

Spontaneous Spawning of Captive Red Snapper, *Lutjanus campechanus*, and Dietary Lipid Effect on Reproductive Performance

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Abstract

Two experiments addressed the spontaneous spawning of red snapper, *Lutjanus campechanus*, under controlled temperatures and photoperiods and the effect of broodstock diets supplemented with oils rich in polyunsaturated fatty acids. In Experiment 1, broodfish were fed a standard diet (ST1) and one enriched with menhaden oil (ER1) over a 355-d period. ER1-influenced egg fatty acid profile, however, did not positively influence egg production. Both diets produced highly viable eggs and larvae but results varied within treatments. In Experiment 2, broodfish were fed either a standard diet (ST2) or one supplemented with oils (ER2) rich in docosahexaenoic and arachidonic acid using a 203-d cycle. Both treatments produced eggs but fertilization rates ranged 0–10%. There was no clear influence of the diets on egg fatty acid profiles. These results indicate that red snapper can spawn spontaneously in tanks under controlled environmental conditions and produce viable eggs and larvae when fed diets based on squid, shrimp, and fish. The fatty acid composition of the diets was reflected in the eggs to some degree, but the oil enrichments did not further enhance the reproductive performance and egg quality under the conditions of this study.

Many marine fish can be held in captivity and will spawn naturally when given a proper environment and an appropriate diet (see reviews by Tucker 1994; Izquierdo et al. 2001; and Watanabe and Vassallo-Agius 2003). Factors that contribute to a proper environment include: tank size, brood density, water quality, photoperiod, and temperature. It is difficult to duplicate the natural setting under which snapper (Lutjanidae) spawn. In the wild, snapper form spawning aggregations of several hundred fish in an open water setting where a female would rise toward the surface followed by several males, with eggs and sperm being released near the surface (Domeier et al. 1996; Heyman et al. 2005). However, snapper will spawn under more confined conditions in captivity where temperature and photoperiod can be controlled. Several species of snapper that have reproduced naturally in captivity include: *Lutjanus argentimaculatus* (Emata 2003; Leu et al. 2003), *Lutjanus campechanus* (Arnold et al. 1978), *Lutjanus kasmira* (Suzuki and Hioki 1979), *Lutjanus stellatus*

(Hamamoto et al. 1992), and *Ocyurus chrysurus* (Soletchnik et al. 1989; Turano et al. 2000).

Providing an appropriate brood diet can be an issue for many fish species. Often, fresh seafood similar to what might be found in the fish's natural diet is adequate. Turano et al. (2000) obtained natural spawning of yellowtail snapper, *O. chrysurus*, feeding the brooders a diet of shrimp, fish, and squid. Emata (2003) found that mangrove red snapper, *L. argentimaculatus*, would spawn under controlled conditions when fed trash fish.

Brood diets have been fortified with vitamins and oils in an attempt to improve spawning success and egg quality (Kent et al. 1993; Lavens et al. 1999; Furuita et al. 2003). Formulated diets often have not proven to be of equal quality to a seafood-based diet (Bell et al. 1997; Morehead et al. 2001; Navas et al. 2001) and must be enriched with highly unsaturated fatty acids (HUFA). Certain HUFA are considered vital components in broodstock diets (review by Izquierdo et al. 2001). Diets deficient in n-3 HUFA have resulted in reduced egg quality (Rodriguez et al. 1998; Almansa et al. 1999). Enrichment of marine broodfish diets with fatty acids has improved spawning success and/or egg

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and larval quality in some species (Fernandez-Palacios et al. 1995; Bruce et al. 1999; Li et al. 2005). Ramos et al. (1993) found that sea bass, *Dicentrarchus labrax*, given diets high in essential fatty acid resulted in higher rates of egg viability and hatching. Bruce et al. (1999) found that a formulated diet enriched with tuna orbital oil gave similar egg quality as a fish-based diet when fed to sea bass. However, in some cases, enrichment may have little added benefit or even a negative effect. Quintio et al. (1996) found that no benefits in enrichment of a brood grouper, *Epinephelus coioides*, diet composed of bycatch, using either cod-liver oil or a commercial HUFA product (SELCO, INVE Aquaculture NV, Baasrode, Belgium). Furuita et al. (2002) found that a high level of n-3 HUFA in broodstock diet negatively affected egg quality of Japanese flounder, *Paralichthys olivaceus*. Li et al. (2005) found that excess dietary n-3 HUFA decreased egg production and egg and larval quality in sweetlips, *Plectorhynchus cinctus*.

Red snapper, *L. campechanus*, is an important species in both the commercial and the recreational fisheries in the Gulf of Mexico that can spawn naturally in a controlled hatchery setting. In the wild, red snapper feed on fish, shrimp, crabs, and squid as well as other invertebrates (McCawley et al. 2003; Szedlmayer and Ouzts 2003). Arnold et al. (1978) obtained natural spawns of red snapper, *L. campechanus*, in May and June under hatchery conditions using wild-caught brooders that had been held approximately 20 mo and fed a diet of frozen shrimp, squid, and fish. However, only a few thousand eggs were obtained.

The objectives of the present study were to test sets of controlled environmental conditions that might be conducive to natural spawning of red snapper, *L. campechanus* and to document the resultant broodfish growth and reproductive success. Emphasis was given to evaluate the contribution of lipid supplementation of the brood diet in regard to egg production, egg quality, and egg fatty acid composition. Menhaden oil (Study 1) and oils rich in docosahexaenoic (DHA) and arachidonic acid (ARA) (Study 2) were tested as supplements in broodstock diets.

Materials and Methods

Systems

These studies were conducted at the Claude Petet Mariculture Center, Gulf Shores, Alabama, from 2001 to 2003. Broodfish were maintained in six identical fiberglass 13.2-m³ tanks. They were caught from the wild by hook and line in October 2000, and upon arrival at the facility were sexed and tagged with passive integrated transponder tags (PIT). Each tank had its own recirculating system that included two 90-cm-diameter TR-140 (0.53 m³/min) sand filters placed in series (Pac-Fab Inc., Sanford, NC, USA), one Perflex EC75 diatomaceous earth (DE) regenerative bump-style filter (Hayward Pool Products, Inc., Elizabeth, NJ, USA), and an 1491-W (2 hp) titanium heat pump with a digital temperature controller (Aqua Logic Inc., San Diego, CA, USA). Once per week, approximately 2–3% of the system volume lost during backwashing of filters was replaced with fresh seawater. The first sand filter functioned both as particulate (>40 µm) and as biological filter, whereas the second was filled with 2- to 3-mm diameter coral particles and was functioning as biological filter and pH buffering aid. The DE filter allowed for very fine particulate removal including protozoan removal (>7 µm). Solar and lunar periods were automatically controlled by a dim up, dim down electronic ballast fluorescent system powered by a Solar-1000 controller (Niche Engineering, Ambler, PA, USA).

