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Growth and Feed Utilization of Hatchery-Reared Juvenile Nassau Grouper Fed Four Practical Diets

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Abstract.—The effects of four practical diets, formulated for carnivorous marine finfish, on growth, feed utilization, and body composition of hatchery-reared juvenile Nassau grouper *Epinephelus striatus* (mean weight ± SD = 2.37 ± 0.75 g) were compared for 56 d in 145-L flow-through seawater (36–38 ‰) aquaria. Diets differed in crude protein (CP) and crude lipid (CL) content as follows: low CP (43.5%), low CL (5.91%) trout grower (LP–LL); midlevel CP (52.7%), high CL (15.2%) salmon grower (MP–HL); midlevel CP (55.6%), low CL (7.79%) Japanese formulation for carnivorous fish (MP–LL); and a high CP (61.8%), high CL (14.2%) research diet for dolphin (mahimahi) *Coryphaena hippurus* (HP–HL). Energy : protein ratios (E:P) of these diets were 35.2, 32.0, 28.3 and 28.9 kJ/g, respectively. Relative growth rate (RGR, % increase in weight) and specific growth rate (SGR, % increase in body weight/day) were strongly correlated ($P < 0.0005$) to dietary CP and E:P ratio and were higher ($P < 0.05$) for fish fed the MP–LL and HP–HL diets (RGR = 454–502%; SGR = 3.05–3.19%/d) than in the LP–LL and MP–HL diets (RGR = 68.6–160%; SGR = 0.93–1.71%). Feed utilization, including conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein retention (ANPR), and apparent net energy retention (ANER) were also highly correlated ($P < 0.0005$) to dietary CP and E:P ratio. These parameters were significantly ($P < 0.05$) higher in the MP–LL diet than in the other diets. No clear relationships between dietary nutrient levels and proximate body composition were observed. Results indicated that cultured juvenile Nassau grouper require a dietary protein level above 55% and an E:P ratio below 28.9 kJ/g for optimum growth.

The Nassau grouper *Epinephelus striatus* is a food fish of high market value in Caribbean countries. This species forms large spawning aggregations, which have been overfished (Colin 1992). Declining natural populations have stimulated interest in the Nassau grouper as a candidate for commercial cultivation or stock enhancement.

Previous work on the culture of Nassau grouper has focused on developing methods for induced spawning (Tucker et al. 1991; Watanabe et al. 1995b; Head et al. 1996) and larval rearing (Watanabe et al. 1995a). Recently, successful rearing of significant numbers of larval Nassau grouper through metamorphosis (W. O. Watanabe, unpublished data) has made experimental culture of juvenile stages possible.

Wild juvenile Nassau grouper are known to be opportunistic carnivores, eating mainly crustaceans and fish (Randall 1967). Cultured groupers are fed primarily trash fish (Chen 1979; Chua and Teng 1980) but accept diets formulated for other species (Teng et al. 1978; Tucker 1992). Little or no information is available on the nutritional requirements of juvenile Nassau grouper as a basis for developing cost-effective feeds. In this study, growth, feed utilization, and body composition of juvenile Nassau grouper hatchery reared on four practical diets are compared. The diets, formulated for carnivorous marine finfish species, had different protein and lipid levels.

Methods

This study was conducted at the Caribbean Marine Research Center on Lee Stocking Island, Exuma Cays, Bahamas, from July through August 1993. Approximately 350 juvenile Nassau grouper of the same cohort (127-d posthatch), produced at the research center's finfish hatchery on the island, were used for the study. Growth experiments were conducted in 145-L indoor glass aquaria (1.2 m long × 0.3 m wide × 0.5 m deep) that were covered on the backs and on both sides by black plastic. Aquaria were supplied with flow-through seawater (salinity, 36–38‰) at a rate of 1 L/min (9.9 exchanges/d) and diffused aeration. Ambient light through laboratory windows provided a natural photoperiod of approximately 12 h light:12 h dark. Supplementary lighting from overhead fluorescent sources was also provided from 0800 to 1700 hours.

Growth, feed utilization, and body composition of juveniles were compared for 56 d on four diets

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TABLE 1.—Proximate composition (% dry matter), gross energy, and energy to protein ratio of experimental diets.

