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Effects of Dietary Lipid Levels and Energy:Protein Ratios on Growth and Feed Utilization of Juvenile Nassau Grouper Fed Isonitrogenous Diets at Two Temperatures

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Abstract.—The growth and feed utilization of juvenile Nassau grouper *Epinephelus striatus* (mean weight = 2.12 g; $N = 9$) were compared for 75 d in laboratory aquaria supplied with flow-through seawater. Fish were fed four isonitrogenous (45% crude protein) diets differing in lipid content (6, 9, 12, or 15% dry weight) and energy:protein (E:P) ratio (28.4, 31.0, 33.5, or 36.0 kJ/g protein) at two temperatures (25°C and 30°C). Specific growth rates and final weights increased ($P < 0.001$) with decreasing dietary lipid and E:P ratios, from a minimum of 1.20% of body weight (bw) per day (5.21 g) for fish fed the 15% lipid diet to a maximum of 2.04% bw/d (9.31 g) for fish fed the 6% lipid diet. The specific growth rate was greater ($P < 0.01$) at 30°C (1.38–2.09%/d) than at 25°C (1.02–1.99%/d). Feed consumption (2.16–3.30% bw/d) was inversely correlated ($P < 0.001$) to dietary lipid content and E:P ratio and was greater ($P < 0.005$) at 30°C than at 25°C. Feed conversion ratio (1.91–2.74) was positively correlated and protein efficiency ratio (1.01–1.45) negatively correlated with dietary lipid content and E:P ratio ($P < 0.01$). Thus, for diets consisting of 45% protein, those with lipid contents of 6–9% produced greater feed consumption, efficiency, and growth of juvenile Nassau grouper than those with 12–15% lipids. Feed consumption and growth were greater at 30°C than at 25°C.

The Nassau grouper *Epinephelus striatus* is a commercially important food fish found in association with coral reefs and grass beds in shallow marine waters of the Caribbean, Bahamas, and southeastern United States. Nassau grouper form large aggregations during the annual spawning period at precise times and locations (Colin et al. 1987; Carter 1989; Colin 1992; Tucker et al. 1993), a characteristic that has led to overfishing of this species throughout the Caribbean region (Smith 1972; Olsen and LaPlace 1978; Colin 1992; Sadovy 1993).

High value and demand, along with declining natural populations, have stimulated interest in the Nassau grouper for aquaculture. Successful spawning of captive broodstock (Tucker et al. 1991; Watanabe et al. 1995a; Head et al. 1996) and culture of larvae through metamorphosis (Tucker and Jory 1991; Watanabe et al. 1996) have recently been achieved. The availability of hatchery-reared postlarval Nassau grouper has made possible the study of environmental (Ellis et al. 1997) and nutritional (Ellis et al. 1996) requirements of juveniles.

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Dietary lipids are the major source of energy among many carnivorous fish species, several of which have a limited ability to utilize high-molecular-weight carbohydrates (Watanabe 1981). Deficiencies of dietary lipid result in the utilization of protein, the single most costly ingredient in feeds (NRC 1983), as an energy source. Increasing the proportion of dietary lipids may allow protein to be used more efficiently for growth by sparing protein from use for energy (Cowey and Sargent 1979 ; NRC 1983; Tacon 1990; Tucker 1992), but excessive dietary lipid can reduce feed intake (Ellis et al. 1996) and growth. Because excessive dietary lipid can lead to fatty livers and flesh, which decreases palatability and dress-out percentage, optimizing the dietary lipid content is also important for maximum product quality.

Besides serving as an energy source, dietary lipids serve as carriers of lipid-soluble vitamins and provide essential fatty acids (EFAs), which play an integral role in the structure of cell membranes (NRC 1983). An EFA deficiency will therefore impair growth, feed conversion, and survival (Stickney 1979; Watanabe 1981; Tacon 1990).

Temperature is the most potent physical factor influencing metabolism in fish (Stickney and Andrews 1971; Phillips 1972; Brett 1979). Recent studies with Nassau grouper have shown that survival of yolk sac larvae (Watanabe et al. 1995b) and growth of juveniles (Ellis et al. 1997) are

TABLE 1.—Feed ingredients of four isonitrogenous, semipractical diets with different lipid levels and energy:protein ratios that were fed to juvenile Nassau grouper for 75 d at 25°C and 30°C; CP stands for crude protein, CL for crude lipids.

