DIET MIXING: NONADDITIVE INTERACTIONS OF DIET ITEMS IN AN OMNIVOROUS FRESHWATER TURTLE[1]

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Abstract. Diet selection and optimization may be affected by nonadditive interactions (or associative effects) among ingested diet items. Associative effects occur when one diet item affects the digestion (either positively or negatively) of another diet item. Feeding trials were conducted with an opportunistic omnivore, the yellow-bellied slider turtle (*Trachemys scripta scripta*), on three diets: duckweed, insect larvae, and duckweed/larvae mix. Diet had a significant effect on digestibilities and intake, but not on transit time.

There was a significant associative effect in the duckweed/larvae diet. For all diet components except lipids, the digestive efficiencies measured in the duckweed/larvae diet were significantly greater than those predicted from the digestive efficiencies for duckweed and larvae diets when fed alone. The presence of larvae in the digesta significantly improved the digestive processing of duckweed, probably by supporting greater numbers or diversity of cellulolytic microbes. A given mass of duckweed provided ≈70% more energy and 20% more nitrogen to the turtle when ingested with insect larvae than when ingested alone.

Associative effects should be incorporated in optimal foraging models and studies of diet selection because the value of a diet item can vary with the foods with which it is ingested. The assigned value must include not only the direct nutritional gain from the item, but also the indirect nutritional gain (or loss) through positive (or negative) effects on digestion of food items with which it is ingested.

Key words: associative effect; diet selection; digestion; foraging ecology; nutrition; omnivory; Trachemys scripta; *turtle.*

INTRODUCTION

Most vertebrate species ingest a mixed diet, but the basis on which these diets are selected is poorly understood. In recent years, ecologists have sought to elucidate the factors that determine diet choice through development and testing of optimal foraging models and in studies that attempt to explain observed feeding patterns in individual species. Westoby (1978) cautioned against attributing a value to a potential diet item that is independent of what other food items are ingested with it. Westoby (1978) also outlined why animals should ingest a mixed diet: to reduce searching costs, to respond to changes in relative diet qualities, to sample potential diet items, and to balance nutrients. Diets can be balanced for toxins (Freeland and Janzen 1974) as well as for nutrients (Rapport 1980, Robbins 1983).

Thus the nutritional value assigned to a potential diet item is not constant and is not independent. The value can change depending on what the animal has already ingested. The value of a phosphorus-rich food, for example, will increase with the length of time that an animal ingests foods low in phosphorus. Balancing nutrients is an additive function; that is, the interdependence of individual diet items can be calculated by summing their individual contribution to the final nu-

trient content of the diet. This additive interdependence of food items rapidly becomes quite complex in herbivores that commonly ingest 15–20 species (Robbins 1983).

An even more complex source of interdependence of individual food items is the nonadditive interactions, or associative effects, between food types. Associative effects occur when one diet item affects the digestion of another diet item either positively or negatively. That is, the nutrient gain to an animal from a mixed diet is either significantly greater or less than would be predicted from summing the nutrient gains from the individual diet items.

The importance of associative effects to the nutrition of some invertebrates has been demonstrated. Termites (Martin and Martin 1978) and beetles (Kukor et al. 1988) ingest fungi that provide enzymes for the digestion of cellulosic diet components. The digestion of the mixed diet is greater than would be predicted from the digestibilities of either diet fed alone.

Associative effects are employed in livestock husbandry to improve utilization of poor quality forage by cattle and sheep. A mixture of urea and molasses is often provided as a supplement to cattle feeding on poor quality forage. Nitrogen in the urea and the rapidly fermentable sugars in molasses support a greater microbial population that will then digest the forage to a greater extent than it would be without the supplement (Church 1977).

The importance of associative effects to nutrition and diet selection in wild vertebrates has not received sufficient attention. One system that has been examined is the effect of plants high in soluble phenolic compounds or tannins on the digestibility of plants low in these compounds. In elk, white-tailed deer, and mule deer, no negative associative effect was found when the animals were fed a diet containing a species high in soluble phenolics or tannins and a species with little phenolics or tannins. The digestion of the mixed diet was not significantly different from the digestibility predicted from values measured for each individual diet (Mould and Robbins 1982, Robbins et al. 1987a, b).

