

THE EFFECTS OF DIETARY PROTEIN AND LIPID ON GROWTH AND BODY
COMPOSITION OF JUVENILE RED SNAPPER, *LUTJANUS CAMPECHANUS*

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Christian Lewis Miller

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THESIS ABSTRACT

THE EFFECTS OF DIETARY PROTEIN AND LIPID ON GROWTH AND BODY COMPOSITION OF JUVENILE RED SNAPPER, *LUTJANUS CAMPECHANUS*

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Two studies were designed and conducted to evaluate the influence of dietary protein and lipid levels on growth and body composition of juvenile red snapper. To evaluate the response to dietary protein, four diets were formulated to contain 44, 40, 36, and 32% protein with practical energy:protein (E:P) ratios. To evaluate the response to dietary lipid levels, four isonitrogenous diets (44% protein) were formulated to contain 14, 12, 10, and 8% lipid. Variable protein diets (four replicates per treatment) were fed to juvenile red snapper (mean initial weight 5.9 g) based on a percentage of body weight, held in rectangular tanks containing 190 l of seawater at 32g/l, $27.0 \pm 0.76^{\circ}\text{C}$ for 10 weeks. Variable lipid (three replicates per treatment) diets were fed to juvenile red snapper (mean initial weight 8.6 g)

based on a percentage of body weight, held in circular tanks containing 1000 l of seawater at 33g/l, $26.3 \pm 1.02^{\circ}\text{C}$ for 10 weeks.

No significant differences ($p < 0.05$) were found in final weights, feed conversion efficiency, survival, whole body lipid, or hepatosomatic index (HSI) in either study. In the study containing diets of variable protein content, there was a significant difference ($p < 0.05$) in the protein conversion efficiency (PCE). Fish fed a diet containing 32-36% protein retained dietary protein at much more efficient rate. In the study containing diets of variable lipid content, there was a significant difference ($p < 0.05$) in the intraperitoneal fat ratio (IPFR) and % whole body protein. Fish fed a diet containing 14% lipid had a significantly greater amount of fat deposition in the body cavity, while those fed a diet containing 10% lipid had a significantly higher percentage of protein based on a whole body composition.

Preliminary results indicate that dietary protein requirements for juvenile red snapper may be as low as 32%. Data also suggests that dietary lipid should not exceed 10% in order to limit the amount of body fat deposition in juvenile red snapper, and promote the sparing of dietary protein for tissue growth.

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I. INTRODUCTION

The Red Snapper, *Lutjanus campechanus*, is the most highly valued species for both recreational and commercial fishing in the Gulf of Mexico (Bradley and Bryan 1976; Moran 1988, Workman and Foster 1994). The wide acceptance of the species as an excellent food fish has led to an accelerated market demand. Poor recruitment, which has been in part linked to shrimp trawl bycatch (Bradley and Bryan 1976; Workman and Foster 1994), combined with intensive fishing pressure have lead to a over harvesting of the snapper fishery (Moran 1988).

Fishery Status

The commercial fishery for red snapper in the Gulf of Mexico had its origins circa 1850 in the area around Pensacola, Florida. By the early twentieth century fishing grounds along the southwestern coast of Florida had been exploited, leading many of the vessels to fish off the Campeche Banks of Mexico (Camber 1955). Currently, the National Marine Fisheries Services reported a total commercial catch of approximately 2,303 metric tons (MT), valued at \$11,920,000 for the 2001 fishing season. This was an increase from the 1,081 MT recorded during the 1991 fishing season. However, these totals are considerably lower than the historical high of 6,389 MT harvested in 1965 (Wilson and Nieland 2001).

An extensive recreational fishery also exists for red snapper, and it is of principal importance in the northern Gulf of Mexico. The recreational red snapper fishery accounted for a total of 1,856 MT reported and estimated to be harvested during the 2001 fishing season. The states of Louisiana, Texas, Alabama, and Florida are the leading states in the harvest of red snapper in both the commercial and recreational fisheries (personal communication from the National Marine Fisheries Service, fisheries statistics and economics division). Perceived over harvesting of the stock, and decreased recruitment has led to increased regulation of both the commercial and the recreational fisheries.

An increased demand on the snapper fishery, and high market value of these species has led to an interest in developing culture methods for commercial production and stock enhancement (Watanabe *et al.* 2001). Species that have shown potential for commercial aquaculture application include these fishes: mutton snapper *Lutjanus analis*, yellow tail snapper *Ocyurus chrysurus*, red snapper *Lutjanus campechanus*, and the mangrove red snapper, *Lutjanus argentimaculatus* (Watanabe *et al.* 2001; Turano *et al.* 2000; Ogle *et al.* 2001; Catacutan *et al.* 2001)

Species Overview

Classification and description

The red snapper (class Osteichthyes; order Perciformes; family Lutjanidae) belongs to a family of fishes known commonly as snappers. This family comprises 21 genera and 125 species that are found in temperate to tropical waters worldwide. They are primarily predacious bottom oriented fishes, inhabiting areas ranging from shallow inshore to deeper

offshore waters (Hoese and Moore 1998). The red snapper ranges from the Gulf of Mexico, from the Bay of Campeche to the Atlantic. The species is seldom encountered north of Cape Hatteras, off the coast of North Carolina. The red snapper is replaced from the Caribbean southward by the caribbean red snapper, *Lutjanus purpureus* (Wilson and Neiland 2001). The red snapper was originally described by Poey (1860), and has several synonyms including *Mesoprion campechanus* (Poey 1860), *Lutjanus campechianus* (Poey 1875), and *Lutjanus blackfordii* (Goode and Bean 1879).

The red snapper is a relatively large fish. The body is compact, and the head is large and slightly pointed with the lower jaw protruding slightly beyond the upper. The eyes are small with a red iris (Moran 1988). The pectoral fins are long, and extend to the anus when compressed against the body. The dorsal fin usually contains nine to ten spines, and there is a slight notch between the first and second dorsal. The anal fin is pointed, and has three spines (Allen 1985). There is a V-shaped patch of vomerine teeth located on the roof of the mouth, and there are usually several large canines on the upper and lower jaws of adults.

As its name indicates, individual coloration varies from pale pink to deep scarlet. Noticeable blue-gray vertical banding may be present on juveniles, along with a dark ocellus near the caudal region, both of which fade as the fish ages. The caudal fin is notched, and a dark band is often present along the margin.

Life history

The red snapper is a relatively large and long-lived fish. Individuals generally grow at a rapid pace initially, and then growth slows as a larger size is reached (Moran 1988).

While Moran indicates a maximum fork length (FL), weight, and age respectively of 845 mm, 12 Kg, and 13 years; recent work by Wilson and Neiland (2001) has placed the maxima for this species at 965mm, 27.8 Kg, and 50+ years. Data from the Wilson and Neiland study also indicated that differential growth is exhibited between the sexes of red snapper, with females generally obtaining larger sizes and older ages than do males.

Habitat

Mature red snapper generally inhabit offshore areas where a hard bottom, or irregular bottom formation is present (Camber 1955; Futch and Burger 1976; Moran 1988; Workman and Foster 1994). Red snapper also are often found around the structures of the offshore drilling platforms scattered across the northern Gulf of Mexico (Moran 1988; Workman and Foster 1994). Studies of red snapper catches conducted by Camber (1955) along the Campeche Banks found that 99% of all red snapper were caught at depths of between 36 and 146 meters. Adult snapper may move into shallow water during the summer, but favor the deep water reefs as the water temperatures cool. Studies have indicated little migratory tendencies in red snapper, although there may be a sufficient amount of movement to facilitate stock mixing within the Gulf of Mexico (Patterson *et al.* 2000).

While the adult red snapper seem to favor deep water over hard bottoms and reefs, juveniles of the species tend to favor much shallower water and bottoms which contain more sand and mud (Bradley and Brian 1976; Moran 1988; Workman and Foster 1994). Juvenile red snapper may be seasonally very abundant in major commercial shrimp fishing grounds.

As a result, shrimp trawl bycatch has been implicated as one of the major causes for mortality in juvenile red snapper (Bradley and Bryan 1976; Workman and Foster 1994).

Workman and Foster (1994) indicated that the greatest incidence of juvenile red snapper activity occurred in water depths ranging from 18 to 28 meters. The juvenile snappers were found in the highest abundance in areas with sand and silt composition. Young snapper also tended to congregate in association with some type of structure. Bradley and Bryan (1976) reported finding juvenile fish at similar depths (15-30 meters), although they reported the juvenile snapper moved to depths of 35-60 meters during the winter months.

While little is known about the early life stages of red snapper in the wild, spawning is assumed to take place off the reefs in large aggregates (Bradley and Bryan 1976; Moran 1988). Snapper larvae are planktonic until settlement, which generally occurs around 25 days post-hatch.

Reproduction

Red snapper reach full sexual maturity at approximately two years of age and between 300-375 mm Fork Length (FL) (Camber 1955; Moran 1988). This species is sexually dimorphic, with females generally reaching larger sizes than males (Wilson and Nieland 2001). Red snapper spawn throughout the summer and fall, but there are several peaks based on geographic distribution. For red snapper in the eastern Gulf of Mexico, spawning peaks around the beginning of July and generally lasts into August. Fish spawning in the waters off of Texas have two peaks, the first from May through July and the second in November (Moran 1988; Bradley and Bryan 1976; Futch and Burger 1976). The actual

spawning has been reported to take place away from the reefs, usually in large aggregations of fish over areas with a firm sand bottom (Bradley and Bryan 1976).

