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INDIVIDUALITY OF GROWTH, APPETITE, METABOLIC RATE AND ASSIMILATION OF NUTRIENTS IN YOUNG GREEN TURTLES (*CHELONIA MYDAS* L.)

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ABSTRACT

Mean appetite and oxygen uptake were highly variable amongst the 12 young green (*Chelonia mydas* L.) turtles studied. Neither appetite nor oxygen uptake had a statistically significant influence on specific growth rate. Amongst the efficiencies of assimilation of nutrients, there were quite wide individual variations in the rates of assimilation of energy, lipid and dry mass, but protein was assimilated with a uniformly high efficiency. Assimilation efficiencies of lipid and dry mass were significantly and positively correlated with specific growth rate. There were also strong positive correlations between the efficiencies of assimilation of different nutrients. There were weak negative correlations between appetite and the assimilation rates for energy and dry mass. These suggest that turtles compensate for a low efficiency of assimilation of these nutrients by an increased rate of food intake. Lipid assimilation in the turtles was lower than for the other nutrients. It was found that fatty acids are not all absorbed to the same extent. Saturated fatty acids and monounsaturated fatty acids were relatively poorly absorbed by comparison with polyunsaturated fatty acids.

INTRODUCTION

This paper reports the second part of an investigation of individuality of growth and nutrition in young green turtles (*Chelonia mydas* L.) fed on satiation rations. The first part (see Davenport & Scott, 1993) established that the specific growth rate of individual hatchling/juvenile turtles was stable, but that there was great variability of growth rate amongst individuals. However, allometric measurements demonstrated that no turtle shape, or pattern of shape/weight change was associated with high or low rates of specific growth. The part of the study reported here was designed to establish whether there were correlations between specific growth rate and various physiological/biochemical variables, viz: appetite, metabolic rate and assimilation of energy, lipid, protein and dry mass. As in the earlier paper, the experimental approach adopted stems from the investigations of individuality of response in marine biology by J.C. Aldrich (Aldrich, 1975, 1986; Aldrich & Regnault 1990) and previous nutritional studies on young green turtles (Davenport & Oxford, 1984; Davenport *et al.*, 1989).

MATERIALS AND METHODS

COLLECTION, MAINTENANCE AND FEEDING

Twelve hatchling green turtles (of unknown but certainly mixed parentage and unknown sex) were sent from the Lara Reserve, Cyprus to the U.K. on 21st October 1989. At this stage they were 40-60 days posthatching. On arrival in the U.K. they were identified by placing hoops of coloured bell wire (four colours available) around the bases of the foreflippers. They were held in a large plastic aquarium (Fastank; 6 x 1 x 1 m) filled with sea water (33‰)at a temperature of 25±1°C. The sea water was gradually replaced by a trickle supply; the contents of the tank were circulated through a biological filter at a flow rate of about 5 l min^{-1}. Throughout the experimental period the animals were fed daily on trout pellets. Great care was taken to ensure that the trout pellets were always offered in satiation amounts (i.e that the animals were always given more food than they could eat), and that no feeding hierarchy could develop to interfere with feeding. The latter objective was achieved by offering trout pellets on large trays suspended a few cm below the water surface in the holding tank. The pellets were spread out over a large area so that each animal could feed in undisturbed fashion. Antagonistic behaviour between the turtles during feeding sessions was rare and did not seem to be related to body size; small turtles were as likely to bite bigger animals as the latter were to be the aggressors.

WEIGHING AND MEASURING

These measurements have been described in detail in Davenport & Scott (1993). Weighing and measuring were carried out over a period of 176 days. Further weights were collected up to 217 days after the start of the experiment.

MEASUREMENT OF APPETITE

For each animal, the size of appetite was measured on six separate occasions (after 26, 82, 89, 107, 117 and 152 days). Appetite was measured for each animal in the following fashion. The animal was weighed, placed in the holding tank for two hours with the day's meal of trout pellets and allowed to feed to satiation. It was removed from the tank and reweighed. Meal size was taken to be the difference between the two weights. In all cases appetite was expressed in weight-specific form (i.e. as % body weight).