Associative effects are more likely to occur in generalist herbivores that ingest a mixture of fruit, flowers, and foliage or in omnivores than in carnivores because of the greater nutritional differences among diet items in the former groups, and because herbivores and omnivores often rely on microbial fermentations. Both of these attributes provide greater opportunity for one food item to affect the digestion of another.

For a study of associative effects, I selected a freshwater turtle, *Trachemys scripta,* the slider turtle. This turtle is an opportunistic omnivore (Parmenter and Avery 1990), is one of the best-studied turtle species (see Gibbons 1990), and has a wide geographic range throughout much of southeastern and south-central United States, through Central America and into South America (Iverson 1986).

The slider turtle shifts from a more carnivorous diet to a more herbivorous diet as it grows (Marchand 1942, Clark and Gibbons 1969, Hart 1983). Juvenile slider turtles grew more rapidly on diets with higher protein contents, when tested on artificial, pelleted diets (Avery 1988). Based on laboratory and field studies, slider turtles, even as adults, prefer a carnivorous diet (Parmenter 1980, Parmenter and Avery 1990).The shift from carnivory to herbivory is assumed to be a result of the great abundance of plants, the greater ease of capture of plants by adult turtles, lower mass-specific nutrient requirements for adults, and, perhaps, a change in foraging habitat, with larger turtles moving to deeper waters with lower densities of animal prey (Parmenter and Avery 1990). In habitats with relatively low availability of insect or vertebrate prey, adult slider turtles continue to ingest small amounts of animal matter (Parmenter 1980, Parmenter and Avery 1990). The fact that adult slider turtles invest the time and effort necessary to capture small quantities of animal matter suggests that even a small quantity may make an important nutritional contribution.

To test whether a small amount of animal matter ingested with plant material confers a nutritional benefit beyond its own, independent value, I conducted a series of feeding trials in which slider turtles were fed a plant diet, an insect diet, and a mixture of the two diets to measure any associative effects. The results demonstrate the need to consider associative effects when evaluating the nutritional value of potential diet items in studies of nutritional ecology, diet selectivity, and diet optimization in vertebrates.

METHODS

Feeding trials

Five adult male yellow-bellied slider turtles (*Trachemys scripta scripta*) were captured in baited traps in small ponds in Alachua County, Florida. Turtles had plastron lengths of 17.6–19.2 cm and body masses of 1.4–1.6 kg. Only males were used, to avoid any effect of egg production on feeding and digestion. Three consecutive feeding trials were conducted with three diets: duckweed (*Spirodela polyrhiza*) alone, *Tenebrio* larvae alone, and 77% (by dry mass) duckweed and 23% *Tenebrio* larvae (duckweed/larvae diet). The 77:23 plant : animal ratio was selected because it approximates the average ratio recorded by Parmenter (1980) for three natural *T. s. scripta* populations in South Carolina. Duckweed was collected from the surface of a local spring and was cleaned of its invertebrate fauna before being fed to the turtles. *Tenebrio* larvae were obtained from a commercial supplier.

During feeding trials, turtles were maintained in individual Nalgene tanks (45 × 60 cm) out of sight of each other. Each tank was equipped with a 20-W full-spectrum natural light fluorescent bulb (Vita-Lite) and a 75-W outdoor flood light for basking. Both lights were on for 12 h each day. Water was drained from the tanks at 0800 to allow the turtles to bask and dry. At 1000, tanks were filled with water to a depth of 15 cm, and a known mass of fresh diet was introduced into each tank. On the duckweed diet and the larvae diet, turtles were fed ad libitum until 1600, when orts (remaining food) were collected. On the duckweed/larvae diet, turtles were fed duckweed ad libitum between 1000 and 1600 as in the duckweed only diet. Orts were collected, and the wet mass of duckweed consumed that day was calculated. Then live *Tenebrio* larvae were placed in the tank. The quantity of larvae offered had a dry mass equal to 30% of the dry mass of the duckweed ingested, resulting in a 77:23 duckweed : larvae diet on a dry matter basis. In all cases, all larvae were consumed by the turtles within 15 min. This daily adjustment in amount of larvae offered to match the amount of duckweed consumed was necessary because turtles vary in intake on a daily basis. Duckweed and larvae could not be introduced simultaneously in the desired mix because turtles would feed selectively. During the 3-d transit time through the digestive tract, the duckweed and larvae became well mixed. Larval exoskeletons were interspersed with duckweed in the feces.

