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Nonadditive interactions between animal and plant diet items in an omnivorous freshwater turtle *Trachemys scripta*

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Abstract

Nonadditive interactions occur when diet items interact with one another such that the net energy or nutrient gain from a mixed diet differs from that predicted by summing the gains from individual diet components. We quantified nonadditive effects between duckweed, *Lemma valdiviana*, and grass shrimp, *Palaemontes paludosus*, in the freshwater turtle *Trachemys scripta*. We fed turtles 100% duckweed, 100% shrimp, and two mixed diets containing 67% duckweed, 33% shrimp and 14% duckweed, 86% shrimp (dry matter basis). During each feeding trial, we measured intake, digestibility, and transit time of the diet, and upon conclusion, short-chain fatty acid concentrations in turtle digestive tracts. Digestibility was lower on the 67% duckweed diet, but higher on the 14% diet. These apparent nonadditive interactions may be due to differences in transit time of duckweed and shrimp. We believe this is the first evidence of two diet items producing opposing nonadditive effects when fed in different ratios.

Keywords: Digestibility; Fermentation; Mixed diets; Nonadditive effects; Associative effects; Nutritional ecology; Freshwater turtle

1. Introduction

Dietary mixing is widespread among animals, commonly occurring in many vertebrate and invertebrate species (Robbins, 1993; Coll and Guershon, 2002). Nonadditive effects between diet items may play an important role in the selection of mixed diets, particularly for diet items that differ radically from each other in nutritional composition or in how they are processed in the digestive tract (Bjorndal, 1991; Bozinovic and Martínez del Rio, 1996). These effects occur when diet items interact with one another such that the net energy or nutrient gain from the mixed diet differs from the net gain predicted by summing the gains from individual diet components. Although many studies have acknowledged the potential importance of nonadditive effects in their study species (Campbell and MacArthur, 1996; Nagy et al., 1998; Spencer et al., 1998; Chen and Lue, 1999; Durtsche, 2000), few have tested for or quantified these effects (Table 1).

The concept of nonadditive effects was first demonstrated in studies of domestic livestock nutrition. Like many herbivorous wildlife species, livestock, such as cattle, use microbial gut symbionts to digest plant material. These symbionts ferment plant cell wall components and produce waste products in the form of short-chain fatty acids (SCFA), which the host absorbs and uses as an energy source. Nonadditive effects found in livestock often result from alterations in microbial fermentation. For example, adding grain to a forage diet depresses digestibility because gut symbionts preferentially attack the easily fermentable grain carbohydrates rather than the structural carbohydrates of the forage. This rapid fermentation produces high concentrations of SCFAs that lower pH of the fermentation chamber and create an unfavorable environment for symbionts (Schneider and Flatt, 1975). However, if urea and a small quantity of easily fermented carbohydrate are added to forage, digestibility increases. This increase is due to extra nitrogen from the urea and readily available energy from the carbohydrate stimulating growth of the microbial population, which can then ferment the forage more efficiently (Pond et al., 1995).

Nonadditive effects have been demonstrated in a diverse array of wild species including insects, turtles, birds, ungulates, and rodents (see Table 1 for summary and references). In some

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Table 1
Summary of studies investigating nonadditive dietary effects in wildlife