Ingredient	Diet (% lipid)			
	6	9	12	15
Herring meal (70% CP; 8% CL)	40.0	40.0	40.0	40.0
Wheat flour	20.0	20.0	20.0	20.0
Solka Floc-40	9.00	6.00	3.00	0.00
Soybean meal (48.5% CP)	5.00	5.00	5.00	5.00
Shrimp meal	5.00	5.00	5.00	5.00
Squid liver powder	5.00	5.00	5.00	5.00
Blood meal (85% CP)	3.50	3.50	3.50	3.50
Menhaden oil	0.00	3.00	6.00	9.00
Abernathy vitamin no. 2	3.00	3.00	3.00	3.00
Feather meal (80% CP; 8% CL)	3.00	3.00	3.00	3.00
Gluten (60% CP)	2.50	2.50	2.50	2.50
Brewer's yeast	1.00	1.00	1.00	1.00
Lecithin	1.00	1.00	1.00	1.00
Monosodium phosphate	1.00	1.00	1.00	1.00
Choline	0.40	0.40	0.40	0.40
Stay-C dry (15%)	0.40	0.40	0.40	0.40
USFW[a] no. 3 minerals	0.20	0.20	0.20	0.20

[a] U.S. Fish and Wildlife Service.

markedly influenced by temperature within an ecological range of 22–31°C.

A preliminary study comparing growth of juvenile Nassau grouper on various practical diets formulated for carnivorous finfish species (Ellis et al. 1997) provided general indications of the protein requirements of this species. The objectives of the present study were to determine the dietary lipid and energy:protein (E:P) ratio requirements of juvenile Nassau grouper fed an isonitrogenous diet and how temperature influenced these requirements.

Methods

This experiment was conducted at the Caribbean Marine Research Center (CMRC) on Lee Stocking Island, Exuma Cays, Bahamas, during May through August 1995. Hatchery-reared juvenile Nassau grouper (~80 d after hatching) originated from hormone-induced spawning of captive broodstock (Watanabe et al. 1995a, 1996). Fish were reared in a flow-through system at ambient seawater temperature (25–28°C) and fed a commercial diet (Nippai; Dainichi Corp., Uwajima, Ehime, Japan) containing 45% crude protein and 10% crude lipid before the study.

Experimental units consisted of twenty-four 145-L aquaria (1.2 m long × 0.3 m wide × 0.5-m high) situated in a controlled-environment laboratory. Aquaria were supplied with flow-through seawater (salinity 36–38 ppt) at a rate of 0.1 L/min, providing approximately one complete exchange of water per day. Aeration to each tank was supplied by a central blower through silica glass diffusers. Three PVC pipe sections (20.3 cm long × 7.6 cm in diameter) were placed in each tank to provide shelter and to minimize aggression between the fish. A 12-h light : 12-h dark photoperiod was maintained by using overhead fluorescent lighting from 0630 to 1830 hours. Temperatures were maintained at 25°C and 30°C by using 500-W immersion heaters to heat water in the presence of an ambient room temperature that was maintained at 21°C.

A 4 × 2 factorial experiment was used to compare growth of juveniles fed four isonitrogenous (45% crude protein) diets that differed in lipid content (6, 9, 12 and 15% dry weight) at temperatures of 25°C and 30°C. A protein content of 45% was selected as a minimum requirement for carnivorous finfish species, such as salmonids (Tacon 1990). Three replicate aquaria were maintained for each treatment. Growth was monitored for 75 d.

Fish were fed semipractical pelleted diets formulated and prepared by a commercial supplier (Zeigler Brothers Inc., Gardners, Pennsylvania); the approximate compositions of the diets are given in Tables 1 and 2. Experimental diets were prepared with a blender, sieved to achieve appropriate particle size (between 1.0 and 2.0 mm), and placed into plastic bags. Diets were then stored frozen until fed. The protein sources were herring meal, shrimp meal, squid liver powder, soybean meal, and blood meal. Animal protein made up 83.8%

TABLE 2.—Proximate composition of four isonitrogenous, semipractical diets with different lipid levels and energy: protein ratios that were fed to juvenile Nassau grouper for 75 d at 25°C and 30°C. The protein, lipid, ash, and fiber values are for dry matter.