Broodfish and Feeds

Captive broodfish were offered fresh squid, shrimp, and herring in a 2:1:1 ratio (Table 1), and to facilitate delivery of selected nutrients, a gelatinized food (gel food) was also given. The gel food was prepared with squid, shrimp, herring, gelatin, vitamin and mineral premix, and various oils in the enriched diets (Table 1–3), grinding the raw ingredients, mixing them with gelatin and hot water, and supplements as required. The gel food was allowed to congeal and then stored in a freezer for later use. The gel food (either enriched or unenriched depending on the treatment protocol) was fed to

TABLE 1. Composition of the standard (ST) and enriched gel food (ER) used in the study over two spawning periods.

Ingredient	ST1		ER1		ST2		ER2	
	Quantity ^a	% DW	Quantity	% DW	Quantity	% DW	Quantity	% DW
Squid (kg)	4	12.6	4	10.9	4	11.9	4	11.5
Shrimp (kg)	2	10.1	2	8.7	2	9.5	2	9.2
Herring (kg)	2	12.3	2	10.6	2	11.6	2	11.2
Gelatin (kg)	2.6	65.0	2.6	56.1	2.6	61.4	2.6	59.2
Hot water (L)	4		4		4		4	
Vitamin and mineral premix ^b (g)			448	10.2	224	5.6	224	5.4
Menhaden oil ^c (g)			160	3.6				
DHA Gold ^{®d} (g)							100	2.4
ARASCO ^{®d} (g)							50	1.2

DW = dry weight.

^a Quantity of ingredients used for the production of gel food.

^b Vitamin and mineral premix (Vitamins and Electrolytes Plus; AgriLabs, St. Joseph, MO, USA) contains as units per 112 g (1 pack): vitamin A, 2,500,000 IU; vitamin D₃, 1,000,000 IU; vitamin E, 2000 IU; riboflavin, 750 mg; d-pantothenic acid, 2000 mg; folic acid, 125 mg; thiamine mononitrate, 375 mg; niacinamide, 5000 mg; pyridoxine HCL, 300 mg; ascorbic acid, 3750 mg; vitamin B₁₂, 2.5 mg; menadione 1000 mg; salt 11%; sodium, 4.7%; potassium, 3.8%; magnesium, 0.26%.

^c Provided by Omega Protein Co., Houston, TX, USA.

^d Provided by Martek Biosciences Co.

the fish three times a week, early in the morning, while the raw squid, shrimp, and herring were fed the same days early in the afternoon to all fish.

In Experiment 1 (EXP1), four females and five males were weighed individually, recording the PIT tag number, stocked into independent recirculating systems, and cultured for 12 mo. The fish in three tanks were fed the standard gel food (no supplement, ST1), and fish in the other three tanks were fed a gel food enriched with menhaden oil and a vitamin and mineral premix (ER1). The photoperiod and temperature manipulation duplicated the natural conditions of the northern Gulf of Mexico. During the 12-mo study, water quality parameters were similar for the two treatments, temperature 22.8 ± 7.99 C, salinity 32.6 ± 0.2 ppt, pH 7.9 ± 0.04 , dissolved oxygen 6.5 ± 0.1 ppm, and total ammonia-nitrogen 0.07 ± 0.03 ppm. The temperature during the spawning season (May to July) was 26.6 ± 0.1 C.

A second experiment (Experiment 2 [EXP 2]) was conducted from August 14, 2002, to February 24, 2003. Broodstock used in the previous trial were randomly restocked across all tanks (four male and four female per tank) and held under a condensed photoperiod and temperature cycle (203 and 109 d, respectively, at

nonspawning conditions) designed to allow spawning in January. All fish were fed for approximately 2 mo the fresh feeds and the standard gel food (no oils were added). In October 17, 2002, broodfish in three tanks were switched to an oil-enriched gel food (ER2) and the other three maintained in the standard diet that had vitamins and minerals added to the gel food but with no supplemental oils added (ST2). Two oils rich in DHA and ARA (DHA Gold[®] and ARASCO[®], respectively) provided by Martek Biosciences Co. (Columbia, MD, USA) were added to make the enriched diet. A mineral and vitamin premix was added in both treatments (Table 1). The two diets were fed for a total of 129 d. In February 24, 2003, the experiment was terminated and fish were weighed. During the 203-d study, water quality parameters were similar for the two treatments: temperature 23.8 ± 0.5 C, salinity 32.0 ± 0.6 ppt, pH 7.8 ± 0.03 , dissolved oxygen 6.4 ± 0.2 ppm, and total ammonia-nitrogen 0.05 ± 0.01 ppm. The temperature during the spawning period (94 d) was 26.5 ± 0.1 C.

Egg Collection, Incubation, and Viability Assessment

Brooders held in the temperature/photoperiod-controlled tanks were allowed to spawn

TABLE 2. Composition (mean \pm SD) of gel feeds.