Component or characteristic	Diet[a]			
	Trout grower (LP–LL)	Salmon grower (MP–HL)	Carnivorous fish diet (MP–LL)	Mahimahi research diet (HP–HL)
Moisture (%)	8.94	8.97	7.58	7.56
Crude protein (CP, %)	43.5	52.7	55.6	61.8
Crude lipid (CL, %)	5.91	15.2	7.79	14.2
Crude ash (CA, %)	15.9	18.4	15 9	11.0
NFE[b] + fiber (%)	34.8	13.8	20.7	12.9
Gross energy (kJ/g diet)[c]	15.3	16.8	15.7	17.8
Energy : protein ratio (kJ/g protein)	35.2	32.0	28.3	28.9

[a] Diet symbols are low (LP), medium (MP), and high (HP) protein and low (LL) and high (HL) lipid.
[b] Nitrogen free extract; calculated by subtraction (100 − [CP + CL + CA]).
[c] Gross energy determined by using physiological fuel values (Phillips 1972).

formulated for carnivorous marine finfish. These diets were obtained from commercial or research sources and varied in crude protein (CP) and crude lipid (CL) content as follows (Table 1): low CP (43.5%), low CL (5.91%) trout grower (LP–LL; Zeigler Brothers, Inc., Gardners, Pennsylvania); midlevel CP (52.7%), high CL (15.2%) salmon grower (MP–HL; Rangen, Inc., Buhl, Idaho); midlevel CP (55.6%), low CL (7.79%) carnivorous fish diet (MP–LL; Dainichi Corp., Uwajima, Ehime, Japan); and high CP (61.8%), high CL (14.2%) research diet for dolphin (mahimahi) *Coryphaena hippurus* (HP–HL; Oceanic Institute, Honolulu, Hawaii). All feeds were nonextruded sinking products; particle diameters ranged from 2.2 to 4 mm; and storage was at 5°C. In the LP–LL diet, 70% of the protein was of animal origin (i.e., fish meal, hydrolyzed feather meal, and blood meal); in the remaining three diets, more than 90% of the protein was from animal sources. Fish were fed the LP–LL diet prior to the start of the study.

In all, 24 aquaria were stocked with 14 fish each; there were 6 replicate aquaria per treatment. Initial weights and standard lengths (means, 2.37 g and 4.41 mm, respectively) did not differ significantly ($P > 0.05$) among treatment groups (Table 2). From day 0, fish were hand fed the treatment diet twice daily (0815 and 1515 hours) to satiation. The daily ration was based on a specified percentage of the tank biomass that was determined at the previous sampling. At each feeding, a level of feed was provided so that a slight excess remained after 10 minutes. The feeding rate was adjusted by 0–1% of tank biomass according to this observation. To monitor growth, fish were individually weighed and measured at the beginning and at the end of the experiment, and fish in each replicate aquarium were mass weighed weekly. Feces and uneaten feed were siphoned daily after the morning feeding. Dead fish were removed as soon as they were discovered. Temperature (range = 28.0–32.5°C), pH (7.9–8.4), and dissolved oxygen (4.9–5.8 mg/L) were monitored twice daily in five tanks chosen at random.

The experimental diets, a sample of 10 fish collected at the start of the experiment, and 12 fish

TABLE 2.—Initial and final weights and standard lengths, relative growth rate (RGR), specific growth rate (SGR), and percent survival, of juvenile Nassau grouper fed four practical diets with different crude protein and crude lipid levels (see Table 1). Values represent means ± SD ($N = 6$). Means along a row without a letter in common were significantly different ($P < 0.05$).

Measure	Diet			
	LP–LL	MP–HL	MP–LL	HP–HL
Weight (g)				
Initial	2.39 ± 0.24 z	2.33 ± 0.23 z	2.39 ± 0.32 z	2.38 ± 0.32 z
Final	4.02 ± 0.39 z	6.07 ± 0.61 y	13.2 ± 1.33 x	14.2 ± 1.55 x
Length (mm)				
Initial	4.44 ± 0.06 z	4.40 ± 0.02 z	4.44 ± 0.07 z	4.37 ± 0.07 z
Final	51.6 ± 1.61 z	58.0 ± 1.61 y	72.9 ± 2.66 x	75.7 ± 2.93 x
RGR (%)	68.6 ± 5.18 z	160 ± 8.37 y	454 ± 22.7 x	502 ± 33.6 x
SGR (%/d)	0.93 ± 0.06 z	1.71 ± 0.06 y	3.05 ± 0.08 x	3.19 ± 0.09 x
Survival (%)	96.4 ± 2.44 z	96.4 ± 2.44 z	92.9 ± 2.61 z	90.5 ± 3.01 z

