C min^{-1} ramp to 250 °C and holding for 10 min.

Diet analysis

Diets were analysed to determine their FA composition following the same procedures described for egg FA 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 per cent moisture before lipid extraction. Per cent 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 6 h in a muffle furnace. Per cent protein for all diets and fibre for the commercially prepared diets were analysed according to AOAC methods (NJ Feed Laboratory, Trenton, NJ, USA).

Data analysis

Fertilization success and hatching success were expressed as percentages. A weighted average from each 24-h spawning period was used to calculate the mean fertilization and hatching success for individual females. Egg lipid was expressed as per cent wet weight, and FA composition was expressed as per cent of total FAs. Quantitative values were expressed as treatment means. All percentage data were arcsine transformed. When two treatments were compared, Bartlett's test was performed to test for homogeneity of variances, and a *t*-test was used to compare means. If assumptions were violated, Wilcoxon'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 analysis of variance (ANOVA), and Tukey's test was used for multiple comparisons among means. When an assumption of ANOVA was violated, means were compared in pairs using Wilcoxon's rank sums test. All statistical analysis was performed using JMP 6.0 statistical software (SAS Institute, Cary, NC, USA).

Results

Spawning results

A total of 30 females were implanted for induced spawning: 12 fish from the silverside treatment, eight from the 20% lipid treatment and 10 from the 12% lipid treatment (Table 3). Mean female weight ranged from 1.2 to 1.7 kg among treatments, with no significant differences (Table 3). 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 3). The mean latency period for induced spawning ranged from 2.3 to 3.9 dpi, 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 one 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 3). Spawning-related mortalities

Table 3 Spawning results for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 20 or 12% lipid

	Dietary treatment		
	SS	**20% lipid**	**12% lipid**
No. of females implanted	12	8	10
LHRHa (50 μg kg^{-1})	3	2	0
LHRHa (75 μg kg^{-1})	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 (dpi)	3.5 ± 0.7	3.9 ± 1.2	2.3 ± 0.2
(range)	(2–6)	(2–10)	(2–3)
No. of spawns per trial (days)	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)

Values represent means ± SE (range).

were seen in all treatments following hormone implantation. Four mortalities were observed in both the SS and the 12% lipid treatments, and three mortalities in the 20% lipid treatment (Table 3). Mortalities were frequently associated with an egg-bound condition characterized by excessive hydration and abdominal swelling. However, one mortality was observed in the 20% lipid treatment following a successful spawn producing fertilized eggs.

Egg production

There were no significant differences ($P<0.05$) in the mean total fecundity (eggs female^{-1}) and relative fecundity (eggs kg^{-1} BW) among treatments (Table 4). The mean total and relative fecundity for the SS treatment were 242.6 ± 87.8 and 192.5 ± 54.7 × 10^3 eggs respectively. The 20% lipid treatment had a mean total fecundity of 155.1 ± 36.6 × 10^3 eggs and a mean relative fecundity of 128.2 ± 29.4 × 10^3 eggs, while the 12% lipid treatment had a mean total fecundity of 173.2 ± 31.9 × 10^3 eggs and a relative fecundity of 116.2 ± 21.7 × 10^3 eggs (Table 4).

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

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

Table 4 Egg production and egg quality for black sea bass fed Atlantic silversides (SS) or pelleted diets containing 20% or 12% lipid

Parameter	SS	20%	12%
Eggs/female (×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. (×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 (×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 (% of fertilized eggs)	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 (no. per female, ×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 (no. per kg BW, ×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

Data represent mean ± SE (range). Means not sharing a common letter are significantly different ($P<0.05$).
ysl, bw.

Total and relative yolk-sac larvae (YSL) production (nos. per female and nos. per kg body weight) in the SS treatment (26.6 ± 12.0 and 21.8 ± 10.3 × 10^3, respectively) were significantly ($P<0.05$) higher than in the 12% lipid treatment (0.3 ± 0.3 and 0.3 ± 0.3 × 10^3 respectively). The 20% lipid treatment was intermediate for both the total and the relative YSL produced (5.1 ± 2.9 and 4.0 ± 2.1 × 10^3 respectively) (Table 4).