Diet items		Study species	Type of interaction	Proposed mechanism	Source
Fungus (species not given)	Wood (species not given)	Termites (species not given)	+	Ingestion of fungus provided cellulytic enzymes which facilitated digestion of plant parts	Martin and Martin, 1978
Browse stems (*Vaccinium* sp.)	Grass hay (*Bromus inermis)*	Elk (*Cervis elaphus*)	+	Transit time of browse increased transit time of grass, facilitating fiber digestion	Baker and Hobbs, 1987
		Mountain sheep (*Ovis canadensis*)	+	Same as above for elk	
Fungus (species not given)	Wood (species not given)	Cerambycid beetles	+	Ingestion of fungus provided cellulytic enzymes which facilitated digestion of plant parts	Kukor et al., 1988
Peach palm pollen (*Bactris gasipaes*)	Palm trichomes (*B. gasipaes*)	Beetle (*Cyclocephala amazona*)	+	Ingestion of highly lignified trichomes crush pollen allowing it to be digested	Rickson et al., 1990
Duckweed plant (*Spirodela polyrhiza*)	Beetle larvae (*Tenebrio* sp.)	Yellow-bellied turtle (*Trachemys scripta*)	+	Nitrogen in larvae stimulated growth of gut microbial population which digested plant fiber more efficiently	Bjorndal, 1991
Fungus (*Boletus edulis*)	Insect larvae (species not given)	Rodent (*Abrothrix longipilis*)	+	Nitrogen in larvae stimulated growth of gut microbial population which digested fungus carbohydrates more efficiently	Bozinovic and Muñoz-Pedreros, 1995
Pollen (*Opuntia echios*)	Nectar (*O. echios*)	Cactus finch (*Geospiza fortis*)	+	Nectar stimulated germination of pollen in the crop, facilitating digestion	Grant, 1996
		Medium ground finch (*Geospiza scandens*)	+	Same as above for cactus finch	
Millipedes (*Alloporus* sp.)	Kale leaves (*Brassica oleracea*)	Hingeback tortoise (*Kinixys spekii*)	–	Transit time of kale reduced transit time of millipedes	Hailey et al., 1998
Milkweed flowers (*Asclepias syriaca*)	Milweed foliage (A. *syriaca*)	Beetle (*Tetraopes etraophthalmus*)	–	Secondary compounds in foliage depressed floral digestion	Matter et al., 1999
Whiting (*Merlangius merlangus*)	Sprat (*Sprattus sprattus*)	Lesser black-backed gulls (*Larus fuscus*)	No effect	None given	Hilton et al., 2000
		Common guillemots (*Uria aalge*)	–	None given	

cases, possible mechanisms underlying these effects mirror those found in domestic livestock. For example, Bjorndal (1991) found a positive nonadditive effect in yellow-bellied slider turtles, *Trachemys scripta*, fed a diet comprised of 77% duckweed, *Spirodela polyrhiza*, and 23% *Tenebrio* larvae (dry matter basis). Adult yellow-bellied slider turtles are opportunistic omnivores that feed primarily on aquatic plants (Parmenter and Avery, 1990), and the ratio of plant to animal material in that study approximated that consumed by a wild population of adult *T. scripta* (Bjorndal, 1991). Bjorndal (1991) hypothesized that the positive nonadditive effect between duckweed and insect larvae was due to nitrogen in the larvae stimulating growth of the microbial symbiont population. She proposed this hypothesis because *T. scripta* use microbial gut symbionts to digest plant material (Bjorndal and Bolten, 1993) and because the cell wall, or fiber, component of the diet was most affected by the nonadditive effect.

The inclusion of animal material in a plant diet, however, does not consistently produce a positive nonadditive effect. For example, an omnivorous tortoise, *Kinixys spekii*, experienced a negative nonadditive effect when fed a diet comprised of 74.2% kale, *Brassica oleracea*, and 25.8% millipedes, *Alloporus* sp. (dry matter basis) (Hailey et al., 1998). This negative effect was attributed to kale, with its relatively short gut transit time, decreasing millipede transit time, thus depressing digestibility. Studies of *K. spekii* and *T. scripta* (Bjorndal, 1991) demonstrate that plant and animal diet items do not always interact in similar ways. Additionally, studies of domestic livestock nutrition have demonstrated that the magnitude of a nonadditive effect can vary with different ratios of diet components (Van Soest, 1994). The direction of the effect could also vary, although that has yet to be demonstrated. Because nonadditive effects can significantly alter diet value, better knowledge of these effects is required to understand more completely the nutritional ecology of animals consuming mixed diets.

The purpose of this study was to quantify nonadditive effects in *T. scripta*, using previously untested diet items, duckweed, *Lemna valdiviana*, and freshwater grass shrimp, *Palaemontes paludosus*. We performed a series of feeding trials in which we fed adult turtles 100% duckweed, 100% shrimp, and two mixed diets containing either 67% duckweed and 33% shrimp or 14% duckweed and 86% shrimp (dry matter basis). During the feeding trials, we measured intake, digestibility, and transit time of the diets. At the conclusion of each trial, we measured SCFA concentrations in the digestive tracts of turtles on each diet. The results of these feeding trials allowed us to (1) determine if nonadditive effects exist between duckweed and shrimp, (2) assess if any existing nonadditive effect varied with the ratio of plant to animal material, and (3) evaluate possible mechanisms underlying these effects.

