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**The Effects of Culture Temperature, Age and
Growth Rate on Protein and Energy Utilization
in Eels (*ANGUILLA* SP.)**

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FOOT COTTON
ANNIVERSARY BOND
FOX RIVER

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ABSTRACT

Temperatures has significant and varying effects on growth and metabolism in eels with different growth rates. Slow-growing and moderately-growing eels grew significantly faster at 25°C or 27°C compared to similar eels cultured at 23°C. However, fast-growing eels grew faster at 23°C compared to 25°C or 27°C. No significant differences in body composition (protein and lipid) were noted. Increasing temperatures (18°C-27°C) increased metabolic rates in all eels regardless of growth rate or size. However, the effect of temperature was more profound in smaller eels. Temperature also affects the optimum protein content of the diet. As temperature increases, optimum protein increases.

When eels were fed diets with varying amounts of protein and energy, slow-growing eels gained very slowly or not at all at all levels of protein or energy. Thus the protein energy ratio does not appear to significantly influence growth in slow-growing eels. However, small slow-growing eels exhibited decreased digestibilities of protein and digestible matter when compared to larger eels of the same year class or to larger eels that were 1-2 years older. Thus the decreased digestibilities appeared to be related to actual size rather than age or growth rate.

Protein and energy content of diets did significantly effect growth and digestibility in larger and faster-growing eels. Our results indicate that protein energy ratio should be at least 70mg protein/kcal-, energy levels at least 4.0 kcal/gram and protein levels 35-40% depending on species.

OBJECTIVES

The specific objectives of the studies carried out in this project were:

1. To evaluate the effects of varying temperatures on growth, metabolism (O_2 consumption), digestion, body composition (protein, lipid, ash) and food conversion of eels fed diets with varying protein/energy ratios.
2. To evaluate the effect of age of fish on growth, metabolism (O_2 consumption), digestion, body composition (protein, lipid, ash) and food conversion of eels fed diets with varying protein/energy ratios.
3. To evaluate the effect of growth rate on growth, metabolism (O_2 consumption) body composition and food conversion (protein, lipid ash) of elvers fed diets with different protein energy ratios.
4. To compare protein and energy utilization in American (A. rostrata) and European (A. Anguilla) eels.

CHAPTER 1¹

EFFECTS OF GROWTH RATE ON PROTEIN AND ENERGY UTILIZATION IN AMERICAN EELS

(ANGUILLA ROSTRATA)

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ABSTRACT

Diets with varying amounts of protein and energy were fed to eels designated as either moderately-, slow-, or very slow-growing. Very slow and slow-growing eels did not grow well on any diet formulation. Faster growing eels grew best on diets containing $4.0 \text{ kcal} \cdot \text{g}^{-1}$ and at least 30% protein in the diet. There were no significant differences in body composition (protein, ash, moisture, lipid) due to growth rate. However, dietary protein and energy levels ($3.0 \text{ kcal} \cdot \text{g}^{-1}$) had significantly higher moisture and protein content, but lower total lipid content. The phenomenon of slow growth rates does not appear to be related to differences in dietary protein or energy contents.

¹This paper will be submitted to the Journal of the World Aquaculture Society.

INTRODUCTION

Body size and weight has a significant effect on energy required for metabolism (Egusa, 1958; Caulton, 1978; and Tarr and Hill, 1978), with metabolic requirements decreasing with increasing weight. For example, in the American eel (Anguilla rostrata), total body protein as a percent of dry weight is negatively correlated to increased body weight, while there is an increase in body lipid content. Therefore, larger eels may grow adequately with less protein in the diet and more lipid (Gallagher, et al. 1984). Page and Andrews (1973) found that when energy levels are adequate in the diet of channel catfish (Octalurus punctatus), there are no improvements in weight gain of fish with initial weights of $114 \pm 3g$ when protein levels were raised from 25 to 35%. Therefore, the optimum protein/energy ratio may change as the metabolism and composition of the fish changes. The effect of size on protein and energy requirements may be more important in eels where there are profound variations in body size and weight in each year class (Degani and Levanon, 1983; Arai, 1976). In addition, these variations in size have been shown to be accompanied by significant changes in metabolic rates (Gallagher and Matthews, 1987).

Therefore, the purpose of this study was to investigate the effects of diets with varying protein and energy contents on the growth and body composition of eels with varying growth rates and at varying ages.

METHODOLOGY

Dietary Trial I

Elvers were caught in March and April in a creek near Newport, North Carolina. They were adapted to artificial food and separated into size

groups by weight at the end of two months, moderately growing = 600 mg, slow growing = 200 mg and very slow growing = 150 mg. Thus, diet groups consisted of eels of approximately the same age but different sizes. Dietary trials were conducted in a closed circulating system consisting of 20L plastic cylindrical tanks equipped with side standpipe which drained from the bottom. Water was delivered to tanks from overhead poly-vinyl chloride pipes. Water was supplied from the Greenville, NC city water system and was dechlorinated by carbon filters and aeration. Elvers were placed randomly in each of 12 diet groups with 3 replications of each group.

Elvers were fed 1/16" pelleted diets shown in Table 1. Diets had varying protein and or energy content. Diets were fed twice daily at 0800 and 1700 h at a rate of 4% of body weight/day.

Total energy in diets was calculated based on 4 kcal.g^{-1} (1 Kcal = 4.184 KJ) for carbohydrate and protein and 9 kcal.g^{-1} for lipid (Klieber 1975). Although the caloric values of nutrients in fish are a matter of some discussion, these caloric values have been used for channel catfish (Garling and Wilson 1976, 1977) rainbow trout, Salmo gairdneri (Lee and Putnam 1973, Yu et al. 1977) common carp, Cyprinus carpio, red sea beam, Chrysophrys major and yellow tail, Seriolo quinqueradiata (Furuichi and Yone 1980) and striped bass, Morone saxatilis (Millikin 1982).

Water quality was monitored weekly for dissolved chlorine, ammonia, nitrate and nitrites. Temperature was measured and recorded daily.

In the closed circulating system used for the commercial diet experiments ammonia levels were always $<1 \text{ mg.L}^{-1}$, nitrate $<1 \text{ mg.L}^{-1}$, nitrite $<1 \text{ mg.L}^{-1}$ and chlorine was $<0.2 \text{ mg.L}^{-1}$. Temperature was $23.6 \pm 1.0^{\circ}\text{C}$ (SD).

Dietary Trial II

Normally feeding elvers from two separate year classes 1985 and 1987 were purchased from Anguilla Farms located in Hastings, Florida. Elvers (1987), were separated into two size classes designated as moderately growing ($> 2g$) and slow growing ($< 1g$).

Two comparisons were made, one using eels of the same size ($> 2g$) from different years (1985 or 1987) and the second using eels of the same year class of different sizes (1987). The second experiment was a rerun of the 1986 experiments.

Diet formulations used in this study are shown in Table 2. Water quality parameters were monitored as described above.

RESULTS AND DISCUSSION

Dietary Trial I

Results of these experiments are shown in Tables 3, 4, and 5. Very slow-growing elvers were characterized by almost no growth over the experimental period. Both slow-growing and moderately growing eels achieved good gains over the experimental period. There were no significant differences in weight gains within size groups due to diet. There was a great deal of variation in growth of eels demonstrated by the high standard deviations. However, there appears to be a trend toward optimum growth rates around 35 or 40% protein in the moderately growing eels. These data are consistent with requirements previously reported (Gallagher, 1981). No such trends are noted in slow or very slow growing eels, indicating that changes in protein and/or energy content do not effect the growth rate of slow-growing eels.

Dietary Trail II

Comparisons of mean growth of slow- and fast-growing eels of the same year class are shown in Table 6, as with the 1986 experiments there were no significant differences in growth related to changes in dietary protein or energy content for eels with varying growth rates (Table 6). Slow growing eels of the same year class demonstrated exceedingly low weight gains and high mortality. Fast-growing eels grew well, but due to the large variation in data, we were unable to demonstrate statistical differences in weight gains. However, the results are consistent with previous findings for Anguilla rostrata where we found no significant increase in growth of elvers fed diets with protein levels above 35%. In addition, in the present study, energy contents of 4.0 kcal-g gave better growth than diets containing lower amounts of energy.

Results from the second series of experiments (Table 7) showed that eels of the same size but at least a year older (1985, 1986 eels) had consistently lower weights than eels of the same size caught in 1987. As shown with faster growing eels in Table 6, eels receiving diets with 4.0 kcal-g achieved better growth than those receiving diets with 3.0 kcal-g. However, there were no significant differences in growth.