Egg FAs

There were no significant differences in the per cent total lipid of eggs between the SS and the 20% lipid treatment. Per cent total lipid of the 12% lipid treatment was also similar; however, it was not included in any statistical comparisons because this represented a single sample (Table 5).

Table 5 Egg fatty acid profile (% TFA) for black sea bass fed: Atlantic silversides (SS) or pelleted diets containing 20% or 12% lipid

		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* b	Tr
16:0	17.5 ± 0.61 b	20.1 ± 0.56 a	20.0
17:0	1.31 ± 0.12 a	Tr b	Tr
18:0	8.83 ± 0.53	9.01 ± 0.59	9.25
19:0	0.67 ± 0.09 a	ND† b	ND
16:1n-7	7.06 ± 0.44 b	8.52 ± 0.32 a	7.93
18:1n-7	3.31 ± 0.25	2.90 ± 0.08	2.79
18:1n-9	13.5 ± 0.58	13.9 ± 0.33	16.7
20:1	Tr	0.51 ± 0.18	0.55
18:2n-6	1.59 ± 0.43 b	9.46 ± 0.38 a	13.5
20:4n-6	1.96 ± 0.11	1.67 ± 0.06	1.23
20:5n-3	6.96 ± 0.16 b	9.19 ± 0.11 a	6.84
22:5n-3	4.64 ± 0.19 a	2.67 ± 0.13 b	2.26
22:6n-3	28.6 ± 1.46 a	17.5 ± 0.56 b	15.6
20:?‡	0.55 ± 0.10 b	0.89 ± 0.03 a	0.83
Unknown	Tr	Tr	0.62
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
Σn-6	3.55 ± 0.41 b	11.1 ± 0.36 a	14.7
Σn-3	40.1 ± 1.70 a	29.4 ± 0.72 b	24.7
EPA/ARA	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
Σn-3/Σn-6	12.0 ± 1.52 a	2.65 ± 0.11 b	1.68

Values represent mean ± SE and letters identify significant differences ($P < 0.05$).
*Trace amounts (< 0.5%) detected.
†Not detected.
‡Polyunsaturated with number of double bonds unidentified.
PUFA, polyunsaturated fatty acids; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Tr, trace amount.

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 (18:1n-9) and DHA (22:6n-3), with DHA comprising the largest percentage in the SS treatment and palmitic acid as the largest percentage in both the 20% and the 12% lipid treatments (Table 5).

Significant ($P < 0.05$) differences were found between the SS and the 20% lipid treatment in 11 FA (Table 5). The 20% lipid treatment eggs contained significantly ($P < 0.05$) more EPA (20:5n-3) and 20:? than the SS treatment, while the SS treatment contained significantly ($P < 0.05$) more DHA and DPA (22:5n-3) than the 20% lipid treatment eggs (Table 5).

The sum of saturated fatty acids (SFA) was significantly ($P < 0.05$) greater in the 20% lipid treatment than in the SS treatment (Table 5). Total SFA in the 12% lipid treatment 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 the 20% lipid treatments, with the SS treatment having a higher sum of n-3 FA and a lower sum of n-6 FA than the 20% lipid treatment. The sums of the n-6 and n-3 series FA in the 12% lipid treatment were 14.7 and 24.7% TFA respectively (Table 5).

The ratios of EFAs and n-3 to n-6 series FA were also significantly different between the SS and the 20% lipid treatments (Table 5). Eggs from the SS treatment contained a significantly ($P < 0.05$) higher ratio of DHA/EPA and n-3/n-6 than the 20% lipid treatment. The 20% lipid treatment had a higher EPA/AA ratio than the SS treatment. The ratios of EPA/AA, DHA/EPA and n-3/n-6 for the 12% lipid treatment were similar to the eggs from the 20% lipid treatment (Table 5).