Name	ST1	ER1	ST2	ER2
Fatty acid profile				
14:0	4.7 \pm 0.3	7.2 \pm 0.1	4.8 \pm 0.2	6.3 \pm 0.1
16:0	24.9 \pm 0.7	19.0 \pm 0.3	23.1 \pm 0.2	25.1 \pm 1.0
16:1	3.9 \pm 0.2	9.7 \pm 0.1	4.3 \pm 0.1	1.7 \pm 0.0
16:2n-4	0.3 \pm 0.1	1.7 \pm 0.0	0.3 \pm 0.0	0.1 \pm 0.1
17:0	1.1 \pm 0.1	0.8 \pm 0.0	1.0 \pm 0.1	0.6 \pm 0.1
18:0	7.0 \pm 0.5	4.5 \pm 0.2	6.6 \pm 0.1	4.8 \pm 0.2
18:1n-9	8.6 \pm 1.4	8.0 \pm 0.4	8.2 \pm 0.2	5.7 \pm 0.1
18:1n-7	3.7 \pm 0.9	3.9 \pm 0.3	3.8 \pm 0.2	1.4 \pm 0.1
18:2n-6	5.2 \pm 0.1	3.0 \pm 0.0	4.4 \pm 0.2	3.3 \pm 0.1
18:3n-3	0.4 \pm 0.6	1.2 \pm 0.1	1.0 \pm 0.1	0.3 \pm 0.0
20:1n-9	2.2 \pm 0.2	1.4 \pm 0.0	1.6 \pm 0.1	0.7 \pm 0.1
20:2	1.0 \pm 0.0	0.5 \pm 0.1	0.8 \pm 0.1	0.3 \pm 0.0
20:4n-6 (ARA)	3.0 \pm 0.1	1.9 \pm 0.0	3.5 \pm 0.1	13.2 \pm 0.4
20:4n-3	0.7 \pm 0.1	1.3 \pm 0.0	0.6 \pm 0.0	0.6 \pm 0.0
20:5n-3 (EPA)	10.0 \pm 0.5	12.9 \pm 0.4	11.3 \pm 0.1	5.0 \pm 0.2
22:5n-3	1.0 \pm 0.1	1.8 \pm 0.0	1.2 \pm 0.2	0.6 \pm 0.0
22:6n-3 (DHA)	20.6 \pm 3.9	15.9 \pm 0.5	21.3 \pm 0.0	27.1 \pm 0.8
DHA/EPA	2.1 \pm 0.3	1.2 \pm 0.0	1.9 \pm 0.0	5.4 \pm 0.1
ARA/EPA	0.3 \pm 0.0	0.1 \pm 0.0	0.3 \pm 0.0	2.6 \pm 0.1
Saturates ^a	39.9 \pm 1.3	33.2 \pm 0.0	38.0 \pm 0.1	39.0 \pm 1.1
MUFA ^b	18.5 \pm 2.4	23.4 \pm 0.8	18.1 \pm 0.2	9.7 \pm 0.2
PUFA ^c	41.5 \pm 3.7	43.4 \pm 0.7	43.9 \pm 0.3	51.3 \pm 1.1
Total n-3 ^d	32.9 \pm 3.7	35.5 \pm 0.8	35.8 \pm 0.2	33.9 \pm 1.1
Total n-6 ^e	8.3 \pm 0.0	5.0 \pm 0.0	7.9 \pm 0.1	17.3 \pm 0.7
n-3/-6	4.0 \pm 0.4	7.1 \pm 0.2	4.5 \pm 0.1	2.0 \pm 0.1
Proximate analysis				
Moisture (%) ^f	73.2 \pm 0.4	72.7 \pm 0.0	72.5 \pm 0.0	71.9 \pm 0.0
Protein (% DW) ^g	83.5 \pm 2.9	79.3 \pm 0.8	77.6 \pm 6.4	77.3 \pm 6.9
Lipids (% DW) ^f	2.4 \pm 0.1	5.6 \pm 0.4	3.3 \pm 0.2	5.9 \pm 0.1
Ash (% DW) ^f	3.1 \pm 0.2	5.4 \pm 0.7	4.6 \pm 0.5	5.0 \pm 0.2
Energy (Kcal/g) ^g	5.06 \pm 0.1	5.13 \pm 0.1	5.09 \pm 0.1	5.14 \pm 0.1

ST = standard; ER = enriched gel food; DW = dry weight; DHA = docosahexaenoic; ARA = arachidonic acid; EPA = eicosapentaenoic; PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid.

^a Includes: 12:0, 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.

^b Includes: 14:1, 15:1, 16:1, 17:1, 18:1n-9, 18:1n-7, 20:1n-9, and 22:1n-9.

^c Includes: 16:2n-4, 16:3n-4, 18:2n-6, 18:3n-6, 18:3n-4, 18:3n-3, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-6, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^d Includes: 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^e Includes: 18:2n-6, 18:3n-6, 20:3n-6, and 20:4n.

^f Analyzed in duplicate.

^g Analyzed in triplicate.

spontaneously. When temperature and photoperiod conditions duplicated the natural spawning season, the external egg collectors of the captive broodstock tanks were checked daily during the evening hours. If there was a spawn, the eggs were collected with a fine mesh net and placed in a plastic 1-L beaker partially filled with water from the tank. The eggs were transferred to the incubation room and the volume of the spawn determined by placing the

eggs in a 1-L volumetric cylinder and allowing them to separate into floating and sinking eggs and the volume of the spawn recorded. The eggs were checked under the stereoscope for fertility, and if they were fertilized, three 0.2-mL scoops of floating eggs were taken and placed in 10-mL beakers filled with seawater for later assessment of fertilization rate. Egg samples were taken and held at -60°C for subsequent lipid and fatty acid analysis.

TABLE 3. Composition (mean \pm SD) of raw ingredients and oils used in the gel food production.

Name	Raw ingredients			Oils		
	Squid	Shrimp	Herring	Menhaden	DHA Gold®	ARASCO®
Fatty acid profile						
14:0	2.0 \pm 0.1	1.7 \pm 0.0	5.6 \pm 0.1	7.7 \pm 0.4	7.1 \pm 0.1	0.9 \pm 0.0
16:0	25.4 \pm 0.2	21.7 \pm 0.5	23.9 \pm 0.7	14.8 \pm 1.0	20.7 \pm 0.2	12.0 \pm 0.1
16:1	0.7 \pm 0.0	3.4 \pm 0.1	5.3 \pm 0.1	11.8 \pm 0.8	0.3 \pm 0.0	0.2 \pm 0.0
16:2n-4	0.1 \pm 0.1	0.3 \pm 0.1	0.9 \pm 0.1	0.3 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
17:0	0.5 \pm 0.0	0.8 \pm 0.0	1.7 \pm 0.0	0.5 \pm 0.0	0.2 \pm 0.0	0.4 \pm 0.0
18:0	3.2 \pm 0.2	7.9 \pm 0.1	7.7 \pm 0.4	3.0 \pm 0.3	0.5 \pm 0.0	8.3 \pm 0.0
18:1n-9	2.3 \pm 0.0	18.3 \pm 0.4	5.4 \pm 0.4	7.8 \pm 0.8	0.4 \pm 0.0	12.3 \pm 0.0
18:1n-7	1.8 \pm 0.1	4.5 \pm 0.0	3.4 \pm 0.2	3.2 \pm 0.3	0.3 \pm 0.0	0.4 \pm 0.0
18:2n-6	0.3 \pm 0.0	12.9 \pm 0.0	2.3 \pm 0.1	1.6 \pm 0.1	0.5 \pm 0.0	6.5 \pm 0.0
18:3n-3	0.3 \pm 0.1	0.7 \pm 0.1	0.9 \pm 0.1	1.6 \pm 0.1	0.1 \pm 0.0	0.1 \pm 0.0
20:1n-9	4.7 \pm 0.1	1.1 \pm 0.0	0.5 \pm 0.0	1.2 \pm 0.0	0.0 \pm 0.0	0.4 \pm 0.0
20:2	0.7 \pm 0.1	1.8 \pm 0.1	0.2 \pm 0.1	0.2 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0
20:4n-6 (ARA)	1.6 \pm 0.1	3.0 \pm 0.0	4.2 \pm 0.1	0.3 \pm 0.0	2.7 \pm 0.0	5.8 \pm 0.1
20:4n-3	0.6 \pm 0.0	0.5 \pm 0.1	0.2 \pm 0.3	1.9 \pm 0.2	0.5 \pm 0.7	0.0 \pm 0.0
20:5n-3 (EPA)	14.5 \pm 0.1	10.9 \pm 0.0	7.6 \pm 0.5	15.2 \pm 1.8	2.5 \pm 0.0	0.1 \pm 0.0
22:5n-6					17.2 \pm 0.3	
22:5n-3	0.4 \pm 0.0	1.3 \pm 0.0	1.3 \pm 0.1	2.8 \pm 0.3	0.7 \pm 0.0	0.0 \pm 0.0
22:6n-3 (DHA)	40.3 \pm 0.1	7.7 \pm 0.1	25.5 \pm 1.4	16.1 \pm 2.2	45.0 \pm 0.7	0.3 \pm 0.0
DHA/EPA	2.8 \pm 0.0	0.7 \pm 0.0	3.3 \pm 0.0	1.1 \pm 0.0	17.9 \pm 0.0	2.0 \pm 0.1
ARA/EPA	0.1 \pm 0.0	0.3 \pm 0.0	0.6 \pm 0.0	0.0 \pm 0.0	1.1 \pm 0.0	354.0 \pm 24.1
Saturates ^a	32.3 \pm 0.1	34.8 \pm 0.6	41.3 \pm 1.0	32.5 \pm 6.9	46.3 \pm 0.1	26.6 \pm 0.1
MUFA ^b	9.6 \pm 0.1	27.5 \pm 0.6	14.8 \pm 0.7	24.3 \pm 1.8	1.1 \pm 0.0	13.3 \pm 0.0
PUFA ^c	58.1 \pm 0.1	37.6 \pm 0.1	43.9 \pm 1.7	43.2 \pm 5.0	52.6 \pm 0.1	60.1 \pm 0.1
Total n-3 ^d	56.1 \pm 0.2	21.3 \pm 0.0	36.3 \pm 1.6	38.6 \pm 4.8	48.8 \pm 0.1	0.5 \pm 0.0
Total n-6 ^e	1.9 \pm 0.1	16.1 \pm 0.0	6.7 \pm 0.0	2.5 \pm 0.1	3.7 \pm 0.0	59.6 \pm 0.1
n-3/6	29.2 \pm 1.3	1.3 \pm 0.0	5.4 \pm 0.2	15.6 \pm 1.2	13.1 \pm 0.1	0.0 \pm 0.0
Proximate analysis						
Moisture (%) ^f	88.0 \pm 0.1	80.9 \pm 3.4	76.7 \pm 0.2			
Protein (% DW) ^g	81.1 \pm 3.2	63.8 \pm 1.1	66.5 \pm 5.3			
Lipids (% DW) ^f	8.1 \pm 0.8	9.0 \pm 0.4	6.5 \pm 1.2			
Ash (% DW) ^f	4.0 \pm 0.0	20.6 \pm 0.2	18.5 \pm 2.5			

