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The effects of dietary protein and lipid on growth and body composition of juvenile and sub-adult red snapper, *Lutjanus campechanus* (Poey, 1860)

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Abstract

To allow for the initial identification of practical diet formulations for red snapper culture, the present study was conducted to evaluate the effects of feeding varying levels of dietary protein and lipid on growth and body composition of juvenile and sub-adult red snapper. Twelve diets were formulated to contain varying levels of dietary protein and lipid. In trial 1, juvenile red snapper (initial mean weight 5.9 g) were offered diets with graded levels of dietary protein (32%, 36%, 40%, 44%) and practical energy to protein ratios. In trial 2, juvenile red snapper (initial mean weight 8.64 g) were offered isonitrogenous diets (44% protein) containing graded levels of dietary lipid (8%, 10%, 12%, 14%). Sub-adult fish (initial mean weight 151.5 g) were used in trial 3 and maintained on diets similar to those of trial 1 (32–44% protein). Sub-adult fish (initial mean weight 178.3 g) in trial 4 were offered isonitrogenous diets containing 32% dietary protein and graded levels of dietary lipid (6%, 8%, 10%, 12%). There were no significant differences in growth, feed efficiency ratio (FER) or survival in juvenile fish. Juvenile fish offered 32% dietary protein exhibited a significantly greater ($P = 0.0497$) protein conversion efficiency (PCE) than fish offered a diet containing 44% dietary protein. Juvenile fish in trial 2 also had significantly higher ($P = 0.005$) intraperitoneal fat ratios (IPFRs) at 14% dietary lipid than fish offered diets containing 8–10% dietary lipid, and displayed trends towards greater protein as a percent of whole-body composition at 8–10% dietary lipid. Sub-adult snapper in trials 3 and 4 showed no significant differences in growth, FER or survival. However, in trial 4 there was a general trend towards increased % weight gain ($P = 0.0615$), FER ($P = 0.0601$) and final mean weight ($P = 0.0596$) with increasing levels of dietary lipid. Fish in trial 4 offered 6% dietary lipid also had significantly lower ($P = 0.0439$) IPFR and PCE ($P = 0.0188$) than fish offered 12% dietary lipid. Based on data obtained from these trials, inclusion of dietary protein at levels of 32–36% appears sufficient to support growth. For this level of protein, dietary lipid should be $\sim$ 10% in order to meet the energetic demands of the fish and to spare dietary protein for growth.

Keywords: red snapper, *Lutjanidae*, protein, lipid, protein conversion efficiency

Introduction

The red snapper, *Lutjanus campechanus* (Poey, 1860), is one of the most important species, in both the commercial and recreational snapper fisheries, in the northern Gulf of Mexico. Their wide acceptance as an excellent food fish and high market value have led to over-harvesting of wild stocks in many areas (Moran 1988). An increased awareness of the status of the red snapper fishery, coupled with high market demand, has led to an interest in the development of culture technologies for commercial production and stock enhancement. However, in order to develop efficient culture strategies for this species, a better understanding of nutritional requirements is required.

With respect to practical diet development, protein is one of the key components when considering the nutritive requirements of any species. Additionally, dietary protein constitutes the principal nutritive cost associated with the formulation of most feeds, and is the primary source of nitrogen waste in culture systems (Catacutan & Coloso 1995; Shiau & Lan 1996; Perez, Gonzalez & Jover 1997; Thoman, Davis & Arnold 1999). Hence, the identification of suitable protein levels and energy to protein ratios (E:P) of the diet are critical. Sparing dietary protein through the optimization of E:P has also been shown to increase growth at a reduced cost (Serrano, Nematipour & Gatlin III 1992; Shiau & Peng 1993; Thoman *et al.* 1999). Consequently, the inclusion of non-protein energy sources such as lipids should be considered.