Body composition analyses are shown in Table 8. There were no significant differences between mean moisture, lipid, protein or ash content of eels classified as either moderately- or slow-growing, therefore, data were combined for further statistical analyses. The moisture contents of eels fed diets with 30, 35, and 40% protein ($3.0 \text{ kcal} \cdot \text{g}^{-1}$) were significantly ($p < 0.05$) higher than all other diets. Eels fed diets with 35 and 45% protein ($4.0 \text{ kcal} \cdot \text{g}^{-1}$) had significantly lower moisture content

The protein content of eels fed diets with 30 and 35% protein ($3.0 \text{ kcal} \cdot \text{g}^{-1}$) was significantly ($p < 0.05$) higher than eels fed all other diets except those fed 35% protein ($4.0 \text{ kcal} \cdot \text{g}^{-1}$). The lipid content of $3.0 \text{ kcal} \cdot \text{g}^{-1}$ diets with 30, 35, and 40% protein were significantly lower than all other diets. The lipid content of eels fed lower energy diets ($3.0 \text{ kcal} \cdot \text{g}^{-1}$) increased as the protein content of the diets increased. In addition, protein content of eels decreased in a similar manner.

These results indicate that faster growing eels achieve better growth on diets containing $4.0 \text{ kcal} \cdot \text{g}^{-1}$ and at least 30% protein. However, the relationship between growth and protein requirements was not clear in this study. The phenomenon of slow growth rates in feeding eels does not appear to be a result of changes in protein or energy utilization in these eels as compared to their faster growing counter parts. In addition, protein and energy ratio in diets do not cause significant changes in body composition of moderately-growing eels as compared to slow-growing eels. However, varying protein energy ratios in diets for eels did significantly influence body composition of eels. Generally, eels fed diets with lower energy content ($3.0 \text{ kcal} \cdot \text{g}^{-1}$) had significantly higher moisture and protein contents and significantly lower lipid content. And eels fed diets with higher energy content ($4.0 \text{ kcal} \cdot \text{g}^{-1}$) had significantly higher lipid content and significantly lower moisture and protein content. The effect of protein level within energy levels was not clear.

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Table 1. Formulations for eels in dietary Trial I.

	Diet #										
	1	2	3	4	5	6	7	8	9	10	11
% Protein	45	40	35	30	45	40	35	30	45	35	40
Kcal/g	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0	4.5	3.5	4.0
TVP/Fishmeal ¹ (%) 1/3 ratio)	71.71	63.74	55.77	47.80	71.71	63.74	55.77	47.80	71.71	55.77	63.74
CHOH Blend ² (%)	0.00	6.57	13.15	19.76	0	6.51	13.15	19.76	5.00	19.76	19.76
Fish Oil (%)	3.00	3.39	3.88	4.37	14.24	14.63	15.12	15.61	16.34	6.25	8.42
Cellulose (%)	20.89	21.88	22.78	23.64	9.65	10.64	11.54	12.40	2.53	13.80	3.66
Vitamin & Mineral ³ Premix (%)	4.42	4.42	4.42	4.42	4.42	4.42	4.42	4.42	4.42	4.42	4.42
P/E (mg/kcal)	150	133	116	100	112	100	87	75	100	100	100

¹TVP = Texture vegetable protein (50% protein, 0.5% fat, 30.5% carbohydrate, 6.5% ash, 9.0% moisture, 3.5% fiber). Fishmeal (67% protein, 8% fat, 2.0% carbohydrate, 15% ash, 8% moisture, 0% fiber)

²33% cellulose, 33% dextrin, 33% cornstarch

³0.4% vitamin premix #30 (Ziegler Bros.), 0.146% choline, 0.075 ascorbic acid, 0.29% lecithin, 3.5% AIN76 mineral mix

Table 2. Diet formulations Dietary Trial II.

Ingredients	#1	#2	#3	#4	#5	#6	#7	#8
Fish meal	22.0	30.0	38.0	44.0	20.0	27.0	36.0	44.0
Blood meal	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0
Poultry/meat meal	14.0	17.0	18.0	20.0	17.0	18.0	18.0	20.0
Wheat middling	30.0	20.0	15.0	8.0	43.0	35.0	25.0	15.0
Fish oil	5.0	5.0	3.5	3.0	10.0	10.0	10.0	10.0
cellulose	20.1	20.1	17.6	17.1	2.1	2.1	3.1	3.1
Vitamin Mix 30	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Mineral Mix								
AIN 76	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Ascorbic Acid	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Choline Chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
g Protein	29.32	34.80	39.90	44.80	31.60	35.60	40.01	45.80
g Fat	10.01	10.60	9.80	9.30	15.70	16.10	16.60	17.10
g CHOH	23.50	17.70	15.10	11.20	32.40	27.70	21.60	15.90
g ASH	6.90	8.10	9.20	10.20	7.70	8.50	9.40	10.50
g Fiber	3.94	3.25	2.90	2.40	5.30	4.70	3.80	3.10
Kcal/100g	301.00	306.00	308.00	308.00	397.00	398.00	396.00	401.00

Table 3. Mean weight (mg $\pm$ SD) of elvers fed diets of different protein content but with a constant energy level of 3.0 kcal·g⁻¹.

	Protein			
	30	35	40	45
Moderately-growing ^a	776 $\pm$ 183	818 $\pm$ 122	1075 $\pm$ 225	643 $\pm$ 101
Slow-growing ^b	328 $\pm$ 36	351 $\pm$ 85	341 $\pm$ 55	330 $\pm$ 75
Very slow-growing ^c	176 $\pm$ 12	160 $\pm$ 3	185 $\pm$ 20	169 $\pm$ 77

^a Initial mean weight = 607mg $\pm$ 203

^b Initial mean weight = 217mg $\pm$ 30

^c Initial mean weight = 153mg $\pm$ 28

Table 4. Mean weight (mg $\pm$ SD) of elvers fed diets of different protein content but with a constant energy level of 4.0 kcal·g⁻¹.

	% Protein			
	30	35	40	45
Moderately-growing ^a	-	1243 $\pm$ 389	845 $\pm$ 343	-
Slow-growing ^b	376 $\pm$ 9	404 $\pm$ 20	363 $\pm$ 44	347 $\pm$ 57
Very slow-growing ^c	145 $\pm$ 13	151 $\pm$ 26	168 $\pm$ 7	158 $\pm$ 4

^aInitial mean weight = 607 mg $\pm$ 203

^bInitial mean weight = 217 mg $\pm$ 30

^cInitial mean weight = 153 mg $\pm$ 28

Table 5. Mean weight (mg $\pm$ SD) of elvers fed diets different protein and energy contents but with a constant protein energy ratio of 100 mg protein/kcal.

	% Protein			
	30	35	40	45
Slow-growing	328 $\pm$ 36	326 $\pm$ 16	351 $\pm$ 37	350 $\pm$ 35
Very slow-growing	176 $\pm$ 12	133 $\pm$ 98	155 $\pm$ 6	151 $\pm$ 14

Table 6. Comparison of mean growth of eels from the same year class with different growth rates fed diets with varying protein and energy contents.

	Diet #							
	1	2	3	4	5	6	7	8
Protein %	30	35	40	45	30	35	40	45
Energy (kcal/g)	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0
Slow-growing eels	810 ± 30	1220 ± 120	740 ± 720	1630 ± 610	1160 ± 300	1120 ± 130	1280 ± 410	910 ± 10
Moderately-growing eels	2730 ± 320	2500 ± 190	2770 ± 510	3100 ± 860	3810 ± 1610	3240 ± 520	3020 ± 290	2830 ± 35

Table 7. Comparison of mean growth in eels from different years (1986 or 1987) of the same size fed diets with varying protein and energy contents.

	Diet #							
	1	2	3	4	5	6	7	8
% Protein	30	35	40	45	30	35	40	45
Energy Kcal/g	3.0	3.0	3.0	3.0	4.0	4.0	4.0	4.0
1985 Eels	2550 ± 620	2540 ± 560	2500 ± 470	2750 ± 620	2600 ± 680	2810 ± 450	2740 ± 550	3140 ± 440
1987 Eels	2760 ± 1120	2710 ± 1020	2940 ± 940	2580 ± 720	2110 ± 630	3950 ± 650	3000 ± 630	3110 ± 1440

Table 8. Mean body composition of moderately- and slow-growing eels fed diets with varying protein and energy ratios.