DW = dry weight; DHA = docosahexaenoic; ARA = arachidonic acid; EPA = eicosapentaenoic; PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid.

^a Includes: 12:0, 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.

^b Includes: 14:1, 15:1, 16:1, 17:1, 18:1n-9, 18:1n-7, 20:1n-9, and 22:1n-9.

^c Includes: 16:2n-4, 16:3n-4, 18:2n-6, 18:3n-6, 18:3n-4, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-6, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^d Includes: 18:3n-3, 18:4n-3, 20:3n-3, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^e Includes: 18:2n-6, 18:3n-6, 20:3n-6, and 20:4n.

^f Analyzed in duplicate.

^g Analyzed in triplicate.

A sample of eggs was also taken and placed on a microscope slide and images were taken for later measurement of egg and oil globule diameter. The remaining eggs were placed into three 60-L aquaria (egg density less than 1.5 eggs/mL), and one 100-mL subsample was taken from each aquarium (total of three) for hatching success assessment.

A total of 20 aquaria were available in a recirculating system that included a 560-W (3/4 hp) submersible pump placed in a 14-m³ vat, one particulate filter, and a 1- μ m polyester cartridge filter. The water in the vat was replaced every 2–3 wk to maintain good water quality. Temperature of the water in the incubation room was regulated through an air conditioning system

and the photoperiod was continuous. Only spawns having a fertilization rate of 10% or higher were monitored further for egg and larval survival.

Morphometrics and Egg Quality Evaluation

An image analysis system was used for accurate measurement of eggs and larvae. A few hundred eggs from each spawn were placed on a microscope slide as soon as possible after fertilization and 2–3 images were taken. The image analysis system included a Camedia digital camera (Olympus Optical Co., Ltd., Central Valley, PA, USA) adjusted to a SZ-PT Olympus microscope and a TV monitor. The Image-Pro® Express image analysis software (MediaCybernetics, Silver Spring, MD, USA) was used for the measurements and was calibrated by using stage micrometer images. The egg and oil globule diameters of approximately 40 eggs from each spawn were measured.

Biochemical Analysis

A modification of the method described by Folch et al. (1957) was used for the lipid proximate analysis. Two replicates (0.2–0.5 g each) for each sample of eggs were accurately weighed using an Ohaus Explorer (Ohaus Co., Pine Brook, NJ, USA) self-calibrating digital balance (+0.01 mg) and then homogenized and the lipids extracted. Lipid content was determined gravimetrically. Once extracted, the lipids were separated into polar and neutral fractions using a Whatman solid phase Silica extraction cartridge (500 µg, No 6804-1705; Whatman Inc., Clifton, NJ, USA). The lipid extract (lipid in 2 mL chloroform) was loaded onto the cartridge with a 10-mL syringe. The neutral fraction was extracted with 3 mL hexane:ethyl ether (1:1, v:v), and the polar fraction was extracted with 5 mL chloroform:methanol (1:1, v:v) followed by methanol.

Lipid fractions were saponified with 1 mL of 0.5 N KOH in methanol (70 C water bath for 30 min) and then esterified with 14% of boron trifluoride (BF₃) in methanol (70 C for 45 min). The tubes were allowed to cool down and 2 mL hexane and 2 mL of sodium chloride (NaCl)-saturated solution were added. The tubes were

vortexed for 1 min, hexane layer was pipetted off into a labeled vial, and stored in an auto freezer at –75 C. Fatty acid methyl esters (FAME) were analyzed with a Shimadzu GC-17A gas chromatograph equipped with a flame ionization detector and Omega Wax 530 capillary column (30 m × 0.53 mm; Supelco, Supelco Park, Bellefonte, PA, USA). Helium was used as the carrier gas and the oven temperature was programmed for a thermal gradient of 140–260 C at a rate of 3 C/min. Methylene chloride was used as the lipid solvent and the extract was transferred into 200-µm vial inserts (Supelco). The integrated peak areas of the FAME were identified by comparison with known standards (FAME and PUFA Sepelco Inc., Bellefonte, PA). The relative concentration of fatty acids was calculated and expressed as percentage of fatty acids identified, and a correction factor was applied to convert the percentages of peak areas into mass percentages of the components.

Proximate analyses of feeds and raw ingredients were conducted using standard methods. The Kjeldahl method was used to measure the protein as it was adapted by Ma and Zuazago (1942) for small samples and the protein content estimated based on an assumed value of 16% N. Ash was determined gravimetrically after combustion at 500 C in an ashing oven. The energy content of the feeds was measured by a Parr 1425 Semimicro Calorimeter (Parr Instrument Company, Moline, IL, USA). Samples were completely oxidized and the released heat was determined. Benzoic acid was used for the calibration of the calorimeter.