The diet of juvenile red snapper in the wild has been fairly well documented (Camber 1955; Bradley & Bryan 1976; Futch & Bruger 1976; Moran 1988; Lee 1998) and consists of prey items with high levels of protein and moderate levels of lipids. This corresponds well with typical practical diets for carnivorous fish, which contain 50–55% protein (Chou, Su & Chen 2000) and moderate-to-high levels of lipids ($\geq$ 10%). Nutritional data regarding levels of crude protein and lipid necessary to promote growth in juvenile *Lutjanidae* are sparse. Species for which information does exist include the mangrove red snapper, *Lutjanus argentimaculatus* (Catacutan, Pagador & Teshima 2001); mutton snapper, *Lutjanus analis* (Watanabe, Ellis & Chaves 2001); the yellowtail snapper, *Ocyurus chrysurus* (Turano, Davis & Arnold 2000) and the red snapper (Wakeman, Arnold, Wohlschlag & Rabalais 1979; Marnell 1999). Catacutan and colleagues (2001) reported that mangrove red snapper offered diets containing 42.5% crude protein showed significantly higher growth rates than those offered diets with 35% crude protein. However, no significant differences in growth were observed between fish offered diets containing 42.5% and 50% crude protein (Catacutan *et al.* 2001). Although dietary protein requirement has not been evaluated for juvenile mutton snapper, maximum growth occurred in fish maintained on diets containing 45% crude protein and between 6% and 9% crude lipid (Watanabe *et al.* 2001).

Although little data exist with regard to the dietary requirements of juvenile snapper, studies have been conducted with other carnivorous marine species such as red drum, *Sciaenops ocellatus* (Williams & Robinson 1988; Thoman *et al.* 1999); cobia, *Rachycentron canadum* (Chou *et al.* 2000); sea basses, family *Serranidae* (Borlongan & Parazo 1991; Catacutan & Coloso 1995; Shiau & Lan 1996; Perez *et al.* 1997); yellowtail, *Seriola quinqueradiata* (Takeda, Shimeno, Hosokawa, Kajiyama & Kaisyo 1975) and hybrid striped bass, *Morone* sp. (Gaylord & Gatlin III 2000). In general, these studies point towards the use of high-protein diets with moderate levels of lipids for most marine species.

Red snapper have been reared in the laboratory on commercial feeds. However, there is limited data with regard to basic nutrient requirements and there is little information that can be utilized for feed selection with this species. Given the high interest in the development of culture protocols for red snapper, this study was designed to initiate the development of practical diets for red snapper and more specifically, to determine the effects of varied levels of dietary protein and lipid on the growth performance of juvenile and sub-adult red snapper.

Materials and methods

Four growth trials were carried out in three independent semi-closed recirculating systems at the Claude Peteet Mariculture Facility in Gulf Shores, AL, USA. Juvenile trials were conducted from August through November. Sub-adult fish were examined March through June. Twelve practical diets were formulated to evaluate the effects of dietary protein and lipid levels on juvenile and sub-adult red snapper. Diet formulations were based on those utilized by Thoman and colleagues (1999) and were designed to meet the known nutrient requirements of the red drum.

Diets were prepared by mixing the dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, OH, USA) for 30 min. Hot water was then blended into the mixture to attain a consistency appropriate for pelleting. The moist mash from each diet was passed through a 3-mm die in a meat grinder, and the pellets were dried in a forced air-drying oven ($<50\,^{\circ}C$) to a moisture content of less than 10%. The protein content of each diet was confirmed using micro-Kjeldahl analysis (Ma & Zuazago 1942). Lipid contents of the basal diets were confirmed using the methods of Folch, Lee and Sloane Stanley (1957). Diets were stored at $-20\,^{\circ}C$, and before use each diet was ground and sieved to an appropriate size.

The culture systems utilized for these studies were semi-closed recirculating systems, each consisting of culture tanks, pumps for water circulation, aeration (provided using a central line, regenerative blower and air diffusers), mechanical and biological filtration, and a submersible heater to maintain minimum water temperatures. Routine system maintenance, such as siphoning of solids and partial water exchanges, was conducted as needed. Systems were treated with chloroquine phosphate (Marex, Aquatronics, Oxnard, CA, USA) at 21.1 mg L^{-1} as a preventative measure against *Amyloodinium ocellateum* infestations. Fish were counted, weighed, dipped in freshwater for 30 s, and culture tanks were scrubbed every 2 weeks. Fish were anaesthetized with dimethylketone α methyl quinoline (Hypno, Jungle Lab, Cibolo, TX, USA) to help reduce stress during the weighing process.

At the termination of each study, group weights, individual weights and total lengths were taken for the fish in each tank. Feed efficiency ratio (FER = wet weight gain × 100/dry weight feed fed) and protein conversion efficiency (PCE = dry protein gain × 100/dry protein fed) were also calculated at the end of the feeding trials. Four fish from each replicate were randomly selected for the determination of hepatosomatic index (HSI = liver weight × 100/fish weight) and intraperitoneal fat ratio (IPFR = intraperitoneal fat weight × 100/fish weight). The fish were then pooled by tank, homogenized and frozen to −60 °C for later analysis. Dry matter was determined by drying to a constant weight at 90 °C. Protein content was determined using the micro-Kjeldahl method. The total lipid content was determined using the method described by Folch and colleagues (1957). All analyses were conducted with triplicate sub-samples from each treatment replicate.