Water temperatures ranged from 24° to 27°C daily during each trial. Air temperature at turtle height under the basking light during the basking period was 31°. The temperature regime was constant throughout the

three trials. The basking light shone directly over the entire tank, and basking platforms were not provided. Turtles were only out of the water during the 2-h basking period. This system avoided differential thermoregulation among individuals or among trials.

Food samples were collected daily as the diet was prepared, dried at 60°, and combined to form one composite sample for the duration of each of the three feeding trials for nutrient analysis. Because an equal quantity of diet was collected each day, the composite diet sample accurately reflects any variation in diet quality throughout the trial. Feces were collected in condoms (Trojan brand nonspermicidal, nonlubricated) that were attached to a harness of Nalgene tubing (3 cm × 2.5 cm internal diameter). The tubing fit snugly between the posterior overhang of carapace and plastron and was held in place by a wire fitted around the tube and through two holes drilled in the posteriormost marginals of the carapace. Spaces between the tubing and the flesh and shell of the turtle were sealed with silicone. The fecal collection unit was durable and prevented loss of feces. The condoms were emptied twice each day. Feces were dried at 60° and combined into one composite sample for each turtle for the duration of each of the three trials.

Feeding trials were conducted from early June to early October. Following a 2-wk acclimation period, each feeding trial was continued until sufficient feces had been collected for analysis, ≈4 wk. At the end of each of the trials, there was no change in body mass in any turtle; for each turtle, change in mass was less than the wet mass of a single defecation.

Sample analyses

For calculations of digestibilities, diet and feces samples were analyzed for a series of components. Replicates of analyses were acceptable within 2% relative error. Dried samples of duckweed diet and feces from duckweed and duckweed/larvae diets were ground to pass through a 1-mm mesh screen in a Wiley mill. To prevent formation of a paste due to high lipid content, samples of larvae diet and feces from larvae diet were ground with dry ice in a mill (C. W. Brabender Instruments). A portion of each sample was dried for 8 h at 105° to determine percent dry matter and then ashed in a muffle furnace for 3 h at 500° to determine percent organic matter.

Percentage of cell walls (cellulose, hemicellulose, lignin and cutin, or neutral detergent fiber ash-free) was measured by the Van Soest technique (Goering and Van Soest 1970) with decalin and sodium sulfite omitted (Golding et al. 1985). Analyses for ligno-cellulose (acid detergent fiber) followed Goering and Van Soest (1970). The values for cell walls and ligno-cellulose in larvae do not represent a measure of cellulose, hemicellulose, lignin, and cutin in the larvae. The fractions in larvae contain the exoskeleton, primarily chitin (Stelmock et al. 1985). However, cell walls and ligno-

cellulose are definitive analyses. That is, the fractions are defined by the chemical treatments to which the substrates are exposed. Neutral detergent fiber is the residue remaining after 1 h extraction in a boiling detergent solution with neutral pH. Acid detergent fiber is the residue remaining following 1 h extraction in a boiling detergent solution with acid pH. The larvae were analyzed for these components to allow comparison with the duckweed and duckweed/larvae diets. Because these fractions in duckweed and larvae are measured by the same procedure, digestion of cell walls and ligno-cellulose fractions in larvae and duckweed can be compared in this study.

Determination of in vitro indigestible cell walls followed Goering and Van Soest (1970). Energy content of food and feces was determined in a bomb calorimeter following standard procedure (Parr Instrument, 1960). Lipids were extracted with ethyl ether in a Goldfisch apparatus for 8 h. Percent concentrations of total (Kjeldahl) nitrogen were measured with a block digester (Gallaher et al. 1975) and an automated Technicon analyzer (Hambleton 1977). Urinary nitrogen is primarily in the form of ammonia and urea (Mahmoud and Klicka 1979). Urea would be converted to ammonia, and the ammonia lost during drying of feces, so that contamination of feces by urine would not affect measurement of nitrogen digestibilities.