	Diet #							
	1	2	3	4	5	6	7	8
Moisture (% of wet weight)								
Moderately-growing eels	73.70±0.76	73.76±0.51	74.18±0.46	73.22±0.21	71.18±0.83	73.11±1.03	43.37±0.89	72.13±0.59
Slow-growing eels	74.77±1.66	73.48±0.40	72.87±0.67	72.54±	72.30±0.29	72.26±0.66	72.63±0.44	71.77±0.46
Group mean	74.34±1.46 ^a	73.50±0.47 ^{ab}	73.39±0.86 ^{ab}	72.82±0.53 ^{bc}	71.85±0.79 ^c	72.60±0.93 ^{bc}	72.83±0.43 ^{bc}	71.93±0.53 ^c
Lipid (% of dry weight)								
Moderately-growing eels	18.43±2.52	18.67±1.97	17.85±1.54	19.69±2.79	25.33±3.62	24.41±0.08	23.13±2.97	25.31±0.03
Slow-growing eels	15.97±5.20	19.78±2.14	21.09±1.48	21.14±2.29	21.61±2.63	23.52±0.38	22.86±1.31	24.94±1.83
Group mean	17.04±5.02 ^a	19.37±2.09 ^{ab}	19.79±2.18 ^{ab}	20.56±2.4 ^{bc}	23.18±3.62 ^{cd}	23.87±0.53 ^d	22.97±2.14 ^{cd}	25.09±1.43 ^d
Protein (% of lipid free dry weight)								
Moderately-growing eels	68.62±0.72	68.09±0.96	61.66±3.13	60.82±2.10	61.81±	67.55±1.06	65.32±4.69	61.78±3.49
Slow-growing eels	67.79±2.21	67.60±2.77	64.83±1.64	62.23±0.06	62.38±3.14	67.24±1.15	62.68±1.23	66.60±4.00
Group mean	68.12±1.82 ^a	67.80±2.24 ^{ab}	63.56±2.82 ^c	61.67±1.50 ^c	62.15±2.45 ^c	67.13±1.13 ^{ab}	63.73±3.37 ^c	64.67±4.48 ^{bc}
Ash (% of lipid free dry weight)								
Moderately-growing eels	13.39±0.64	14.95±1.44	14.38±0.13	14.60±0.17	13.33±0.03	12.62±0.36	12.67±0.45	13.50±1.67
Slow-growing eels	13.44±2.65	13.61±0.25	14.10±0.07	14.62±0.70	13.87±0.93	13.07±0.25	13.83±0.30	13.24±0.28
Group mean	13.42±2.09	14.14±1.14	14.21±0.17	14.61±0.55	13.65±0.77	12.87±0.37	13.37±0.61	13.34±1.08

¹Mean with different superscripts are significantly different from one another at $p \leq 0.05$.

CHAPTER 2¹

EFFECTS OF TEMPERATURES AND DIETARY PROTEIN ENERGY RATIO ON GROWTH, BODY COMPOSITION AND FEED UTILIZATION OF SLOW-GROWING JUVENILE EELS

(ANGUILLA ANGUILLA L.)

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ABSTRACT

The influence of dietary protein energy ratios on growth, body composition and feed utilization of slow-growing elvers maintained at three different temperatures (23°C, 25°C and 27°C) was studied.

The maximum growth of eels was dependent both on temperature and protein energy ratios. At temperatures of 25°C or 27°C the higher level of protein (45% or 50%) in the diet had more effect on growth than the lower percentages of protein. At a low temperature (23°C) the effect of protein level on growth was minimal. Similar relationships were found in the diet with respect to energy. For maximum growth, higher temperatures (27°C) and higher energy in the diet are required.

¹This paper has been submitted to Aquaculture.

INTRODUCTION

Several studies have investigated the optimum artificial diet for various species of eels. Nose and Arai (1972) and Arai et al. (1971, 1972) studied the protein requirements and optimum level of protein of A. japonica. Degani et al. (1984, 1985) examined the level of dietary protein needed to produce maximum growth in A. anguilla and the effect on growth of different sources of protein in the diet. Gallagher (1984) found that the optimum protein energy ratio for A. rostrata was 140 mg protein/kcal of energy.

Lipid and carbohydrates have also been studied. A. anguilla can utilize several sources of carbohydrates (Spannhof and Kuhne, 1977; Degani et al. 1986; Degani and Levanon, 1986; Gallagher and Pollock, 1984). Fatty acid in body composition of A. japonica was reported by Arai et al. (1971, 1972) and that of A. rostrata was reported by Lovern (1938) and Gallagher et al. (1984). A. anguilla was studied by Dave et al. (1976, 1974) and Degani (1986). The effect of dietary oil level effect on growth rate of glass eels was studied by Degani (1986).

However, the growth of cultured eels fed artificial diets is not only effected by feed composition. Other factors, such as water temperature, size of fish and age of fish, significantly affect rate of growth. The aim of the work reported here was to evaluate the relationship of temperatures and protein energy ratios in the diet on the growth of slow-growing juvenile eels.

MATERIALS AND METHODS

Glass eels (Anguilla anguilla) were collected from coastal water of France and brought to Israel at the end of February 1985. The larvae weighed about 0.3 grams and measured approximately 7 cm. in length. The elvers were held for 3 months in indoor containers at the constant temperature of $23^{\circ}\text{C} \pm 1^{\circ}\text{C}$, as described previously (Degani and Levanon, 1983). The elvers were fed food ad libitum from commercially available food powder mixed with raw chicken offal and water (Degani and Levanon, 1983) (Table 1). After 3 months, marked differences in growth were observed.

Elvers which has learned to eat artificial feed, grew slowly and increased their body weight during the initial 3 months by as much as 1 gram were used in the study. The elvers were housed in groups of 20 in indoor open containers (40 x 20 x 20 cm). There was no significant difference in weight among elvers (t-test). There were three replication containers of each experimental group. Each group received a different diet (Table 1) and/or was held at a different temperature (23°C , 25°C and 27°C). The temperatures were controlled as described previously (Degani and Levanon, 1983).

All the elvers were weighed every three weeks using an electronic balance with an accuracy of 0.019 gram.

At the end of the experimental period, all the elvers from each container were taken for analysis of body composition (protein, fat, ash) as described in detail by Degani and Dosoretz, 1986. Food conversion (FC), protein efficiency ratios (PER), protein production value (PPV), energy retention values (ERV), and specific growth rates (SGR) were calculated according to Degani et al. 1986.

Data on the body weight of fish were stored in a Casio personal computer and the mean weight of each experimental group was calculated. Differences between means were statistically evaluated using student's t-test.

RESULTS

Eels fed a diet with gross energy of 4.73 kcal/g and maintained at 27°C grew significantly faster than the eels fed a diet with 4.58 kcal/g and 4.88 kcal/g (Table 2). However, no significant differences were found in weight gain when eels were fed the same diets at 23°C and 25°C. There were no significant differences among body moisture, percent protein, and percent lipid of eels fed these diets at various temperatures (Table 3).

The food conversion of slow-growing eels maintained at 27°C was significantly lower than the slow-growing eels maintained at 23°C and 25°C for most diets. At 27°C, the food conversion of eels fed a diet with 4.73 kcal/g was lower than the eels fed diets with 4.58 kcal/g. However, PER, PPV, and ERV was higher in these eels than in those fed diets with other amounts of gross energy (Table 4).

The percent weight gain of eels fed diets with different protein levels (30%, 35%, 40%, 45% and 50%) and the same gross energy (4.75 kcal/g) are shown in Table 5. The SGR was significantly higher in eels maintained at 27°C than in eels maintained at 25°C or 23°C when diets containing 30%, 35%, or 40% protein were fed. However, when dietary protein was fed at 45% or 50%, eels maintained at 25°C and 27°C had significantly higher SGRs than the other experimental groups.

There was a significantly lower percent of fat in the bodies of eels maintained at 23° compared to eels maintained at 25°C fed 45% or 50% protein or 27°C in all the experimental groups. The highest percent fat was found in eels maintained at 25°C and fed a high protein level (45% or 50% of their diet) (Table 6). There were no differences among mean percent ash and percent protein.

The food conversion (FC), PER, PPV, and ERV were significantly better in eels maintained at 25°C or 27°C than in eels maintained at 23°C. The most efficient utilization was in eels maintained at 25°C and fed a high level of protein.

The relation between the protein energy ratio, temperature, and SGR is shown in Table 8. The best SGR was achieved at 25° with a diet of 144 protein energy ratio.

DISCUSSION

The result of this study supports the hypothesis that the maximum growth of eels is dependent on temperature and level of protein and energy. In other words, both protein energy ratio and temperature have an effect on eels' growth.

Previous studies investigating optimum level of protein in artificial diets (Yamazaki, 1966; Arai et al., 1971, 1972; Nose and Arai, 1972; Degani et al., 1985; Gallagher, 1986), have shown that eels required about 45% protein in their diet for maximum growth. The results of this study are in general agreement with these findings. In smaller eels optimum growth was found both at 25°C and 27°C in a diet of 45% of protein. However, eels maintained at 23°C fed a diet with 50% protein grew significantly faster than eels maintained at the same temperature and fed 45% protein in their

diet. Therefore, temperature can have a significant effect of optimum protein requirements. Moreover, not only the protein level for maximum growth was effected, but also the energy level for maximum growth.

Higher energy levels in the diet had more of an effect on growth of small eels maintained at higher temperatures (25°C or 27°C) than on the growth of eels maintained at the lower temperature (23°C).

The results are in agreement with previous studies of the same authors. Degani and Levanon (1986) found that slow-growing eels fed the same level of protein but a higher level of glucose grew faster than eels fed a lower level of glucose. The difference between the rate of growth was higher in eels maintained at 27°C than in eels maintained at 23°C. In this study the energy source was fat, but the result was similar. It is clear from these studies that dietary composition and temperature have interactive effects on growth of eels.

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Table 1. Formulation and analysis of the test diet (percent).