Trial 1. Response of juvenile red snapper to varying levels of dietary protein

Juvenile red snapper of the same cohort (65 days post hatch) were graded to a uniform size (mean weight 5.9 g) and stocked at a density of 15 fish per tank (190 L). Four practical diets (Table 1) were formulated to contain graded levels of protein (32–44%) with practical energy to protein ratios. Each of the four diets was randomly assigned to four replicate tanks per treatment. A daily ration was divided into two equal feedings and offered to the fish in the morning and evening. Daily ration was based on a percentage of the mean weight per treatment replicate. The initial ration was offered at 8% body weight, and was reduced as the fish grew. System temperature (27.02 ± 0.76 °C), dissolved oxygen (5.70 ± 0.56 mg L^{-1}), salinity (32.09 ± 1.58 g L^{-1}) and pH (7.78 ± 0.11) were monitored once daily using a YSI model 556 MPS (Yellow Springs, OH, USA). Nitrite-nitrogen (0.02 ± 0.04 mg L^{-1}) was monitored weekly using a model PLN code test kit from LaMotte (Chestertown, MD, USA). Total ammonia-nitrogen (0.06 ± 0.08 mg L^{-1}) was monitored twice weekly using the method described by Boyd (1979).

Table 1 Composition (g 100 g^{-1} dry weight) of practical diets containing varying levels of protein and used to rear juvenile and sub-adult snapper in growth trials 1 and 3 respectively

Ingredient	32% protein	36% protein	40% protein	44% protein
Menhaden fish meal*	21.8	24.5	27.3	30
Poultry meal†	7.3	8.2	9.1	10
Soybean meal‡	20.3	23.5	26.5	29.8
Menhaden fish oil§	4.2	4.45	4.6	4.9
Wheat starch¶	23.03	15.98	9.13	1.93
Whole wheat¶	20	20	20	20
Vitamin premix‖	1.8	1.8	1.8	1.8
Trace mineral premix**	0.5	0.5	0.5	0.5
Stay C††	0.07	0.07	0.07	0.07
Choline chloride¶	0.2	0.2	0.2	0.2
CaP dibasic¶	0.8	0.8	0.8	0.8
Formulated to contain				
Protein	32	36	40	44
Lipid	8	8.7	9.3	10

*Special Select™, Omega Protein, Hammond, LA, USA.
†Griffin Industries, Cold Springs, KY, USA.
‡De-hulled solvent-extracted soybean meal, Southern States Cooperative, Richmond, VA, USA.
§Omega Protein, Reedville, VA, USA.
¶United States Biochemical, Cleveland, OH, USA.
‖g 100 g^{-1} premix: cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250; ferrous sulphate, 4.0; magnesium sulphate heptahydrate, 28.398; monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193; α-cellulose, 53.428.
**g kg^{-1} premix: thiamin HCl, 0.5; riboflavin, 3.0; pyridoxine HCl, 1.0; DL Ca-pantothenate, 5.0; niacin, 5.0; biotin, 0.05; folic acid, 0.18; vitamin A acetate (20 000 IU g^{-1}), 5.0; vitamin D_3 (400 000 IU g^{-1}), 0002; DL-α-tocopheryl acetate (250 IU g^{-1}), 8.0; α-cellulose, 865.266
††Stay C® (L-ascorbyl-2-polyphosphate 35% active C), Roche Vitamins, Parsippany, NJ, USA.