MEASUREMENT OF OXYGEN CONSUMPTION

Oxygen uptake was measured in animals at rest in humid air at 25°C as described by Davenport *et al.* (1982). Three separate recordings were made from each animal between days 138 and 142 of the study. Each animal was weighed after oxygen uptake had been recorded.

ASSIMILATION OF NUTIRENTS

For each turtle, three estimates of the efficiency of assimilation of nutrients were made during the following periods of the study:- 14-22 days, 59-71 days, 111-128 days. To do this,

turtles were fed on trout pellets mixed with an indigestible marker, chromic oxide. Large quantities of trout pellets were ground up with the green coloured marker (2% w/w), thoroughly blended with a little water, extruded from a large syringe and dried. Turtles were fed continuously on the labelled pellets until they had produced green faeces for 4 days. A faecal collection was then carried out by placing each turtle on a chicken mesh floor in a separate plastic vessel filled with filtered sea water. The turtles were inspected at hourly intervals so that fresh, intact faecal pellets could be collected. Food and faecal samples were freeze-dried for subsequent analysis.

Chromic oxide content of samples was analysed by wet oxidation to dichromate and subsequent spectrophotometric determination by the diphenylcarbazide reaction (McGinnis & Casting, 1964). Energy content was established by wet oxidation, while lipid content was measured gravimetrically after extraction by the method of Folch *et al.* (1957). Protein content was determined by the micro-Kjeldahl technique. Dry organic matter content was established by ashing freeze-dried food and faecal samples of known weight at 680°C for six hours and reweighing.

Absorption of Fatty Acids

Since lipid assimilation had not been studied previously in green turtles, further analysis was performed to investigate absorption of fatty acids from the trout pellet diet. The fatty acid composition of the diet and of faeces from three turtles (collected for gravimetric analysis of lipid as described above) was determined as follows. The lipid of each sample was extracted by the method of Folch *et al.* (1957). The extract was incubated in methanol:dichloromethane solvent under nitrogen at 100°C for 1 h. The resultant material was mixed with a pentane:distilled water mixture and shaken. The upper (pentane) phase was collected, evaporated to dryness and dissolved in hexane. Samples (1 ml) of the solution of fatty acids in hexane were passed through a Carla Erba gas chromatograph and the fatty acid profiles displayed on a Hewlett Packard 3390A integrator. Identification of fatty acids was carried out by comparison with commercial standards. Quantification of data was achieved by measuring the area beneath each fatty acid peak and expressing that area as a percentage of the total area beneath the fatty acid trace.

Turtle No.	Wt (g) (day 0)	Wt (g) (day 217)	Mean specific growth rate (day 0-176)
1	33.5(12)	338.9(12)	0.01358 (10)
2	65.4(2)	839.3(2)	0.01470 (2)
3	58.9)5)	711.9(4)	0.01444 (3)
4	51.4(9)	415.7(10)	0.01152 (11)
5	56.7(6)	803.8(3)	0.01552 (1)
6	64.7(3	681.9(5)	0.01407 (7)
7	42.0(11)	454.8(9))	0.01383 (8)
8	64.3(4)	645.2(7)	0.01365 (9)
9	49.9(10)	350.9(11)	0.01100 (12)
10	70.5(1)	840.2(1)	0.01444 (4)
11	52.3(8)	633.2(8)	0.01440 (6)
12	56.6(7)	669.9(6)	0.01441 (5)

Figures in parentheses represent rank orders of weight and growth rate, with 1 being highest and 12 lowest.

TABLE 1. Weight changes and mean specific growth rates of individual young green turtles (*Chelonia mydas*). From Davenport & Scott (1993).

RESULTS

Growth Rates

Specific growth rates were calculated as follows:

$$g = \frac{\ln (W_t/W_o)}{t}$$

where g = specific growth rate, t = elapsed time between weight measurements, W_o = initial weight, W_t = weight after time t.

Values of specific growth were calculated for each interweighing interval, and then an overall mean value was obtained (see Table I).