2. Material and methods

2.1. Experimental animals and diets

Two feeding trials were conducted to compare how turtles process plant, animal, and mixed diets. In the first trial, turtles were fed pure diets of either duckweed, *L. valdiviana* ($n=6$ males; $n=1$ female), or a freshwater grass shrimp, *P. paludosus* ($n=5$ males). In the second trial, different turtles were fed a mixed diet by dry mass of either 67% duckweed, 33% shrimp ($n=1$ male, $n=4$ females) or 14% duckweed, 86% shrimp ($n=2$ males, 2 females). These ratios of plant to animal material are within ranges measured for natural populations of *T. scripta* (Parmenter, 1980; Parmenter and Avery, 1990). During the first trial, duckweed was collected from a local pond in Gainesville, Florida. Because this pond dried up before the onset of the second trial, duckweed for that trial was purchased from an aquarium store. Grass shrimp for all trials were purchased from a bait shop that obtained the shrimp from Gainesville area lakes. Because some turtles did not eat the anterior most portion of the shrimp containing the eyes and antennae or the posterior portion containing the caudal fin, these parts were removed before shrimp were fed to turtles. This ensured all animals consumed the same diet. Diet nutrient composition is described in Table 2.

Turtles were collected from ponds located at Savannah River Ecology Laboratory and Audubon Society's Silver Bluff Sanctuary in Aiken County, South Carolina. Before the trials, turtles were maintained on a mixture of aquatic plants (primarily duckweeds) and invertebrates collected from a local pond. At the onset of the first trial, turtles were switched to the experimental diet; no turtle demonstrated difficulty with this switch. Both trials consisted of a two-week acclimation period followed by a three-week experimental period during which daily food intake and feces production were quantified. Mean turtle mass at the beginning of the first and second trials was 995.4 g (range: 375.2–1451.1 g) and 1340.2 g (range: 812.2–1810.9 g), respectively.

2.2. Experimental protocol

Turtles were housed individually in square Nalgene tanks (45 × 60 cm) equipped with a 75-W floodlight and a 20-W full spectrum natural light fluorescent bulb. They experienced a 12-h photoperiod and temperatures between 25–26 °C. To determine digestibility, we collected and quantified all feces produced during the experimental periods. Turtles were fitted with fecal collection devices as described in Bouchard (2004).

During the trials, water was drained from tanks every morning at 0800 h so turtles could bask for the same amount of time each day and differential thermoregulation could be controlled. At 1000 h, feces were collected, and tanks were refilled with water. At 1100 h, turtles were fed a known mass of either duckweed or shrimp, with turtles on the mixed diet receiving only the duckweed fraction of their diet. Turtles fed ad libitum for 6 h until 1700 h when orts (remaining food) were collected and weighed. Turtles on the mixed diets were then fed a quantity of shrimp that resulted in the appropriate ratio of duckweed to shrimp depending on the amount of duckweed consumed that day. This ensured they consumed a constant ratio of plant to animal matter despite daily fluctuations in duckweed intake. Turtles on the mixed diet immediately consumed all shrimp offered at the end of the day. Analysis of feces and digestive tract contents indicated that shrimp and duckweed diet components mixed thoroughly after consumption. Feces were collected again at 1700 h. Note that we collected feces over the same time period that food was consumed even though diet transit time was three to five days. This timing is justified because turtles were acclimated to the diets for two weeks before the onset of the trial, so we are confident all feces collected resulted from the experimental diets. Additionally, all turtles ate each diet and produced feces consistently throughout the experimental period.

Table 2
Nutrient composition of duckweed and shrimp fed to *T. scripta*. All values except energy are presented on a percent dry matter basis

	Duckweed (from pond)	Shrimp	Mixed diets	
			Duckweed (from store)	Shrimp
Organic matter (%)	85.5	87.1	75.9	87.0
Fiber (%)[a]				
NDF	45.2	–	42.9	–
ADF	21.4	4.8	15.0	4.7
Nitrogen (%)	4.1	12.6	4.1	12.2
Lipids (%)	13.2	15.1	12.0	15.8
Energy (kJ g^{-1} dry matter)	17.4	20.9	14.5	20.8

Note that shrimp values are for shrimp with anterior and posterior portions removed.

[a] Neutral detergent fiber (NDF) represents cellulose, hemicellulose, lignin and cutin, whereas acid detergent fiber (ADF) represents cellulose, lignin, and cutin of duckweed. ADF represents the chitin component of shrimp.

