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The effects of ration level on food retention time in juvenile lemon sharks, *Negaprion brevirostris*

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Synopsis

Fecal production was monitored to observe the effects of meal size on retention time of food in the digestive tracts of lemon sharks, *Negaprion brevirostris*. Initial appearance of feces occurred more rapidly when ration level was increased. The onset of fecal production was negatively correlated with rate of intake. Production of feces continued for a longer period of time when meal size was increased. Retention time of food was directly related to feeding rate, suggesting that the rate of digestion was constant. The correlation between retention time and intake on a percentage body weight basis was greater than the correlation between retention time and intake on an energy density basis. The use of agar to bind food may have delayed digestion and prolonged food passage for sharks fed an experimental diet.

Introduction

Rate of food passage and retention time of food in the digestive tract are important determinants and indicators of consumption and production rates of fish (Windell 1978, Hofer et al. 1982). Development of effective ration and associated feeding techniques for cultured fish also requires a thorough understanding of the mechanisms and physiology of digestion (Ross & Jauncey 1981, Buddington & Christofferson 1985). The extent to which food is digested, and ultimately utilized by an organism, is dependent upon the retention time of food (Ash 1985). The longer a meal is in the digestive tract of a fish, the longer it is subject to the processes of enzymatic digestion and absorption (Schneider & Flatt 1975, Ash 1985). Thus, retention time of food is a component of the digestive balance of fish, and is important in determining the availability of nutrients in the food presented (Fauconneau et al. 1983, Lassuy 1984).

Retention time of food may be dependent upon rate of consumption, energy density and composition of food (Tyler 1970, Beamish 1972, Jobling 1981, Fauconneau et al. 1983, Jobling 1987). Although the total amount of time required to process a meal increases with feeding rate, the rate at which larger meals are processed may also increase (Jobling et al. 1977, Grove et al. 1978, Windell 1978). At high rates of food intake, the efficiency with which nutrients are absorbed from food may decline (Solomon & Brafield 1972, Elliott 1976, Windell et al. 1978). Thus, a reduction in absorption efficiency at high feeding levels may be associated with an increased rate of food processing, as there is not sufficient time for fish to maximize extraction of energy from food (Kinne 1960).

This study was conducted in conjunction with a broader study examining the effects of meal size on absorption efficiency for lemon sharks, *Negaprion brevirostris*. The present experiments were conducted to determine the effects of meal size on the retention time of food in the digestive tract of lemon sharks. This study was also aimed at improving the overall understanding of the digestive physiology of elasmobranchs, and relationships between rate of consumption, rate of digestion, and rate of growth in sharks. Retention time was also measured to establish appropriate feeding regimens for captive lemon sharks to be used in further experiments. Finally, we attempted to establish whether inclusion of an alginate binder in the diet of lemon sharks affected retention time of food.

Material and methods

Lemon sharks were caught with rod and reel in the Florida Keys near Lower Matecumbe Key, and transported to the laboratory where they were held for approximately two months at 25° C, and 30‰ salinity in a 4800 l aquarium. Water was changed monthly and a natural photoperiod was simulated (12 h light/12 h dark). The aquarium was separated with fiberglass screen into 8 equal compartments, 48 × 66 × 230 cm, each containing approximately 60 l of water. Four sharks of each sex between 1.6 and 2.1 kg (66–74 cm total length), were used in experiments. Sharks were placed in compartments two weeks prior to the beginning of experiments, and were fed at the same time of day, 3 times per week, with filets of blue runner, *Caranx crysos*.

Experimental diets were prepared by mixing 200 g skinless, blue runner filet and 50 ml distilled water in a food processor. As part of experiments on absorption efficiency, 2 g of celite (a form of diatomaceous earth, silicon dioxide, Johns-Manville), an inert marker was also added to the diet. A 7 g block of agar was completely dissolved in 360 ml boiling distilled water and cooled. Blue runner paste and agar were then poured into an enamel coated, steel pan and mixed with a hand blender for 5 minutes. The food mixture was cooled in a refrigerator for 1 h, and subsamples of the food were placed in preweighed drying dishes for analysis of water, ash and energy content.