Ingredient	Nutritional composition (%)			
Moisture	8.77	8.65	8.53	8.41
Crude protein	44.9	44.9	44.9	44.9
Crude lipids	6.13	9.13	12.1	15.1
Crude ash	8.00	7.99	7.98	7.97
Crude fiber	10.3	7.53	4.72	1.91
Nitrogen-free extract[a]	30.6	30.5	30.3	30.1
Gross energy (kJ/g diet)[b]	15.2	16.3	17.4	18.5
Energy:protein ratio (kJ/g)	33.9	36.3	38.8	41.2

[a] Calculated as 100 minus the percentages for the previous four ingredients.
[b] Calculated using physiological fuel values (Phillips 1972).

of the total protein in each diet. Lipid content was increased by adjusting the ratios of menhaden oil and a nonnutritive cellulose filler in each diet. The E:P ratios (kilojoules of gross energy per gram of crude protein; kJ/g protein) of the diets with lipid contents of 6, 9, 12, and 15% were calculated and were 28.4, 31.0, 33.5, and 36.0, respectively.

To begin the study, we stocked each of the 24 aquaria with nine juvenile Nassau grouper. The initial weight (and standard length) of the juveniles averaged 2.12 g (40.5 mm) and the coefficient of variation (SD/mean) of the initial weights (lengths) averaged 0.14 (0.4), with no significant ($P > 0.05$) differences observed among treatment groups (Table 3). Fish were stocked at 28°C and acclimated to the experimental temperatures at a rate of 1°C/d.

Fish were fed twice daily (at 0715 and 1515 hours) to apparent satiation. At each feeding, the amount of feed provided was such that a slight excess remained after 10 min but was all consumed within 20 min. The feeding rate was adjusted daily according to this observation. The tank ration was increased by 0.5% of tank biomass if the entire ration was consumed within 10 min at both daily feedings; conversely, if excess feed was observed at both daily feedings, tank ration was decreased by 0.5% of the tank biomass. The tank ration was not changed if excess feed was observed at only one of the daily feedings. To monitor growth, we weighed and measured the fish (standard length) individually with the aid of an anesthetic (75 mg/L MS-222) on day 0 (3 d after stocking) and on day 75, when the experiment was terminated. Fish from each replicate aquaria were weighed as a group on days 15, 30, 45, and 60 to avoid excessive handling stress, which can lead to increased mortality and reduced growth.

Water temperature, measured twice daily, averaged 25.0 ± 0.2°C and 30.0 ± 0.3°C for the two treatments used. Salinity (36–38 ppt), dissolved oxygen (5.7–6.3 mg/L) and pH (7.9–8.2) were measured daily; total ammonia nitrogen (0–0.20 mg/L) was measured weekly. Each of the aquaria was siphoned daily to remove fecal material and uneaten feed.

Relative growth rate (RGR; percent increase in weight) was calculated as [(wet final weight − wet initial weight)/(wet initial weight)] × 100. Specific growth rate (SGR; percent increase in body weight per day) was calculated as $\{[\log_e(\text{wet final weight}) - \log_e(\text{wet initial weight})]\text{time in days}\} \times 100$. Daily weight gain (DWG), in grams, was calculated as (wet final weight − wet initial weight)/time in days.

Feed consumption (FC) for a sampling interval (15 d) was calculated as a percentage of the average daily biomass during the interval; that is, FC = dry weight of feed eaten/average fish weight. Consumption for the entire experiment was calculated as the average over all intervals. Feed conversion ratio (FCR) was calculated as dry weight of feed/wet weight gain. Protein efficiency ratio (PER) was calculated as wet weight gain/dry weight protein fed.

Treatment means were compared by a two-way analysis of covariance (ANCOVA), with survival as a covariate, except for FC, which was analyzed using a standard two-way analysis of variance (ANOVA; Sokal and Rohlf 1995). When an ANOVA or ANCOVA result was significant, differences between treatment means were analyzed for significance ($P < 0.05$) by the Ryan−Einot−Gabriel−Welsch multiple range (Day and Quinn 1989). Regression equations were calculated to relate growth and feed utilization parameters to a diet's lipid content and E:P ratio.

Results

Growth rates were similar among treatments through day 30 (Figure 1) but showed differential

TABLE 3.—Initial and final weights, coefficient of variation (CV = SD/mean) of initial and final weights, relative growth rates (RGR), specific growth rates (SGR), and survival of juvenile Nassau grouper fed four isonitrogenous (45% protein) diets with different lipid levels and energy:protein ratios at 25°C and 30°C. Values are means ± SDs ($N = 3$).