	Diet #							
	1	2	3	4	5	6	7	8
I. Basic Ingredients								
Fish Meal	35.00	35.00	35.00	-	-	3.44	23.47	43.50
Poultry meat meal	30.00	30.00	30.00	38.81	48.99	55.53	42.47	29.94
Wheat meal	23.00	18.00	13.00	40.91	31.74	21.46	15.27	9.08
Vitamin premix	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Guar gum	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Milk meal	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Chicken oil	5.00	10.00	15.00	12.27	12.27	12.57	11.52	10.48
II. Analysis (% DM) ¹								
Protein	45.84	44.32	43.12	30.00	35.00	40.00	45.00	50.00
Gross energy								
(kcal/g) ²	4.58	4.74	4.88	4.78	4.82	4.84	4.75	4.71

¹ % of Dry Matter

² Calculated

Table 2. Growth of eels fed different energy in the diet and maintained at different temperatures.

Dietary Energy cal/kg	Temperature	Weight Gain %	Specific Growth Rate
4.58	23	15.75	0.35
4.73	23	16.26	0.36
4.88	23	12.09	0.27
4.58	25	18.74	0.41
4.73	25	18.00	0.40
4.88	25	23.23	0.50
4.58	27	26.08	0.55
4.73	27	43.66	0.86
4.88	27	30.68	0.64

¹Means with different superscripts are significantly different from one another at $p \leq 0.05$ within a temperature range.

Table 3. Body composition of eels fed different levels of energy at the end of the experiment.

Temperature	Energy	% Moisture	Protein	Lipid
			% of Dry Weight	
23	4.58	75.85	68.44	20.46
23	4.73	75.32	67.42	18.64
23	4.88	76.16	61.64	19.00
25	4.58	76.63	63.16	20.05
25	4.73	79.62	63.02	19.05
25	4.88	77.48	61.79	20.23
27	4.58	76.64	71.50	20.40
27	4.73	76.41	59.07	20.40
27	4.88	77.31	61.70	18.29

Table 4. Feed utilization of food of diet of different energy.

Temperature	Energy	Food Conversion	PER	PPV	ERV
23	4.58	5.60 ^a	41.67	28.52	31.46
23	4.73	5.25 ^a	43.99	19.36	28.35
23	4.88	7.00 ^a	33.22	19.37	18.58
25	4.58	4.48 ^b	49.58	31.96	35.82
25	4.73	4.67 ^b	48.70	30.68	30.20
25	4.88	3.65 ^b	63.68	39.35	36.02
27	4.58	3.23 ^{ab}	68.99	49.00	53.23
27	4.73	1.92 ^{bc}	1.18	69.78	66.97
27	4.88	2.74 ^c	.85	52.41	46.59

¹Mean with different superscripts are significantly different from one another at $p \leq 0.05$ within a temperature range.

Table 5. Growth of eels fed different protein levels in diet, and maintained in different temperatures.

Protein Level %	Temperature °C	Weight Gain %	Specific Growth Rate ¹
30	23	5.72	0.12 ^a
30	25	10.14	0.20 ^a
30	27	15.82	0.31 ^b
35	23	6.07	0.13 ^a
35	25	7.85	0.17 ^a
35	27	15.34	0.31 ^b
40	23	6.07	0.13 ^a
40	25	11.34	0.23 ^a
40	27	12.23	0.25 ^b
45	23	6.40	0.14 ^a
45	25	28.32	0.55 ^c
45	27	22.84	0.44 ^c
50	23	13.26	0.27 ^a
50	25	24.03	0.49 ^c
50	27	21.76	0.42 ^c

$$^1\text{SGR} = \frac{\ln W_e - \ln W_o}{\text{days}} \times 100$$

days

²Means with different superscripts are significantly different from one another at $p \leq 0.05$.

Table 6. Body composition of eels fed different levels of protein.

Temperature °C	% Protein in Diet	% Moisture	% Ash	Protein	Lipid
				% of Dry Weight	
23	30	76.42	2.94	58.76	21.33 ^{a1}
23	35	75.50	3.03	59.72	21.83 ^a
23	40	79.29	3.21	56.73	23.93 ^a
23	45	75.41	3.00	56.51	21.23 ^a
23	50	76.35	2.87	56.95	21.05 ^a
25	30	75.84	2.00	63.31	19.16 ^a
25	35	74.89	2.09	58.96	21.83 ^a
25	40	77.48	2.39	58.85	21.04 ^a
25	45	74.14	2.14	55.78	29.72 ^b
25	50	75.54	2.32	52.10	26.46 ^b
27	30	73.33	2.68	46.90	26.14 ^b
27	35	74.99	2.39	57.75	26.22 ^b
27	40	74.62	2.85	50.85	25.09 ^b
27	45	74.89	2.30	52.93	24.36 ^b
27	50	75.64	2.37	55.64	24.74 ^b

¹Means with different superscripts are significantly different from one another at $p \leq 0.05$.

Table 7. Feed utilization by eels fed a diet with different protein levels and maintained at different temperatures.

Temperature °C	% Protein in diet	Food conversion	PER	PPV	ERV
23	30	16.01 ^a	19.23	7.46	7.11
23	35	19.55 ^a	18.37	12.65	11.09
23	40	15.55 ^a	16.08	9.00	10.14
23	45	3.24 ^{bc}	68.59	38.41	42.91
23	50	7.07 ^b	28.29	15.87	18.27
25	30	5.89 ^b	25.46	29.71	26.32
25	35	5.31 ^b	57.54	59.67	59.93
25	40	5.88 ^b	19.10	20.51	21.61
25	45	1.66 ^c	60.19	61.30	93.22
25	50	1.39 ^c	63.89	61.52	79.56
27	30	5.08 ^b	65.59	42.59	49.19
27	35	5.60 ^b	49.60	60.32	54.45
27	40	5.55 ^b	20.25	33.20	29.84
27	45	4.80 ^b	46.30	28.09	24.15
27	50	6.21 ^b	53.24	36.92	35.90

¹Means with different superscripts are significantly different from one another at $p \leq 0.05$.

Table 8. The effect of temperature on Specific growth rate (SGR) of slow-growing eels fed different protein energy ratio.

Temperature	PER mg/kcal	Diet	SGR
23	151	1	0.35
23	144	2	0.36
23	137	3	0.27
25	151	1	0.41
25	144	2	0.40
25	137	3	0.50
27	151	1	0.55
27	144	2	0.86
27	137	3	0.67
23	62	4	0.12
23	72	5	0.13
23	82	6	0.13
23	94	7	0.14
23	106	8	0.27
25	62	4	0.20
25	72	5	0.17
25	82	6	0.23
25	94	7	0.55
25	106	8	0.49
27	62	4	0.31
27	72	5	0.31
27	82	6	0.25
27	94	7	0.44
27	106	8	0.42

CHAPTER 3¹

THE RELATIONSHIP BETWEEN GROWTH, FOOD CONVERSION, BODY SIZE, BODY COMPOSITION AND TEMPERATURE IN THE EUROPEAN EEL (ANGUILLA ANGUILLA L.)

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ABSTRACT

The influence of body size and temperature on food conversion and growth of European eels (Anguilla anguilla) was studied. The mean weight of small eels (SE) (0.6g body weight) maintained at 23°C or 27°C was significantly lower than that of SE maintained at 25°C or 27°C. No significant differences were found at the end of the experimental period between SE maintained at 25° and 27°C. Similar results were obtained among the moderate size eels (ME) (1.5g body weight). However, the average weight increase maintained at 25°C was significantly higher than that of large eels (LE) (3.25g body weight) maintained at 23° or 27°C.

¹ This paper has been accepted for publication in Aquaculture and Fisheries Management.

INTRODUCTION

The first year of eel culture is characterized by high mortality and varying growth rates. Previous studies have shown marked differences in growth of eels after 12 months (Degani and Levanon, 1983; Degani et al., 1984). The relationship between size and body composition of American eels (Anguilla rostrata) (Gallagher et al., 1984) and of European eels (A. anguilla) (Degani et al., 1986) was studied previously.

In both species the protein content of the whole eel -- calculated on a dry-weight basis -- decreased, and the total fat content increased with the size of the eels. The results of previous studies with different eel species -- A. japonica [Egusa, 1958], A. mosambica, A. momorata, A. nebulosulabita [Tarr and Hill, 1979], A. rostrata [Degani and Gallagher, 1985] -- show that oxygen consumption is affected by the temperature and body size of the eels.

Previous studies in our laboratory of eel (A. anguilla) nutrition (Degani, 1986) concerned themselves with the different effect of temperature on growth during the various stages of eel growth. Besides this, very little attention has been focused on the relationship between temperature and growth of fish from the same species but of different sizes.

The purpose of this study was to assess the relationships of growth, food conversion (FC), and temperature in European eels of various sizes.

MATERIALS AND METHODS

Glass eels of Anguilla anguilla were obtained from the coastal water of France in February 1986. Glass eels were placed in indoor containers at a density of 2 kg-m². They were maintained for 4 months and fed ad libitum

(Degani and Levanon, 1983). At the end of 4 months the elvers which had learned to eat were used in the study. The elvers were divided into three groups: Group 1 -- Small eels (SE) with a mean weight of 0.6g, Group 2 -- Moderate eels (ME) with a mean weight of 1.5g and Group 3 -- Large eels (LE) with a mean weight of 3.25g.