Appetite, Oxygen Uptake and Assimilation of Nutrients

Data for all of these variables are displayed in Table 2. Assimilation efficiencies were calculated after Maynard and Loosli (1969) as:

$$E = 100 \times 1 - \frac{(c^d/n^d)}{(c^f/n^f)}$$

where E = assimilation efficiency (%), c^d = chromic oxide content of diet, n^d = nutrient content of diet, c^f= chromic oxide content of faeces, n^f = nutrient content of faeces.

Mean appetite was highly variable amongst the turtles (range 2.05-3.98 % body wt d^{-1}) as was mean oxygen uptake (range 0.127-0.249 ml g^{-1} h^{-1}). However, neither appetite nor oxygen uptake had a statistically significant relation to specific growth rate (see Table 3). Amongst the efficiencies of assimilation of nutrients, there were quite wide inter-individual variations in the rates of assimilation of energy, lipid and dry mass, but protein was assimilated at a uniformly high level of efficiency (Table 2), significantly greater ($P<0.05$) than for all other nutrients. It was therefore not surprising to find that there was no significant correlation between the efficiency of protein assimilation and specific growth rate. Assimilation efficiencies of two nutrients (lipid and dry mass) were significantly and positively correlated with specific growth rate (i.e. the higher the assimilation rate, the higher the specific growth rate). Amongst the nutrient data there was a general trend towards strong positive correlations between the efficiencies of assimilation (i.e. a high rate of assimilation of one nutrient was associated with high rates of assimilation of other nutrients). Again the narrow range of protein assimilation efficiencies recorded tended to be an exception to this rule, but even so the correlations between protein assimilation efficiency and assimilation efficiency for dry mass, lipid and energy were all close to significance at the 5% level.

Two other regression analyses are of interest; those between appetite and the assimilation rates for energy and dry mass. In both cases there are negative correlations

Turtle No.	Specific growth rate	Mean assimilation rate of nutrients (%*)				Mean Appetite (%* bw d^{-1})	Mean Oxygen Uptake (ml O_2 $g^{-1}h^{-1}$)
		energy	protein	lipid	dry mass		
1	0.01358	66.87	86.43	58.30	66.65	3.395	0.148
2	0.01470	74.64	91.12	68.72	74.50	2.050	0.204
3	0.01444	64.64	88.25	61.09	66.36	2.193	0.200
4	0.01152	68.48	90.31	56.63	63.50	2.438	0.179
5	0.01552	76.51	89.39	71.53	71.70	2.332	0.187
6	0.01407	72.43	88.58	67.67	72.07	2.222	0.192
7	0.01383	67.69	89.21	60.07	60.68	3.912	0.127
8	0.01365	64.11	87.84	47.22	64.65	2.435	0.162
9	0.01100	60.04	87.12	49.72	54.26	2.857	0.206
10	0.01444	70.77	92.26	68.44	70.77	2.937	0.249
11	0.01440	71.27	91.07	60.92	71.12	2.670	0.175
12	0.01441	57.23	88.57	59.23	59.66	3.978	0.183
mean	0.01380	68.02	89.28	60.90	66.43	2.750	0.184
SD	0.00130	5.88	1.89	7.62	6.13	0.700	
Coeff. of variation	9.4%	8.6%	2.1%	12.5%	9.2%	25.5%	16.8%

* mean and standard deviations were calculated after arcsin transformation

TABLE 2. Specific growth rate, assimilation of nutrients, appetite and metabolic rate (measured as oxygen uptake rate) in individual young green turtles (*Chelonia mydas*)

(significant at the 10% level, but not at the 5% level) which suggest that turtles compensate for a low efficiency of assimilation of these nutrients by increasing their rate of food intake.

Individuality of Nutrition

The picture that emerges from the regression analyses is that growth rate is predominantly controlled by the efficiency of assimilation of nutrients, rather than by size of appetite or level of metabolic rate. This is highlighted when we consider individual turtles that exhibited particularly low or high specific growth rates. From the data for the slowest-growing animal (9), growth rate was ranked 12th, energy assimilation efficiency 11th, protein assimilation 11th, lipid assimilation 11th and dry mass assimilation 12th. In contrast, its metabolic rate and appetite were above average (5th and 2nd respectively).