Fish sampled from Florida indicated fecundity ranges from 0.2 million in a 3 year old fish (386 mm FL) to 9.3 million eggs from a 12 year old fish (754 mm FL) (Moran 1988). The eggs of the red snapper are pelagic, spherical, translucent, and usually contain a single oil globule. Eggs average approximately 0.82 mm in diameter (Rabalais *et al.* 1980). Laboratory studies by Rabalais (1980) on larval development of red snapper indicated that 50% of the eggs had hatched within 25 hours post fertilization. Newly hatched larvae averaged 2.2 mm standard length (SL), and began feeding on algae and rotifers three days after hatching.

Feeding Habits

Little is known about the feeding habits of larval red snapper before settlement, but it is assumed that larval fish prey on small zooplankton once eye pigmentation is complete and the mouth parts have formed. Zooplankton was found to be the primary prey item of juvenile red snapper up to 150 mm FL, although fish may start to prefer larger prey starting at around 100 mm FL (Bradley and Bryan 1976).

The stomachs of juvenile red snapper collected from the Gulf of Mexico throughout the year were found to contain primarily shrimp (Camber 1955; Bradley and Bryan 1976; Futch and Burger 1976), although squid and octopus were found in a greater percentage of stomachs in the summer and fall (Bradley and Bryan 1976).

Upon reaching maturity, the diet of adult red snapper shifts to reflect a more piscivorous lifestyle. Examination of the gut contents of adult red snapper revealed that the animals were preying upon shrimp, crabs, fish, and gastropods (Camber 1955; Bradley and Bryan 1976). Bradley and Bryan (1976) found that fish constituted the highest percentage of prey items of adult snapper throughout the year except during the summer, when crabs were found with the greatest frequency.

Diet Formulation

Goals of practical diet formulation

The primary goal in the formulation of practical feeds is to meet the nutritional needs in a manner which supports growth, maintenance, and reproduction of an animal at an acceptable cost. The formulation should be palatable to the fish, and not contain antinutrients at levels that would interfere with the overall performance. The diet needs to be relatively stable in water, and should not foul water quality in a greater means than the culture system is capable of handling (National Research Council 1993).

In formulating a diet, the nutrient content must be managed in a way that meets all physiological demands, and allows for a maximal amount of growth. Knowledge of the basic physiological processes and dietary composition of wild snapper (Table 1) provide a starting point for practical diet formulation. The diet of juvenile red snapper, an opportunistic carnivore, has been fairly well documented (Camber 1955; Bradley and Bryan 1976; Futch and Burger 1976; Moran 1988; Lee 1988). Proximate composition of common prey items are also established (Lytle and Lytle 1992; USDA FNIC).

Proteins

Protein is one of the key components when considering the nutritive requirements of any species. Proteins are polymers of various single amino acid monomers, which are joined together by peptide bonds. Amino acids are short chained fatty acids which contain a basic amino group (-NH₂) and an acidic carboxyl group (-COOH), 22 of which are commonly found in proteins. Proteins are classified by their basic structure and their amino acid profile. Proteins make up the bulk composition of the dry matter in fishes, they are used as an energy source, and they play a regulatory role as enzymes and hormones (Halver and Hardy, 2002). From an economics standpoint, dietary protein constitutes the principle nutritive cost associated with the formulation of most feeds, and is also the primary source of nitrogen waste within a given culture system (Catacutan and Coloso 1995; Shiao and Lan 1996; Perez *et al.* 1997; Thoman *et al.* 1999).

The optimization of dietary protein levels would help reduce nitrogen loading and cut production costs as well as increasing nutrient retention in the culture animal (Thoman *et al.* 1999). Dietary proteins also serve roles as attractants, feeding simulators, warning signals, osmoregulatory functions, and flavor (Halver and Hardy, 2002).

The dietary requirements for protein in fish has two parts. First, there is a need for the organism to acquire the indispensable amino acids that it either cannot synthesize entirely, or at a rate commensurate to meet the physiological demands for that organism. Secondly, the protein must supply sufficient dispensable amino acids or a source of amino nitrogen so that they may be synthesized (National Research Council 1993).

Table 1. Approximate percentage of total volume¹ and proximate composition² of a general diet for wild caught *L. campechanus* in the northern Gulf of Mexico.

Prey Item	% of total volume	composition % protein	composition % lipid
fish	14-20	20-30	1-3
shrimp	41-75	20	1.7
crab	7-10	16	0.8
copepod	9	-	-
octopus	45	15	1
squid	41	16	1.4

¹ From Bradley and Bryan, 1976.

² From Lytle and Lytle, 1992.

When determining the dietary requirements of protein for an organism, several factors must be considered. The quality of the protein source is determined by its bioavailability to the organism. Protein level, digestibility, palatability, presence of anti-nutrients, and amino acid profile, all must be taken into account when determining the quality of the protein source in relation to practical diet formulation.

Due to the high variability of amino acid profiles among different sources of protein, understanding these profiles is a major determining factor when formulating a diet. Synthesis of amino acids requires an expenditure of energy, so utilization of proteins which most closely correlate to the amino acid requirements of a species will result in the most efficient growth (National Research Council 1993).

Lipids

Lipids are a diverse group of organic molecules whose only shared trait is their insolubility in water, and solubility in organic solvents. Lipids include fats, phospholipids, steroids, and waxes. Dietary lipids are mainly in the form of glycerol, to which fatty acid chains are bonded. These molecules are hydrolyzed in the body by digestive enzymes and are absorbed to be used in the synthesis of cellular components or catabolized to meet energetic requirements. As a dietary component, lipids are important as sources of energy and essential fatty acids (EFA). Lipids also serve to allow for certain fat soluble nutrients to be absorbed by the body, and an attractant in feeds.

Essential fatty acids are fatty acids which must be present in the diet. These EFAs can either not be synthesized entirely, or at a level necessary to meet the physiological demands of the animal. EFAs have a generalized role in the maintenance of the structural integrity of cellular membranes, as well as serving as precursors to a highly active group of biological hormones known as eicosanoids (Sargent *et al.* 1999). Sargent (1999) indicated that the n-6 and n-3 fatty acids are extremely important in the early developmental stages of larval and juvenile fishes. This study also illustrated the importance of considering the proper ratios of EFAs when formulating diets. The requirement for EFAs in fish is somewhat related to their ability to modify these fatty acids metabolically (National Research Council 1993). Given the abundance of highly unsaturated fatty acids (HUFA) in the prey of marine species, differences in EFA requirements among fishes may reflect varying dietary metabolic adaptations to different ecological environs (Sargent *et al.* 1999).

Requirements of dietary proteins and lipids

Protein constitutes one of the highest dietary feed costs. Protein is also the primary source of ammonia, as ammonia is released from its breakdown, and as a result dietary protein is the primary source of nitrogen loading in systems. Typically, the dietary demand of protein in carnivorous fish range from 50-55% (Chou *et al.* 2000). The feeding habits of these fishes, combined with their fast rates of growth, are reflected by a requirement for high amounts of dietary protein. A high protein to energy ratio (i.e. an inadequate amount of non-protein energy) could lead to the usage of protein to meet physiological energetic demands, and excess nitrogen loading in the systems.

Lowering the protein to energy ratio is one way to optimize dietary requirements in fish. Increasing the lipid content in a diet will allow energetic demands to be met, while allowing for the sparing of dietary protein to be used for tissue building by the organism. Tekeda (1975) found that by increasing content of dietary lipids, the protein content could be decreased from 70 to 55% without any reduction in growth rates in juvenile yellowtail, *Seriola quinqueradiata*. Dietary lipid concentrations of up to 20% may give optimal results of feed efficiency (FE) and growth (Chou *et al.* 2000). However, an excess of dietary lipid may result in an imbalance of the digestible energy to crude protein ratio and excessive fat deposition, or intraperitoneal fat ratio (IPFR).

Little is known about the nutritional requirements of red snapper, however the general requirements can be ascertained by examining the proximate composition of common prey items (Table 1) of wild caught red snapper. Based on seasonal availability, the diet of juvenile red snapper in the wild contains approximately 20-30% protein and between 1-3% lipid, which translates to approximately 50-70g of protein and 2-7g of lipid per 100 g of dry matter. Laboratory studies with subadult red snapper fed a diet of chopped fish, squid, and penaeid shrimp over a period of 300 days indicate a daily growth rate of 0.45% body weight and a daily dietary requirement of 1.5% body weight (Wakeman *et al.* 1979). Wakeman also found feed conversion efficiency to be around 30%

Although little data exists in regards to the dietary requirements of juvenile red snapper, studies have been conducted with other carnivorous marine species such as mutton snapper, *Lutjanus analis* (Watanabe *et al.* 2001); mangrove red snapper, *Lutjanus argentimaculatus* (Catacutan *et al.* 2001); red drum, *Sciaenops ocellatus* (Williams and

Robinson 1988; Thoman *et al.* 1999); coxia, *Rachycentron canadum* (Chou *et al.* 2000); sea basses, family Serranidae (Borlongan and Parazo 1991; Catacutan and Coloso 1995; Shiau and Lan 1996; Perez *et al.* 1997); and hybrid striped bass, *Morone* sp. (Gaylord and Gatlin 2000).