There were three replicated containers in each experimental group which were treated with different temperatures (23^o, 25^o, and 27^o) , as described by Degani and Levanon (1983) and Degani (1986). The diet was administered in pellet form (4% of the body weight) twice daily, at 7:00 and 15:00 h. The feed composition was 56% protein, 16.2% fat, and organic matter 91.63%. Elvers were kept at densities of 4.5kg (SE and ME) and 6.5kg (LE) per container (2 x 0.4 x 0.4m).

Every month 150 elvers from each experimental group were weighed individually on a balance with an accuracy of ± 0.01 g and the total weight of each container was also recorded.

At the end of the experimental period (90 days) 20 fish from each container were removed for analysis of body composition. The samples were frozen and later homogenized for analysis. Sample preparation was carried out according to Boetius and Boetius (1980) with a simple modification (Degani and Dosoretz, 1986). The eels were cut into slices of about 0.5cm and homogenized for 45 seconds (4,500 rpm) in a Polytron homogenizer. For moisture determination a sample of the homogenate was dried overnight in a Heraeus vacuum oven at 45^oC and <25 mmHg. After drying, the sample was ground using a mortar and pestle for further analysis. The ash content was measured by incineration at 600^oC for 12h, total nitrogen by the Kjeldahl

technique (protein = $N \times 6.25$), and total lipid according to Folch et al. (1957).

The differences between the group means were evaluated by analysis of variance (ANOVA) and the standard t-test.

RESULTS

The growth of SE maintained at 23° had a significantly ($P < 0.001$, ANOVA and t-test) lower body weight at the end of the experimental period than SE maintained at 25° or 27°C. However, no significant difference was found at the end of the experimental period between elvers maintained at 25° and 27°C (Table 1).

Similar results were obtained among the ME. ME maintained at 25° or 27° grew significantly faster than ME maintained at 23°C ($P < 0.05$, t-test) (Table 1). However, the average weight increase of LE maintained at 23°C was significantly higher ($P < 0.05$, t-test) than of those maintained at 25° or 27°C (Figure 1).

The effect of temperatures on eel growth and production is presented in Table 1. Food conversion after 34 days is shown in Figure 2. The body composition of different experimental groups is presented in Table 2. No significant differences were found among the experimental groups in % protein or % fat content of the eels.

DISCUSSION

In this study we found that the optimum temperature for LE -- 23°C is lower than that for SE (27°). Previous studies in our laboratory (Degani, 1986) showed that elvers maintained at 27°C grew faster than glass eels maintained at 25°C; 23°C encouraged the slowest growth.

The explanation for these results may be found in the life history of eels. Glass eels migrate from the sea to fresh water (Tesch, 1977) and the water temperature may change during this migration.

Another explanation which can add to our understanding of the difference between optimum temperatures between LE and SE may be found in the dramatic change in body composition of eels during the growth period.

In general, the percentage of total fat increased and that of protein, glucose and moisture decreased (Degani et al., 1986). Moreover, there was a rapid change in body composition when the body weight changed from 1g to 100g, the sizes used in this study.

In conclusion, it was found that there are different optimum growth temperatures for European eels during their different stages of growth and development.

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Table 1. Effect of temperature on eel growth during three experimental periods.

Group	Temperature (°C)	Weight Gains (g/m ²)			Total	Production (g/m ² .days) (90 days)
		0-34	34-59	59-90		
SE ¹	23	2364.25	18.13	2095.25	4441.25	49.35
SE	25	2419.25	594.00	2041.75	5055.00	56.17
SE	27	3997.88	791.00	1443.63	6232.51	69.25
ME	23	2440.38	1129.25	2522.88	6052.51	67.25
ME	25	3084.25	427.13	2792.38	6303.76	70.04
ME	27	3935.25	621.38	2424.63	6981.26	77.57
LE	23	2598.25	1439.50	3439.75	7477.50	83.08
LE	25	3039.00	1139.50	2670.25	6848.75	76.10
LE	27	3722.00	1021.11	1884.25	6627.66	73.64

SE = small eels
ME = moderate eels
LE = large eels

Table 2. Body composition of eels at the end of the experiment.

Group	Temperature (°C)	% Moisture	% Wet Weight		% Dry Weight		
			Protein	Lipid	Ash	Protein	Lipid
SE ¹	23	67.15	17.91	12.95	2.12	54.53	39.45
SE	25	64.78	17.22	15.66	1.63	48.86	44.41
SE	27	63.17	17.71	16.39	1.70	48.15	44.41
ME	23	66.49	17.48	13.68	1.64	52.18	39.06
ME	25	63.48	17.07	16.68	1.46	46.84	45.70
ME	27	65.19	16.94	15.20	1.63	48.66	43.58

SE = small eels

ME = moderate eels

FIGURE LEGENDS

Fig. 1. Food conversion of various sized elvers maintained at different temperatures (SE = small eels, ME = moderate eels, LE = large eels).

CHAPTER 4¹

THE INFLUENCE OF BODY SIZE AND TEMPERATURE OF THE METABOLISM OF EUROPEAN EELS (ANGUILLA ANGUILLA)

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ABSTRACT

The effect of temperature and body size on oxygen consumption of European eels (Anguilla anguilla) was studied. The oxygen consumption of large eels was lower at all temperatures than that of small eels. The effect of temperature on metabolism was greater on small eels than on large eels.

The relationship between oxygen consumption and body size is described by the equation $M = aW^b$, where M is metabolism (O_2/h) and W is body weight (in g) and a, b are constant. Increased temperature caused an increase in a and a decrease in b.

¹ This paper has been submitted to Environmental Biology of Fish.

INTRODUCTION

Metabolism measured as oxygen consumption can yield important information on the metabolic rate in fish. Bassas metabolism rate has been the subject of investigation in many fish species (Attman and Dittmar, 1974), in regard to the effect of both temperature (Schollander et al., 1953; Wohschlag, 1960, 1964; Caulton, 1978) and fish size (Egusa, 1958; Caulton, 1978; Tarr and Hill, 1979).

Metabolism is a very important parameter whcih can be used for calcuating the energy requirement of fish. This information can be used on adjust the energy supply in fish feed, which is a very important parameter for moder aquaculture. Many factors have an effect on metabolism, e.g. age of fish, time of day, body size, activity of fish, breeding season, light, time of feeding, and feed composition. For these reasons it is important to develop a metabolic system which can measure oxygen consumption over a long period and under conditions as close as possible to the situation in aquaculture.

The energy requried for swimming has been determined for fish (Beamish, 1978; Brett and Glass, 1973).

The culture of various species of eels (Anguilla anguilla, A. japonica, A. mossambica, A. rostrata) has been the subject of many investigations over the past few years (Angel and Jones, 1974; Tarr and Hill, 1979; Forest, 1976; Liewes, 1978; Otwell and Richard, 1981, 1982; Degani and Levanon, 1983; Degani and Gallagher, 1985; Degani et al., 1985, 1986).

In the culture of the eel A. anguilla marked growth variation was found without physical interaction with other elvers an without competition

for food (Degani and Levanon, 1983; Wickens, 1985). The relation between eel body size (9 - 420g) and body composition was described in detail by Degani et al. (1986). Relative amounts of protein and glucose were found to decrease with increasing weight of the eels, but the percent fat was higher in large eels than in small ones.

Extensive studies have been made of the oxygen consumption of various eel species: A. mosambica, A. momorta, A. nebulosulabita (Tarr and Hill, 1979); A. japonica (Egusa, 1958); and A. anguilla, and A. rostrata (Degani and Gallagher, 1985). However, little information has been published on the relationship between water temperature, body weight and oxygen consumption of European eels.

MATERIALS AND METHODS

European glass eels (Anguilla anguilla) were caught in February 1985, during winter migration in the coastal waters off France, when they were 7 cm in length and weighed 0.35g. They were maintained indoors in open containers (1m³) for 14 months. Water temperature was $25^{\circ} \pm 1^{\circ}\text{C}$ (Degani and Levanon, 1983; Degani et al., 1986). In order to prevent cannibalism, the eels were separated according to size every 3 months.

The eels were fed 4% of their body weight each day, divided into two meals, at 7:00 and 15:00. The food was prepared from commercially available chicken offal powder mixed with fish meal (Degani et al. 1986), and placed on a net at one location in each container; any unconsumed food was removed from the net after 1h.

Oxygen consumption was measured in a closed system described in detail in Figure 1; the system was controlled by a computer. All the data were stored in the computer for subsequent analysis.

Eels of four different sizes, 10 ± 5 g, 90 ± 4 g, 150 ± 6 g, and 480 ± 10 g, were used. They were acclimated to the metabolism system for 3 days and the measurements for each temperature were carried out for a period of 1 week. The lowest oxygen consumption recorded was taken as the metabolic oxygen consumption of the eels. A preliminary measurement of metabolism lasting 24h showed the lowest 3h measurement for eel oxygen consumption.

RESULTS

The weight and specific oxygen consumption rate of eels of different sizes maintained at various temperatures are shown in Figure 2. The oxygen consumption of larger eels was lower at all temperatures than that of small eels. Moreover, the effect of temperature on oxygen consumption was greater in small eels than in large eels. The equation comparing oxygen consumption in various sized eels maintained at different temperatures is shown in Table 1. A linear correlation was found between oxygen consumption and water temperature.