Discussion

Spawning results

In this study, there were no significant effects of dietary lipid treatments on total egg production, contrary to what has been reported in other studies. Duray *et al.* (1994) found that in rabbitfish *Siganus guttatus* (Bloch), a broodstock dietary lipid level of 18% yielded a higher relative fecundity (1487 eggs g^{-1} BW) compared with a diet of 12% lipid (1210 eggs g^{-1} BW). Cerdá *et al.* (1995) also found reduced fecundity in European sea bass *Dicentrarchus labrax* L. fed commercially prepared diets (20% and 11% lipid) when compared with a diet of frozen fish. In this study, the total egg production for all dietary treatments was similar to results reported previously for volitional spawning of BSB fed commercially prepared diets (44–54% protein, 9–15% lipid) (Watanabe *et al.* 2003) (149×10^3 eggs $female^{-1}$) or a mixed diet of commercially prepared pellets (50% protein, 12% lipid) of Atlantic silversides, smelt, squid and krill (197×10^3 eggs $female^{-1}$), using a comparable hormone dose (50 μg kg^{-1} BW LHRHa) (White 2004). The total lipid levels in the commercially prepared as well as natural fish diets in this study were adequate for gonadal formation and egg production, but these diets produced differences in egg quality as described below.

A commonly used indicator of egg quality in marine finfish is the percentage of buoyant eggs from a spawn (McEvoy 1984; Carillo, Bromage, Zanuay, Serrano &

Prat 1989; Kjorsvik, Mangor-Jensen & Holmefjord 1990; Sargent *et al.* 1995). In this study, spawned egg quality, measured by egg buoyancy, varied within and among dietary treatments. Previous studies in BSB also reported a high variability in egg buoyancy (0–100%) in successive spawns induced by LHRHa (Watanabe *et al.* 2003; Berlinsky *et al.* 2005). Despite this high variability, the SS treatment 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 diet (Table 2), which was reflected in high egg levels (Table 5). Watanabe *et al.* (1984) found that in red sea bream *Pagrus major* (Temminck and Schlegel) broodstock, a diet deficient in EFA reduced egg buoyancy but did not affect the total egg production.

Fertilization success (% overall), another measure of egg quality, was also highly variable (0–48%) in this study, consistent with earlier findings. White (2004) reported fertilization success ranging from 0% to 100% in BSB fed a mixed diet of raw fish and commercially prepared pellets and induced to spawn with LHRHa (5–100 μg kg^{-1} BW). Watanabe *et al.* (2003) also reported a high variability in the 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^{-1} BW). A similar variability (0–98% fertilization success) was obtained following strip spawning of BSB implanted with LHRHa (6.3–50 μg kg^{-1} BW) (Berlinsky *et al.* 2005). High variability in fertilization success during induced spawning of marine teleosts is common and is likely a result of many interacting factors including stress, egg overripening, sperm quality and hormone dose (Bromage, Bruce, Basavaraja & Rana 1994; Coward, Bromage, Hibbitt & Parrington 2002). Mate selectivity and genetic compatibility of brooders are also likely to play a major role in fertilization success (Nordeide 2007).

In this study, the mean fertilization success (% overall) 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 possibly due to elevated dietary levels of DHA (22:6n-3) found in SS (9.30% TFA) compared with the commercially prepared 20% lipid diet (6.72% TFA) and the 12% lipid diet (4.92% TFA). A similar trend was seen in spawned eggs from the silverside treatment (28.6% TFA) and the commercially prepared dietary treatments (20% lipid diet = 17.5% TFA and 12% lipid diet = 15.6% TFA). Docosahexaenoic acid is an important FA in reproduction and early development in marine finfish. A significant reduction in fecundity, fertilization success and hatching success was observed in gilthead seabream *Sparus aurata* L. broodstock fed an n-3 HUFA-deficient diet (Rodriguez *et al.* 1998). The poor spawning success was primarily attributed to low levels of DHA and EPA found in female gonads and eggs.

The hatching success of fertilized eggs 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^{-1} 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^{-1} BW), similar to the hatch rates found in this study.