Work done by Thoman (1999) found that red drum require crude protein levels of 44% to achieve optimum growth. Weight gain and FE were found to increase with protein and energy contents of the diets. An increase of lipid content in diets containing 44% protein resulted in increased FE, but also significantly increased IPFR. Red drum fed diets containing 40% protein were found to optimize growth, feed conversion ratio (FCR), and survival at dietary lipid levels of 7.4 and 11.2%. Fish fed a diet containing 18.8% lipid were found to have lower weight gains and higher feed conversion ratios than fish fed lower levels of lipid (Williams and Robinson 1988).

Juvenile coxia were found to have peak weight gain and FCR at a dietary protein level of 44.5%. The growth rate was slowest in fish fed diets containing 3% lipid, while weight gain was significantly higher in fish fed a diet with lipid levels increasing up to 5.76% (Chou *et al.* 2000). The total crude lipid was formulated to contain between 0.8-1.2% EPA and DHA. The author concluded that since growth did not increase significantly when lipid levels were raised past 5.76%, that the EFA requirements of coxia is within the 0.8 - 1.2% range and energy was not limiting.

Several studies with different species of the Serranidae family of sea basses indicate that optimum growth, FE, and protein efficiency (PE) is achieved when crude dietary protein is between 42.5-48%, while in conjunction with dietary lipid concentration of 10-14%

(Borlongan and Parazo 1991; Catacutan and Coloso 1995; Shiao and Lan 1996; Perez *et al.* 1997). Borlongan and Parazo (1991) found that a combination of cod oil and soybean oil was adequate to meet EFA demands, while diets containing a majority of coconut oil as the source of crude lipid had the lowest rates of growth and survival.

Little nutritional data exists regarding levels of crude protein and lipid necessary to promote growth in juvenile lutjanid snappers. Catacutan (2001) has indicated that crude protein levels for optimum growth of laboratory reared mangrove red snapper is greater than 40%. Fish fed diets containing 42.5% crude protein showed significantly higher growth rates than those fed diets with 35% crude protein. However, no significant differences in growth were observed between fish fed diets containing 42.5 and 50% crude protein. Likewise, work with juvenile mutton snapper has shown dietary requirements of crude protein being greater than 40%. Maximum growth of laboratory reared mutton snapper occurred in fish fed diets containing 45% crude protein and between 6-9% crude lipid (Watanabe *et al.* 2001).

Due to limited information and the interest to culture, this research was designed to determine the requirements of dietary protein and lipid necessary to promote growth in juvenile red snapper.

II. EFFECTS OF DIETARY PROTEIN ON GROWTH AND BODY COMPOSITION OF JUVENILE RED SNAPPER (*LUTJANUS CAMPECHANUS*)

ABSTRACT: The present study was designed to evaluate the influence of dietary protein on growth and body composition of juvenile red snapper. An eight-week feeding trial was conducted in a semi-closed recirculating system. Diet treatments were formulated to contain 44, 40, 36, and 32% protein, each diet was offered to four replicate groups of juvenile red snapper (initial mean weight 5.9 g) maintained in 190 L tanks at a density of 15 fish per tank. Fish were fed a ration twice daily based on percentage body weight, and were weighed bi-weekly. Water quality parameters were maintained within acceptable standards, and system maintenance was conducted as needed. At the conclusion of the growth trial fish were group weighed, and four fish per replicate were randomly selected for subsequent proximate analysis. Based on statistical analyses there were no significant effects of the treatments on weight gain, survival, feed conversion efficiency, intraperitoneal fat ratio, hepatosomatic index, or proximate composition of the fish. Fish fed a diet consisting of 32-36% protein exhibited an increased efficiency ($P = 0.0115$) in retaining dietary protein.

INTRODUCTION

The red snapper, *Lutjanus campechanus*, is one of the most important species, both commercially and recreationally, in the northern Gulf of Mexico. Their wide acceptance as an excellent food fish and high market value has led to over harvesting of wild stock in many areas (Morran 1988; Workman and Foster 1994). An increased awareness of the red snapper fishery has led to an interest in the development of culture methods for commercial production and stock enhancement. Little information exists on nutritional requirements for juvenile red snapper. Gaining an understanding of the nutritional requirements for juvenile red snapper, and the development of a practical diet for this species is an area which must be addressed.

Protein is one of the key components when considering the nutritive requirements of any species. Proteins make up the bulk composition of the body, they are used as an energy source, and they play a regulatory role as enzymes and hormones (Halver and Hardy, 2002). From an economics standpoint, dietary protein constitutes the principle nutritive cost associated with the formulation of most feeds, and is also the primary source of nitrogen waste within a given culture system (Catacutan and Coloso 1995; Shiao and Lan 1996; Perez *et al.* 1997; Thoman *et al.* 1999). The optimization of dietary protein levels would help reduce nitrogen loading and cut production costs as well as increasing nutrient retention in the culture animal (Thoman *et al.* 1999).

Typically, the dietary demand of protein in carnivorous fish range from 50-55% (Chou *et al.* 2000), as the feeding habits of these fish are reflected in this requirement for

high amounts of dietary protein. This necessity for high levels of dietary protein is reflected in the proximate composition of common prey items. The diet of juvenile red snapper has been fairly well documented (Camber 1955; Bradley and Bryan 1976; Futch and Bruger 1976; Moran 1988; Lee 1998). Proximate composition of common prey items are also established (Lytle and Lytle 1992; USDA FNIC). Based on seasonal availability, the diet of juvenile red snapper in the wild contains approximately 20-30% protein and between 1-3% lipid, which translates to approximately 50-70g of protein and 2-7g of lipid per 100 g of dry matter.

Little nutritional data exists regarding levels of crude protein necessary to promote growth in juvenile lutjanid snappers. Catacutan (2001) has indicated that crude protein levels for optimum growth of laboratory reared mangrove red snapper, *Lutjanus argentimaculatus*, is greater than 40%. Fish fed diets containing 42.5% crude protein showed significantly higher growth rates than those fed diets with 35% crude protein. However, no significant differences in growth were observed between fish fed diets containing 42.5 and 50% crude protein. Likewise, work with juvenile mutton snapper, *Lutjanus analis*, has shown dietary requirements of crude protein being greater than 40%. Maximum growth of laboratory reared mutton snapper occurred in fish fed diets containing 45% crude protein and between 6-9% crude lipid (Watanabe *et al.* 2001). Laboratory studies with sub-adult red snapper fed chopped fish, squid, and penaeid shrimp over a period of 300 days indicate a daily growth rate of 0.45% of body weight and a daily dietary requirement of 1.5% of body weight (Wakeman *et al.* 1979). Wakeman also found feed conversion efficiency to be around 30%

Although little data exists in regards to the dietary requirements of snappers, studies have been conducted with other carnivorous marine species such as red drum, *Sciaenops ocellatus* (Williams and Robinson 1988; Thoman *et al.* 1999); cobia, *Rachycentron canadum* (Chou *et al.* 2001); sea basses, family Serranidae (Borlongan and Parazo 1991; Catacutan and Coloso 1995; Shiau and Lan 1996; Perez *et al.* 1997); and hybrid striped bass, *Morone* sp. (Gaylord and Gatlin 2000).

Thoman (1999) found that red drum require crude protein levels of 44% to achieve optimum growth. Weight gain and FE were found to increase with protein and energy contents of the diets. An increase of lipid content in diets containing 44% protein resulted in increased FE, but also significantly increased IPFR. Juvenile cobia were found to have peak weight gain and FCR at a dietary protein level of 44.5%. The growth rate was slowest in fish fed diets containing 3% lipid, while weight gain was significantly higher in fish fed a diet with lipid levels increasing up to 5.76% (Chou *et al.* 2000). Several studies with different species of the Serranidae family of sea basses indicate that optimum growth, FE, and protein efficiency (PE) is achieved when crude dietary protein is between 42.5-48%, while in conjunction with dietary lipid concentration of 10-14% (Borlongan and Parazo 1991; Catacutan and Coloso 1995; Shiau and Lan 1996; Perez *et al.* 1997).

Currently, little information exists regarding the nutritional demands of juvenile red snapper. Since protein is a critical component in the formulation of practical feeds in relationship to an economic and nutritional standpoint, this study was designed to evaluate the response of juvenile red snapper to varying levels of dietary protein.

MATERIALS AND METHODS

This study was conducted over an eight week period at the Claude Peteet Mariculture Facility in Gulf Shores, AL, from August through October 2002. Four practical diets (Table 1) were formulated to contain graded levels of protein (44-32%) with practical energy to protein ratios. Diet formulations were based on work conducted by Thoman (1999) with red drum.

Diets were prepared by mixing the dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, OH, USA) for 30 minutes. 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 allowed to dry to a moisture content of less than 10%. Protein content was confirmed using micro-Kjeldahl analysis (Ma and Zuazago, 1942). Diets were stored in a -20° C freezer, and prior to use each diet was ground and sieved to an appropriate size.