The relationship between oxygen consumption and body size of eels maintained at different temperatures is shown in Table 2.

DISCUSSION

The results of this study show for the first time that the oxygen consumption of European eels is affected differently by temperature in small eels than in large eels.

The results are in agreement with those of previous studies which showed a dramatic difference between the body composition of large and small eels. The protein content of whole eels was higher in the small eels than in the large ones. However, total fat content increased with size of

the eels (Degani, et al., 1986). It is possible that a decrease in protein synthesis and an increase in fat synthesis had an effect on metabolism. Moreover, enzyme activity (aldolase) per g of tissue decreased with an increase in eel body weight. This enzyme is involved in glycolysis and therefore very important in determining the energy requirements of eels.

The low metabolism found in large European eels may help the eels in their biological cycle. For spawning, the European eel migrates to the region of the Bermuda Islands in the Atlantic Ocean (Schmidt, 1922). It is possible that during the migration, low metabolism is not greatly affected by temperature.

In this study the eels at different temperatures (20, 24, and 27°C) show a high correlation to the body weight, which can be described by the equation $M = aW$ (where M is oxygen consumption and W is body weight). A low correlation was found at 18°C.

The results of this study are in agreement with those of previous studies (see Table 2) and the relationship between oxygen consumption and body weight is similar in different species. We measured the metabolism at different temperatures. There are very similar slopes of the regression curve among the American eels (A. rostrata) at 25°C AND 23°C, Japanese eels (A. japonica) at 25°C, and European eels (A. anguilla) at 24°C. Moreover, the slopes in this study are not dramatically different at 20, 24, and 27°C. The only difference in slope was found between the above three species of eels and A. mossambicus (Table 2). There is no difference between the allometric equation of European eels and Japanese eels. However, it is known that Japanese eels adapt to higher temperatures than

European eels (Tesch, 1977; Usiu, 1979), and this difference may represent the different adaptation to water temperature.

In conclusion, it was shown that there is an effect of temperature and body size on oxygen consumption in European eels which may explain many aspects of the biology and culture of eels. The optimum temperature for these eels changes from when they are small (27°C) to when they grow in size (25°C) (Degani and Levanon, 1986; Dosoretz and Degani, 1987). Moreover, the energy supplied in feed also had a dramatic effect on growth and the latter's dependence on temperature.

ACKNOWLEDGEMENT

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Table 1. Comparison of oxygen consumption rates in different sizes and species of eel (Anguilla anguilla) at various temperatures.

M = oxygen consumption (mg/g.h); T = temperature (°C)

<u>Mean Weight (g)</u>	<u>Allometric Equation</u>	<u>r</u>	<u>P</u>
10	$M=0.0203T - 0.2656$	$r=0.99$	<0.01
90	$M=0.0095T - 0.1169$	$r=0.97$	<0.05
150	$M=0.0084T - 0.1154$	$r=0.99$	<0.01

Table 2. Comparison of oxygen consumption rate in various eel species at different temperatures. (M = oxygen consumption mg/h; W = body weight.

<u>Species</u>	<u>Temp. (°C)</u>	<u>Allometric Equation</u>	<u>r</u>	<u>Data Source</u>
<u>A. rostrata</u>	25	$M=0.56W^{0.64}$	0.94	Degani & Gallagher, 1985
<u>A. rostrata</u>	23	$M=0.638W^{0.525}$	0.95	Gallagher, et al., 1984
<u>A. mossambica</u>	25	$M=0.396W^{1.0969}$	0.99	Tarr & Hill, 1979
<u>A. japonica</u>	25	$M=0.23W^{0.707}$	0.99	Egusa, 1958
<u>A. anguilla</u>	18	$M=0.0038W^{1.291}$	0.92	This study
<u>A. anguilla</u>	20	$M=0.2132W^{0.773}$	0.99	This study
<u>A. anguilla</u>	24	$M=0.4660W^{0.6781}$	0.99	This study
<u>A. anguilla</u>	27	$M=0.5583W^{0.6886}$	0.99	This study

FIGURE LEGENDS

Fig. 1. The metabolism system.

Fig. 2. The oxygen consumption of eels (Anguilla anguilla) at different weights (n=30).

METABOLISM SYSTEM

CHAPTER 5¹

DIGESTIBILITY OF CRUDE PROTEIN AND ORGANIC MATTER IN DIETS WITH VARYING PROTEIN AND ENERGY CONTENTS FOR EELS WITH VARYING GROWTH RATES

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INTRODUCTION

Protein in diets for fish acts as both a source of essential amino acids and as an energy source. In addition, protein digestion coefficients remain high when the diet contains protein at levels either above, below, or at optimal levels (Ash, 1985) and protein digestibility does not appear to be limited by the inability to assimilate changing protein levels (Cho and Kaushik, 1985). However, a number of factors may influence the digestibility of proteins, including the source of protein with fish meal generally being a superior source when processed and handled properly. In addition, sources and types of non-protein energy in the diet have been shown to influence digestibility of protein. The protein energy ratio must also be considered. Temperature decreases digestibility apparently due to an increase in gut transit time. Size of fish has also been reported to influence digestibility. However, there is not any general agreement as to these effects. For example, rainbow trout were reported to have the same apparent protein digestibility when size varied between 10 and 100g. However, in other work, digestibility of white fish meal and casein decreased in fish weighing less than 6g. The purpose of the following study was to evaluate the effect of size and rate of growth on digestibility of protein and organic matter in eels.

¹This paper will be submitted to Aquaculture.

MATERIALS AND METHODS

Eels

All eels for this study were purchased from Anguilla Farms, Hastings, Florida. Eels were shipped by air freight to our laboratories at the Institute for Coastal and Marine Resources. Eels were separated into three groups designated as: 1) moderately-growing 1987 (>5g), 2) slow-growing 1987 (<2.5g), and 3) slow-growing 1985-86 (>5g). After sizing, eels were placed in each 40-L aquaria with 3 replications of each size. The number of eels in each replication varied between 3 and 11, depending upon the size of the eels. In addition, four larger sized eels of unknown age were also used.

Diets

Diet formulations used in this study are shown in Table 1. After formulation, approximately 1% chromic oxide was added to each diet. Water was added to form a dough and pellets were produced by extruding the dough through a 50cc syringe, drying and breaking dried strands into pellets. Pellets were stored dry in sealed plastic freezer containers.

Procedure

Digestibility was determined using the chromic oxide marker method described by Windell (1978) where

$$\% \text{ Digestibility} = 100 - \left(\frac{\% \text{Cr}_2\text{O}_3 \text{ in diet}}{\% \text{Cr}_2\text{O}_3 \text{ in feces}} \times \frac{\% \text{nutrient in feces}}{\% \text{nutrient in diet}} \times 100 \right).$$

Eels were fed diets for 48 hours before collection of feces began. Eels in each aquaria were fed all the feed they would consume in 30 minutes each morning for 5 days. Eighteen hours after each feeding, feces were collected into aluminum weighing pans by suction and dried at 60°C. Each

diet was fed to each replication of eels for a period of 5 days with a 48-hour pre-collection feeding period between each diet collection period.

Chromic oxide content of feces and diet was determined using the sodium molybdate method described by Fenton and Fenton (1979). Crude protein was determined by measuring nitrogen content using the colorimetric method described by Scheiner (1976) and multiplying nitrogen content by 6.25 to obtain crude protein. Organic matter was determined where sufficient sample was available by ashing samples to a constant weight at 600°C and subtracting total ash from sample weight to obtain total organic matter. Analysis of Variance and Least Significance Difference test were used to test for significant differences in mean protein and organic matter digestibility among diets and size groups of eels.

RESULTS

The mean percent digestibilities of protein with varying growth rates are given in Table 2. Generally, protein digestibility declined as protein levels increased in both 3.0 kcal/g diets (1-4) and 4.0 kcal/g diets (5-8). In moderately-growing eels, digestibilities were significantly higher in diets with 30 and 35% protein as compared to 40 and 45% protein diets with 4.0 kcal/g. Digestibility of the 35% protein diet was also significantly higher than 40 and 45% protein diets with 3.0 kcal/g. In slow-growing eels (1987) and slow-growing eels (1985-1986) these trends were still apparent but not significant. In all diets digestibility of protein in slow-growing eels (1987) was lower than either moderately-growing eels (1987) or slow-growing eels (1985-1986). However, this difference was only significant for diets 4 and 7. Digestibilities for diet 1 were significantly lower than all other diets for both slow-growing eels (1987) and (1985-1986).

When eels of different sizes were compared, no statistical differences were found due to size, so all data were combined for statistical analyses. In these larger eels, all protein digestibilities were high. Diet 1 had significantly lower % digestibility than all other diets. Diet 2 was significantly higher than diet 5.

The mean percent digestibilities of organic matter for eels with varying growth rates are shown in Table 2. Generally, organic matter was significantly more digestible in diets containing high energy levels than in those containing lower energy levels, especially in moderately growing eels. These data are not surprising in view of the higher levels of cellulose in the 3.0 kcal/g diets.