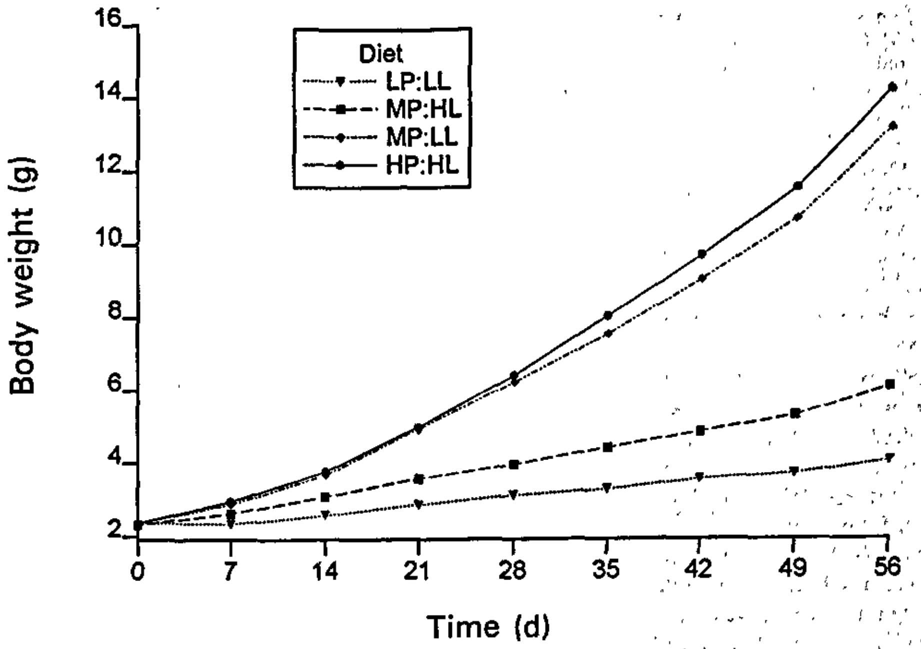

FIGURE 1.—Growth of juvenile Nassau grouper fed four practical diets with different crude protein and energy levels: LP–LL, 43.5% crude protein (CP) and 5.91% crude lipid (CL); MP–HL, 52.7% CP and 15.2% CL; MP–LL, 55.6% CP and 7.79% CL; HP–HL, 61.8% CP and 14.2% CL. Plotted points represent means of six replicate tanks.

from each treatment at the end of the study were analyzed for proximate composition using standard AOAC methods (AOAC 1984). Dietary gross energy (E) content of each diet was calculated from these results by using published physiological fuel values (Phillips 1972).

Relative growth rate (% increase in weight) was calculated as RGR = 100 × (wet final weight − wet initial weight)/(wet initial weight). Specific growth rate (% increase in body weight per day) was calculated as SGR = 100 × $\log_e$(wet final weight) − $\log_e$(wet initial weight)/(time in days).

Feed consumption during a sampling interval was determined as a percentage of average daily biomass during the interval. Consumption for the duration of the experiment was taken as the average over all sampling intervals. Feed conversion ratio was calculated as FCR = (dry weight of feed fed, g)/(wet weight gain, g).

Protein efficiency ratio was calculated as PER = (wet weight gain, g)/(dry weight protein fed, g). Apparent net protein retention was calculated as ANPR = 100 × [(final wet weight, g) × (final body protein %)] − [(initial wet weight, g) × (initial body protein, %)]/(dry weight protein fed, g). Apparent net energy retention was calculated as ANER = 100 × [(final wet weight × final kJ energy/g body weight) − (initial wet weight × kJ energy/g body weight)]/(kJ energy fed on dry weight basis).

Treatment means were compared by one-way analysis of variance (Sokal and Rohlf 1969). Differences between means were analyzed for significance ($P \leq 0.05$) with Tukey's *w*-procedure. Percentage data were arcsine-transformed and FCR data were log-transformed prior to analysis. Growth rates (RGR, SGR) and feed utilizations (FCR, PER, ANPR, ANER) for all replicates were compared, by linear regression (Sokal and Rohlf 1969), with dietary protein level and E:P ratio.