A striking result of this study was the low egg viability seen in the low-lipid (12%) treatment. Only two of the six fish in this treatment that spawned produced fertilized eggs, and only one fish produced enough fertilized eggs to yield data on hatching success and biochemical composition (Table 4). The overall fertilization success from the low-lipid treatment (mean = 0.6%, range = 0–2.6%) was considerably lower than the majority of spawns from the 20% lipid treatment (mean = 4.8%, range = 0–15.2%), and much lower than the SS treatment (mean = 22.4%, range = 0.7–71.5). Furthermore, per cent hatching success and FA composition in the 12% lipid treatment represented only one successful spawning. The evidence clearly indicated that the 12% lipid treatment resulted in poor egg production and viability, whereas the 20% lipid treatment and the SS treatment produced moderate to high amounts of viable eggs and YSL respectively.

In this study, a high proportion of LA (18:2n-6) in the 12% lipid diet (18.8% TFA) may have also contributed to poor spawning performance and egg quality, which was almost twice the percentage in the 20% lipid diet (9.66% TFA) and was not detected in the SS diet. Linoleic acid 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 used in both commercial diets and that represented a large proportion of the lipid in the low-lipid diet. Lane and Kohler (2006) found that white bass *Morone chrysops* (Rafinesque) broodstock fed a

diet high in LA-produced eggs containing elevated LA, resulting in poor hatching success. Vassallo-Agius, Imaizumi, Watanabe, Yamazaki, Satoh and Kiron (2001) found that striped jack *Pseudocaranx dentex* (Bloch and Schneider) broodstock fed a raw fish diet yielded higher egg production and fertilization success than fish fed a pelleted feed containing soybean and corn meal. 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. Marine finfish are not capable of effectively converting LA to AA (Sargent *et al.* 2002). Linoleic acid may have a negative effect on egg quality by interfering with the functions of AA, DHA and EPA (Sargent *et al.* 1995).

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 substantially less n-6 FA in the SS diet than in the commercially prepared diets (Table 2). 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 SS diet (21.1) than in the 12% lipid diet (0.71) (Table 2), and may have resulted in higher fertilization success in the SS treatment than in the 12% lipid treatment. It is well documented that, compared with terrestrial animals, marine finfish have a higher dietary requirement for the n-3 than for the n-6 series FA (Sargent *et al.* 1995).

The dietary intake of the n-6 series FA must be considered along with the ratio of n-3/n-6 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 PGE2, thus reducing eicosanoid activity (Lands 1991; Valentine & Valentine 2004). Hence, the ratio of EPA/AA is important due to their interactions in the production of eicosanoids; however, the appropriate ratio is species dependent (Bell, Ghioni & Sargent 1994; Sargent *et al.* 2002). In this study, eggs from the silverside treatment contained the lowest EPA/AA ratio of all the dietary treatments (3.60), with eggs from the commercial diet treatments containing much higher ratios (5.51–5.58) (Table 5). In European sea bass, an EPA/AA ratio of 3.7 was better for hatching and larval survival compared with eggs with a ratio of 9.7 (Bruce *et al.* 1999).

In conclusion, dietary lipid level and source can have a strong influence on the egg FA profile and spawning performance in BSB when fed 3 months before spawning. Broodstock BSB fed commercially prepared diets formulated with menhaden oil and soy oil showed a significant reduction in the percentage of fertilized eggs compared with fish fed a natural diet of Atlantic silversides. The results suggested that low-dietary DHA levels and high n-6 series FA in the commercial diets had a negative effect on fertilization success. Although a dietary lipid level of 12% was clearly inadequate for use as a BSB broodstock feed, the poor performance of this diet may have been due to a small amount of soy oil used in the diet formulations that increased the proportion of LA (and reduced the proportion on n-3 FA) compared with the 20% lipid diet. Further investigations on BSB broodstock lipid nutrition should emphasize lipid sources as well as lipid levels, including adequate levels of DHA, and low levels of LA. The use of fish oil similar in composition to Atlantic silversides may improve the spawning performance of BSB broodstock fed a prepared diet.

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