There were no significant differences between eels of the same size but different ages (moderately-growing, 1987, vs. slow-growing 1985-1986) at any dietary level. Due to insufficient sample size, 1987 slow-growing eels could not be subjected to statistical analyses.

In the larger eels studied, digestible organic matter (DOM) varied widely in diets containing 3.0 kcal/g. Diet 3 with 40% protein had significantly lower percent digestible organic matter than all other diets and diet 1 had significantly lower DOM than either diet 2 or 4. There were no significant differences in DOM for diets with 4.0 kcal/g. There was a trend toward higher percent DOM as size increased; however, this was not significant.

As with protein digestibility, organic matter digestibility was extremely variable and significantly lower in diet 1 compared to all other diets.

DISCUSSION

The results of these studies indicate that size of eel may have a significant influence on digestibility of protein. Eels caught in the same year but growing at a slower rate had reduced protein digestibilities. However, these same eels also had reduced protein digestibilities when compared to slow-growing eels that were a year older, indicating that actual size, rather than rate of growth, is the key factor.

Percent digestibility values for protein are similar to those reported by others. As pointed out by Ash (1985), most protein used in fish diets have high (84-99%) digestibilities even when consumed in levels above, below or near the optimal level. However, Cho and Kauskih (1985) have noted that efficiency does decrease as dietary protein increases. This phenomenon is apparent from the decreasing protein digestibilities in Table 2.

Organic matter digestibilities are generally lower than those reported for eels by Schmitz et al (1984). Low OMD would be expected in the diets containing 3.0 kcal, since they are relatively high in cellulose, which has a digestibility of 0.11% (Schmitz et al. 1982) in eels. The explanation for the poor performance of diet 1 is unclear. However, this diet does contain the highest amounts of both cellulose and wheat middlings which, in combination, could have caused decreased digestibility of both protein and organic matter. Inaba et al (1963) showed that starch is more digestible in diets for trout when fed in low levels.

From these results we conclude that eel size may significantly influence digestibility of protein and organic matter in eels. In addition, at protein levels above 35%, digestibility is decreased, especially

in eels growing at a moderate rate.

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Table 1: Eel diet formulations used for digestibility studies.

Ingredients	Diet Number							
	#1	#2	#3	#4	#5	#6	#7	#8
Fish meal	22.00	30.00	38.00	44.00	20.00	27.00	36.00	44.00
Blood meal	3.00	3.00	3.00	3.00	3.00	3.00	3.00	3.00
Poultry/meat meal	14.00	17.00	18.00	20.00	17.00	18.00	18.00	20.00
Wheat middling	30.00	20.00	15.00	8.00	43.00	35.00	25.00	15.00
Fish oil	5.00	5.00	3.50	3.00	10.00	10.00	10.00	10.00
L. cellulose	20.10	20.10	17.60	17.10	2.10	2.10	3.10	3.10
Vitamin Mix 30	0.50	0.50	0.50	0.50	0.50	0.50	0.50	0.50
Mineral Mix AIN 7.6	3.50	3.50	3.50	3.50	3.50	3.50	3.50	3.50
Ascorbic Acid	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Choline Chloride	0.15	0.15	0.15	0.15	0.15	0.15	0.15	0.15
g Protein	29.32	34.80	39.90	44.80	31.60	35.60	40.01	45.80
g Fat	10.01	10.60	9.80	9.30	15.70	16.10	16.60	17.10
g CHO	23.50	17.70	15.10	11.20	32.40	27.70	21.60	15.90
g ASH	6.90	8.10	9.20	10.20	7.70	8.50	9.40	10.50
g Fiber	3.94	3.25	2.90	2.40	5.30	4.70	3.80	3.10
Kcal/100 g	301.00	306.00	308.00	308.00	397.00	398.00	396.00	401.00

Table 2: Mean digestibility of crude protein by moderate- or slow-growing eels fed diets with varying protein energy contents.

Eel Group	% Digestibility							
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Moderate-growing 1987	91.59 ^{bc} ¹	94.57 ^a	91.04 ^c	91.67 ^{bc}	94.53 ^a	94.11 ^a	91.77 ^{bc}	92.52 ^b
Slow-growing 1987	71.93 ^a	91.94 ^b	88.79 ^b	85.55 ^b ²	93.99 ^b	92.92 ^b	86.27 ^b ²	92.44 ^b
Slow-growing 1985-1986	84.98 ^a	94.99 ^c	93.73 ^{bc}	92.38 ^{bc}	94.90 ^{bc}	93.88 ^{bc}	93.02 ^{bc}	92.07 ^b

¹Means with different superscripts are significantly different from one another at $p < 0.05$ within rows.

²Mean is significantly lower than fast-growing eels from 1987 or slow-growing eels from 1985-1986 at $p < 0.05$.

Table 3: Mean digestibility of organic matter by moderate- or slow-growing eels fed diets with varying protein energy levels.

Eel Group	% Digestibility							
	Diet 1	Diet 2	Diet 3	Diet 4	Diet 5	Diet 6	Diet 7	Diet 8
Fast-growing 1987	55.74 ^a +7.23	73.50 ^c +1.07	61.99 ^{ab} +1.30	64.18 ^b +3.29	72.57 ^c +3.70	73.97 ^c +3.94	72.67 ^c +0.91	76.67 ^c +3.06
Slow-growing 1987	--	75.86 +1.85	--	71.72 +7.21	70.90	78.63	55.90 +11.20	73.39
Slow-growing 1985-1986	20.09 ^a +18.30	69.30	64.59 ^b +0.28	64.81 ^b +0.15	74.20 ^c +2.87	68.14 ^b +6.15	72.29 ^c +0.91	76.03 ^c +0.53

¹ Means with different superscripts are significantly different from one another at $p < 0.05$ within rows.

Table 4: Mean digestibility of crude protein and organic matter by eels of varying sizes fed diets with varying protein varying contents.

Diet	Eel #1 (wt=17.2g)			% Digestibility			Eel #3 (wt=70g)			Eel #4 (wt=300g)			Total	
	Crude Protein			Crude Protein			Crude Protein			Crude Protein			Crude Protein	
	Matter			Matter			Matter			Matter			Matter	
1	88.50	33.06	85.10	24.11	89.62	41.99	92.24	58.54	88.86 ^a	2.56	39.43 ^b	12.72		
2	87.09	76.27	94.74	73.80	94.30	73.36	92.86	69.58	92.25 ^b	3.06	73.25 ^{cd}	2.39		
3	92.70	18.60	92.78	7.19	93.69	30.40	93.46	52.49	93.16 ^{bc}	0.43	27.17 ^a	16.76		
4	96.62	65.37	92.79	69.02	92.26	68.73	94.30	74.56	92.99 ^{bc}	0.78	69.42 ⁺	3.30 ^c		
5	94.93	72.74	93.53	78.42	94.14	77.99	95.12	83.05	94.43 ^c	0.64	78.05 ⁺	3.65 ^{cd}		
6	93.31	69.16	92.10	77.67	94.88	80.76	93.92	82.11	93.55 ^{bc}	1.01	77.34 ⁺	4.38 ^{cd}		
7	92.05	70.44	91.24	76.97	92.44	79.83	94.07	83.40	92.45 ^{bc}	1.03	77.66 ⁺	4.75 ^{cd}		
8	92.45	78.37	93.04	80.53	92.67	82.56	93.83	83.83	93.00 ^{bc}	0.53	81.32 ⁺	2.07 ^d		

CHAPTER 6¹

EFFECT OF DIETARY PROTEIN AND ENERGY LEVELS ON GROWTH OF EUROPEAN EELS

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ABSTRACT

The influence of the protein/energy ratio in a purified diet on the growth of large European eels (Anguilla anguilla) (40-120g) was examined. The eels fed a diet with a low protein/energy ratio (58 mg/kcal or 68 mg/kcal) had a significantly lower ($P < 0.05$ / t-test) body weight than those fed with a high protein/energy ratio (72 mg/kcal, 83 mg/kcal and 100 mg/kcal). The optimum growth was obtained with the diet of 100 mg/kcal.

Comparing this study to previous research on small European eels, we come to the conclusion that large eels can achieve quite good growth when the protein/energy ratio is above 70 mg/kcal.

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INTRODUCTION

Various studies on artificial diet have investigated the optimum protein level of Anguilla anguilla (Degani et al., 1985) and A. japonica (Arai, 1972 and Arai et al., 1971, 1972).

Eels can utilize fat as an energy source (Arai et al., 1971, 1972; Lavurn, 1933, 1940; Degani, 1986) as well as carbohydrates (Spannhof and Kuhne, 1977; Degani et al., 1986; Degani and Levanon, 1986; Degani and Viola, 1987).

The growth of eels is not affected only by protein level but also by the energy level of the diet, or in other words, by the protein/energy ratio. Moreover, the body composition of eels from different species A. rostrata (Gallagher et al., 1984), A. anguilla (Degani et al., 1986) and A. japonica (Ochiai et al., 1981) show that the total protein level decreases and the total fat level increases during the growth period.