Variable	Lipids (%) + energy:protein ratio					
	6.0% + 28.4		9.0% + 31.0		12.0% + 33.5	
	25°C	30°C	25°C	30°C	25°C	30°C
Initial weight (g)	1.96 ± 0.10	2.04 ± 0.39	2.10 ± 0.12	2.31 ± 0.21	2.05 ± 0.24	2.26 ± 0.60
CV, initial weight	0.19 ± 0.03	0.12 ± 0.01	0.13 ± 0.01	0.14 ± 0.04	0.12 ± 0.05	0.12 ± 0.04
Final weight (g)	8.85 ± 2.02	9.76 ± 1.76	6.90 ± 0.67	10.32 ± 2.90	5.15 ± 0.58	7.48 ± 4.73
CV, final weight	0.65 ± 0.07	0.61 ± 0.10	0.58 ± 0.26	0.74 ± 0.04	0.54 ± 0.02	0.51 ± 0.19
RGR (%)	351.0 ± 93.8	392.4 ± 134.9	228.9 ± 32.7	349.0 ± 134.7	151.1 ± 3.9	219.2 ± 146.6
SGR (%)	1.99 ± 0.28	2.09 ± 0.41	1.58 ± 0.13	1.96 ± 0.43	1.23 ± 0.02	1.46 ± 0.57
Survival (%)	55.6 ± 7	77.8 ± 11	82.0 ± 3	59.3 ± 21	82.0 ± 3	67.7 ± 17

growth trends thereafter. Significant differences in body weight among treatments were evident from day 45. By day 75, final weights ranged from 5.05 to 10.32 g, and standard lengths ranged from 53.7 to 65.0 mm among treatments (Table 2) and generally increased with decreasing dietary lipid content and E:P ratios and with increasing temperature. On day 75, significant effects of dietary lipid ($P < 0.01$) and temperature ($P < 0.05$) on body weight and length were also observed, but there was no significant interaction between these effects.

The RGR increased with decreasing dietary lipid content and E:P ratio and with increasing temperature from a minimum of 115.5% in fish fed the 15% lipid diet at 25°C to a maximum of 392.4% in fish fed the 6% lipid diet at 30°C (Table 3). Significant effects of both dietary lipid ($P < 0.005$) and temperature ($P < 0.05$) on RGR were observed, but there was no significant interaction between these effects.

The SGR increased with decreasing dietary lipid content and E:P ratio and with increasing temperature from a minimum of 1.02% bw/d in fish fed the 15% lipid diet at 25°C to a maximum of 2.09% bw/d in fish fed the 6% lipid diet at 30°C (Table 3). Significant effects of both dietary lipid ($P < 0.001$) and temperature ($P < 0.01$) on SGR were observed, but there was no interaction between these effects. Growth rates (RGR, SGR, DWG) were negatively correlated ($P < 0.001$) to dietary lipid content and to E:P ratio (Table 4).

Survival of fish in all treatments averaged 74% and did not differ significantly among treatments (Table 3). Cannibalism was observed in all treatments and probably accounted for approximately 70% of all mortalities, given that escape was prevented by a tank lid and screened drain and that the fish appeared to be in robust health throughout the study.

The consumption of feed (FC) generally increased with decreasing dietary lipid content and increasing temperature from a minimum of 2.16% bw/d in fish fed the 15% lipid diet at 25°C to a maximum of 3.30% bw/d in fish fed the 6% lipid diet at 30°C (Table 5). Highly significant effects of both dietary lipid ($P < 0.001$) and temperature ($P < 0.005$) on FC were observed, but there was no interaction between these effects. At both temperatures, FC was negatively correlated ($P < 0.05$) with dietary lipid content and E:P ratios.

The FCR decreased with decreasing dietary lipid and E:P ratio from a maximum of 2.97 in fish fed the 15% lipid diet at 25°C to a minimum of 1.82 in fish fed the 6% lipid diet at 30°C (Table 5). No significant effects of dietary lipid or temperature on FCR were observed, and there was no interaction between these effects. At 25°C, FCR was positively correlated ($P < 0.05$) with dietary lipid content and E:P ratios, whereas at 30°C, no significant correlation was evident (Table 4).