The culture system utilized for this study was a semi-closed recirculating system consisting of two adjoining vats, with a total volume of 15,000 liters, containing sixteen rectangular (61x61x61 cm) polyethylene tanks (Polytank Inc., Litchfield, MN, USA) with an individual volume of 190 liters. Each tank had approximately 20 cm freeboard, and was covered in order to prevent the animals from jumping out. Subsurface aeration was provided by two diffusers per tank and a common regenerative blower. Water was circulated using an Ebara model 32z707au submersible pump (Ebara Int. Corp. Rock Hill, SC, USA), and flow rates were set at a mean rate of 4.1 liters a minute per culture unit. Five vertical filter plates

(91 cm x 122 cm) were utilized for biological filtration. Water temperature was maintained at 27° C by a model c302-LT submersible heater (Process Technologies Inc. Mentor, OH, USA). Photoperiod was maintained on a 12:12 hour light:dark cycle, and lighting was provided by overhead fluorescent fixtures.

Juvenile red snapper, *L. campechanus*, of the same cohort (65 days post-hatch) were acquired from environmentally manipulated natural spawns from brood fish at Claude Petet. Prior to the initiation of the trial, juvenile red snapper were graded to a uniform size, and fifteen fish (mean weight 5.9 g) were stocked into each culture tank. A random sub-sample of fish was collected at initiation and frozen at -60° C for proximate analysis.

Four diets were 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. Routine system maintenance, such as siphoning of solids and partial water exchanges, were conducted as needed throughout the duration of the trial. System temperature, dissolved oxygen, salinity, and pH were monitored once daily using a YSI model 556 MPS (Yellow Springs, OH, USA). Nitrite-nitrogen was monitored weekly using a model PLN code test kit from LaMotte (Chestertown, MD, USA). Total ammonia-nitrogen was monitored twice weekly using the Nessler method (Boyd, 1979). The system was also treated with Marex brand chloroquine phosphate from Chemaqua (Oxnard, CA, USA) at 3.1 mg/l as a preventative measure against *Amyloodinium ocellatum*.

Table 1. Composition of experimental diets (g/100g dry weight)

Ingredient	32% Protein	36% Protein	40% Protein	44% Protein
Menhaden Fish Meal ¹	21.8	24.5	27.3	30
Poultry Meal ²	7.3	8.2	9.1	10
Soybean Meal ³	20.3	23.5	26.5	29.8
Menhaden Fish Oil ⁴	4.2	4.45	4.6	4.9
Wheat Starch ⁵	23.03	15.98	9.13	1.93
Whole Wheat ⁵	20	20	20	20
Vitamin Premix ⁶	1.8	1.8	1.8	1.8
Trace Mineral Premix ⁷	0.5	0.5	0.5	0.5
Stay C ⁸	0.07	0.07	0.07	0.07
Choline Chloride ⁵	0.2	0.2	0.2	0.2
CaP dibasic ⁵	0.8	0.8	0.8	0.8
Formulated to contain:				
Protein	32	36	40	44
Measured to contain:				
Protein	31.7	34.5	39.0	44.3
% Dry matter	94.8	94.6	94.2	96.7

¹ Special Select™, Omega Protein™, USA Inc., Randeville, LA, USA

² Griffin Industries, Inc. Cold Springs, KY, USA

³ Solvent extracted, Producers Cop, Bryan, TX, USA

⁴ Omega Protein™, Reedville, VA, USA

⁵ United States Biochemical Company, Cleveland, OH, USA

⁶ g 100g⁻¹ Premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferros sulphate 4.0, magnesium sulphate heptahydrate 28.398, monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428

⁷ g Kg⁻¹ Premix: Thiamin Hcl 0.5, riboflavin 3.0, pyridoxine HCL 1.0, DL Ca-pantothenate 5.0, nicotinc acid 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

⁸ StayC®, (L-ascorbyl-2-polyphosphate 35% active C) Roche Vitamins Inc., Parsippany, NJ, USA

Fish were counted, weighed, dipped in freshwater for approximately 30 seconds, and culture tanks were scrubbed bi-weekly. To minimize handling stress at the conclusion of the growth trial, fish were treated with 0.34 ml/l of Hypno brand dimethylketone alpha methyl quinoline (Jungle Lab Corp. Cibolo, TX, USA).

At the termination of the study, group weights, individual weights, and total lengths were taken for each tank. Individual lengths and weights were taken to determine the length to weight ratio. Feed utilization (FCE) was also calculated (wet weight gain*100/dry weight feed offered). Four fish from each treatment replicate were randomly selected, scaled, and dissected in order to determine the hepatosomatic index (HSI = liver weight x100/fish weight) and intraperitoneal fat ratio (IPFR = intraperitoneal fat weight x 100/fish weight). The fish and dissected material was pooled by tank, then homogenized and frozen to -60° C for later proximate analysis. Dry matter was determined by drying to a constant weight at 90° C. Protein content was determined by the micro-Kjeldahl method. Total lipid content was determined using methods described by Folch (1957). All analyses were conducted with triplicate sub-samples from each treatment replicate.

All data was subjected to a one way analysis of variance to determine significant differences between the treatment means. The Student-Neuman Keuls' multiple range test was used to distinguish significant differences among treatment means. All statistical analyses were conducted using the SAS system for windows (version 8.0 SAS Institute, Cary, NC USA).

RESULTS AND DISCUSSION

The snapper used in this study initially adapted readily to the culture system and experimental protocol. Results through week six indicated good growth, and little evidence of aggression was witnessed. However, depressed feed consumption and aggression were observed with increasing frequency starting in week seven of the trial. Water quality parameters (mean \pm standard deviation) were as follows: temperature, $27.02 \pm 0.76^{\circ}$ C; salinity, 32.09 ± 1.58 g/l; dissolved oxygen, 5.70 ± 0.56 mg/l; total ammonia-nitrogen, 0.06 ± 0.08 mg/l; nitrite-nitrogen, 0.02 ± 0.04 mg/l; pH, 7.78 ± 0.11 .

At the conclusion of the eight week trial there were no significant differences (Table 2) in weight gain, survival, feed utilization (FCE), lipid stores (IPFR), or proximate whole body composition. However, fish fed a diet containing 32-36% protein retained dietary protein (Figure 1) at a much more efficient rate ($P = 0.0115$). At week six, a possible tank effect was observed, irrespective to the dietary treatments. Mean FCE and growth rate (Figure 2) dropped throughout the course of the trial, and there was a significant drop in FCE noticed at the weighing six weeks into the trial (Table 3). The coefficients of variation ranged from 33.5% to 28.2%, and were not significantly different ($P = 0.3852$). Beginning in week five, fish were observed showing aggressive behavior irrespective of dietary treatment. At termination, evidence of fin nipping was prevalent, and sixteen total fish were noted as having severely

Table 2. Response of juvenile red snapper (mean initial weight 5.9 g) offered diet treatments over an eight week growth trial¹

Diet	% weight gain ²	FCE ³	IPFR ⁴	Survival ⁵	PCE ⁶	% Protein ⁷	% Lipid ⁸
32% protein	392.90 ^a	11.93 ^a	0.44 ^a	98.33 ^a	26.12 ^a	50.70 ^a	1.39 ^a
36% protein	410.59 ^a	14.05 ^a	0.45 ^a	98.33 ^a	24.34 ^a	53.58 ^a	1.76 ^a
40% protein	435.10 ^a	15.40 ^a	0.77 ^a	100.00 ^a	19.58 ^{ab}	54.41 ^a	1.79 ^a
44% protein	436.49 ^a	15.78 ^a	0.88 ^a	95.00 ^a	16.48 ^b	55.59 ^a	1.95 ^a
PSE ⁹	24.55	2.06	0.32	1.99	1.69	1.42	0.22

¹ Means of four replicates (P<0.05).

² % weight gain = (final mean weight - initial mean weight)/initial mean weight * 100.

³ FCE = (final mean weight - initial mean weight)/feed offered * 100.

⁴ IPFR = intraperitoneal fat weight * 100/fish weight.

⁵ (Final number fish per treatment/initial number of fish per treatment)*100

⁶ (Protein gain/protein fed)*100

⁷ % protein = % nitrogen * 6.25.

⁸ % lipid = [(lipid dry weight/sample wet weight)*100]* % dry matter.

⁹ Pooled standard error.

Table 3. Feed Conversion Efficiency (FCE) of juvenile red snapper (mean initial weight 5.9 g) offered diet treatments over an eight week growth trial¹

	32% protein	36% protein	40% protein	44% protein
FCE ² Week 2	65.46	78.26	91.83	88.97
FCE Week 4	65.32	63.69	70.81	63.01
FCE Week 6	47.14	47.67	41.55	45.37
FCE Week 8	58.20	29.91	15.73	15.11
Trial FCE ³	15.80	15.39	14.08	11.91

¹ Means of four replicates.

² FCE = (final mean weight - initial mean weight)/feed offered * 100.

³ P = 0.5635

damaged fins, which was defined as having greater than 50% loss of pectoral and caudal fins. Several fish with severely damaged fins were also noted as being in an emaciated state.

Growth also dramatically slowed down, and evidence of possible tank effect on growth can be seen by comparing the growth rates of fish being fed two similar diets (Figure 3) running concurrently in separate systems. Diet 1, a treatment in the protein trial, was being offered to fish in 190 L rectangular culture tanks. Diet 7, a treatment in a separate trial, was being offered to fish in 1,000 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 in both this system and a similar system utilizing the same model of culture tanks. The growth rates were similar for all trails, with decreased growth and increased incidence of aggression noticed when fish exceeded an average weight of approximately 25g irregardless of the densities. Hence, density or biomass loading is not likely the major contributing factor to increased aggression. More than likely, there is a social interaction accentuated by tank size.