Sharks were not force fed or handled during the course of any experiment. They were fed at levels of 2.0, 1.7, 3.4, 2.6, and 4.3 percentage body weight per day (% bwd^{-1}) in that order. Cubes weighing approximately 50 g each were placed directly in front of oncoming sharks with wooden tongs until each shark had consumed the desired amount. This manner of food presentation enabled sharks to swallow most cubes whole, with no food loss to the surrounding water. In the few cases when cubes were bitten, uneaten food was recovered and offered again with the tongs, or weighed and another cube of equal weight was substituted for the uneaten quantity. All sharks consumed the required quantities of food within a 30 min feeding period. Sharks were fed once every 96 h for a total of 16 days. Meals administered on the 16th day of a feeding trial were regarded as test meals at each feeding level, and feces resulting from these meals were removed from the aquarium tank. Sharks fed at 2.6 and 3.4 % bwd^{-1} continued to produce feces beyond 96 h, and were not fed respective test meals until 98 and 102 h following the previous meal. To compare retention time of the experimental food with a natural prey item, sharks were fed filets of blue runner at a rate of 1.0 % bwd^{-1}, and fecal production was monitored.

To remove feces which had accumulated during the preliminary feeding period, the experimental tank was thoroughly cleaned shortly after feeding of sharks had been completed. Each compartment was checked for the presence of feces hourly, from 10 h after feeding until the conclusion of fecal production. When feces were observed they were removed from each compartment. When feces had not been produced by any of the eight sharks for three consecutive hours, fecal production was considered to be complete. The final hour during which feces were produced was taken as the total transit time of the meal. Minimum transit time was considered as the hour during which feces were first observed. In no case were feces produced prior to 12 h following the test meal. This indicates that all feces removed from the tank after administration

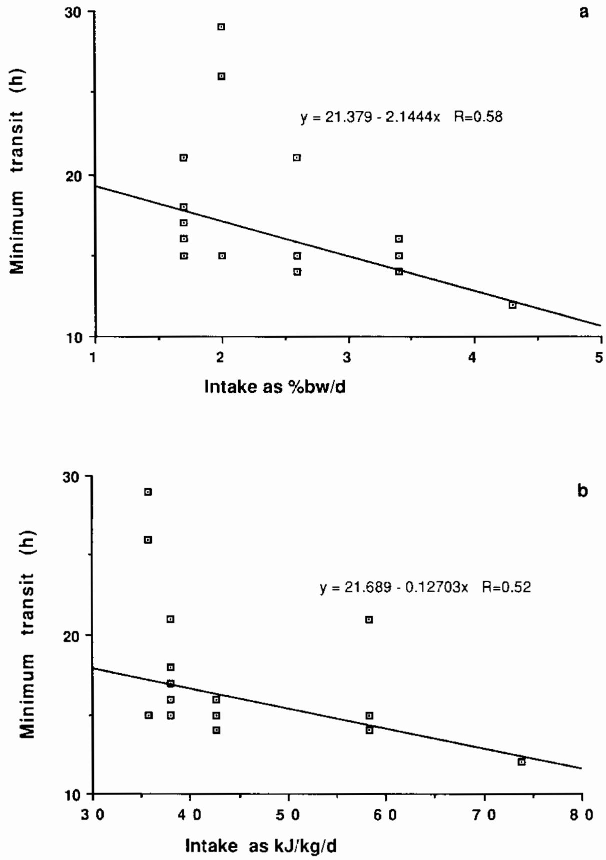

Fig. 1. Minimum food transit time for juvenile lemon sharks at 25° C as a function of rate of intake: a – Intake as percentage body weight per day (% bwd^{-1}). b – Intake as energy (kJ $kg^{-1}d^{-1}$) (n = 8 for each intake level).

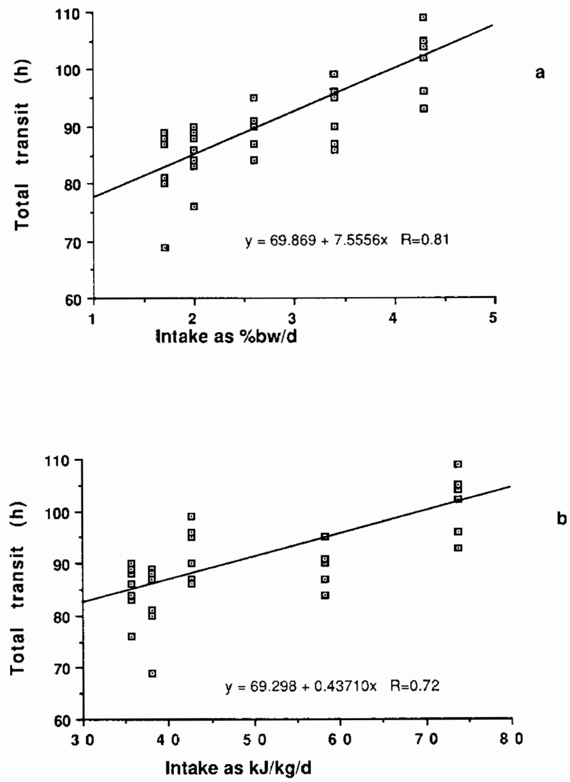

Fig. 2. Total food transit time for juvenile lemon sharks at 25° C as a function of rate of intake: a – Intake as percentage body weight per day (% bwd^{-1}). b – Intake as energy ($kJkg^{-1}d^{-1}$) (n = 8 for each intake level).