Statistical Analysis

Statistical analysis was performed using SPSS version 11.0 (SPSS, Chicago, IL, USA). The comparison of the treatments was made by one-way ANOVA and differences were considered significant when $P < 0.05$. Data were checked with Levene's homogeneity of variances test and for normality, the Mann-Whitney test and the Kruskal-Wallis test were conducted to analyze nonparametric data. Pearson correlation analysis was used to examine the existence of correlations. Data are expressed as means ± SD.

TABLE 4. Growth and reproductive performance (mean \pm SD) of red snapper broodstock in Experiment 1, maintained in six recirculating tanks and fed either a standard diet (ST1) or a supplemented diet with menhaden oil and a vitamin and mineral premix (ER1).

Parameter	ST1	ER1
Average initial weight (kg)		
Males	2.8 \pm 0.2	2.6 \pm 0.3
Females	3.0 \pm 1.3	2.8 \pm 0.2
Relative growth (%)		
Males	53.1 \pm 32.7	52.2 \pm 23.8
Females	52.3 \pm 33.2	50.0 \pm 31.9
Overall	57.0 \pm 24.8	55.4 \pm 26.5
Number of spawns	70	23
Total egg production (10 ⁶)	13.9	1.3
Eggs per spawn (10 ³)	198	57.2
Mean egg production (10 ⁶)	4.5 \pm 4.8	0.39 \pm 0.35
Number of fertilized spawns	32	3
Total fertilized egg production (10 ⁶)	8.7	0.19
Eggs per fertilized spawn (10 ³)	272	63
Number of larvae produced (10 ⁶)	2.9	0.1
Fertility (%)	83.9 \pm 20.9	99
Hatch rate (%)	81.6 \pm 21.4	88.2
Cumulative survival at 36 hours post-hatch (%)	49.1 \pm 17.6	70
Egg diameter (μ m)	803.2 \pm 11.9	798.1 \pm 21.6
Oil globule diameter (μ m)	139.1 \pm 6.10	138.5 \pm 2.90

Results

Experiment 1

The average relative growth of broodfish was not different between the two treatments (Table 4). However, high variation in growth within treatments was observed. Eggs were collected from five of six tanks, but both quantity and quality of eggs were variable among tanks. Fertilized eggs were collected from three tanks. Seventy spawns (fertilized and unfertilized totaling 13.9 million eggs) were collected from the standard treatment (ST1) and 23 spawns (total of 1.3 million eggs) from the enriched treatment (ER1) over a 3-mo spawning period. The mean number of eggs per spawn was much greater from brooders given the unenriched diet compared to those given the enriched diet (133,977 \pm 112,232 vs. 36,070 \pm 31,500, $P = 0.0002$). Thirty-two fertilized spawns (8.7 million eggs) were collected from the ST1

and three fertilized spawns (0.19 million eggs) from the ER1, but only one spawn from the latter treatment had over 10% fertility and was monitored for egg and larval viability. Overall, 2.9 million larvae were produced from the ST1 and 0.1 million from the ER1. Both treatments resulted in highly viable eggs. Fertility of fertilized spawns ($\geq 10\%$) was more than 80% and hatch more than 75% in both treatments. The egg and oil globule diameters were similar between the two treatments. One tank of the standard treatment produced more than 25 large fertilized spawns but no particular pattern existed in the sequence of spawning. At the end of the spawning season, there was a decrease of the spawn size but no apparent decrease of fertility.

Only one spawn from the ER1 had more than 10% fertility and it was the only spawn from this treatment analyzed for fatty acid profiles. The percentage of several fatty acids, fatty acid fractions, and fatty acid ratios of this one spawn was outside of the confidence intervals for the same parameter of SR1 eggs, especially in the polar fraction (Table 5). The menhaden oil supplementation resulted in higher DHA, eicosapentaenoic (EPA), palmitic, polyunsaturated fatty acid (PUFA), n-3, and n-3/n-6 ratio; and lower stearic, oleic, ARA, DHA/EPA, ARA/EPA, total saturated, and monounsaturated fatty acids in the polar fraction based on the confidence intervals of the ST1. There was less dietary influence on the fatty acid composition in the neutral fraction of eggs, but still ST1 had lower PUFA, n-3, and n-3/n-6 ratio. The comparison of recently released fertilized and unfertilized floating eggs from EXP1 ($n = 4$) showed only limited differences in fatty acid profiles.

Experiment 2

No significant differences ($P > 0.05$) were found in relative growth of broodfish given the standard (ST2) or enriched (ER2) treatments, 29.6 \pm 17.3% and 32.1 \pm 24.9%, respectively. Twenty-two spawns (462,300 eggs) were collected from the ST2 and four spawns (64,500 eggs) from the ER2 (Table 6). However, the fertility was very low ($< 10\%$) in both treatments and most spawns were totally unfertilized. No differences in major fatty acid concentration

were revealed when fertilized and floating unfertilized eggs from EXP1 were compared. Therefore, it was considered valid to analyze newly released, floating, unfertilized eggs in EXP2. The mean egg production per tank was $154,100 \pm 138,600$ and $21,500 \pm 37,200$ for the ST2 and ER2, respectively. The mean egg and oil globule diameters were not different between the two treatments. The egg fatty acid profiles of the two treatments were similar. The only differences that were detected were C18:1n-7 in both lipid fractions and C16:0 and C22:5n-3 in the polar fraction (Table 7).

Discussion

Reproductive Characteristics

Spontaneous spawning of red snapper, *L. campechanus*, was first reported in 1978 (Arnold et al. 1978), who obtained seven spawns of a few thousand eggs each. The present study is the first report of several, large (198×10^3 eggs per spawn), highly viable (>80% hatch) spontaneous spawns of red snapper when maintained in relatively small tanks (13.2 m^3) recirculating system under temperature and photoperiod manipulation. Brooders were successfully held for 18 mo and taken through two winter/summer protocols and spawns were obtained with each protocol. As demonstrated in EXP2, it was possible to condense the natural yearly cycle of temperature and photoperiod and obtain snapper spawning out of the natural spawning season. There the cycle was reduced to a total of 190 d with 93 d at a temperature and photoperiod conducive to spawning. Red snapper will reproduce multiple times within a season as evidenced from one tank of four females in EXP1 where 28 fertilized spawns with an average of 295×10^3 eggs were obtained over a 34-d period. This supports the conclusions based on gonadal observations of wild populations that red snapper may spawn multiple times over a several month period (Beaumariage and Bullock 1976; Collins et al. 1996).

In both experiments, spawning took place when temperature was above 26 C and at photoperiods equivalent to those found in the northern Gulf of Mexico from May to August. This is

similar to the conditions associated with the natural spawning period reported by Collins et al. (1996) for red snapper in Northwest Florida.