The PER generally increased with decreasing dietary lipid content and E:P ratio and ranged from 0.99 in fish fed the 15% lipid diet at 25°C to 1.45 in fish fed the 9% lipid diet at 30°C (Table 4). A significant ($P < 0.01$) effect of dietary lipid on PER was observed, but the effect of temperature was not significant, and there was no interaction between these effects. At both temperatures, PER was negatively correlated ($P < 0.005$) with dietary lipid and E:P ratios (Table 4).

Discussion

Growth rates of juvenile Nassau grouper fed isonitrogenous (45% protein) diets were markedly affected by dietary lipid contents within the range of 6–15% (E:P ratios 28.4–36.0 kJ/g), the greatest growth being observed at the lower lipid values

TABLE 3.—Extended.

Variable	Lipids (%) + energy:protein ratio 15.0% + 36.0	
	25°C	30°C
Initial weight (g)	2.35 ± 0.18	1.91 ± 0.09
CV, initial weight	0.16 ± 0.00	0.13 ± 0.02
Final weight (g)	5.05 ± 0.51	5.38 ± 0.52
CV, final weight	0.47 ± 0.06	0.48 ± 0.06
RGR (%)	115.5 ± 15.8	182.0 ± 32.0
SGR (%)	1.02 ± 0.10	1.38 ± 0.15
Survival (%)	88.9 ± 6	77.8 ± 6

(6–9%) and E:P ratios (28.4–31.0 kJ/g). These results support a preliminary study (Ellis et al. 1996) with juvenile Nassau grouper in which practical diets with E:P ratios of 28.3–28.9 kJ/g protein were found superior to diets containing 32.0–35.3 kJ/g protein. The results are also similar to those reported for the estuary grouper *E. salmoides*, in which E:P ratios of 27.3–29.3 kJ/g were optimal (Lim 1985).

The FC decreased with increasing dietary lipid content and E:P ratio. This suppression of appetite by high dietary lipid has also been demonstrated in other marine finfish, including sea bass *Dicentrarchus labrax* (Hidalgo and Alliot 1988), red drum *Sciaenops ocellatus* (Williams and Robinson 1988), and Nassau grouper (Ellis et al. 1996). Lower FC by fish fed the higher lipid content (12% and 15%) diets resulted in slower growth, probably related to an insufficient ingestion of protein and a decreased efficiency of protein use, consistent with the trend toward lower PER with increasing lipid content. High amounts of dietary lipid and energy are apparently unnecessary for the Nassau grouper, which is a sedentary species.

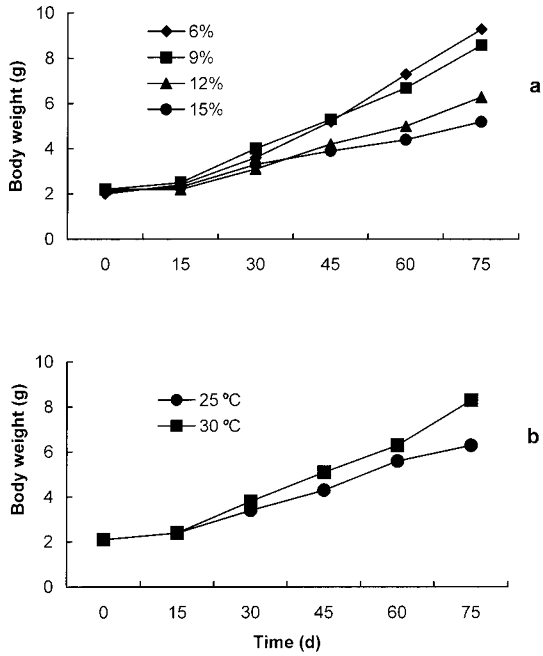

FIGURE 1.—Growth of juvenile Nassau grouper fed four isonitrogenous (45% crude protein), semipractical diets with different crude lipid content (6, 9, 12, or 15%) and E:P ratio (28.4, 31.0, 33.5, or 36.0 kJ/g protein) at 25°C and 30°C. Panel **(a)** shows growth at different lipid values averaged across both temperatures, panel **(b)** growth at both temperatures averaged across the four dietary lipid values. Plotted symbols represent means (n = 6 in panel [a] and n = 12 in panel [b]).