Trial 2. Response of juvenile red snapper to varying levels of dietary lipid

Juvenile red snapper of the same cohort (74 days post hatch) were graded to a uniform size (mean weight 8.6 g) and stocked at a density of 20 per tank (1000 L). Four practical diets (Table 2) were formulated to be isonitrogenous (44% protein) and contain 8–14% crude dietary lipid. Each of the diets were randomly assigned to three replicate tanks per

Table 2 Composition (g 100 g^{-1} dry weight) of practical diets containing 44% protein and varying levels of lipids use to rear juvenile red snapper in growth trial 2

Ingredient	8% lipid	10% lipid	12% lipid	14% lipid
Menhaden fish meal*	30	30	30	30
Poultry meal†	10	10	10	10
Soybean meal‡	30.6	30.6	30.6	30.6
Menhaden fish oil§	2.93	4.93	6.93	8.93
Wheat starch¶	1.1	1.1	1.1	1.1
Whole wheat¶	16	16	16	16
Trace mineral premix‖	0.5	0.5	0.5	0.5
Vitamin premix**	1.8	1.8	1.8	1.8
Choline chloride¶	0.2	0.2	0.2	0.2
Stay C††	0.07	0.07	0.07	0.07
CaP dibasic¶	0.8	0.8	0.8	0.8
Cellufil¶	0	2	4	6

*Special Select™, Omega Protein, Hammond, LA, USA.
†Griffin Industries, Cold Springs, KY, USA.
‡De-hulled solvent-extracted soybean meal, Southern States Co-operative, Richmond, VA, USA.
§Omega Protein, Reedville, VA, USA.
¶United States Biochemical, Cleveland, OH, USA.
‖g 100 g^{-1} premix: cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250; ferrous sulphate, 4.0; magnesium sulphate heptahydrate, 28.398; monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193; α-cellulose, 53.428.
**g kg^{-1} premix: thiamin HCl, 0.5; riboflavin, 3.0; pyridoxine HCl, 1.0; DL Ca-pantothenate, 5.0; niacin, 5.0; biotin, 0.05; folic acid, 0.18; vitamin A acetate (20 000 IU g^{-1}), 5.0; vitamin D3 (400 000 IU g^{-1}), 0002; DL-α-tocopheryl acetate (250 IU g^{-1}), 8.0; α-cellulose, 865.266.
††Stay C® (L-ascorbyl-2-polyphosphate 35% active C), Roche Vitamins, Parsippany, NJ, USA.

treatment. A daily ration, based on a percentage of the mean weight per treatment replicate, was divided into two equal feedings and offered to the fish in the morning and evening. Water-quality parameters, temperature (26.29 ± 1.02 °C), dissolved oxygen (6.27 ± 0.32 mg L^{-1}), salinity (32.09 ± 0.92 g L^{-1}), pH (7.95 ± 0.19), nitrite-nitrogen (below delectable levels) and total ammonia-nitrogen (0.10 ± 0.09 mg L^{-1}), were within the acceptable ranges for this species.

Trial 3. Response of sub-adult red snapper to varying levels of dietary protein

Sub-adult red snapper of the same cohort were acquired from animals used in trials 1 and 2. Sub-adult red snapper were graded to a uniform size (mean weight 151.53 g) and stocked at 12 fish per tank (1000 L). Four diets (Table 1) were formulated to contain graded levels of protein (32–44%) with practical E:P's. Each diet was randomly assigned to three replicate tanks per treatment. A daily ration was divided into two equal feedings and offered to the fish in the morning and evening. The initial ration was offered at 3% body weight. System temperature (25.76 °C ± 1.36), dissolved oxygen (6.25 ± 0.33 mg L^{-1}), salinity (32.00 ± 1.48 g L^{-1}) and pH (7.81 ± 0.14) were monitored once daily. Total ammonia-nitrogen (0.09 ± 0.09 mg L^{-1}) was monitored twice weekly.

Trial 4. Response of sub-adult red snapper to varying levels of dietary lipid

Sub-adult red snapper of the same cohort were acquired from animals used in trials 1 and 2. Sub-adult red snapper were graded to a uniform size (mean weight 178.27 g) and were stocked at nine fish per tank (1000 L). Four practical diets (Table 3) were formulated to be isonitrogenous (32% protein) and

Table 3 Composition (g 100 g^{-1} dry weight) of practical diets designed to contain 32% protein and varying levels of lipids and used to rear sub-adult snapper in trial 4

Ingredient	6% lipid	8% lipid	10% lipid	12% lipid
Menhaden fish meal*	21.8	21.8	21.8	21.8
Poultry meal†	7.3	7.3	7.3	7.3
Soybean meal‡	20.3	20.3	20.3	20.3
Menhaden fish oil§	2.2	4.2	6.2	8.2
Wheat starch¶	24.03	22.03	20.03	18.03
Whole wheat¶	20	20	20	20
Trace mineral premix‖	0.5	0.5	0.5	0.5
Vitamin premix**	2.7	2.7	2.7	2.7
Choline chloride¶	0.3	0.3	0.3	0.3
Stay C††	0.07	0.07	0.07	0.07
CaP dibasic¶	0.8	0.8	0.8	0.8