2.3. Nutrient analyses

During the experimental periods, duckweed and shrimp diet samples as well as feces and orts from each turtle were collected daily. All samples were dried overnight at 60 °C. Daily fecal and ort samples were combined to obtain a composite fecal and ort sample for each individual turtle. Daily diet samples were also combined in a composite sample. All samples were ground to pass through a 1 mm screen in either a Wiley Mill or coffee grinder (Mr. Coffee, Model IDS 57). All samples were analyzed for dry matter, organic matter, neutral detergent fiber (NDF), acid detergent fiber (ADF), nitrogen, lipid, and energy content. Orts were analyzed and compared to diet samples to test if turtles fed selectively.

Dry matter content was determined by drying subsamples overnight at 105 °C. Ash, or mineral, content was then determined by combustion of subsamples at 500 °C for three hours. The difference between these two measures represents the organic matter component of the sample. NDF and ADF were determined by sequentially refluxing subsamples in neutral detergent and acid detergent solutions (Goering and Van Soest, 1970) in an Ankom^{200} Fiber Analyzer according to the guidelines supplied with the equipment (Ankom Technology, 1998, 1999). NDF represents the cell wall component of duckweed (cellulose, hemicellulose, lignin and cutin), and ADF represents the lignocellulose and cutin component. The ADF component of shrimp

represents the exoskeleton (primarily chitin) fraction of the diet (Stelmock et al., 1985). Lipid content was determined with a Soxhlet extractor, using diethyl ether and petroleum ether as the solvent. Nitrogen content of the samples was determined using a Carlo Erba elemental analyzer. Energy content was determined with a Parr bomb calorimeter (Parr Instrument, 1960).

All samples were analyzed in duplicate. Dry and organic matter and energy duplicates were accepted within 2% relative error. Nitrogen duplicates were accepted within 1% absolute error, and duplicates for lipid, NDF and ADF were accepted within 3% absolute error.

2.4. Digestive processing calculations

Dry and organic matter intakes were calculated as the difference between the amount of food offered and orts remaining each day multiplied by the fraction of dry and organic matter in the diet. Because nutrient composition of the orts was similar to that of the diet (Bouchard, 2004), no adjustments were necessary to account for selective feeding. Digestibility of dry and organic matter, NDF, ADF, lipid, energy and nitrogen was determined using the following equation:

$$\text{digestibility} = (\text{intake} - \text{feces})/\text{intake}$$

where intake was total grams of the dietary component consumed during the experimental period, and feces was total grams of that component in the feces produced. Digestible intakes (dry and organic matter, energy, and nitrogen) were calculated by multiplying daily intake of the component by its digestibility. On the shrimp diet, digestible dry matter intakes were less than digestible organic matter intakes, suggesting that either the quantity of ash in the diet was underestimated or the quantity in the feces was overestimated. Because of this discrepancy, organic matter digestibilities and digestible intakes are not presented. Transit time of the diet was time elapsed from when a 3 mm round piece of plastic flagging was fed to turtles to when it appeared in feces. This flagging approximated the size of duckweed fronds that were oblong and ranged 2–4 mm in length and 1.0–1.5 mm in width. Because of unequal variances, differences in all digestive parameters between treatments were evaluated with Kruskal–Wallis tests with post hoc analyses according to Conover (1980).

To test for nonadditive effects between diet items, we compared digestibilities and digestible intakes of turtles fed mixed diets with values predicted based on results from turtles fed pure diets. Predicted digestibilities for each component were calculated with the equation used by Bjorndal (1991):

$$V_p = (V_D \times F_D) + (V_S \times F_S)$$

V_P predicted digestibility
V_D actual digestibility of component in 100% duckweed diet
F_D fraction of that component contributed to the mixed diet by duckweed
V_S actual digestibility of component in 100% shrimp diet
F_S fraction of that component contributed to the mixed diet by shrimp

Predicted digestible organic matter intakes were calculated by multiplying V_p for organic matter by mass-specific intake of the mixed diet and organic matter content of the mixed diet (Bjorndal, 1991). Predicted energy and nitrogen digestible intakes were calculated the same way using the appropriate values for energy and nitrogen.

For each digestibility, V_D and V_S were random numbers generated from distributions with means and standard deviations as determined from turtles fed pure duckweed and shrimp diets. Random numbers from those distributions were used rather than means to maintain variance in predicted values. Reduced variance in predicted values could increase the likelihood of finding a significant difference between actual and predicted values. All percentage data were first arcsine transformed before random numbers were generated from the distributions. Random numbers from those distributions were converted back to percentages to calculate predicted values.