Although not statistically significant, FCR appeared to decrease with dietary lipid content and E:P ratios, the best conversion ratios (1.91–1.94)

TABLE 4.—Linear regressions of dietary lipids as a percentage of diet and energy:protein ratios (E:P; kJ/g protein) on relative growth rate (RGR), specific growth rate (SGR), feed conversion ratio (FCR), protein efficiency ratio (PER), and feed consumption (FC; % body weight per day). For RGR, SGR, and FC, regression analyses were performed separately for each temperature (25°C and 30°C); those for FCR and PER were based on data for both temperatures combined. Each regression was based on 12 observations except FCR and PER, which were based on 24 observations; $P < 0.05$*, $P < 0.005$**, $P < 0.001$***, $P < 0.0001$****.

Dependent variable	Dietary lipid		E:P ratio	
	Equation	r^2	Equation	r^2
RGR (25°C)	−26.15L + 486.20	0.76***	−31.11(E:P) + 1,213.87	0.76****
RGR (30°C)	−25.37L + 552.03	0.36*	−30.14(E:P) + 1,256.73	0.36*
SGR (25°C)	−0.109L + 2.596	0.85***	−0.129(E:P) + 5.616	0.85***
SGR (30°C)	−0.088L + 2.641	0.35*	−0.104(E:P) + 5.075	0.35*
FC (25°C)	−0.113L + 3.758	0.79****	−0.134(E:P) + 6.894	0.79****
FC (30°C)	−0.052L + 3.487	0.32*	−0.623(E:P) + 4.94	0.33*
FCR	0.094L + 1.235	0.29****	0.112(E:P) − 1.389	0.29**
PER	−0.045L + 1.708	0.29**	−0.053(E:P) + 2.949	0.29**

TABLE 5.—Feed consumption (FC), feed conversion ratios (FCR), and protein efficiency ratios (PER) of juvenile Nassau grouper fed four isonitrogenous (45% protein) diets with varying lipid levels and energy:protein ratios at 25°C and 30°C. Values are means ± SEs (N = 3). Diet costs ($/kg of feed) and feed costs per unit fish weight are also shown.

	Lipids (%) + energy:protein ratio					
	6.0% + 28.4		9.0% + 31.0		12.0% + 33.5	
Variable	25°C	30°C	25°C	30°C	25°C	30°C
FC (% body weight/d)	3.18 ± 0.24	3.30 ± 0.38	2.63 ± 0.22	2.82 ± 0.10	2.32 ± 0.03	2.88 ± 0.11
FCR	2.01 ± 0.48	1.82 ± 0.20	1.86 ± 0.29	2.02 ± 0.43	2.16 ± 0.24	2.43 ± 0.69
PER	1.31 ± 0.23	1.39 ± 0.20	1.32 ± 0.22	1.58 ± 0.31	1.21 ± 0.13	1.06 ± 0.35
Diet cost	1.75	1.75	1.70	1.70	1.66	1.66
Cost per unit fish weight[a]	3.52	3.19	3.16	3.43	3.59	4.03

[a] Computed as diet cost × feed conversion ratio.

occurring in fish fed the 6% and 9% lipid diets. The FCRs in the present study are greater than that (FCR = 0.94) reported earlier for juvenile Nassau grouper fed a commercially available diet containing 8% lipid and 55.6% protein (Ellis et al. 1996). The 45% dietary protein level used in this study may therefore have been suboptimal.

Growth rates for juvenile Nassau grouper were greater at 30°C than at 25°C, probably as a result of the increased FC at 30°C, because neither FCR nor PER values differed significantly between temperatures. These results are consistent with an earlier study in which increased growth rates were observed with increasing temperature over a range of 22–31°C (Ellis et al. 1997).

In the present study, FC was greater at 30°C than at 25°C, probably reflecting an increased metabolic rate at the higher temperature, a phenomenon reported in many carnivorous fish species (Hidalgo et al. 1987; Woiwode and Adelman 1991; Ellis et al. 1997). However, temperature had no significant effect on either FCR or PER. This is in agreement with results of the earlier study of juvenile Nassau grouper (Ellis et al. 1997) in which temperatures within the range of 22–31°C did not affect FCR.