of test meals were derived solely from the test meals, and not from previous meals.

For determination of water content, four samples of the diet were dried to constant weight (72 h) at 60° C in a drying oven. Dried food was ground in a Wiley mill (20 mesh) and the resulting powder was stored in a desiccator until analyzed. Ash content was established by burning 2 g of sample in a muffle furnace for 16 h at 550° C, modified from Buddington (1980). For determination of energy content, powdered samples were pressed into pellets approximately 0.1 g in weight. Pellets were weighed to the nearest 0.01 mg and combusted in a Parr semimicro, oxygen bomb-calorimeter and energy content recorded (Parr Instrument Company, Manual 160). Blue runner filets were similarly analyzed for water, ash, and energy content to allow comparison of composition of the experimental diet with a natural prey item. Results were subjected to analysis of variance and compared using multiple comparison analysis (Sokal & Rohlf 1981).

Results

Composition of experimental diets varied significantly between experiments (Table 1). Percentage water in food ranged from 87.81 to 92.80%, and ash from 6.92 to 9.72%. Energy content of experimental diets on a dry weight basis, ranged from 19.28 to 20.36 kJg^{-1}, and from 20.71 to 22.40 kJash-free-g^{-1}. Blue runner filets had a slightly higher energy content (kJg^{-1}) than experimental diets, but expressed as $kJash\text{-}free\text{-}g^{-1}$, energy content was nearly identical to experimental diets. The variation in energy density of experimental diets was such that an increase in meal size (% bwd^{-1}) did not correspond with increases in intake on the basis of $kJkg^{-1}d^{-1}$ in all cases (Table 1). As a result, retention time was compared with intake both on the basis of weight (% bwd^{-1}), and energy density ($kJkg^{-1}d^{-1}$).

As rate of energy intake was increased, minimum transit time decreased (Fig. 1a). Minimum transit time was also negatively correlated with feeding rate expressed as % bwd^{-1} (Fig. 1b). The relationship between minimum transit time and rate of intake expressed both as energy and as weight were similar ($r = 0.52$ and 0.58 respectively). The longest average minimum transit time (18.12 h) was observed at the lowest rate of energy intake, which corresponded to the second smallest meal size on a % bwd^{-1} basis (Table 2). The most rapid average minimum transit time (12.00 h) was recorded at the highest rate of feeding both in terms of specific energy content and specific weight.

A linear regression was fitted to data for rate of intake and total transit time, and a significant correlation was found (Fig. 2). The coefficient of determination for the regression of total transit time and meal size as % bwd^{-1} ($r^2 = 0.65$) was higher than for intake on an energy basis, as $kJkg^{-1}d^{-1}$ ($r^2 = 0.50$). Analysis of partial sum of squares in regression analysis showed that weight explained more of the variation in total transit time than energy content. The lowest average value of total transit time (82.62 h) was recorded at the second lowest rate of energy intake, corresponding to the