*Special Select™, Omega Protein, Hammond, LA, USA.
†Griffin Industries, Cold Springs, KY, USA.
‡De-hulled solvent extracted soybean meal, Southern States Co-operative, Richmond, VA, USA.
§Omega Protein, Reedville, VA, USA.
¶United States Biochemical, Cleveland, OH, USA.
‖g 100 g^{-1} premix: cobalt chloride, 0.004; cupric sulphate pentahydrate, 0.250; ferrous sulphate, 4.0; magnesium sulphate heptahydrate, 28.398; monohydrate, 0.650; potassium iodide, 0.067; sodium selenite, 0.010; zinc sulphate heptahydrate, 13.193; α-cellulose, 53.428.
**g kg^{-1} premix: thiamin HCl, 0.5; riboflavin, 3.0; pyridoxine HCl, 1.0; DL Ca-pantothenate, 5.0; niacin, 5.0; biotin, 0.05; folic acid, 0.18; vitamin A acetate (20 000 IU g^{-1}), 5.0; vitamin D3 (400 000 IU g^{-1}), 0002; DL-α-tocopheryl acetate (250 IU g^{-1}), 8.0; α-cellulose, 865.266.
††Stay C® (L-ascorbyl-2-polyphosphate 35% active C), Roche Vitamins, Parsippany, NJ, USA.

contain 6–12% crude dietary lipid. Each diet was randomly assigned to three replicate tanks per treatment. A daily ration was divided into two equal feedings and offered to the fish in the morning and evening. Initial ration was offered at 2.5% body weight. System temperature (26.99 °C ± 1.33), dissolved oxygen (6.31 ± 0.46 mg L^{-1}), salinity (31.34 ± 1.73 g L^{-1}) and pH (7.92 ± 0.14) were monitored once daily. Total ammonia-nitrogen (0.16 ± 0.17 mg L^{-1}) was monitored twice weekly.

Statistical analysis

All data were subjected to a one-way analysis of variance to determine significant ($P \leq 0.05$) differences among the treatment means. Student–Neuman Keuls' multiple range test was used to distinguish significant differences among treatment means. In the case of the third and fourth growth trials, regression analyses were conducted as a descriptive statistic to determine if there was a significant increase in final mean weights relative to dietary protein or lipid levels respectively. All statistical analyses were conducted using SAS system for windows (version 8.0 SAS Institute, Cary, NC, USA).

Results

Response of juvenile red snapper to varying levels of dietary protein

At the conclusion of the 8-week trial there were no significant differences (Table 4) in weight gain ($P = 0.5547$), survival ($P = 0.3814$), FER ($P = 0.1429$), IPFR ($P = 0.4192$), whole-body % protein ($P = 0.1872$) or whole-body % lipid ($P = 0.2618$). However, based on PCE values, fish fed diets containing 32–36% protein retained a higher percentage of the dietary protein ($P = 0.0497$) than those offered the 44% protein diet. Mean FER and growth rate decreased throughout the course of the trial; however, a noticeable decrease in FER was observed at week 6.

Response of juvenile red snapper to varying levels of dietary lipid

There were no significant differences (Table 5) in weight gain ($P = 0.9351$), FER ($P = 0.8943$) or percentage body lipid ($P = 0.7162$) among treatments. However, a significantly greater amount of body fat deposition (IPFR) was observed in fish offered a ration containing 14% dietary lipid ($P = 0.005$). Fish of-

Table 4 Responses of juvenile red snapper (mean initial weight 5.9 g) offered practical diets containing graded levels of protein over an 8-week growth trial*

Dietary protein	% weight gain†	FER‡	IPFR§	Survival¶	PCE‖	Whole body % protein**	Whole body % lipid††
32%	392.9[a]	59.15[a]	0.44[a]	98.33[a]	27.40[a]	59.10[a]	19.4[a]
36%	410.6[a]	52.43[a]	0.45[a]	98.33[a]	25.30[a]	56.49[a]	21.8[a]
40%	435.1[a]	48.50[a]	0.77[a]	100.00[a]	20.44[ab]	61.32[a]	22.9[a]
44%	436.5[a]	47.83[a]	0.88[a]	95.00[a]	19.48[b]	60.02[a]	17.6[a]
PSE‡‡	24.55	3.34	0.32	2.29	2.04	1.49	1.95