Calculations and statistical analyses

Two methods were used to calculate digestibility, or the disappearance of nutrients along the digestive tract. Digestibility measures in trials with the duckweed/larvae diet and the larvae diet were based on total collection of feces and calculated using the equation:

$$D = (\text{intake} - \text{feces})/\text{intake}, \qquad (1)$$

where intake (in grams per day) was calculated as the difference between the dry mass of food offered and the dry mass of orts collected each day, and feces (in grams per day) is the dry mass of feces collected each day.

Because 3 of the 5 turtles tore their fecal collection bags during the duckweed diet trial, it was not possible to calculate digestibility based on total collection in all animals. Instead, in vitro indigestible cell walls (IVICW) was used as an indigestible marker in the duckweed diet trial. Indigestible markers allow digestibilities to be calculated using the equation:

$$D = 100 - [(M_D/N_D) \times (N_F/M_F)], \qquad (2)$$

where D is digestibility expressed as percent, M_D and M_F are the concentrations of the marker in the diet and feces, respectively, and N_D and N_F are the concentrations of the nutrient component in the diet and feces, respectively, expressed as percent of dry matter or, in the case of energy, as kilojoules per gram dry matter.

In the two turtles for which digestibilities of duckweed could be calculated by both Eqs. 1 and 2, the

TABLE 1. Nutrient composition of three diets fed to captive *Trachemys scripta scripta*.

	Diet		
	Duck-weed	Larvae	Duck-weed/larvae
Dry matter (% fresh mass)	8.6	40.7	16.0
Organic matter (% dry mass)	88.6	96.7	90.5
Energy (kJ/g dry mass)	18.1	28.5	20.5
Cell walls (% dry mass)	38.5	13.6	32.8
Ligno-cellulose (% dry mass)	20.2	5.9	16.9
Lipids (% dry mass)	5.1	38.1	12.7
Nitrogen (% dry mass)	5.1	7.2	5.6

difference between the digestibilities was <2% relative error for each animal for all diet components. This difference is within the experimental error because replicates of analyses were accepted within 2% relative error. Thus, IVICW is an accurate marker for the duckweed diet, and results of Eqs. 1 and 2 are comparable for duckweed. IVICW has been tested in several reptiles on a range of herbivorous diets and found to be a reliable marker: in *T. scripta* feeding on the aquatic plant *Hydrilla verticillata* (K. A. Bjorndal and A. B. Bolten, *unpublished manuscript*), in *Gopherus polyphemus* on a foliage diet (Bjorndal 1987), and in *Geochelone carbonaria* and *G. denticulata* on two fruit and one foliage diets (K. A. Bjorndal, *unpublished manuscript*). However, IVICW was not an accurate marker for the larvae diet, perhaps because chitin, which is a major component of IVICW in mealworms, is digested to a greater extent in turtles than by rumen microflora. Therefore, Eq. 2 could not be used to compare among diets, but comparisons of digestibilities calculated using Eq. 1 for duckweed/larvae and larvae diets and using Eq. 2 for duckweed are appropriate.

Intake was calculated as the difference between the dry mass of food offered and the dry mass of orts collected each day, for all diets. Daily gains of energy and nitrogen were calculated as the product of intake (in grams of dry mass per day), diet composition (in kilojoules per gram or percent N), and digestibility (percent).

Transit time was measured twice for each turtle during each of the feeding trials. A piece of plastic flagging the size of a duckweed frond was placed in the mouth of each turtle, and feces were inspected until the flagging appeared. The time elapsed between ingestion and defecation is transit time.