The reproductive cycle of many asynchronous spawning temperate and tropical reef fish is influenced by the lunar cycle. Gray snapper, *Lutjanus griseus*, and cubera snapper, *Lutjanus cyanopterus*, spawn primarily around the dark of the moon (Domeier et al. 1996; Heyman et al. 2005), while mutton snapper, *Lutjanus analis*, spawn during a full moon (Burton et al. 2005). Captive mangrove red snapper, *L. argente-maculatus*, were reported by Emata (2003) to spawn more frequently 3 d before or after the last quarter moon (43.4% of the monthly spawns) and near a new moon (37.1%). However, Leu et al. (2003) obtained 71 natural spawns of mangrove red snapper from May 21 to September 15, 1999, with eggs collected for 25 consecutive days during the first spawning period.

Even though natural lunar cycles were simulated in our studies, no specific spawning pattern was observed that corresponded to a given lunar phase. For example, fish in one tank of the first study gave 29 spawns over a 65-d period producing several sets of 2–6 consecutive spawns following by 1- to 9-d interval of no spawning.

Spawning occurred mainly during the late afternoon/evening hours. Several fertilized spawns with egg development at the blastula stage were collected in the late afternoon to early evening hours indicating that spawning occurred between 1700–1900 h, before lights start dimming off (approximately at 1900 h). Spawning behavior was observed several times that corresponded with egg releases. In brief, a female fish with swollen abdomen was followed by several males, which approached with their snout close to female's abdomen. Spawning behavior included fish schooling and slow swimming interrupted by sudden fast swimming and rushing toward the water surface. This pattern was repeated several times. Fertilized eggs were found in the egg collector 30 min to 2 h after the spawning behavior had been observed. The spawning behavior was similar to the spawning behavior of *L. stellatus* as described in detail by Hamamoto et al. (1992), but the pattern of

TABLE 5. Major PUFA fractions (% by weight of total fatty acids identified) and ratios in neutral and polar lipids of red snapper eggs from broodstock fed in Study 1, either an unenriched diet (ST) or a diet enriched with menhaden oil (ER).

Lipid fraction diet	Neutral		Polar	
	ST	ER	ST	ER
14:0	2.9 ± 0.5	2.7	1.0 ± 0.1	1.1
16:0	20.7 ± 1.2	20.9	19.9 ± 0.2	22.6 ^a
16:1	4.9 ± 0.5	5.2	2.1 ± 0.1	2.2
17:0	1.3 ± 1.2	0.6	0.7 ± 0.1	0.6
18:0	11.2 ± 0.9	9.7	17.4 ± 0.7	12.3 ^a
18:1n-9	22.3 ± 1.8	16.5	11.0 ± 0.8	7.5 ^a
18:1n-7	4.3 ± 0.2	4.3	3.0 ± 0.2	2.5
18:2n-6	1.5 ± 0.7	1.4	1.2 ± 0.3	1.4
18:3n-3	0.9 ± 0.5	0.7	0.5 ± 0.2	0.5
20:4n-6	4.7 ± 0.3	4.4	1.6 ± 0.5	0.9 ^a
20:5n-3	3.2 ± 0.8	2.3	7.2 ± 1.0	9.1 ^a
22:5n-3	1.5 ± 0.2	2.0	1.6 ± 0.1	1.7
22:6n-3	18.1 ± 1.5	25.3	26.4 ± 0.6	29.6 ^a
DHA/EPA	6.1	11.3	3.8	3.2 ^a
ARA/EPA	0.6	0.4	0.7	0.5 ^a
SATU ^b	37.7	36.7	39.9	37.9 ^a
MUFA ^c	32.5	27.9	16.6	13.0
PUFA ^d	29.9	36.4 ^a	43.5	49.2 ^a
n-3 ^e	24.9	31.4 ^a	36.2	41.8
n-6 ^f	3.4	2.8	6.3	6.1
n-3/n-6	7.6	11.1 ^a	5.7	6.8 ^a

DHA = docosahexaenoic; EPA = eicosapentaenoic; ARA = arachidonic acid; PUFA = polyunsaturated fatty acid; SATU = saturates; MUFA = monounsaturated fatty acid.

^a Outside the confidence interval for that parameter in eggs from fish given the unenriched diet.

^b Includes: 12:0, 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.

^c Includes: 14:1, 15:1, 16:1, 17:1, 18:1n-9, 18:1n-7, and 20:1n-9.

^d Includes: 16:2n-4, 16:3n-4, 18:2n-6, 18:3n-4, 18:3n-3, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-6, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^e Includes: 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^f Includes: 18:2n-6, 18:3n-6, 20:3n-6, and 20:4n-6.

the school swimming in a spiral formation as they reported was not observed. This swimming pattern may have been restricted by tank size (13.2 vs. 242 m³ in Hamamoto et al. 1992).

Tank size and brood density may be factors influencing natural spawning success; however, other snappers have also spawned in captivity under a range of conditions. Spontaneous spawning of 2.2–4.7 kg mangrove red snapper has occurred in 85-m³ cages at densities of 12–22 fish per cage (Emata 2003). Leu et al. (2003) re-

TABLE 6. Growth and reproductive performance (mean ± SD) of red snapper broodstock in Experiment 2, maintained in six recirculating tanks and fed either a standard diet (ST2) or a supplemented diet with DHA and ARA rich oils (ER2).

Parameter	ST2	ER2
Average initial weight (kg)		
Males	4.0 ± 0.7	3.9 ± 0.6
Females	4.6 ± 1.4	3.6 ± 1.2
Average final weight (kg)		
Males	5.8 ± 0.7	4.7 ± 0.6
Females	4.9 ± 0.5	5.1 ± 0.4
Relative growth (%)		
Males	43.9 ± 12.4	21.6 ± 14.3
Females	13.1 ± 29.0	52 ± 53
Overall	29.35 ± 17.3	32.1 ± 24.9
Number of spawns	22	4
Total egg production (10 ³)	462.3	64.5
Eggs per spawn (10 ³)	21.02	16.13
Mean egg production (10 ⁶)	154.1 ± 138.6	21.5 ± 37.2
Egg diameter (µm)	796.4 ± 7.9	810.9 ± 3.6
Oil globule diameter (µm)	139.1 ± 8.0	141.2 ± 3.9

DHA = docosahexaenoic; ARA = arachidonic acid.

ported spontaneous spawning of mangrove red snapper (6.9–8.4 kg female weight) in a 3000-m³ concrete tank when stocked at 30 per tank. Star snapper, *L. stellatus*, have spawned spontaneously in a 242-m³ aquarium tank (Hamamoto et al. 1992). Smaller snappers have spawned spontaneously in smaller tanks. Bluestripe snapper, *L. kasmira* (0.51–0.67 kg), spawned in a 4.6-m³ concrete aquarium tank (Suzuki and Hioki 1979) and yellowtail snapper, *O. chrysurus* (0.31 kg mean weight), spawned in a temperature- and photoperiod-controlled 16-m³ tank (Turano et al. 2000). In our study, spawning took place in 13.2-m³ tanks at fish densities of 8 fish per tank with a biomass of 2.9 kg/m³.