The observed reduction in growth could also be attributed to other factors such as gas supersaturation within the system, and/or a low grade infestation of *Amyloodinium ocellatum*. In a previous trial in the same system, gas supersaturation was likely present. Juvenile snapper under possible supersaturation also exhibited diminishing growth after several weeks into the trial. While corrective measures were taken to remove possible sources of supersaturation, it can not be ruled out for this trial. Similarly, the presence of *Amyloodinium ocellatum* is known to have occurred in previous trials as well. In trials where *A. ocellatum* was known to have occurred, juvenile red snapper exhibited a dramatically

reduced feeding response. While preventative measures were taken to reduce the possible impact of infestation in this trial, a minor infestation can also not be ruled out.

Although growth may not have been optimal during the last two weeks, there were no significant differences in growth at week six, or any strong indications of differential growth. While results of this study did not show a positive correlation between increasing levels of dietary protein and increased growth, this effect has been demonstrated with other warm water carnivorous fishes (Shiau and Lan 1996; Thoman *et al.* 1999; Chou *et al.* 2000; Catacutan *et al.* 2001). Evidence also suggests that the red snapper has a slower growth rate relative to other warm water fish, such as the cobia and red drum, and as a result may not require such a high level of dietary protein for optimum growth.

Research with the yellowtail snapper, *Ocyurus chrysurus*, indicates a similar growth rate and a resulting low dietary protein requirement. Turano (2000) concluded a protein level of 36% was sufficient for optimum growth in yellowtail snapper. A similar growth rate has been exhibited for juvenile red snapper, and may also indicate a lower dietary protein requirement. The increased ability to retain dietary protein (PCE) displayed by the fish offered 32-36% protein, also intimates that the requirements for dietary protein in juvenile red snapper may be much lower relative to other marine fishes. In practical diet formulations for fish, protein is one of the major costs associated with the nutrients and ingredients. To minimize costs, it would be beneficial to optimize both the dietary level of protein and its retention in the fish.

Figure 1. Protein conversion efficiency (PCE) of juvenile red snapper (mean initial weight 5.9 g) offered four diets with varying levels of dietary protein, and similar E:P ratios, over an eight week growth trial

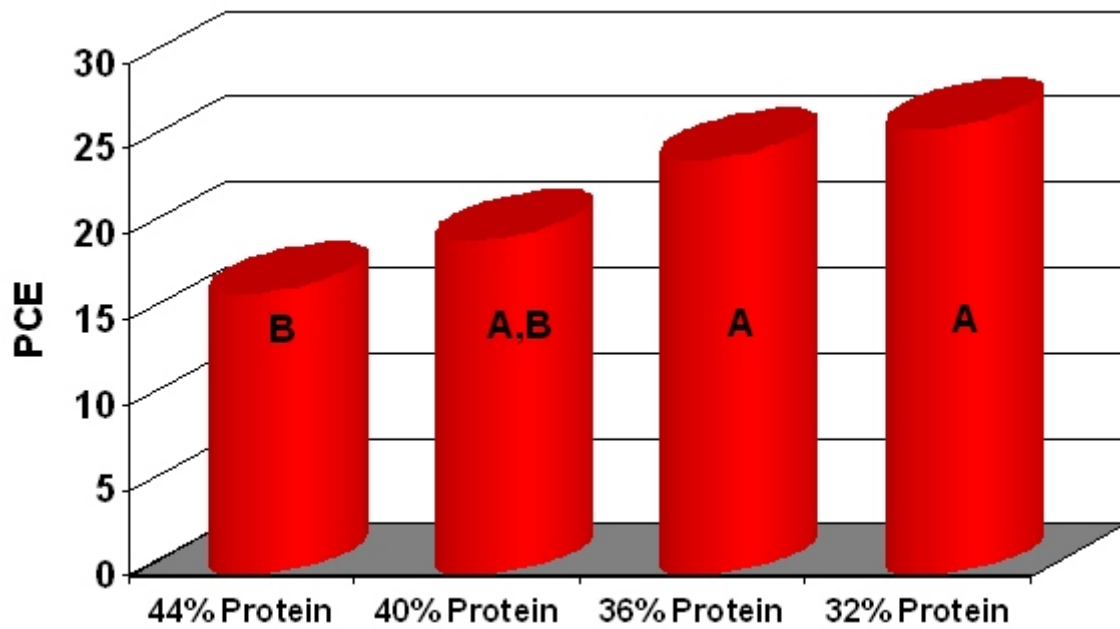


Figure 2. Growth of juvenile red snapper (mean initial weight 5.9 g) offered four diets with varying levels of dietary protein, and similar E:P ratios, over an eight week growth trial

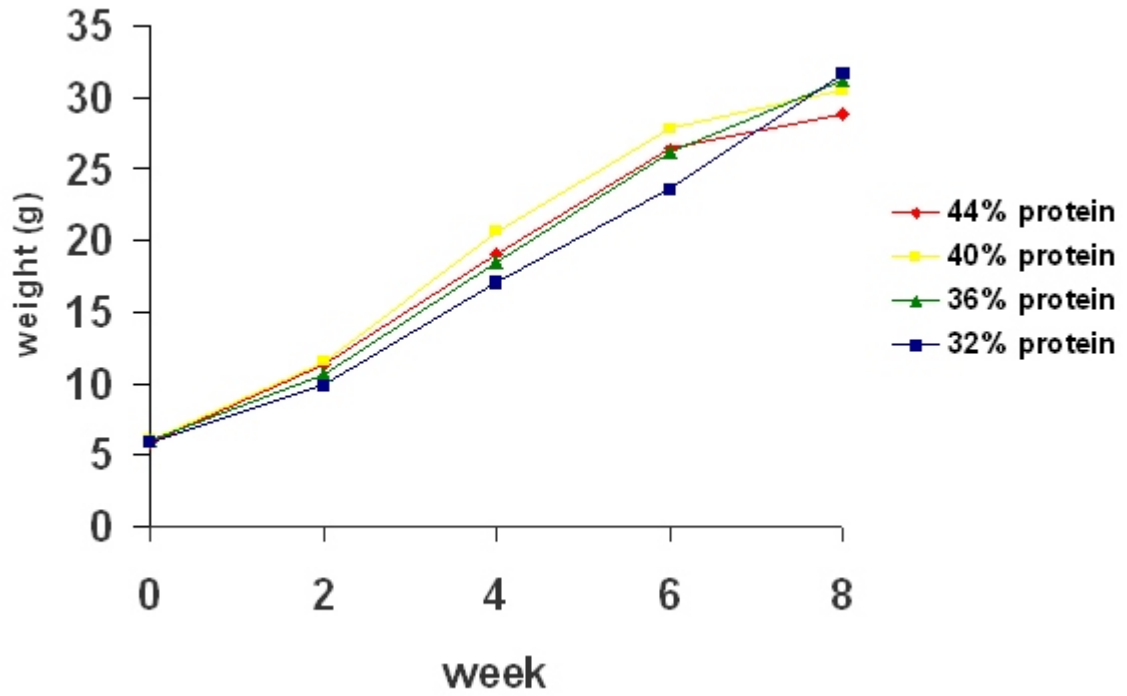
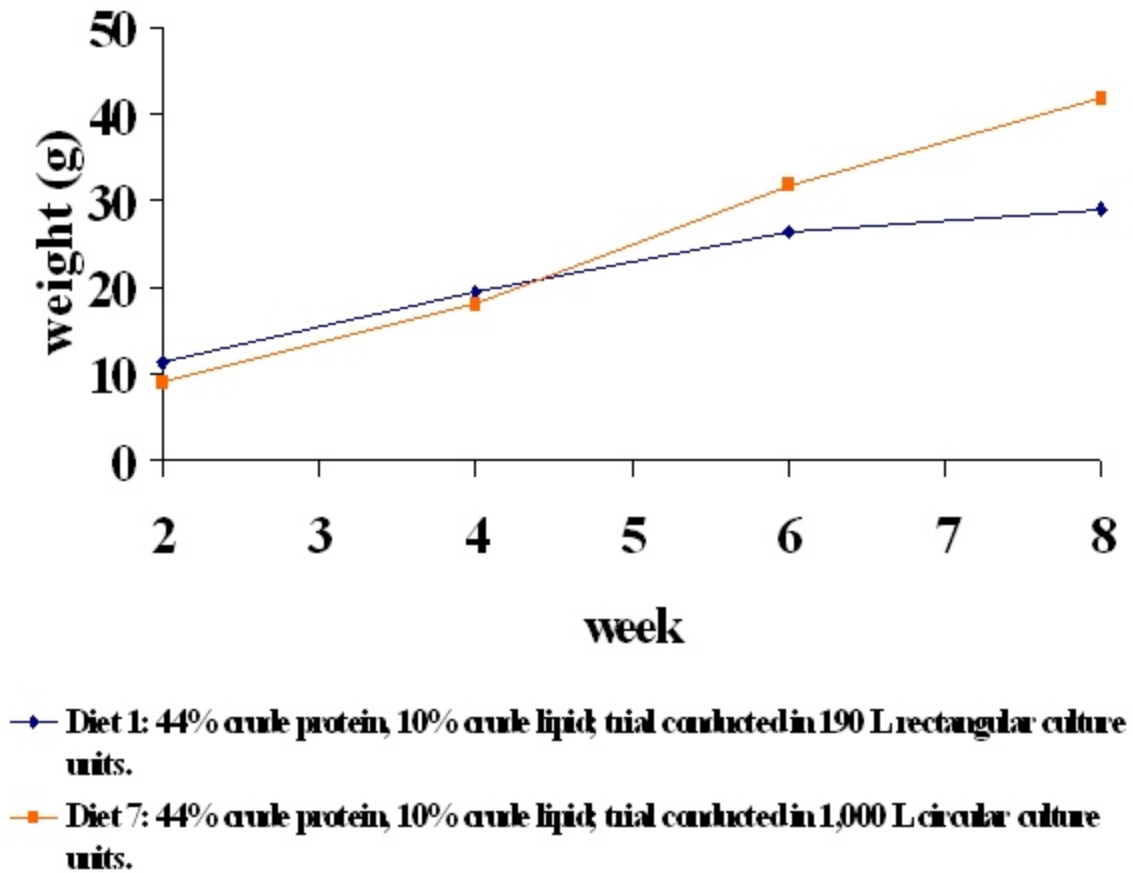