In this study, mortalities were caused primarily by cannibalism, a phenomenon well documented in serranid fishes, including estuary grouper (Chua and Teng 1980), black-spotted grouper (Chen and Tsai 1994), and greasy grouper (Chen 1979). Because cannibalism mortalities primarily removes the smaller individuals, the result is an artificial increase in the average weight (within an aquarium or within a treatment group). In addition, cannibalism effectively adds high-quality protein to the diet of the surviving individuals. Cannibalism was probably promoted in this study by large size variation (Hecht and Pienaar 1993) within replicate tanks at the time of stocking (Table 2).

Costs of the semipractical diets used in this study increased with decreases in the dietary lipid content from $1.62 for the 15% lipid diet to $1.75 for the 6% lipid diet (Table 5). Despite this trend, however, feed costs per kilogram of fish produced were generally lower for the 6% and 9% lipid diets (range = $3.16 to $3.52/kg) than for the 12% and 15% lipid diets ($3.59 to $4.81/kg; Table 5). Costs of low-lipid diets will probably be less than this in practical diets, which would omit expensive, nonnutritive fillers. However, these values must be interpreted with caution because we did not analyze body composition of the fish, and their visceral weight is unknown.

In conclusion, feed conversion and growth of juvenile Nassau grouper fed a diet containing 45% protein and 6% to 15% lipid (E:P ratios of 28.4–36.0 kJ/g) were greater at the lower dietary lipid values (6–9%; E:P ratios of 28.4–31.0 kJ/g). Feed costs per kilogram of fish produced were also lowest for the 6% and 9% lipid diets. Growth rates and FC were greater at 30°C than at 25°C, but no differences in food efficiency were observed. To further improve growth and feed costs, studies are needed to determine the optimal protein proportion at a constant E:P ratio of 28.4–31.0 kJ/g.

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TABLE 5.—Extended.

Variable	Lipids (%) + energy:protein ratio 15.0% + 36.0 25°C	30°C
FC (% body weight/d)	2.16 ± 0.20	2.75 ± 0.20
FCR	2.97 ± 0.87	2.51 ± 0.50
PER	0.99 ± 0.14	1.03 ± 0.08
Diet cost	1.62	1.62
Cost per unit fish weight[a]	4.81	4.07

References

Brett, J. R. 1979. Environmental factors and growth. Pages 599–675 *in* W. S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, volume 8. Academic Press, New York.

Carter, J. 1989. Grouper sex in Belize. Natural History 98 (10):60–69.

Chen, F. Y. 1979. Progress and problems of net cage culture of grouper (*Epinephelus tauvina*) in Singapore. Proceedings of the World Mariculture Society 10:260–271.

Chen, H., and J. Tsai. 1994. Optimal dietary protein level for the growth of juvenile grouper, *Epinephelus malabaricus*, fed semipurified diets. Aquaculture 119:265–271.

Chua, T. E., and S. K. Teng. 1980. Economic production of estuary groupers, *Epinephelus salmoides*, reared in floating net cages. Aquaculture 20:187–228.

Colin, P. L. 1992. Reproduction of the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae) and its relationship to environmental conditions. Environmental Biology of Fishes 34:357–377.

Colin, P. L., D. Y. Shapiro, and D. Weiler. 1987. Aspects of the reproduction of two groupers, *Epinephelus guttatus* and *E. striatus* in the West Indies. Bulletin of Marine Science 40:221–230.

Cowey, C. B., and J. R. Sargent. 1979. Fish nutrition. Pages 1–69 *in* W. S. Hoar, D. J. Randall, and J. R. Brett, editors. Fish physiology, volume 8. Academic Press, New York.

Day, R. W., and G. P. Quinn. 1989. Comparisons of treatments after an analysis of variance in biology. Ecological Monographs 59:433–463.

Ellis, S. C., G. Viala, and W. O. Watanabe. 1996. Growth and feed utilization of hatchery-reared juvenile Nassau grouper fed four practical diets. Progressive Fish-Culturist 58:167–172.

Ellis, S. C., W. O. Watanabe, and E. P. Ellis. 1997. Temperature effects on feed utilization and growth of post-settlement stage Nassau grouper (*Epinephelus striatus*). Transactions of the American Fisheries Society 126:309–315.

Head, W. D., W. O. Watanabe, S. C. Ellis, and E. P. Ellis. 1996. Hormone-induced multiple spawning of captive Nassau grouper broodstock. Progressive Fish-Culturist 58:65–69.