*Means of four replicates. Means within the same column with different superscripts are significantly different ($P < 0.05$).
†% weight gain = (final mean weight − initial mean weight)/initial mean weight × 100.
‡FER = (final mean weight − initial mean weight)/dry weight feed offered × 100.
§IPFR = intraperitoneal fat weight × 100/fish weight.
¶Survival = (final number fish per treatment/initial number of fish per treatment) × 100.
‖PCE = (protein gain/protein intake) × 100.
**% protein (% dry matter) = % nitrogen × 6.25.
††% lipid (% dry matter) = [(lipid dry weight/sample wet weight) × 100]/% dry matter.
‡‡Pooled standard error.
FER, feed efficiency ratio; IDFR, intraperitoneal fat ratio; PCE, protein conversion efficiency.

Table 5 Responses of juvenile red snapper (mean initial weight 8.6 g) offered practical diets containing varying levels of lipid over a 10-week growth trial*

Dietary lipid	% weight gain†	FER‡	IPFR§	Survival¶	PCE‖	Whole body % protein**	Whole body % lipid††
8%‡‡	716.13[a]	62.31[a]	2.31[b]	98.33[a]	14.83[a]	52.70[ab]	33.4[a]
10%	676.44[a]	62.33[a]	2.10[b]	100.00[a]	15.60[a]	58.57[a]	38.7[a]
12%	671.10[a]	63.32[a]	2.95[ab]	98.33[a]	13.15[a]	51.35[ab]	38.5[a]
14%	683.0[a]	62.15[a]	3.43[a]	98.33[a]	13.80[a]	48.27[b]	41.7[a]
PSE§§	54.67	1.46	0.52	1.44	0.96	1.49	4.35

*Means of three replicates. Means within the same column with different superscripts are significantly different ($P < 0.05$).
†% weight gain = (final mean weight − initial mean weight)/initial mean weight × 100.
‡FER = (final mean weight − initial mean weight)/dry weight feed offered × 100.
§IPFR = intraperitoneal fat weight × 100/fish weight.
¶Survival = (final number fish per treatment/initial number of fish per treatment) × 100.
‖PCE = (Protein gain/protein intake) × 100.
**% protein (% dry matter) = % nitrogen × 6.25.
††% lipid (% dry matter) = [(lipid dry weight/sample wet weight) × 100]/% dry matter.
‡‡Diets formulated to be isonitrogenous (44% protein).
§§Pooled standard error.
FER, feed efficiency ratio; IDFR, intraperitoneal fat ratio; PCE, protein conversion efficiency.

fered a diet containing 8–10% dietary lipid had a greater amount of protein based on a percentage of whole-body composition than those offered 12–14% dietary lipid. The fish offered the diet containing 10% lipid exhibited a significantly greater ($P = 0.0219$) amount of protein based on a percentage of whole-body composition.

Response of sub-adult red snapper to varying levels of dietary protein.

The sub-adult snapper in trials 3 (as well as 4) adapted well to the culture system and experimental protocol. Some aggression among fish was noted; however, behaviour such as that observed in the smaller culture system was not evident. Table 6 summarizes the response of the red snapper to the various diets. At the conclusion of the 10-week growth trial, there were no significant differences in growth ($P = 0.1941$), survival ($P = 0.2192$) or FER ($P = 0.2170$) because of shifts in dietary protein. Additionally, regression analyses of percent weight gain data did not indicated a significant influence of dietary protein.

Response of sub-adult red snapper to varying levels of dietary lipid

Table 7 summarizes the response of sub-adult snapper to the various diets. Fish fed diets containing 8–12% lipid had a significantly higher ($P = 0.0439$) amount of body fat deposition than fish offered diets containing 6% lipid. Fish offered the 12% lipid diet also exhibited a significantly greater ($P = 0.0188$) ability to retain dietary protein compared with the snapper offered a 6% lipid diet. Although not significant, fish in trial 4 exhibited trends in relation to increased % weight gain ($P = 0.0615$), FER ($P = 0.0601$) and final mean weight ($P = 0.0596$) with escalating levels of dietary lipid. Regression analyses indicate a significant curvilinear response of final weight ($P = 0.0197$, Adj $R^2 = 0.48$) or weight gain ($P = 0.0251$, Adj $R^2 = 0.4611$) to dietary lipid levels. There were no significant treatment effects on % protein based on whole-body analysis ($P = 0.2509$).