Snapper are social animals and the proper combination of brood density, space, and other factors may be needed for successful spawning. In each trial, unfertilized spawns were common. In the first trial, fertility ranged from 0 to 99% with the majority of spawns being either highly fertile (>80% fertility) or totally unfertilized. In every trial, two or three tanks performed much better than the rest. Within the same diet

TABLE 7. Major PUFA fractions (% by weight of total fatty acids identified) and ratios in neutral and polar lipids of red snapper eggs from broodstock fed in Experiment 2, either an unenriched diet (ST) or a diet enriched with menhaden oil (ER).

Lipid fraction diet	Neutral		Polar	
	ST	ER	ST	ER
14:0	3.7 ± 0.3	3.9 ± 0.7	1.0 ± 0.1	1.4 ± 0.5
16:0	21.3 ± 1.4	23.0 ± 1.3	20.6 ± 0.2	22.5 ± 0.5*
16:1	5.4 ± 0.7	5.3 ± 0.6	2.0 ± 0.3	2.2 ± 0.3
17:0	0.9 ± 0.2	0.7 ± 0.1	0.5 ± 0.1	0.4 ± 0.1
18:0	10.9 ± 2.1	11.7 ± 1.1	14.0 ± 0.9	13.2 ± 0.6
18:1n-9	29.0 ± 2.2	27.1 ± 2.6	10.3 ± 1.7	10.6 ± 0.8
18:1n-7	4.0 ± 0.3	3.2 ± 0.2**	2.1 ± 0.1	1.7 ± 0.1*
18:2n-6	3.3 ± 0.5	3.2 ± 0.5	2.3 ± 0.2	1.7 ± 0.2
18:3n-3	1.8 ± 0.7	1.3 ± 0.7	0.6 ± 0.2	0.4 ± 0.2
20:4n-6	0.7 ± 0.1	1.0 ± 0.3	4.7 ± 0.5	5.2 ± 0.2
20:5n-3	1.7 ± 0.3	1.8 ± 0.6	6.3 ± 0.2	6.0 ± 0.5
22:5n-3	1.1 ± 0.2	0.6 ± 0.4	2.1 ± 0.2	1.1 ± 0.1*
22:6n-3	7.7 ± 1.3	9.1 ± 0.9	27.7 ± 1.7	28.4 ± 1.7
DHA/EPA	4.6	5.6	4.4	4.7
ARA/EPA	0.4	0.5	0.8	0.9
SATU ^a	37.4	39.8	38.0	38.5
MUFA ^b	41.1	37.8	15.2	15.8
PUFA ^c	21.3	22.4	46.8	45.7
n-3 ^d	14.3	15.2	37.6	36.7
n-6 ^e	4.5	4.5	7.1	7.2
n-3/n-6	3.2	3.4	5.3	5.1

DHA = docosahexaenoic; EPA = eicosapentaenoic; ARA = arachidonic acid; PUFA = polyunsaturated fatty acid; MUFA = monounsaturated fatty acid.

^a Includes: 12:0, 14:0, 16:0, 17:0, 18:0, 20:0, 22:0, and 24:0.

^b Includes: 14:1, 15:1, 16:1, 17:1, 18:1n-9, 18:1n-7, and 20:1n-9.

^c Includes: 16:2n-4, 16:3n-4, 18:2n-6, 18:3n-4, 18:3n-3, 18:4n-3, 20:3n-6, 20:3n-3, 20:4n-6, 20:4n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^d Includes: 18:3n-3, 18:4n-3, 20:3n-3, 20:5n-3, 22:5n-3, and 22:6n-3.

^e Includes: 18:2n-6, 18:3n-6, 20:3n-6, and 20:4n-6.

Significant difference within neutral or polar fraction at * $P \leq 0.001$, at ** $P \leq 0.01$.

treatment, one tank gave 28 spawns of which 23 were fertilized, while another replicate of that treatment gave 23 spawns, but none were fertilized. However, environmental conditions included water quality parameters (temperature, dissolved oxygen, pH, salinity, and total ammonia–nitrogen concentration) and photoperiod were similar among tanks, and overall, no difference in rearing conditions was apparent between treatments. In the second trial, one replicate of the same diet protocol gave 25 spawns, while another replicate gave no spawns. The high frequency of unfertilized spawns and the variability between tanks make natural spawning an unpredictable method for providing viable eggs in a hatchery.

Influence of Diet

Growth and Reproductive Characteristics. Diet enrichment had little effect on snapper growth. In the first experiment, the relative growth of brooders was 57.0 and 55.4% for fish given the unenriched and enriched diets, respectively. In the second study, the relative growth was 29.35 and 32.1% with the unenriched and enriched diet, respectively.

Enrichment of a seafood-based diet with n-3 HUFA sources general negatively impacted red snapper spawning success. In EXP1, fish given the unenriched diet gave a total of 70 spawns with 45% of the spawns being fertile, while fish given the enriched diet gave a total of 23 spawns

of which 13% were fertile. In Study 2, fish given the non-HUFA enriched diet gave 82% more spawns than the fish given the enriched diet. A similar pattern was seen in the average number of eggs per spawn. Fish given the unenriched diet gave 432 and 130% more eggs per spawn in EXPs 1 and 2, respectively, than fish given the enriched diets. Brood diet had no influence on egg or oil globule diameter in either trial.

Enrichment of a seafood-based diet may not be necessary if the seafood used is similar to the natural diet and is of good quality. Qunitio et al. (1996) found no benefits in enrichment of a brood grouper, *E. coioides*, diet composed of bycatch, using either cod-liver oil or a commercial HUFA product (SELCO). This also appears true for red snapper when a diet of squid, shrimp, and fish is given. Enrichment of a seafood-based diet with menhaden oil or DHA Gold and ARASCO had no apparent effect on enhancing red snapper reproduction.

Effects of Diet Enrichment of Egg Biochemistry

Enrichment of a seafood-based diet with HUFA sources did alter some aspects of the fatty acid profile of red snapper eggs. Based on the feed consumption of the fresh seafood and gelatinized supplement, in the first study, the dietary intake of lipids was approximately 5.9% of the feed consumed for the unenriched diet and 6.9% with the enriched diet (a 17.2% increase). In the second trial, the percentages of lipids were approximately 6.1% for the unenriched diet and 7.1% with the enriched diet (a 15.7% increase). If only the seafood ingredients are considered, that is, similar to the wild diet, the total lipid concentration is 7.9%. These levels of lipids are lower than those of formulated diets used with other marine broodfish: halibut, *Hippoglossus hippoglossus*, 16.4% (Mazorra et al. 2003); Japanese flounder, *P. olivaceus*, 14–15% (Furuita et al. 2002); and sea bass, *D. labrax*, 20.3% (Bruce et al. 1999). A practical diet with 8.6% lipids (DW) resulted in good reproductive performance in mangrove red snapper (Emata and Borlongan 2003). Li et al. (2005) found that sweetlips, *P. cinctus* (Lutjanidae), reproduced successfully when given trash fish with a total lipid value of 14.2% or when given formulated

diets with 2.4 and 3.7% n-3 HUFA where the total lipids were approximately 19.8%. They found that lower or higher n-3 HUFA levels negatively impacted egg and larval quality. In our study, fish given an unenriched diet where the total lipid intake was approximately 5.9% (DW) of the feed consumed spawned successfully giving many large, well-fertilized spawns.