Digestibilities were predicted for the duckweed/larvae diet based on the values measured for duckweed and larvae when fed alone. A predicted digestibility value for each diet component was calculated with the equation:

$$D_P = (D_D \times C_D) + (D_L \times C_L), \qquad (3)$$

where D_P is the predicted digestibility of a given component, D_D is the digestibility of that component measured in the duckweed diet trial, C_D is the fraction of the component in the duckweed/larvae diet contributed by the duckweed portion, D_L is the digestibility of the component measured in the larvae diet trial, and C_L is the fraction of the component in the duckweed/larvae diet contributed by the larvae portion.

Predicted daily gain of energy was calculated as the product of D_P for energy, intake measured in the duckweed/larvae feeding trial, and energy content of the duckweed/larvae diet. Predicted daily gain of nitrogen was calculated in the same manner using values for nitrogen digestibility and nitrogen content.

Digestibilities, intake, and transit time for the three diets were compared with repeated-measures ANOVA

TABLE 2. Results of a feeding experiment in which five captive *Trachemys scripta scripta* were fed three different diets. Data are means ± SD. Within each row, means with different letter superscripts are significantly different (repeated-measures ANOVA, Tukey's test).

	Diet			F
	Duckweed	Larvae	Duckweed/larvae	
Digestibilities (%)				
Dry matter	[A]32 ± 4.4	[B]86 ± 2.4	[C]57 ± 2.3	132
Organic matter	[A]35 ± 3.6	[B]88 ± 2.5	[C]61 ± 1.7	174
Energy	[A]29 ± 4.9	[B]88 ± 3.4	[C]61 ± 2.0	144
Cell walls	[A]25 ± 1.8	[B]78 ± 2.9	[C]48 ± 3.1	122
Ligno-cellulose	[A]9 ± 1.1	[B]70 ± 3.1	[C]36 ± 3.3	171
Lipids	[A]14 ± 9.8	[B]85 ± 6.1	[C]69 ± 3.9	52
Nitrogen	[A]53 ± 6.6	[B]90 ± 1.4	[C]73 ± 1.8	41
Intake				
Absolute (g/d)	[A]4.4 ± 1.3	[B]9.3 ± 0.6	[A]4.4 ± 0.9	13
Mass-specific* (g·kg⁻¹·d⁻¹)	[A]3.0 ± 0.9	[B]6.3 ± 0.7	[A]3.0 ± 0.6	12
Daily gain†				
Energy (kJ·kg⁻¹·d⁻¹)	[A]16 ± 7.5	[B]157 ± 14.2	[C]37 ± 6.4	111
Nitrogen (g·kg⁻¹·d⁻¹)	[A]0.08 ± 0.04	[B]0.41 ± 0.04	[A]0.12 ± 0.02	46
Transit time (h)	[A]72 ± 26	[A]71 ± 15	[A]85 ± 7	1

* Dry matter intake per unit fresh body mass per day.
† Energy or nitrogen gain per unit fresh body mass per day.

TABLE 3. Digestibilities and daily gains for turtles fed the duckweed/larvae diet as measured in the feeding trial (actual) and as predicted from digestibilities of duckweed diet and larvae diet. Means ($\pm$ SD) were compared with an a priori orthogonal contrast using a t statistic.

	Actual	Predicted	t	P
Digestibilities (%)				
Dry matter	57 ± 2.3	45 ± 3.8	3.481	<.01
Organic matter	61 ± 1.7	48 ± 3.3	3.964	<.01
Energy	61 ± 2.0	48 ± 4.3	3.365	<.01
Cell walls	48 ± 3.1	30 ± 1.8	5.249	<.001
Ligno-cellulose	36 ± 3.3	14 ± 1.1	8.927	<.001
Lipids	69 ± 3.9	63 ± 6.3	0.947	>.05
Nitrogen	73 ± 1.8	64 ± 4.8	2.481	<.05
Daily gain*				
Energy ($kJ \cdot kg^{-1} \cdot d^{-1}$)	37 ± 6.4	30 ± 7.9	4.918	<.01
Nitrogen ($g \cdot kg^{-1} \cdot d^{-1}$)	0.12 ± 0.02	0.10 ± 0.03	3.158	<.05

* Energy or nitrogen gain per unit fresh body mass per day.

to control for interindividual variation (SAS 1982, Zar 1984). Differences between the digestibilities measured for the duckweed/larvae diet and those predicted from digestibilities of the duckweed diet and larvae diet were compared with an a priori orthogonal contrast using a t statistic (Kirk 1982). Unless otherwise stated, alpha = .05.