For the fastest growing turtle (5) assimilation rate rankings were as follows; energy 1st, protein 5th, lipid 1st and dry mass 3rd. Its appetite was below average (9th) and metabolic rate about average (6th).

Comparison of animals 7 and 2 (the second slowest and fastest growers respectively) provides further support for the view that slow growing animals have low assimilation efficiencies for nutrients other than protein, yet have a relatively large appetite. Fast growers have high assimilation rates and relatively low appetites.

Absorption of Fatty Acids

Fatty acid compositions for food and faeces are displayed in Table 4. From these data it is obvious that different fatty acids are not all absorbed to the same extent. The results vary somewhat between the three turtles, but it is evident that saturated fatty acids (SFAs; 14:0, 16:0, 18:0) and monounsaturated fatty acids (MUFAs; 16:1, 18:1, 20:1, 22:1) were relatively poorly absorbed by comparison with the polyunsaturated fatty acids (PUFAs; 18:2, 18:4, 20:5, 22:5, 22:6), all of which appeared to be completely assimilated.

DISCUSSION

The levels of appetite, oxygen uptake and assimilation efficiencies for energy, protein and dry mass recorded in this study are consistent with those reported in earlier studies upon captive green turtles (Wood & Wood, 1981; Davenport *et al*, 1982; Hadjichristophorou & Grove, 1983; Davenport & Oxford, 1984; Davenport *et al*., 1989). Lipid assimilation efficiencies have not previously been recorded for sea turtles, so it is interesting to note that the values presented in this study are lower than for the other nutrients. This difference probably results from the variable capacity for absorption of individual fatty acids. The fatty acid assimilation data presented here are limited, but it appears that the turtles are relatively efficient at assimilating PUFAs, but less efficient in assimilating SFAs and MUFAs. This differential capacity for fatty acid assimilation presumably limits total lipid assimilation from trout pellets (mean = 60.9%). Comparisons with other species are consequently very difficult. For example, although Sargent *et al.* (1979) reported that herring (*Clupea harengus*) could assimilate 99.4% of dietary lipid, the fish were being fed a zooplankton diet very rich in wax esters. A diet rich in PUFAs could be devised for green turtles to see if higher levels of lipid assimilation could be achieved.

Variables				
y	x	equation	r^2 (%)	P
Appetite	S.G.	$y' = 10.2 - 52\,x$	0.0	>0.05
O_2 uptake	S.G.	$y = 0.162 + 1.65\,x$	0.0	>0.05
Energy	S.G.	$y' = 37.6 + 1304\,x$	15.5	>0.05
Protein	S.G.	$y' = 65.1 + 422\,x$	1.4	>0.05
Lipid	S.G.	$y' = 18.5 + 2380\,x$	43.8	<0.05
Dry mass	S.G.	$y' = 27.6 + 1953\,x$	42.8	<0.05
Lipid	Dry mass	$y' = 1.0 + 0.922\,x$	53.1	<0.01
Lipid	Energy	$y' = -1.70 + 0.954\,x$	53.4	<0.01
Lipid	Protein	$y' = -52.8 + 1.47\,x$	25.0	<0.10>0.05
Protein	Energy	$y' = 56.2 + 0.265\,x$	23.1	<0.10>0.05
Dry mass	Protein	$y' = -29.2 + 1.18\,x$	23.2	<0.10>0.05
Dry mass	Energy	$y' = 4.90 + 0.894\,x$	71.3	<0.001
Appetite	Dry mass	$y' = 19.0 - 0.173\,x$	25.0	<0.10>0.05
Appetite	Lipid	$y' = 12.7 - 0.061\,x$	0.0	>0.05
Appetite	Protein	$y' = 19.15 - 0.140\,x$	0.0	>0.05
Appetite	Energy	$y' = 19.0 - 0.101\,x$	21.6	<0.10>0.05
Appetite	O_2 uptake	$y' = 12.3 - 15.1\,x$	9.1	>0.05

Notes: 'Energy', 'Protein', 'Lipid', 'Dry Mass' refer to the assimilation efficiencies (%) of these nutrients, in this table arcsin transformed to degrees; 'S.G.' = specific growth rate; 'Appetite' = mean appetite (% body wt d^{-1} again arcsin transformed to degrees); 'O_2 uptake' = mean O_2 uptake (ml $O_2 g^{-1} h^{-1}$).