Figure 3. Comparison of growth in juvenile red snapper, in separate growth trials, over a concurrent six week period being offered similar diets



Under these reported conditions, there were no significant differences in weight gain, feed utilization (FCE), lipid stores (IPFR), or proximate whole body composition. Although this study showed no correlation between increased protein levels and growth, it has been documented in other warm water carnivorous marine fishes. Possible tank effects were witnessed beginning at week six, and a trend towards increased aggression was seen beginning in week five. Similar growth trials conducted with other lutjanid snappers indicate protein requirements may be lower than previously thought, and as a result the lowest level (32%) used in this study may have been sufficient to meet the protein requirements of this species. The increased retention of dietary protein in the fish offered diets containing 32-36% dietary protein also indicate dietary protein requirements may be as low as 32%.

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III. EFFECTS OF DIETARY LIPID ON GROWTH AND BODY COMPOSITION OF JUVENILE RED SNAPPER (*LUTJANUS CAMPECHANUS*)

ABSTRACT: The present study was designed to evaluate the influence of dietary lipid on growth and body composition of juvenile red snapper. A ten-week feeding trial was conducted in a semi-closed recirculating system. Diet treatments were formulated to be isonitrogenous (44% protein) and contain 14, 12, 10, and 8% lipid. Each diet was offered to three replicate groups of juvenile red snapper (initial mean weight 8.6 g) maintained in 1000 L tanks at a density of 20 fish per tank. Fish were fed a ration twice daily based on percentage body weight, and were weighed bi-weekly. Water quality parameters were maintained within acceptable standards, and system maintenance was conducted as needed. At the conclusion of the growth trial fish were group weighed, and four fish/replicate were randomly selected for subsequent proximate analysis. Based on statistical analyses there were no significant effects of the treatments on weight gain, survival, feed conversion efficiency, protein retention, hepatosomatic index, or percent whole body lipid. There was, however, a significant treatment effect in regards to IPFR, and percent protein based on whole body composition. Fish fed a ration containing 14% dietary protein had a significantly greater amount of intraperitoneal fat deposition ($P = 0.0052$). Fish offered diets containing 10%

dietary lipid showed an increased percentage of protein based on whole body composition (P = 0.0224).

INTRODUCTION

The red snapper, *Lutjanus campechanus*, is one of the most important species, both commercially and recreationally, in the northern Gulf of Mexico. Their wide acceptance as an excellent food fish and high market value has led to over harvesting of wild stock in many areas. An increased awareness of the red snapper fishery has led to an interest in the development of culture methods for commercial production and stock enhancement. Little information exists on nutritional requirements for juvenile red snapper. Gaining an understanding of the nutritional requirements for juvenile red snapper, and the development of a practical diet for this species is an area which must be addressed if this species is to be cultured.

As a dietary component, lipids are important as sources of energy and essential fatty acids (EFA). Lipids also serve to allow for certain fat soluble nutrients to be absorbed by the body, and as an attractant in feeds. Retention and accretion of protein is influenced by several factors including the dietary energy to protein (E:P) ratios. Protein is one of the key components when considering the nutritive requirements of any species, it makes up the bulk composition of the body, is used as an energy source, and plays a regulatory role as enzymes and hormones (Halver and Hardy, 2002).

From an economic standpoint, dietary protein constitutes the principle nutritive cost associated with the formulation of most feeds, and is also the primary source of nitrogen waste within a given culture system (Catacutan and Coloso 1995; Shiao and Lan 1996; Perez

et al. 1997; Thoman *et al.* 1999). Optimization of E:P ratios is an established method for spare dietary protein, resulting in increased growth at a reduced cost (Lee and Putnam, 1973; Machiels and Henken, 1985; Ellis and Reigh, 1991; Serrano *et al.*, 1992; Shiao and Peng, 1993; Thoman *et al.* 1999). Tekeda (1975) found that by increasing content of dietary lipids, the protein content could be decreased from 70 to 55% without any reduction in growth rates in juvenile yellowtail, *Seriola quinqueradiata*. Dietary lipid concentrations of up to 20% may give optimal results of feed efficiency (FE) and growth (Chou *et al.* 2000). However, an excess of dietary lipid may result in an imbalance of the digestible energy to crude protein ratio and excessive fat deposition, or intraperitoneal fat ratio (IPFR).

Little nutritional data exists regarding levels of crude protein and lipid necessary to promote growth in juvenile red snapper, however the diet of juvenile red snapper in the wild has been fairly well documented (Camber 1955; Bradley and Bryan 1976; Futch and Bruger 1976; Moran 1988; Lee 1998). Proximate composition of common prey items are also established (Lytle and Lytle 1992; USDA FNIC). Based on seasonal availability, the diet of juvenile red snapper in the wild contains approximately 20-30% protein and between 1-3% lipid, which correlates to approximately 50-70g of protein and 2-7g of lipid per 100 g of dry matter.

Although little data exists in regards to the dietary requirements of red snapper, studies have been conducted with other carnivorous marine species. Thoman (1999) found that weight gain and FE were found to increase with protein and energy contents of the diets of red drum, *Sciaenops ocellatus*. An increase of lipid content in diets containing 44% protein resulted in increased FE, but also significantly increased IPFR. The growth rate in

juvenile cobia, *Rachycentron canadum*, was slowest in fish fed diets containing 3% lipid, while weight gain was significantly higher in fish fed a diet with lipid levels increasing up to 5.8% (Chou *et al.* 2000). Several studies with different species of the Serranidae family of sea basses indicate that optimum growth, FE, and protein efficiency (PE) is achieved when crude dietary protein is between 42.5-48%, while in conjunction with dietary lipid concentration of 10-14% (Borlongan and Parazo 1991; Catacutan and Coloso 1995; Shiau and Lan 1996; Perez *et al.* 1997). Maximum growth in laboratory reared mutton snapper, *Lutjanus analis*, occurred when fish were fed a ration containing 45% crude protein in conjunction with 6-9% crude lipid (Watanabe *et al.* 2001).

Currently, little information exists regarding the nutritional demands of juvenile red snapper. Dietary lipid is a critical component in the formulation of practical feeds. Inclusion of lipids in practical feeds is important in order to meet energetic demands, provide EFA, allow transport of fat-soluble vitamins, serve as an attractant, and to spare protein for tissue building. Due to limited information and the interest to culture, this study was designed to evaluate the ability of juvenile red snapper to utilize dietary lipid.

MATERIALS AND METHODS

This study was an eight week feeding trial conducted at the Claude Petet Mariculture Facility in Gulf Shores, AL, from August through November 2002. Four practical diets (Table 1) were formulated to be isonitrogenous (44% protein) and contain 8-14% crude dietary lipid. Diet formulations were based on work done by Thoman (1999) with red drum.

Diets were prepared by mixing the dry ingredients and menhaden fish oil in a food mixer (Hobart, Troy, OH, USA) for 30 minutes. 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 allowed to dry to a moisture content of less than 10%. Protein content was confirmed using micro-Kjeldahl analysis (Ma and Zuazago, 1942). Diets were stored in a -20° C freezer, and prior to use each diet was ground and sieved to an appropriate size.