RESULTS

Nutrient composition of the three diets is shown in Table 1. The composition of the duckweed diet from the duckweed only trial was not different from that of the duckweed diet from the duckweed/larvae trial at the level of 2% relative error accepted for replication of analyses.

Diet had a significant effect on digestibility of all diet components (Table 2; repeated-measures ANOVA, P < .0001; Tukey's test). Diet also had a significant effect on intakes, both absolute and mass specific, with intakes of larvae significantly higher than those of duckweed or duckweed/larvae (Table 2; repeated-measures ANOVA, P < .001; Tukey's test). Daily gain of energy and nitrogen was significantly affected by diet (Table 2; repeated-measures ANOVA, P < .0001; Tukey's test). Transit times are not significantly different for the three diets (Table 2; repeated-measures ANOVA, P = .417).

Given the greater energy gain on the larvae diet (Table 2), it is surprising that the mass of turtles did not change during any of the trials. This lack of variation may be due to differences in internal water storage, the relatively short duration of each trial, or differences in activity level of turtles among the trials. However, no marked change in turtle activity was noted among the trials.

Predicted digestibilities for the duckweed/larvae diet, calculated from digestibilities of the duckweed diet and larvae diet (Eq. 3), are significantly lower than the actual values measured for the duckweed/larvae diet for all components except lipids (Table 3; a priori orthogonal contrast using a t statistic). The percent increase

in digestibility is higher for cell walls and ligno-cellulose than for the other components (Table 3). Daily gains of energy and nitrogen measured in the duckweed/larvae diet are also significantly higher than predicted (Table 3; a priori orthogonal contrast using a t statistic).

The next question is whether the difference between the actual digestibilities and predicted digestibilities of the duckweed/larvae diet (Table 3) can be attributed to an improved digestibility of either the duckweed or the larvae portion of the diet. To determine if the digestion of duckweed was increased, predicted digestibilities were recalculated assuming 100% digestion of the larvae ($D_L = 1.00$ in Eq. 3). The difference between these recalculated digestibilities and the actual digestibilities (Table 4) is the minimum increase that can be attributed to an improved digestion of duckweed. For all components except lipids, the majority of the difference between actual and predicted digestibilities is accounted for by an increase in the duckweed digestibility (Table 4).

None of the differences between the actual and predicted digestibilities of the duckweed/larvae diet can

TABLE 4. Predicted mean percent digestibilities for duckweed/larvae diet calculated from values measured when each of the diets was given alone (see Table 3), by assuming digestion of larvae is 100% ($D_L = 1.0$), and by assuming digestion of duckweed is 100% ($D_D = 1.0$). The difference between actual and predicted digestibilities (actual − predicted) is shown in parentheses following predicted digestibilities.

	Digestibilities (%)		
	Predicted	Predicted if $D_L = 1.0$	Predicted if $D_D = 1.0$
Dry matter	45 (12)	48 (9)	97 (−40)
Organic matter	48 (13)	51 (10)	97 (−36)
Energy	48 (13)	52 (9)	96 (−35)
Cell walls	30 (18)	33 (15)	98 (−50)
Ligno-cellulose	14 (22)	16 (20)	98 (−62)
Lipids	63 (6)	73 (−4)	90 (−21)
Nitrogen	64 (9)	67 (6)	97 (−24)

be attributed with certainty to an increase in digestion of larvae. The recalculation of predicted digestibilities, assuming that digestion of duckweed was 100% (D_D = 1.00 in Eq. 3), yielded all negative differences (Table 4).

DISCUSSION

Effect of diet on digestive efficiency

The significant differences in digestibilities among the diets (Table 2) were expected. In comparisons of digestion of insects and plant matter in other reptiles, insects were digested to a greater degree than was plant matter (Johnson and Lillywhite 1979, Ruppert 1980). Digestive efficiency for energy (88%) in slider turtles on a diet of *Tenebrio* larvae fell within the range of values (83–93%) measured for a number of lizard species on the same diet (Johnson and Lillywhite 1979, review in Zimmerman and Tracy 1989).