Results

A clear departure in growth rates was observed by day 21 among fish fed the different diets (Figure 1). By day 56, fish weights among treatments ranged from 4.02 to 14.2 g and standard lengths ranged from 51.6 to 75.7 mm; values generally increased ($P < 0.05$) with increasing dietary protein level from a minimum in the LP–LL diet to a maximum in the MP–LL and HP–HL diets (Figure 1, Table 2). Survival of fish under all treatments was high (range = 90.5–96.4%) and did not differ significantly among treatments (Table 2). Feed consumption (% body weight/day) ranged

TABLE 3.—Feed consumption (FC), food conversion ratio (FCR), protein efficiency ratio (PER), apparent net protein retention (ANPR), and apparent net energy retention (ANER) of juvenile Nassau grouper fed four practical diets with different crude protein and energy levels (see Table 1). Values represent means ± SD (N = 6). Means along a row without a letter in common were significantly different (P < 0.05).

Variable	Diet			
	LP–LL	MP–HL	MP–LL	HP–HL
FC (% body weight/d)	5.90 ± 0.26 z	3.59 ± 0.05 w	4.12 ± 0.11 x	5.54 ± 0.18 y
FCR	5.55 ± 0.39 z	1.60 ± 0.06 y	0.94 ± 0.02 w	1.32 ± 0.03 x
PER	0.42 ± 0.07 z	1.17 ± 0.09 y	1.91 ± 0.12 x	1.21 ± 0.07 y
ANPR (%)	8.14 ± 1.11 z	14.7 ± 1.18 y	29.4 ± 1.83 w	20.8 ± 1.07 x
ANER (%)	5.05 ± 0.71 z	17.8 ± 1.17 y	28.4 ± 1.73 w	21.6 ± 1.10 x

from 3.59 on the MP–HL diet to 5.90 on the LP–LL diet, with significant (P < 0.05) differences observed among all treatments. Feed conversion ratio (FCR) was lower for the MP–LL diet (0.94) than for the HP–HL or MP–HL diets (1.32 and 1.60, respectively) and was highest for the LP–LL diet (5.55; Table 3). Significant differences (P < 0.05) were observed among all treatments. Protein efficiency ratio (PER) was significantly higher (P < 0.05) for the MP–LL diet (1.91) than for the MP–HL and HP–HL diets (1.17 and 1.21, respectively; Table 3); PER was significantly lower (P < 0.05) for the LP–LL diet (0.42). Apparent net protein retention (ANPR) and apparent net energy retention (ANER) were higher for the MP–LL diet than for the MP–HL and HP–HL diets and were lowest for the LP–LL diet. Significant differences (P < 0.05) were observed among all treatments (Table 3).

Growth rate (RGR, SGR) and feed utilization (FCR, PER, ANPR, ANER) were highly correlated (P < 0.005) with dietary protein level and E:P ratio (Table 4). The RGR, SGR, PER, ANPR and ANER were positively correlated with dietary protein concentration; FCR was negatively correlated. The RGR, SGR, PER, ANPR and ANER were negatively correlated with E:P ratio, and FCR was positively correlated. No clear relationships between dietary and body levels of crude protein or crude lipid were evident (Table 5).

Discussion

The results demonstrate a high protein requirement for juvenile Nassau grouper. Maximum growth rates were obtained at levels of 55.6% (MP–LL) and 61.8% (HP–HL) crude protein. These two diets differed markedly in crude lipid levels (7.79 versus 14.2%), but the E:P ratios (28.3 and 28.9 kJ/g protein) were similar and lower than in the other diets (32.0–35.2 kJ/g). This suggests the importance of providing a proper balance of dietary energy and protein, as well as sufficient dietary protein. This also suggests that dietary protein may be reduced by lowering dietary lipid while maintaining an E:P ratio of 28–29 kJ/g protein. For *Epinephelus salmoides,* optimum growth has been reported on diets with protein levels of 40–50% and E:P ratios of 27.3–29.3 kJ/g protein (Teng et al. 1978; Lim 1985). For *E. tauvina,* a diet containing 57% crude protein supported optimum growth (as opposed to diets containing 25, 31, 35 and 45% protein), but the lowest E:P ratio tested was 42.1 kJ/g protein (El-Dakour and George 1981).

Because growth rates of fish fed the MP–LL and HP–HL diets were not significantly different, the

TABLE 4.—Linear regressions of dietary crude protein level (CP) and energy : protein ratio (E:P ratio) with growth and feed utilization variables. Each regression was based on 24 observations.