The culture system utilized for this study was a semi-closed recirculating system consisting of 12 fiberglass culture tanks and two sumps, with a total volume of approximately 12,800 liters. Each tank was approximately 122cm in diameter, had approximately 20 cm freeboard, and was covered in order to prevent the animals from jumping out. Subsurface aeration was provided by two diffusers per tank and a common regenerative blower. Water was circulated using a one horsepower centrifugal pump and flow rates were set at a mean rate of 17.2 liters a minute per culture unit. Two bead filters (Aquaculture Systems Technology LLC, New Orleans, LA, USA) were utilized for biological filtration. Water temperature was

Table 1. Composition of experimental diets (g/100 g Dry Weight)

Ingredient	8% Lipid	10% Lipid	12% Lipid	14% Lipid
Menhaden Fish Meal ¹	30	30	30	30
Poultry Meal ²	10	10	10	10
Soybean Meal ³	30.6	30.6	30.6	30.6
Menhaden Fish Oil ⁴	2.93	4.93	6.93	8.93
Wheat Starch ⁵	1.1	1.1	1.1	1.1
Whole Wheat ⁵	16	16	16	16
Trace Mineral Premix ⁶	0.5	0.5	0.5	0.5
Vitamin Premix ⁷	1.8	1.8	1.8	1.8
Choline Chloride ⁵	0.2	0.2	0.2	0.2
Stay C ⁸	0.07	0.07	0.07	0.07
CaP dibasic ⁵	0.8	0.8	0.8	0.8
Cellufil ⁵	0	2	4	6
Formulated to contain:				
Protein	44	44	44	44
Lipid	8	10	12	14

¹ Special Select™, Omega Protein™, USA Inc., Randeville, LA, USA

² Griffin Industries, Inc. Cold Springs, KY, USA

³ Solvent extracted, Producers Cop, Bryan, TX, USA

⁴ Omega Protein™, Reedville, VA, USA

⁵ United States Biochemical Company, Cleveland, OH, USA

⁶ g 100g⁻¹ Premix: cobalt chloride 0.004, cupric sulphate pentahydrate 0.250, ferros sulphate 4.0, magnesium sulphate heptahydrate 28.398, monohydrate 0.650, potassium iodide 0.067, sodium selenite 0.010, zinc sulphate heptahydrate 13.193, filler 53.428

⁷ g Kg⁻¹ Premix: Thiamin Hcl 0.5, riboflavin 3.0, pyridoxine HCL 1.0, DL Ca-pantothenate 5.0, nicotinc acid 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

⁸ Stay C®, (L-ascorbyl-2-polyphosphate 35% active C) Roche Vitamins Inc., Parsippany, NJ, USA

maintained at 27° C by a model c165-P2-HOC submersible heater (Clecco. Cleveland, OH, USA) in each sump. Photoperiod was maintained on an ambient light:dark cycle, a result from the system being housed in a greenhouse.

Juvenile red snapper, *L. Campechanus*, of the same cohort (74 days post-hatch) were acquired from environmentally manipulated natural spawns from brood fish at Claude Petet. Prior to the initiation of the trial, juvenile red snapper were graded to a uniform size, and twenty fish (mean weight 8.6 g) were stocked into each culture tank. A random sub-sample of fish was collected at initiation and frozen at -60° C for proximate analysis.

Four diets were 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. Daily ration was based on a percentage of the mean weight per treatment replicate.

The initial ration was offered at eight percent body weight, and was reduced as the fish grew. Routine system maintenance, such as siphoning of solids and partial water exchanges, were conducted as needed throughout the duration of the trial. System temperature, dissolved oxygen, salinity, and pH were monitored once daily using a YSI model 556 MPS (Yellow Springs, OH, USA). Nitrite-nitrogen was monitored weekly using a model PLN code test kit from LaMotte (Chestertown, MD, USA). Total ammonia-nitrogen was monitored twice weekly using the Nessler method (Boyd, 1979). The system was also treated with Marex brand chloroquine phosphate from Chemaqua (Oxnard, CA, USA) at 3.1 mg/l as a preventative measure against *Amyloodinium ocellateum*. Fish were counted, weighed, dipped in freshwater for 30 seconds, and culture tanks were scrubbed bi-weekly.

At the termination of the study, group weights, individual weights, and total lengths were taken for each tank. To minimize handling stress at the conclusion of the growth trial, fish were treated with 0.34 ml/l of Hypno brand dimethylketone alpha methyl quinoline (Jungle Lab Corp. Cibolo, TX, USA). Individual lengths and weights were taken to determine the length to weight ratio. Four fish from each treatment replicate were randomly selected, scaled and dissected to determine the hepatosomatic index ($HSI = \text{liver weight} \times 100 / \text{fish weight}$) and intraperitoneal fat ratio ($IPFR = \text{intraperitoneal fat weight} \times 100 / \text{fish weight}$). The fish and dissected material was pooled by tank, then homogenized and frozen to -60°C for later proximate analysis. Dry matter was determined by drying to a constant weight at 90°C . Protein content was determined by the micro-Kjeldahl method. Total lipid content was determined using methods described by Folch (1957). All analyses were conducted with triplicate sub-samples from each treatment replicate.

All data was subjected to a one way analysis of variance to determine significant differences between the treatment means. The Student-Neuman Keuls' multiple range test was used to distinguish significant differences among treatment means. All statistical analyses were conducted using the SAS system for windows, (version 8.0 SAS Institute, Cary, NC USA).

RESULTS AND DISCUSSION

The snapper in this study adapted readily to the culture system and experimental protocol. Water quality parameters (mean \pm standard deviation) were as follows; temperature, $26.29 \pm 1.02^\circ \text{C}$; salinity, $32.90 \pm 0.92 \text{ g/l}$; dissolved oxygen, $6.27 \pm 0.32 \text{ mg/l}$; total ammonia-nitrogen, $0.10 \pm 0.09 \text{ mg/l}$; nitrite-nitrogen, 0 mg/l ; pH, 7.95 ± 0.19 . Growth (figure 1) was relatively consistent throughout the course of the trial, from an initial mean weight of 8.6 g. At the conclusion of the trial there were no significant differences (Table 2) in weight gain, feed utilization (FCE), or percentage lipid based on whole body composition. There was, however, a significantly greater amount of body fat deposition (IPFR) in fish offered a ration containing 14% dietary lipid (Figure 2). Fish offered a diet containing 8-10% dietary lipid showed a greater percentage protein based on whole body composition than those offered 12-14% dietary lipid (Figure 3). The fish fed the treatment containing 10% dietary lipid exhibited a significantly greater ($P = 0.0224$) amount of protein based on a percentage of whole body composition.

Although evidence of a protein sparing effect was not seen in relation to increasing levels of dietary lipid, it has been documented in other species (Lee and Putnam, 1973; Machiels and Henken, 1985; Ellis and Reigh, 1991; Serrano *et al.*, 1992; Shiau and Peng, 1993; Thoman *et al.* 1999). One of the possible reasons for a lack of a treatment effect is the possibility that dietary protein was available in excess of the requirement. Similar trials conducted with juvenile red snapper indicate that this species has a relatively slow growth

Table 2. Response of juvenile red snapper (mean initial weight 8.6 g) offered diet treatments over a ten week growth trial¹

Diet	% weight gain ²	FCE ³	IPFR ⁴	Survival ⁵	PCE ⁶	% Protein ⁷	% Lipid ⁸
8% Lipid ¹⁰	716.13 ^a	78.17 ^a	2.31 ^b	98.33 ^a	13.45 ^a	47.75 ^{ab}	3.01 ^a
10% Lipid	676.44 ^a	76.97 ^a	2.10 ^b	100.00 ^a	14.98 ^a	53.09 ^a	4.05 ^a
12% Lipid	671.10 ^a	76.73 ^a	2.95 ^{ab}	98.33 ^a	12.66 ^a	46.58 ^{ab}	3.53 ^a
14% Lipid	683.0 ^a	77.23 ^a	3.43 ^a	98.33 ^a	12.80 ^a	43.79 ^b	4.72 ^a
PSE ⁹	0.9351	1.202	0.446	1.25	0.93	1.56	0.623

¹ Means of three replicates (P<0.05).

² % weight gain = (final mean weight - initial mean weight)/initial mean weight * 100.

³ FCE = (final mean weight - initial mean weight)/feed offered * 100.

⁴ IPFR = intraperitoneal fat weight * 100/fish weight.

⁵ (Final number fish per treatment/initial number of fish per treatment)*100

⁶ (Protein gain/protein fed)*100

⁷ % protein = % nitrogen * 6.25.

⁸ % lipid = [(lipid dry weight/sample wet weight)*100]* % dry matter.

⁹ Pooled standard error.

¹⁰ Diets formulated to be isonitrogenous (44% protein).