To compare actual and predicted values, we used the following procedure in the statistical and programming language *R* (Ihaka and Gentleman, 1996). For each digestibility, random numbers ($n=4$ for 67% duckweed diet; $n=3$ for 14% duckweed diet) were generated from the corresponding duckweed and shrimp distributions. Using the preceding equation, random numbers were used to calculate a predicted digestibility for each turtle in the treatment. These predicted digestibilities were then used to calculate the corresponding predicted digestible intakes. Differences between predicted and observed values for each turtle were calculated, and the mean difference for all turtles in the treatment was determined. This entire procedure was repeated 1000 times with new random numbers generated each time. The mean difference for each iteration was then plotted in a histogram, and 95% confidence intervals were determined. Actual values were considered different from predicted values if confidence intervals did not overlap with zero.

2.5. Short-chain fatty acid concentrations

At the conclusion of the trials, turtles were euthanized with an intramuscular injection of sodium pentobarbital. Three additional individuals (one fed duckweed and two fed shrimp) were also included in this portion of the study. Although intake and digestibility were not measured for these animals, they were maintained under feeding trial conditions for five weeks before being euthanized. Although turtles were euthanized and dissected throughout the day, all animals were able to feed ad libitum in the time before euthanization.

Turtles were dissected and digesta was analyzed for short-chain fatty acid concentrations (SCFA). Whenever sufficient digesta was present, samples were collected from five gut sections (stomach, anterior and posterior small intestine, and anterior and posterior large intestine) and preserved in 20% phosphoric acid, which stopped fermentation and the production of SCFAs. Samples were centrifuged and SCFA concentrations of the supernatants were measured using a Shimadzu gas

Table 3
Digestive processing of duckweed, shrimp, and mixed diets by *T. scripta*

	100% duckweed	67% duckweed 33% shrimp	14% duckweed 86% shrimp	100% shrimp	*H*	*p*
	(*n*=7)	(*n*=4)	(*n*=3)	(*n*=5)		
Intake (mg g turtle^{-1} day^{-1})						
Dry matter	2.0 (1.3–3.2)	1.4 (0.7–2.7)	2.1 (2.0–2.4)	3.2 (2.0–4.7)	6.518	0.089
Organic matter	1.8 (1.1–2.8)	1.1 (0.5–2.2)	1.8 (1.7–2.1)	2.8 (1.8–4.1)	7.341	0.062
Digestibility (%)						
Dry matter	64.9^{a} (58.3–75.8)	62.1^{a} (56.1–67.2)	85.0^{b} (79.2–88.6)	77.0^{b} (66.2–85.6)	11.632	0.009
NDF	74.5^{a} (59.4–82.8)	60.7^{b} (56.3–67.1)	81.3^{c} (72.1–87.5)	94.1^{c} (91.7–94.3)	14.472	0.002
ADF	65.0^{a} (41.9–78.2)	32.2^{b} (23.2–38.5)	74.5^{a} (55.7–79.3)	83.3^{c} (74.6–84.9)	13.687	0.003
Lipid	60.8^{a} (49.4–72.4)	59.2^{a} (52.8–68.5)	88.5^{b} (84.5–88.5)	77.8^{b} (76.6–87.6)	13.961	0.003
Energy	65.8^{a} (59.1–75.8)	68.6^{a} (64.6–73.6)	92.5^{b} (88.6–93.4)	90.1^{b} (85.5–94.5)	13.288	0.004
Nitrogen	74.6^{a} (70.7–81.5)	87.1^{b} (86.5–92.4)	96.5^{c} (92.4–97.2)	94.0^{c} (90.2–95.0)	15.517	0.001
Digestible intake						
Dry matter (mg g turtle^{-1} day^{-1})	1.4^{a,b} (0.8–2.1)	0.8^{a} (0.5–2.0)	1.9^{b,c} (1.6–2.1)	2.6^{c} (1.9–4.6)	10.645	0.014
Energy (kJ g turtle^{-1} day^{-1})	24.4^{a} (15.4–34.0)	15.4^{a} (8.6–36.1)	39.9^{b} (35.7–44.9)	56.5^{b} (38.4–86.9)	13.596	0.004
Nitrogen (mg g turtle^{-1} day^{-1})	0.05^{a} (0.04–0.08)	0.08^{a} (0.04–0.18)	0.23^{b} (0.21–0.26)	0.36^{b} (0.24–0.55)	14.519	0.002
Transit time (hours)	170.5 (94.8–199.5)	91.0 (55.5–126.5)	135.0 (114.5–143.8)	72.5 (37.5–143.8)	4.407	0.221
Samples sizes for transit time	(*n*=5)	(*n*=2)	(*n*=3)	(*n*=5)		