Table 6 Responses of sub-adult red snapper (mean initial weight 151.5 g) offered practical diets containing graded levels of protein over a 10-week growth trial*

Dietary protein	% weight gain†	FER‡	Survival¶
32%	36.60[a]	19.58[a]	100.00[a]
36%	40.58[a]	21.64[a]	97.22[a]
40%	50.90[a]	26.38[a]	100.00[a]
44%	39.49[a]	21.08[a]	94.45[a]
PSE§	4.42	2.03	1.96

*Means of three replicates. Means within the same column with different superscripts are significantly different ($P < 0.05$).
†% weight gain = (final mean weight − initial mean weight)/initial mean weight × 100.
‡FER = (final mean weight − initial mean weight)/dry weight feed offered × 100.
§Pooled standard error.
¶Survival = (final number fish per treatment/initial number of fish per treatment) × 100.
FER, feed efficiency ratio.

Table 7 Responses of sub-adult red snapper (mean initial weight 178.3 g) offered practical diets containing varying levels of lipid over a 10-week growth trial*

Dietary lipid	% weight gain†	Final mean wt‡	FER§	IPFR¶	Survival ‖	PCE**,††
6%‡‡	14.81[a]	206.33[a]	12.24[a]	2.32[a]	92.60[a]	12.07[b]
8%	25.2[a]	229.47[a]	20.70[a]	3.65[b]	96.30[a]	18.28[ab]
10%	35.81[a]	236.03[a]	27.94[a]	3.50[b]	88.89[a]	20.61[ab]
12%	32.59[a]	232.97[a]	25.9[a]	3.97[b]	92.60[a]	25.80[a]
PSE§§	4.85	6.97	3.62	0.63	5.55	2.32

*Means of three replicates. Means within the same column with different superscripts are significantly different ($P < 0.05$).
†% weight gain = (final mean weight − initial mean weight)/initial mean weight × 100.
‡Final mean wt (g) = final mean weight of the treatment replicates (g).
§FER = (final mean weight − initial mean weight)/dry weight feed offered × 100.
¶IPFR = intraperitoneal fat weight × 100/fish weight.
‖Survival = (final number fish per treatment/initial number of fish per treatment) × 100.
**PCE = (protein gain/protein intake) × 100.
††% protein (% dry matter) = % nitrogen × 6.25.
‡‡Diets formulated to be isonitrogenous (32% protein).
§§Pooled standard error.
FER, feed efficiency ratio; IDFR, intraperitoneal fat ratio; PCE, protein conversion efficiency.

Discussion

The water-quality parameters measured in these trials were all within acceptable ranges, and no disease problems were noted during the course of these trials. Growth in all four trials was also similar to what is known for this species. In trial 1, the fish initially adapted readily to both culture systems and experimental protocol; however, aggression in the smaller tanks was apparent at the conclusion of the growth trial.

Results through week 6 indicated good growth, and little evidence of aggression was witnessed.

However, at the conclusion of the 8-week growth trial, evidence of fin nipping, which was observed with increasing frequency as the fish exceeded a size of 20–25 g, was prevalent. A total of 16 total fish were noted as having severely damaged fins, i.e. having greater than 50% loss of pectoral and caudal fins. Several fish with severely damaged fins were also in an emaciated state. Growth slowed down starting at week 6, and evidence of a possible tank effect was seen by comparing the growth rates of fish being fed two similar diets (Fig. 1) running concurrently in separate systems. Diet 1, a treatment in trial 1, was being offered to fish in 190-L rectangular culture tanks. Diet 7, a treatment in trial 2, was being offered to fish in 1000-L circular tanks. Both diets contained 44% crude protein and 10% crude lipid. Water-quality parameters were similar for both systems. Similar trials have also been conducted with juvenile red snapper at varying densities utilizing the same model of culture tanks. The growth rates were similar for all trials, with decreased growth and increased incidence of aggression noticed when fish exceeded an average weight of 20–25 g regardless of the densities. Thus, density or biomass loading is not likely the major contributing factor to increased aggression. More likely, there was a social interaction accentuated by the smaller tank size or shape.