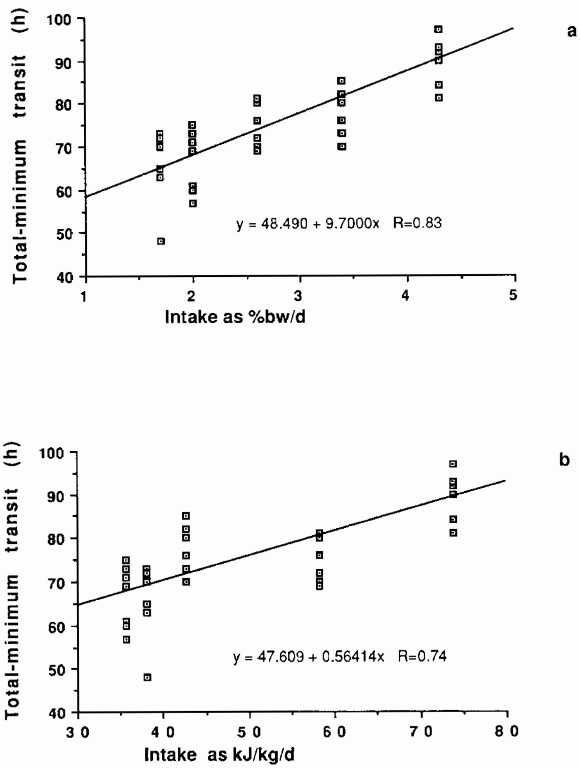

Fig. 3. Difference between total food transit time and minimum food transit time for juvenile lemon sharks at 25° C as a function of rate of intake: a – Intake as percentage body weight per day (% bwd^{-1}). b – Intake as energy ($kJkg^{-1}d^{-1}$) (n = 8 for each intake level).

Table 1. Composition of experimental diets fed to lemon sharks at five levels of intake expressed on the basis of percentage body weight per day (% bwd^{-1}) and as energy ($kJkg^{-1}d^{-1}$). Values within columns with the same superscript are not significantly different (mean of 4 samples for each intake level ± SD).

Weight intake	Energy intake	% water	% ash	kJg^{-1}	kJ Ash-free-g^{-1}
2.0	35.66	88.90 ± 0.27[a]	8.81 ± 0.15[a]	19.80 ± 0.09[a]	21.72 ± 0.10[a]
1.7	38.05	87.81 ± 0.98[b]	6.92 ± 0.61[b]	19.28 ± 0.08[b]	20.71 ± 0.08[b]
3.4	42.68	92.80 ± 0.49[c]	9.72 ± 0.15[c]	19.65 ± 0.14[ac]	21.45 ± 0.14[c]
2.6	58.25	88.18 ± 0.22[ac]	9.11 ± 1.12[ac]	20.36 ± 0.05[d]	22.40 ± 0.06[d]
4.3	73.89	90.11 ± 0.67[c]	9.65 ± 0.53[c]	19.61 ± 0.27[c]	21.70 ± 0.25[ac]
Average		89.56 ± 1.73	8.84 ± 1.08	19.71 ± 0.38	21.62 ± 0.56

smallest meal size on a % bwd^{-1} basis. The longest average value of total transit time (103.37 h) was observed at the highest rate of intake both as $kJkg^{-1}d^{-1}$ and % bwd^{-1} (Table 2). The shortest total transit time observed in any of the experiments was 69 h, and the longest observed was 109 h. Sharks fed blue runner filets at a rate of 56.15 $kJkg^{-1}d^{-1}$ (1.0% bwd^{-1}) completely emptied their digestive tracts within 72 h of feeding, while comparable meals of the experimental diet (58.25 $kJkg^{-1}d^{-1}$ or 1.7% bwd^{-1}) required 90.37 h to be completely eliminated.

The relation between duration of fecal production (total – minimum transit time) and feeding rate (both as $kJkg^{-1}d^{-1}$ and as % bwd^{-1}) was linear (Fig. 3). Duration of fecal production was better correlated with intake expressed on a % bwd^{-1} basis ($r^2 = 0.69$) than on the basis of $kJkg^{-1}d^{-1}$ ($r^2 = 0.53$). Feces were produced for the shortest time (an average of 65.62 h) at the second lowest rate of energy intake, corresponding to the smallest meal size on a % bwd^{-1} basis (Table 2). The maximum average value for duration of fecal production (91.37) occurred at the highest rate of intake, both as $kJkg^{-1}d^{-1}$ and as % bwd^{-1}. Multiple regression analysis showed that there was no significant interaction between intake as weight and intake as energy for any of the values measured. Results of a Student's t-test indicated that differences between values obtained for males and females were not significant.

Discussion

More rapid appearance of feces at high rates of intake indicates that initial processing of food proceeds at a faster rate, or begins sooner as ration size is increased. Hofer et al. (1982) also found that fecal production occurred more rapidly when roach, *Rutilus rutilus*, were fed larger meals. The linear relation between minimum transit time and rate of intake implies that minimum transit time decreases at a constant rate as intake is increased. Higher variability of minimum transit time at low rations may be a result of stimulus (volume of food) dependent digestion. Davies (1964) postulated that small meals may not provide sufficient stimulus on the digestive tract to initiate or fully stimulate digestive processes. Since total transit time and meal size are linearly related, total transit time appears to change at a constant rate as meal size is increased. Thus, larger meals are processed at the same rate as smaller meals, but require a longer period of time to be eliminated from the digestive tract.