TABLE 3. Results of linear regression analysis of the data shown in Table 2 (after arcsin transformation to degrees in the case of percentages).

The most important findings of the study concern the relationships between growth rate, appetite, metabolic rate and assimilation of nutrients. For such young animals, in which little energy will be diverted into the development of gonadal material, energy flow equations (see Crisp, 1984) can be simplified thus:

$$A = G + R + U + F \quad [1]$$

where A = appetite (food consumption), G = growth, R = respiration, U = excretory output, F = faecal loss of material.

$$\text{Absorption, } Ab = A - F = G + R + U \quad [2]$$

Absorption of organic material is obviously a function of assimilation of nutrients across the gut wall.

Equations [1] and [2] can be rearranged as:

$$G = A - R - U - F \quad [3]$$

$$G = Ab - R - U \quad [4]$$

A priori, it would be expected that high growth rates would be associated with high appetite (high A), low metabolic rate (low R) and high rates of assimilation of nutrients (high Ab, low F). The results derived from the investigation presented here contradict several of these predictions. No systematic relationships between appetite, respiration rates and growth rates were evident. To some extent this may be an artifact of a captive existence in which little energy was required to capture food; in the field, an animal would have to expend more energy in satisfying a large appetite.

Protein assimilation rates were uniformly high despite great variations in growth rate. This is surprising since poor growth (albeit in animals suffering from 'runt syndrome') was recently attributed to impaired protein assimilation in salt-water crocodiles (Davenport *et al.*, 1990). However, the present study clearly demonstrated that fast growing turtles had high rates of assimilation of energy, lipid and dry mass whereas assimilation rates were low in the slow growers. In the case of assimilation of energy and dry mass, there were

Fatty acid	Food composition (% total fatty acids)	Faecal composition (% total fatty acids)		
		Turtle 2	Turtle 3	Turtle 7
14:0	7.6	6.1	12.5	5.2
16:0	20.3	32.9	69.8	24.5
18:0	3.6	4.1	12.2	6.2
16:1ω7	6.5	7.0	5.6	3.5
18:1ω9	13.7	7.9	0.0	6.6
18:1ω7	5.1	5.9	0.0	11.4
20:1ω9	8.4	14.9	0.0	17.0
22:1ω11	10.0	21.1	0.0	25.5
18:2ω6	3.5	0.0	0.0	0.0
18:4ω3	2.8	0.0	0.0	0.0
20:5ω3	10.5	0.0	0.0	0.0
22:5ω3	0.8	0.0	0.0	0.0
22:6ω3	7.1	0.0	0.0	0.0

TABLE 4. Absorption of fatty acids from a diet of trout pellets

strong indications that low rates of assimilation were associated with compensatory large appetites (and vice versa). This makes sense; there is evidence from mammalian studies that the circulating levels of carbohydrates and fatty acids control appetite (low levels being stimulatory).

It is also interesting that high rates of assimilation of one nutrient are positively correlated with the ability to assimilate other nutrients at high rates. There is no obvious biochemical reason why this should be so, since the assimilation of different nutrients will depend upon different enzyme systems and carrier molecules, and different nutrients may be assimilated in different parts of the gut. However, since all exchange systems are influenced by the surface area over which exchange can take place; if turtles vary in gut length and surface area, this probably contributes to the observed variations in assimilation rate.

This study, and the earlier study of Davenport & Scott (1993), demonstrate considerable individual variability of growth, metabolic rate and nutritional physiology in young green turtles. The experimental animals were all incubated together and were from the same geographical population (though of mixed parentage). The sex of the turtles was unknown, but is in any case determined by incubation temperature. It is therefore probable that the observed variability reflects underlying genetic differences rather than environmental influences.

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