Figure 1. Growth of juvenile red snapper (mean initial weight 8.6 g) offered four isonitrogenous diets (44% protein) with varying levels of dietary lipid over a ten week growth trial

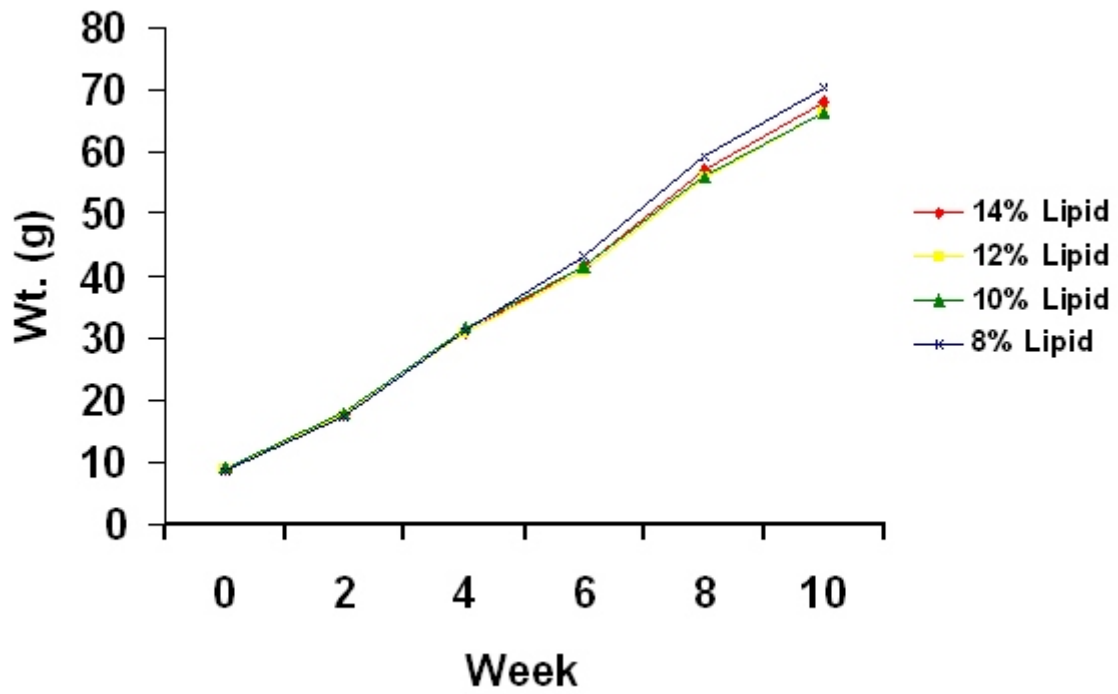


Figure 2. Intraperitoneal fat ratio of randomly sampled juvenile red snapper (mean initial weight 8.6 g) fed an isonitrogenous diet (44% protein) and four levels of dietary lipid.

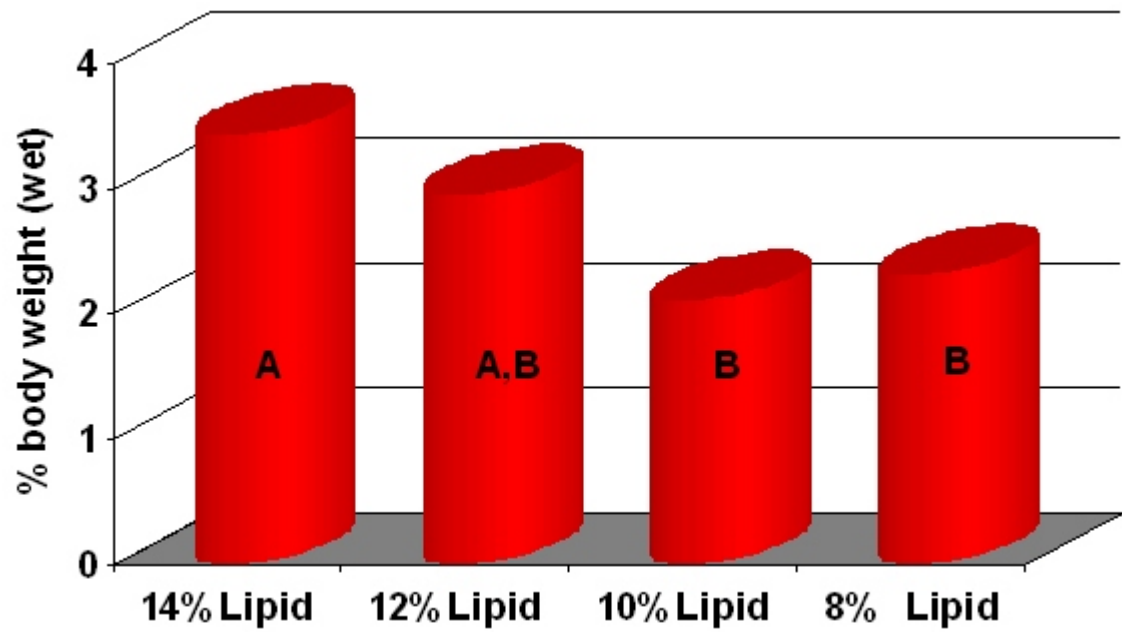
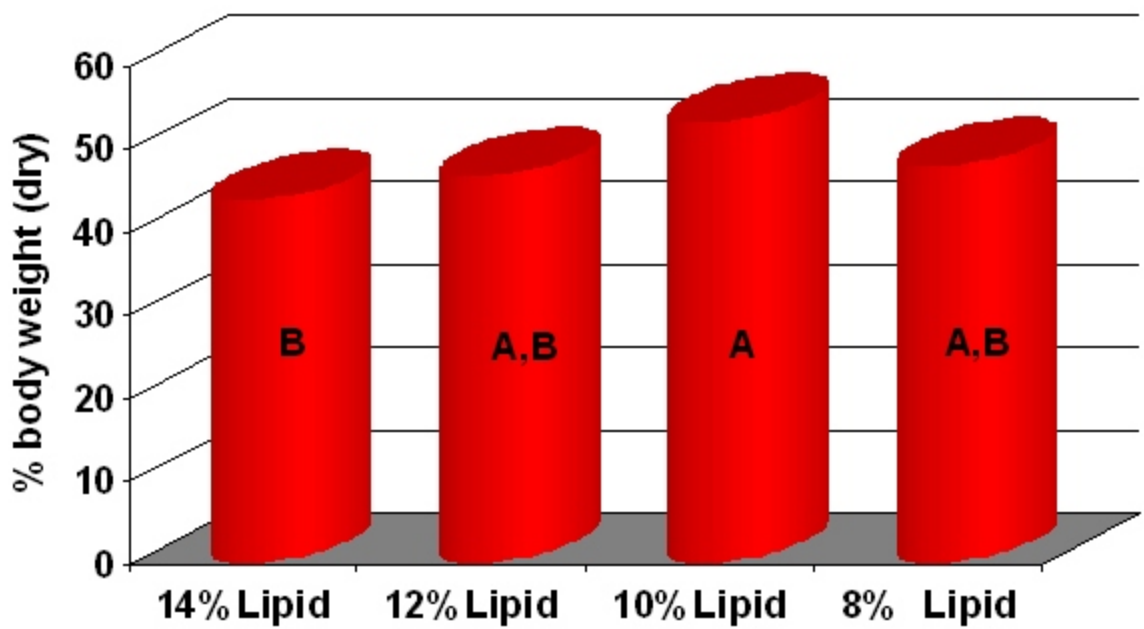


Figure 3. Percentage protein based on whole body composition of randomly sampled juvenile red snapper (mean initial weight 8.6 g) fed an isonitrogenous diet (44% protein) and four levels of dietary lipid



rate, and as a result dietary protein requirements for this species may be significantly less than previously thought.

The yellowtail snapper, *Ocyurus chrysurus*, exhibits a relatively slow growth rate similar to that of the red snapper. Turano (2000) indicated no significant differences in growth and FCE in yellowtail snapper offered rations ranging from 44-36% dietary protein, and recommended a protein requirement of 36% for optimum growth. Watanabe (2001) found that at a dietary protein level of 45%, juvenile mutton snapper displayed optimum 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 in diets containing 6-9% dietary lipid than in those including 12-15% lipid.

Levels of dietary protein and lipid were similar to those used by Watanabe (2001), and growth rates of juvenile red snapper were commensurate with those of yellowtail snapper (Turano 2000) and mutton snapper (Watanabe 2001). The inclusion of dietary lipid at a rate of 14% was also found to be in excess of nutritional requirements for juvenile red snapper. In the present experiment, there was little change in IPFR values at 8-10% lipid, and there were significantly greater rates ($P = 0.0552$) associated with juvenile red snapper offered 14% lipid diets. These diets may provide protein in amounts in excess of what is necessary to meet the nutritional demands of juvenile red snapper. Excess protein could then be used to meet to meet the energetic demands of the animal. Because protein was in excess of the requirement, and E:P ratios were relatively high, protein sparing in relation to increasing levels of dietary lipid may be not be apparent in this growth trial.

Under these experimental conditions, juvenile red snapper did not display improved growth, feed utilization (FCE), or proximate whole body composition at increased levels of dietary lipid. No palatability problems were observed, and feeding response was generally good irrespective of the treatment offered. There was minimal aggression observed among cohorts during feedings, and the ration was distributed equally throughout the culture tanks to minimize competition.

In conclusion, under these experimental conditions, juvenile red snapper did not require elevated levels of lipid. Fish offered treatments containing 8% dietary lipid displayed the best growth. Fish offered feed containing 10% dietary lipid exhibited a significantly higher amount of protein based on a percentage of whole body composition than those fish offered a diet containing 14% dietary lipid. Fish offered a ration containing 12-14% dietary lipid had elevated levels of body fat deposition (IPFR) compared to those sampled from the lower lipid treatments. Juvenile snapper showed no response in growth (% weight gain), feed utilization (FCE), liver weight (HSI), survival, or percentage lipid based on whole body composition relative to the level of dietary lipid.

The fish in this study displayed a growth rate at levels lower than previously thought, however corresponding with those found in similar studies with both with red snapper and other species of lutjanid snappers. Given the growth rate, and the lack of variation due to increasing amounts of dietary lipids, the possibility exists that the requirement of dietary protein needed to promote optimal growth in this species is lower than what was utilized in this study. At this level of dietary protein, 14% dietary lipid exceeds the energetic demands

for juvenile red snapper, and should probably remain in the 10% range in order to prevent large amounts of body fat deposition.

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