The variation in energy density of the experimental diet created a situation where increased meal size on a % bwd^{-1} basis did not necessarily result in an increase in rate of intake on an energy basis. When meal size was increased from 1.7 to 2.0% bwd^{-1} and from 2.6 to 3.4% bwd^{-1}, rate of intake expressed as $kJkg^{-1}d^{-1}$ actually decreased. Although weight of food ingested and the amount of energy ingested were related, the correlation ($r^2 = 0.62$) was not absolute. Thus, rate of intake in our experiments can be related to retention time of food in two different ways; consumption on a

Table 2. Minimum transit time, total transit time, and difference (total-minimum) for lemon sharks fed at five levels of intake expressed on the basis of percentage body weight per day (% bwd^{-1}) and energy ($kJkg^{-1}d^{-1}$). Values within columns with the same superscript are not significantly different (mean of 8 samples for each intake level ± SD).

Intake as % bwd^{-1}	Intake as $kJkg^{-1}d^{-1}$	Minimum transit time (h)	Total transit time (h)	Total-minimum time (h)
2.0	35.66	18.12 ± 5.84^{a}	85.25 ± 4.43^{a}	67.13 ± 6.77^{a}
1.7	38.05	17.00 ± 2.00^{a}	82.62 ± 6.65^{a}	65.62 ± 8.18^{ab}
3.4	42.68	14.37 ± 0.74^{b}	93.50 ± 5.15^{b}	79.13 ± 5.57^{c}
2.6	58.25	15.37 ± 2.33^{ab}	90.37 ± 3.70^{ab}	75.00 ± 4.38^{bc}
4.3	73.89	12.00 ± 0^{c}	103.37 ± 6.12^{c}	91.37 ± 6.12^{d}

weight basis, and consumption on an energy basis. The higher correlation between total transit time and intake expressed on a % bwd^{-1} basis, as well as analysis of the partial sums of squares in regression analysis, indicate that weight, rather than energy, is a more important stimulus on the digestive system in terms of influencing retention time of a meal. Fange & Grove (1979) stated that large meals may stimulate the stomach of fish to a greater degree, which results in increased rate of digestion.

The energy density of food may also influence retention time, as total transit time was also correlated with rate of energy intake. However, the correlation between rate of energy intake and total transit time may be a result of the correlation between weight of food ingested and rate of energy intake. In several studies with teleosts, lowered energy density of food resulted in an increased rate of gastric evacuation (Grove et al. 1978, Flowerdew & Grove 1979). An increased rate of gastric evacuation allows a fish to process low energy food more rapidly, which means that more food may be consumed and energy demands of the fish can be met in this way (Lee & Putnam 1973, Page & Andrews 1973). To determine the effects of energy density on retention time in the lemon shark, it would have been desirable to specifically formulate experimental diets so that weight of food and energy density were not correlated.

The duration of fecal production combines the trends of decreased minimum transit time and increased total transit time into one measurement. This measure is visible evidence that food is being processed, or that digestion is influenced by changes in the rate of intake. Change in the duration of fecal production with increased ration is more significant than change in either minimum or total transit time alone (Table 2). As meal size was increased, duration of fecal production also increased. Again, there was a higher correlation between duration of fecal production and feeding rate expressed as % bwd^{-1}, suggesting that weight influences retention time to a greater degree than energy density. Regardless of the manner in which the rate of intake is expressed, food was completely eliminated from the digestive tracts of lemon sharks more slowly at higher rates of intake.

Experimental diets required a longer period of time to be completely emptied from digestive tracts than meals of blue runner. The difference between total transit time for sharks fed blue runner filets and those fed the experimental diet could be related to differences in weight of food consumed. Although the amount of energy consumed was similar (56.15 versus 58.25 $kJkg^{-1}d^{-1}$), the amount of food consumed on a weight basis was nearly doubled (1.0 versus 1.7% bwd^{-1}). However, transit time of the experimental diet could also have been delayed by the inclusion of agar in food as a binding agent. Storebakken (1985) suggested that alginates may continue to bind food in the stomachs of fish, making the food more resistant to digestion, delaying evacuation. Wetherbee et al. (1987) monitored the transit of filets of blue runner through gastrointestinal tracts of lemon sharks using an x-ray technique. They found that digestive tracts of sharks were completely empty 68–82 h after a meal. The present study confirms that retention of food in the digestive tracts of lemon sharks is substantially prolonged in comparison to most teleosts.

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