The extent of duckweed cell wall digestion indicates that the slider turtle harbors a microbial fermentation in its gut. All herbivorous reptiles that have been studied have been found to rely on symbiotic gut microflora to degrade plant cell walls (review in Zimmerman and Tracy 1989, Bjorndal and Bolten 1990).

In Florida, the slider relies heavily on duckweed for food (Marchand 1942, Auth 1975). However, the digestibilities of the duckweed diet are low compared with most values for reptiles feeding on herbivorous diets (review in Zimmerman and Tracy 1989, Bjorndal and Bolten 1990). The low value may reflect the difference in digestive efficiency between the omnivorous slider turtle and the more herbivorous reptiles in the above studies. Or it may result from differences among the diets. The digestibility of duckweed has not been measured in any other reptile, and it is difficult to draw conclusions on relative digestive efficiency among herbivorous reptiles fed different diets (Bjorndal 1989).

The low digestibility may be attributed to the structure of the duckweed. The great majority of the duckweed fronds are ingested whole. Because reptiles do not chew their food, many of the duckweed fronds pass through the digestive tract entire. Inspection with scanning electron microscopy of cross sections of whole duckweed fronds from feces revealed the presence of microbes in some fronds, although many of the fronds had not been invaded by bacteria. The integrity of the cutin cover on the duckweed fronds apparently limited access to the digestible portions of the plant. The surface area of food particles open to microbial invasion is an important limiting factor in reptilian herbivores that do not reduce particle size of their food (Bjorndal et al. 1990).

Associative effects in the duckweed/larvae diet

In the duckweed/larvae diet, which approximated the plant : animal diet ratio of three natural *T. s. scripta*

populations (Parmenter 1980), there were significant associative effects. The digestibility of all diet constituents except lipids was significantly greater in the duckweed/larvae diet than predicted from the individual duckweed and larvae diets (Table 3). For each diet component except lipids, the majority of the increased digestion can be attributed to an increase in the digestibility of the duckweed portion of the diet (Table 4). Therefore, the associative effects are a result of the presence of larvae in the digesta enhancing digestion of duckweed.

The diet component that had the greatest increase in digestibility was the ligno-cellulose fraction (Table 3). The increase in cellulose fermentation was probably the result of increased microbial activity in the gut. Stimulation of the microbial population may have resulted from the increase in available nitrogen or other nutrients from the larvae. Duckweed has relatively high nitrogen levels (Table 1), but the nitrogen may be largely unavailable to the gut microflora until the cutin envelope of the duckweed frond is penetrated. By having nitrogen from the larvae available in the digesta, the microbial populations could build to greater numbers more rapidly, and more rapidly degrade duckweed fronds. This mechanism would explain the increased digestibility of all components. Because cell walls are a major fraction of the duckweed diet (Table 1), an increase in cell wall digestibility would increase digestion of dry matter, organic matter, and energy. Because a greater number of duckweed fronds would be penetrated by microbes, more cell contents would be exposed to digestion, increasing nitrogen digestion, as well as those of dry matter, organic matter, and energy.

In the typical vertebrate digestive tract, most of the digestible nitrogen in the larvae would be absorbed in the small intestine. Larvae nitrogen would have a greater chance of supporting rapid microbial growth if the slider turtle harbors a microbial fermentation in its small intestine, which may be the case. The only other freshwater turtle studied to date, *Pseudemys nelsoni*, does maintain a microbial fermentation in its small intestine (Bjorndal and Bolten 1990). *Pseudemys nelsoni* and *T. scripta* are both in the family Emydidae, have similar gross gut morphology, and share habitats in northern Florida.

Digestion of the lipid fraction in the duckweed/larvae diet was not significantly increased by an associative effect. This lack of an associative effect reinforces the idea that the enhanced digestion of the duckweed/larvae diet was largely due to an increased digestion of duckweed. The lipid fraction was the only diet component in the duckweed/larvae diet derived mainly from the larvae (Table 1).