Evidence of increased growth was not seen in relation to increasing levels of dietary protein and lipid, although documented in other species (Lee & Putnam 1973; Machiels & Henken 1985; Ellis & Reigh 1991; Serrano *et al.* 1992; Shiau & Peng 1993; Shiau & Lan 1996; Thoman *et al.* 1999; Chou *et al.* 2000; Catacutan *et al.* 2001). Similar trials conducted with juvenile red snapper indicate that this species has a relatively slow growth rate (as compared with other warm water marine fish such as the red drum), and as a result dietary protein requirements for this species may be significantly less than previously estimated.

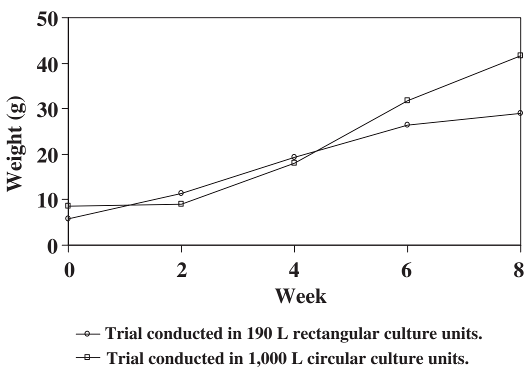

Figure 1 Comparison of growth in juvenile red snapper, in trials 1 and 2, over a concurrent 6-week period being offered similar diets.

Growth rates of juvenile red snapper reared in this study were typical of what we have observed in the laboratory on commercial feeds and were similar to those of juvenile yellowtail snapper (Turano *et al.* 2000), juvenile mutton snapper (Watanabe *et al.* 2001) and sub-adult red snapper (Marnell 1999). Turano and colleagues (2000) found no significant differences in growth and FER in yellowtail snapper offered diets ranging from 36% to 44% protein, and recommended a protein level of 36% for optimum growth. These results would indicate that the physiological demands for growth were being met and that excess protein was probably being utilized as an energy source. This is reflected in trial 1 by the decrease in PCE values as protein levels increased by treatment (Table 4).

In addition to protein being limited, poor growth could be as a result of an imbalance or inadequacy of dietary energy. Quite often, because of the expense of protein as well as a desire to minimize nitrogen pollution, non-protein energy values are elevated to spare dietary protein. Watanabe and colleagues (2001) reported that juvenile mutton snapper fed diets with varied E:P displayed good growth at the lowest levels of inclusion of dietary lipids. Final weight, final length, specific growth rate, daily weight gain and relative growth rates were all observed to be statistically greater for fish fed diets containing 6–9% dietary lipid than those including 12–15% lipid.

The inclusion of dietary lipid at a level of 14% was found to exceed the energetic demands for juvenile red snapper. In the present experiment, there was little change in IPFR values at 8–10% dietary lipid yet; when fish were offered diets containing 14% lipid, there were significant increases in the IPFR. In the sub-adult snapper (trial 4), there was little change in the IPFR values associated with fish offered diets containing 8–12% lipid. However, the IPFR was significantly less in the snapper offered 6% dietary lipid. This, in conjunction with the trends in growth and FER, indicates that 32–36% dietary protein is adequate in conjunction with $\sim$10% dietary lipid.

The increased retention of dietary protein (PCE) displayed by the fish in trial 1 offered 32–36% protein also indicates that the requirements for dietary protein in juvenile red snapper may be much lower relative to other marine fishes. Mutton snapper offered similar diets (45% crude protein; 6–12% crude lipid) also displayed protein-retention efficiencies consistent with those found for juvenile red snapper offered high-protein diets, indicating that dietary protein (45%) may have been offered in excess of the requirements for juvenile mutton snapper. Watanabe and colleagues (2001) found no significant differences in apparent net protein retention, which ranged from 15.8% to 17.1%, in relation to E:P.

The fish in this study displayed a growth rate at levels lower than expected. However, growth rates correspond with those found in similar studies with both red snapper and other species of *Lutjanid* snappers. Given the growth rate and the lack of an effect on growth and FER in relation to dietary protein and lipid in trials 1 through 3, the possibility exists that the requirement for dietary protein needed to promote maximum growth in this species is lower than what was utilized in these studies. In diets containing 44% protein, 14% dietary lipid exceeds the energetic demands for juvenile red snapper, and should probably remain in the 10–12% range in order to prevent large amounts of body fat deposition. Based on data obtained from these trials, inclusion of dietary protein at levels of 32–36% appears sufficient. For this level of protein, dietary lipid should be $\sim 10\%$ in order to meet the energetic demands of the animal and to spare dietary protein for growth.

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