Hecht, T., and A. G. Pienaar. 1993. A review of cannibalism and its implications in fish larviculture. Journal of the World Aquaculture Society 24:247–261.

Hidalgo, F., and E. Alliot. 1988. Influence of water temperature on protein requirement and protein utilization in juvenile sea bass, *Dicentrarchus labrax*. Aquaculture 72:115–129.

Hidalgo, F., E. Alliot, and H. Thebault. 1987. Influence of water temperature on food intake, food efficiency and gross composition of juvenile sea bass, *Dicentrarchus labrax*. Aquaculture 64:199–207.

Lim, P. E. 1985. Prepared diets in cultured groupers. Pages 381–400 *in* Proceedings of the 2nd international conference on warm water aquaculture—finfish. Brigham Young University, Laie, Hawaii.

NRC (National Research Council). 1983. Nutrient requirements of warmwater fishes and shellfishes. National Academy Press, Washington, D.C.

Olsen, D. A., and J. A. LaPlace. 1978. A study of a Virgin Islands grouper fishery based on a breeding aggregation. Proceedings of the Gulf and Caribbean Fisheries Institute 131:130–144.

Phillips, A. M., Jr. 1972. Calorie and energy requirement. Pages 1–28 *in* J. E. Halver, editor. Fish nutrition. Academic Press, New York.

Sadovy, Y. 1993. The Nassau grouper, endangered or just unlucky? Reef Encounter: Newsletter of the International Society for Reef Studies 13:10–12.

Smith, C. L. 1972. A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). Transactions of the American Fisheries Society 101:257–261.

Sokal, R. R., and F. J. Rohlf. 1995. Biometry. Freeman, San Francisco.

Stickney, R. R. 1979. Principles of warmwater aquaculture. Wiley, New York.

Stickney, R. R., and J. W. Andrews. 1971. Combined effects of dietary lipids and environmental temperature on growth metabolism and body composition of channel catfish (*Ictalurus punctatus*). Journal of Nutrition 101:1703–1710.

Tacon, A. G. J. 1990. Standard methods for the nutrition and feeding of farmed fish and shrimp. Argent Laboratories Press, Redmond, Washington.

Tucker, J. W., Jr. 1992. Marine fish nutrition. Pages 25–40 *in* G. L. Allan and W. Dall, editors. Proceeding of the aquaculture nutrition workshop. NSW Fisheries Brackish Water Fish Culture Research Station, Salamander Bay, Australia.

Tucker, J. W., Jr., P. G. Bush, and S. T. Slaybaugh. 1993. Reproductive patterns of Cayman Islands Nassau grouper (*Epinephelus striatus*) populations. Bulletin of Marine Science 52:961–969.

Tucker, J. W., Jr., and D. E. Jory. 1991. Marine fish culture in the Caribbean. World Aquaculture 22:10–27.

Tucker, J. W., Jr., J. E. Parsons, G. C. Ebanks, and P. G. Bush. 1991. Induced spawning of Nassau grouper *Epinephelus striatus*. Journal of the World Aquaculture Society 22:187–191.

Watanabe, T. 1981. Lipid nutrition in fish. Comparative Biochemistry and Physiology 73B:3–15.

Watanabe, W. O., S. C. Ellis, E. P. Ellis, W. D. Head,

C. D. Kelley, A. Moriwake, C.-S Lee, and P. K. Bienfang 1995a. Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) broodstock by hormone induction. Aquaculture 138:205–219.

Watanabe, W. O., C. Lee, S. C. Ellis, and E. P. Ellis. 1995b. Hatchery study of the effects of temperature on eggs and yolksac larvae of the Nassau grouper *Epinephelus striatus*. Aquaculture 136:141–147.

Watanabe, W. O., S. C. Ellis, E. P. Ellis, V. Gracia-Lopez, P. Bass, J. Ginoza, and A. Moriwake 1996. Evaluation of first-feeding regimens for larval Nassau grouper (*Epinephelus striatus*) and preliminary pilot-scale culture through metamorphosis. Journal of the World Aquaculture Society 27:323–331.

Williams, C. D., and E. H. Robinson. 1988. Response of red drum to various dietary levels of menhaden oil. Aquaculture 70:107–120.

Woiwode, J. G., and I. R. Adelman. 1991. Effects of temperature, photoperiod, and ration size on growth of hybrid striped bass x white bass. Transactions of the American Fisheries Society 120: 217–229.