Associative effects and diet selection

Nonadditive interactions of diet items have not received sufficient attention. Students of diet selection and optimal foraging have struggled with the com-

plexity of mixed diets and lack of independence of individual food items, but associative effects have not been addressed. Studies of nutrition and digestive processing in nondomesticated animals have been largely limited to single-species diets or to diets of a constant mix. Such studies will not reveal the potential importance of associative effects to the nutrition of the animal.

The nutritional value of both duckweed and larvae increases when ingested together. The value of each is equal to its individual value plus the increased gain resulting from the associative effects. To a turtle that has just ingested insects or that has a good probability of capturing insects in the near future, the quality of duckweed is greater than it is to a turtle that has not recently ingested insects or has little chance of ingesting insects. In this study, a turtle gained between 8 and 9 kJ energy and between 31 and 34 mg nitrogen from 1 g of duckweed in the duckweed/larvae diet, but gained only 5 kJ energy and 27 mg nitrogen from 1 g of duckweed in the duckweed diet. This represents an increase of ≈70% in energy gain and an increase of ≈20% in nitrogen gain from duckweed when it is ingested with larvae. (The ranges for the duckweed/larvae diet are calculated by assuming all increased energy and nitrogen digestibility of the duckweed/larvae diet was a result of improved digestion of duckweed [maximum value] or that only the increased energy and nitrogen digestibility above 100% digestion of larvae resulted from improved digestion of duckweed [minimum value].)

Therefore, a small amount of animal matter in the diet of *T. s. scripta* confers a nutritional benefit beyond its own, independent value. This increased value for animal matter would increase the search effort the turtle should expend to capture relatively rare and/or elusive prey.

Associative effects can be difficult to predict, and the extent of the associative effect may change as the ratio of diet items changes (Van Soest 1982). A duckweed/larvae diet with a 50:50 ratio may have a different associative effect than a 77:23 diet mix. To completely quantify the associative effects between two diet items, digestibility measurements are needed for a series of diets with ratios from 0:100 to 100:0.

In livestock nutrition, associative effects are considered to be very common, and often result in over- or underestimates of the nutritional value of a diet supplement (Van Soest 1982). Additional studies are needed to determine how widespread such interactions may be in natural populations. Associative effects may be significant in the nutrition of primates feeding on mixtures of fruit and foliage or in rodents that ingest seeds and foliage or insects and plant parts. The great disparity among nutrient composition and structure of the various food items in the diets of omnivores and generalist herbivores makes them the most likely to have their nutrition significantly affected by associative

effects, particularly if they rely heavily on symbiotic gut microflora.

For example, two species of South American tortoises *Geochelone carbonaria* and *G. denticulata* have a varied diet and combine fruit, flowers, and foliage in single meals (Moskovits and Bjorndal 1990). Feeding trials with these two tortoise species revealed significant differences in intake, transit time, digestibility, and extent of cell wall fermentation between fruit and foliage diets, when fed separately (Bjorndal 1989). Intake was 4 times greater and transit time was 4 times shorter on a guava (*Psidium guajava*) fruit diet than for foliage of *Lantana urticifolia*. Although the two diets had similar cell wall percentages (58% in guava fruits, 53% in lantana foliage), cell wall fermentation was essentially abandoned in the guava diet (7% cell wall digestion), whereas significant cell wall fermentation occurred in the lantana diet (39% cell wall digestion).

If these diets were consumed in a mixture, an interaction between the two diets would be expected such that intake, transit time, and digestion could be affected in a nonadditive manner. Because the digestive processing of the two diets is very different, the "compromise" processing of the mixture could result in less efficient nutrient gain than from either individual diet, resulting in a negative associative effect. Or, the nutrient contents of the two diets might complement each other in such a way that digestive efficiency of the mixture is improved, resulting in a positive associative effect.

Such nonadditive diet interactions may help to explain complex patterns of diet selection. A diet item may be selected or rejected not on the basis of its own nutritional value alone, but on the effect it may have on other foods with which it is ingested. Associative effects are another level of complexity in the interaction of diet items that should be incorporated in optimal foraging models and studies of diet choice.

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