Most of the nutrition studies on eels concern eels smaller than 10g body weight. It seems from these studies that eels need a high protein level diet for maximum growth (Arai, 1972; Arai et al., 1971; Degani et al., 1985, 1986). However, in larger eels the diet formula may be different.

The purpose of this study was to assess the level of protein/energy ratio in the diet of large eels (40-120g).

MATERIALS AND METHODS

Animal and Feed Patterns

Fifteen outdoor containers of 1m² were allocated to five diet groups. An experimental group of 50 eels (Anguilla anguilla) of 56g mean initial weight was stocked in each container. The water temperature was $25 \pm 4^{\circ}\text{C}$

and the water exchange rate was 300 l/day. All the eels were fed 2% (as 80% dry matter, D.M.) of their body weight each day, half at 7:30 and the other half at 15:30. The ratio was equal for all the experimental groups and was based on the fastest growing group. The diets were in paste form (Degani et al., 1986) which were placed on a net in each container. All the eels were weighed individually on day 1, 21, 43, 66, 90, and 105.

Diets

The composition of the five experimental diets is given in Table 1. The diets contained different levels of protein (between 32-45%) and about the same level of energy. The protein/energy ratios of the diets were varied from 58 to 100 mg/kcal. The diets were composed of fish meal, poultry meal, fish fat, milk meal, wheat meal and vitamins. This composition has shown a good effect on eel growth in previous studies in our laboratory (Degani et al., 1985; Degani et al., 1986; Degani, 1986; Dororetz and Degani, 1987).

RESULTS

The growth of eels which were fed different protein/energy ratio levels is shown in Fig. 1. Eels fed a diet with a protein/energy ratio below 58 and 68 mg/kcal showed significantly less growth ($P < 0.001$ t-test) than those eels fed diets with a higher protein/energy ratio. In addition, eels fed a diet with a higher protein/energy ratio (100 mg/kcal) grew significantly faster than those eels fed on a diet with a lower protein/energy ratio (72 and 82 mg/kcal).

The % weight gain, production and food conversion of eels fed different protein/energy ratios are shown in Table 2. Again, the best

production, % weight gain and food conversion were according to the protein/energy ratios of 100, 83, 72, 68, and 58 respectively.

DISCUSSION

The results of this study support the idea that the maximum growth of eels is dependent not only on the level of protein (Yanazaki, 1986; Arai et al., 1971, 1972; Nose and Arai, 1972; Degani et al., 1985) but also on the protein/energy ratio.

Previous studies on the optimum level of protein in a purified diet have worked with Japanese eels (Anguilla japonica) (Nose and Arai, 1972) or European eels (Degani et al., 1985). These studies showed that eels required about 45% protein in their diet for maximum growth. In this study it was found that the other parameter of energy is very important. Eels fed with a diet of 35% or 42% protein grew almost the same because the protein/energy ratio was similar (72 and 83 respectively).

On the other hand, eels fed a much lower protein/energy ratio (58 and 68) grew significantly less than those fish fed a diet with a higher protein/energy ratio. The highest growth rate was found in eels fed a diet with 45% protein and a protein/energy level of 100 mg/kcal. A similar result was found in previous studies. However, small eels fed a diet with 35% protein grew much slower than the eels in this study (Degani et al., 1985). It seems that the protein/energy ratio is different for small eels than for large eels.

The main sources of protein in eels' diet is chicken meal and fish meal (Degani et al., 1986). Most studies of eel diet involved fish meal as the source of protein. However, replacing 50% of the fish meal in the diet with chicken meal reduced the costs and produced a similar yield (Degani et

al., 1986).

Eels have been shown to digest a considerable amount of carbohydrates, which is a potential energy source (Spanhof and Kuhne, 1977; Hagihara et al., 1967; Degani et al., 1986). Eels fed a diet with 45% protein and 30% of different carbohydrates (wheat meal, bread meal, soluble corn starch, native potato starch and sorghum starch) showed different growth rates. It seems that this difference is due to the different protein/energy ratios among the experimental diets, as was found in this study.

Another very important energy source in the eels' diet is fat, mainly animal fat such as chicken fat or fish fat (Degani, 1986). Glass eels fed animal lipids (poultry) grew faster than those fed on plant lipids (soya bean).

Gallagher et al. (1984) found a significant correlation between the percentage of body fat and the body size of American eels (Anguilla rostrata) and Degani et al. (1986) found a similar result in European eels (Anguilla anguilla). The meaning of these results is that fat is not only used as an energy source but also for body composition. In this study it was found that high fat in the diet (40.8%) and low protein (32.5%), or in other words, a low protein/energy ratio (58 mg/kcal) is not the optimum relationship between protein and fat for growth of eels at the size of between 40-120, a higher protein/energy ratio of about 100 mg/kcal is needed.

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Table 1. Formulation of test diets.

Diet Group No.	<u>% FEEDSTUFF (AS FED)</u>				
	1	2	3	4	5
Basic Ingredients					
Fish meal	51.27		59.09	66.04	
Poultry meal	0.06	44.83	4.07		64.00
Fish fat	40.82	14.34	31.17	25.04	0.24
Milk meal	4.90	5.10	4.90	4.90	5.10
Wheat meal		32.81			27.80
Guar gum	2.00	2.00	2.00	2.00	2.00
Vitamin premix	1.00	1.00	1.00	1.00	1.00
Gross energy (kcal/g)	5.53	5.09	5.26	5.06	4.49
Protein	32.50	35.00	38.00	42.10	45.10
Protein/energy ratio					
(mg/kcal)	58.00	68.00	72.00	83.10	100.00

Table 2. Growth and food conversion of eels fed diet with different protein/energy ratios.

<u>Protein/energy ratio</u> (mg/kcal)	<u>Production</u> g/m ₂ /day	<u>% weight gain/day</u>	<u>Food conversion</u>
58	0.33	0.50	3.95
68	0.14	0.29	6.80
72	0.50	1.00	1.98
83	0.49	0.88	2.26
100	0.51	1.16	1.71

Figure 1. Weight gain (%) increase of fish fed different experimental diets.

Feed 1. 32% protein and 5.53 kcal/g;

Feed 2. 35% protein and 5.09 kcal/g;

Feed 3. 38% protein and 5.26 kcal/g;

Feed 4. 42.1% protein and 50.1 kcal/g;

Feed 5. 45.1% protein and 4.49 kcal/g.

DESCRIPTION OF COOPERATION

Both the Institute for Coastal and Marine Resources at East Carolina University and MIGAL - Galilee Technological Center, Kiryat, Shmona, have had a continuing interest in aspects of eel culture because of its high market value and potential in aquaculture. Therefore the work described in this report was a joint effort to investigate problems associated with the large variation in growth rate associated with eels of the Anguilla sp. Various factors which could influence or be influenced by growth rate were investigated, including dietary protein energy ratios, digestibility of dietary ingredients, metabolic rates at varying temperatures, body composition, and food conversions.

Metabolism and temperature studies were conducted by MIGAL, because of the ability of that laboratory to run experiments at several temperatures simultaneously. Digestibility studies were conducted at East Carolina University because it had the equipment and appropriate safety facilities to conduct digestibility trials. Both laboratories used eels of varying sizes and growth rates and conducted feeding trials and body composition studies. Extensive discussions of results, progress and technical problems allowed the investigators to maintain direction and overcome problems.

EVALUATION OF RESEARCH ACHIEVEMENTS

The purposes of the work described in this report were to produce information of practical use in the culture of eels as well as basic data concerning the significance of temperature and dietary protein and energy ratios in the culture of eels.

The following conclusions can be drawn from the work reported:

1. Increasing temperatures increases protein and energy requirements in eels. This increase is presumably due to an increase in metabolic rate due to an increase in metabolic rate due to increased temperature. Therefore, in practical application the advantages of increased growth at higher temperatures must be balanced against increased dietary requirements for total energy and, most importantly, for protein.
2. Increasing temperatures do not effect slow-growing eels in the same way as faster-growing eels. Specifically, increasing temperatures consistently increases growth in slow-growing eels. Increased temperatures also increase metabolic rate significantly more in slow-growing eels as compared to faster-growing eels. Therefore, there appears to be a difference in optimum culture temperature of faster-growing eels as compared to slower-growing eels. The practical significance of this difference is not clear, but should be taken into account when rearing eels of different sizes.
3. Digestibility of protein and organic matter and overall growth is significantly reduced in slow-growing eels compared to faster-growing eels, regardless of dietary protein and energy content. Therefore, manipulation of dietary ingredients would not be of practical value in dealing with slow-growing eels.

4. The body composition of all size eels and both Anguilla rostrata and A. anguilla were significantly effected by the protein and energy content of the diet. Generally, diets with energy contents of $4.0 \text{ kcal} \cdot \text{g}^{-1}$ produced eels with significantly higher fat content (a desirable outcome) compared diets with lower energy levels but with the same protein content.
5. Dietary protein and energy contents should be at least 35% and $4.0 \text{ kcal} \cdot \text{g}^{-1}$, respectively for both Anguilla rostrata and A. anguilla.

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ANNIVERSARY BOND

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