Lipid enrichment of brood diets often alters the fatty acid profile of the eggs. Fernandez-Palacios et al. (1997) tested four increasing rates of n-3 HUFA in formulated diets for brood sea bream and found that n-3 HUFA and EPA in the eggs increased in response to increased rates in the diet but DHA and ARA did not. Additions of n-3 HUFA reduced the DHA:EPA and ARA:EPA ratios. Furuita et al. (2002) found that with Japanese flounder, the addition of n-3 HUFAs to levels of 15, 30, and 45% of the total fatty acids in the diet increased the relative abundance in the eggs of n-3 HUFAs, DHA, and EPA in the neutral fraction but made little changes in the relative abundances in the polar fraction.

In the present study, the type of enrichment material did affect the relative abundance of several fatty acids in the eggs. When fertilized spawns from the unenriched diet are compared to the one fertilized spawn obtained from the enriched diet, the relative abundance of DHA increased 10%, EPA 26.4%, and the n-3/n-6 ratio increased 40.5% in the polar fraction of the enriched diet eggs while ARA decreased. These changes resulted in reduced DHA/EPA and ARA/EPA.

In the neutral fraction, the relative abundance of DHA increased 39.8% above that found in the unenriched diet, EPA was reduced 28.1%, and ARA increased 43.8%. These changes gave a resultant increase in DHA:EPA ratio from 6.1:1 to 11.3:1 and ARA:EPA from 0.5:1 to 0.6:1. In both the polar and the neutral fractions, n-3 increased with a slight decrease in n-6 gave an increased n-3:n-6 ratio.

In the second experiment, using DHA Gold and ARASCO oils, the level of total lipids consumed in the diet was increased 15.7% above that of the unenriched diet, but there was little change in the polar fraction in the relative abundance of DHA, ERA/ARA, or their ratios in the

eggs. Greater shifts were observed in the neutral fraction where eggs from fish given the enriched diet had a relative increase of 18.2% in DHA and 42.9% for ARA when compared to eggs from fish given an unenriched diet. There was little change in EPA. These shifts resulted in slight shifts on DHA:EPA ratios in the polar or neutral fraction, 4.4:1 to 4.7:1 and 4.6:1 to 5.6:1, respectively. ARA:EPA ratios in the polar and nonpolar fractions of eggs differed little among fish given the two diets.

Imbalances among HUFAs associated with HUFA enrichment can have negative impacts (Bell et al. 1997; Farndale et al. 1999; Furuita et al. 2003). Increase of dietary n-3 HUFA levels reduced ARA abundance in egg polar lipids of Japanese flounder and reduced egg quality (Furuita et al. 2002). Lavens et al. (1999) also found that feeding turbot, *Psetta maxima*, broodstock with HUFA-enriched raw fish decreased egg ARA content. They found a strong positive correlation between the ARA concentration in eggs and the hatching rate. Fernandez-Palacios et al. (1995) found that the percentage of unfertilized eggs decreased as the percentage of EPA of the total lipids in sea bream diets increased. Navas et al. (2001) concluded that higher hatch rates of sea bream eggs were associated with higher DHA:EPA and ARA:EPA ratios. Bell et al. (2003) suggests that pigmentation of turbot and halibut can be improved when DHA:EPA and ARA:ERA ratios are 2:1 and 0.2:1, respectively.

Sargent et al. (1999) discussed how both concentration and ratios of DHA, EPA, and ARA are important to larval fish nutrition and suggests that appropriate ratios are in the range of 2:1 DHA:EPA and 0.2:1 ARA:EPA. In the present study, red snapper eggs from brooders given either enriched or unenriched diets had DHA:EPA ratios of >3:1 and ARA:EPA ratios of >0.4:1. These ratios are below that found in eggs from wild-caught, hormone induced to spawn, red snapper of 5.3:1 DHA:EPA and 0.9:1 ARA:EPA (Papanikos 2004), or eggs from naturally spawned captive mangrove red snapper fed raw fish with ratios of 5.6:1 DHA:EPA and 1.1:1 ARA:EPA (Ogata et al. 2004).

Menhaden oil, used for enrichment in the first study, is rich in EPA and did reduce the ARA:EPA ratio slightly in favor of EPA but still remained $\geq 0.4:1$. Prostaglandin, a derivative of ARA, is involved in fish ovulation and sexual behavior (Mustafa and Srivastava 1989; Tamaru et al. 1992; Murdoch et al. 1993; Sorensen and Goetz 1993). It reaches high levels at the time of ovulation and its metabolites function as pheromones and stimulate male sexual behavior when they are released into the water (Sorensen and Goetz 1993). In the present study, more spawns and larger spawns were obtained from brooders given the unenriched diet, that is, the higher ARA:EPA ratio. However, there was considerable variation among replicates in fertilization success for brooders given the unenriched diet with 23 of 28 spawns being fertilized in one replicate and 0 of 26 in another. This variation does not help support a hypothesis that a reduction in ARA:EPA ratio and any subsequent consequences on prostaglandin contributed to the reduced spawning observed from fish given the enriched diet.

In the second study, enrichment with oils rich in DHA and ARA reduced the relative abundance of EPA in the polar fraction, but no positive effects on spawning were seen. Brooders given the unenriched diet produced a total of 22 spawns with an average number of eggs per spawn of 21,020 compared to brooders given the enriched diet where four spawns averaging 16,130 eggs per spawn were obtained. High variability in spawning again occurred among replicates for brooders given the unenriched diet with 18 spawns in one replicate and none in another. Successful snapper spawning is more than a function of diet as evidenced in the variability observed.

Conclusions

This study has demonstrated that red snapper maintained in tanks under temperature and photoperiod manipulation and fed diets based on squid, shrimp, and fish can spawn spontaneously and produce highly viable eggs and larvae. However, spontaneous spawning was variable and unpredictable. Unfertilized spawns were common. Dietary fatty acids influenced egg

fatty acid composition but did not enhance broodfish reproductive performance in terms of egg production and egg quality, under the conditions of this study. It is likely that factors other than nutrition did not allow red snapper broodstock to reach their full potential for consistent egg production. Factors such as male reproductive performance, fish density, and tank size should be investigated along with broodstock nutrition as factors contributing to the variation in spawning success of red snapper.

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