Variable[a]	Dietary protein		E:P ratio	
	Formula	r^2	Formula	r^2
RGR	2526 (CP) − 1051	0.772	−64.2 (E:P) + 2,291	0.861
SGR	13.3 (CP) − 4.83	0.842	−0.34 (E:P) + 127	0.941
FCR	−25.1 (CP) + 15.8	0.736	0.63 (E:P) − 17.1	0.786
PER	5.39 (CP) − 1.70	0.449	−0.17 (E:P) + 6.54	0.794
ANPR	85.0 (CP) − 27.1	0.510	−2.68 (E.P) + 101	0.872
ANER	104 (CP) − 37.2	0.647	−2.97 (E:P) + 111	0.915

[a] All variables regressed were significant at P < 0.005; RGR = relative growth rate (%); SGR = specific growth rate (% body weight/day); SGR = specific growth rate (% body weight/day); FCR = feed conversion ratio; PER = protein efficiency ratio; ANPR = apparent net protein retention (%); ANER = apparent net energy retention (%).

TABLE 5.—Initial and final proximate composition (% dry matter) of juvenile Nassau grouper fed four practical diets with different crude protein and energy levels (see Table 1 for diet abbreviations and compositions).

Component or characteristic	Initial	Final for diet: LL–PL	MP–HL	MP–LL	HP–HL
Moisture (%)	79.4	75.4	75.8	74.5	73.9
Crude protein (%)	64.1	64.0	53.0	58.7	63.3
Crude lipid (%)	9.98	11.7	27.8	26.2	19.8
Crude ash (%)	10.8	6.50	10.6	12.1	15.3
Gross energy (kJ/g fish)[a]	17.0	18.2	20.8	20.2	18.3

[a] Gross energy determined by using physiological fuel values (Phillips 1972).

higher lipid and protein levels in the HP–HL diet were probably excessive, resulting in poorer FCR, PER, ANPR and ANER. Efficiency of protein utilization for growth in fish is reduced when optimum dietary protein level is exceeded (Halver 1976). Similar observations have been made for other brackish water or marine piscivores, including *E. salmoides* (Teng et al. 1978); striped bass *Morone saxatilis* (Millikin 1982); *Dicentrarchus labrax* (Hidalgo and Alliot 1988); *Siganus guttatus* (Parazo 1990) and red drum *Sciaenops ocellatus* (Ellis and Reigh 1991).

Although the MP–HL and MP–LL diets contained similar crude protein levels (range = 52.7–55.6%), growth was slower on the MP–HL diet, which had higher crude lipid levels. This was related, in part, to lower consumption (Table 3), because feed consumption in fish is inversely related to dietary energy level (Halver 1976). Growth retardation by high dietary lipid and energy levels has been recorded for other marine piscivores, such as red drum (Williams and Robinson 1988) and *Seriola quinqueradiata* (Shimeno et al. 1979). Since cultured Nassau grouper are sedentary, energy requirements are probably lower than in most marine piscivores.

The poor performance of fish fed the LP–LL diet can be attributed to several factors. In addition to having the lowest protein level and highest E:P ratio, this diet contained high levels of plant proteins, which are more poorly assimilated than animal proteins by marine fish (NRC 1983). Furthermore, the LP–LL diet had a much higher concentration (34.8%) of carbohydrate (nitrogen free extract plus fiber) than recommended for marine fish (10–20%) (New 1986; Tucker 1992), which cannot efficiently use carbohydrate as an energy source (Yone 1976; Shimeno et al. 1977; NRC 1983; Ellis and Reigh 1991). High carbohydrate levels may also interfere with protein digestibility (Page and Andrews 1973; Cowey et al. 1974; Shimeno et al. 1977).

Differences between the four diets were manifested in feed utilization and growth rates, but no clear relationships between dietary nutrient levels and proximate body composition were observed. Although correlations between dietary and body levels of protein and lipid have often been reported in other cultured fish (Shimeno et al. 1980; Millikin 1982; Parazo 1990; Ellis and Reigh 1991), body composition also varies with fish size (Halver 1976), which ranged considerably among fish fed the different diets in this study.

In summary, of the four diets compared, cultured Nassau grouper juveniles showed superior growth and feed utilization on a diet with a crude protein level of 55.6%, a crude lipid level of 7.79%, and an E:P ratio of 28.9 kJ/g protein. Excellent feed conversion (FCR, 0.94) and superior ANPR and ANER values observed on the MP–LL diet indicate an adequate amino acid balance and E:P ratio for Nassau grouper.

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