Differences between treatments were determined by Kruskal–Wallis tests and post hoc tests according to Conover (1980). Values are medians (range), and different superscripts across rows indicate significant differences between treatments.

chromatograph (Model GC-9 AM) with a Perkin Elmer Computing Integrator (LCI-100).

SCFA concentrations of different gut regions were compared between turtles fed duckweed (n=5) and shrimp (n=6) using a repeated-measures analysis of variance. SCFA concentrations in each gut region of the same turtle were used as the repeated-measure. Measurements from one turtle fed duckweed were not included in the analysis because SCFA concentrations were over

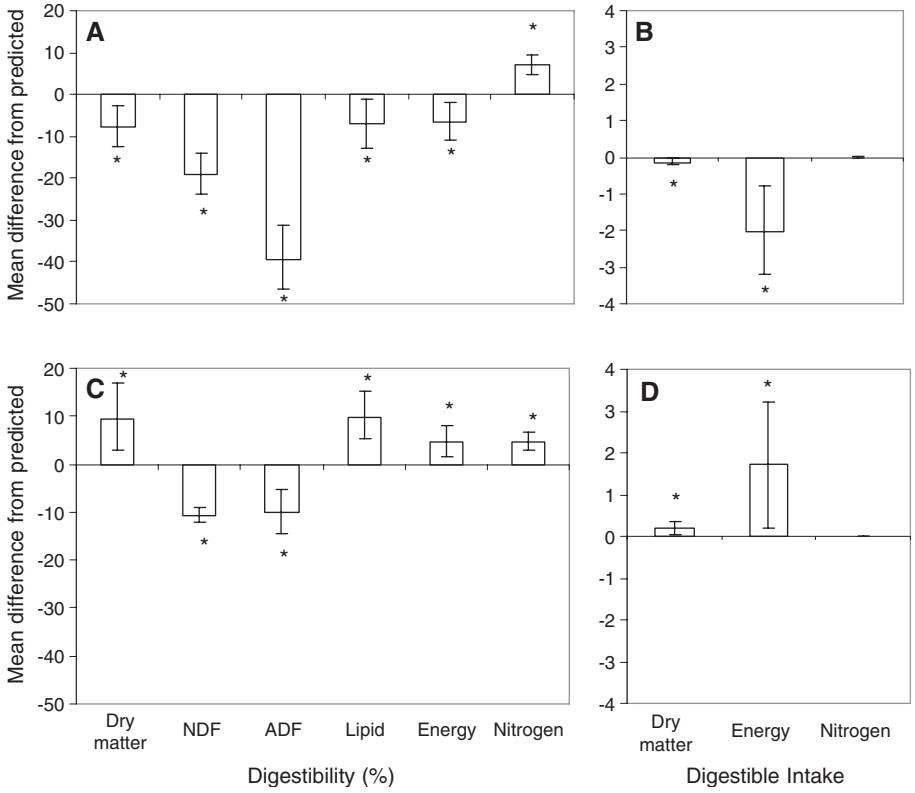

Fig. 1. Differences between actual and predicted digestibilites and digestible intakes of *T. scripta* fed two mixed diets of duckweed and shrimp. Parts A and B refer to turtles fed the 67% duckweed, 33% shrimp diet, and parts C and D refer to turtles fed the 14% duckweed, 86% shrimp diet. Bars represent 95% confidence intervals, and asterisks indicate those confidence intervals that do not overlay zero and are therefore significantly different from predicted values. The units for digestible intake are mg g turtle^{-1}day^{-1} for dry matter and nitrogen and kJ g turtle^{-1} day^{-1} for energy.

four standard deviations lower than the average. These extremely low levels were probably due to a sample collection problem as this turtle was able to digest the ADF component of diet to the same extent as were other turtles (65.0%). An additional turtle fed duckweed and one fed shrimp were also not included in the analysis because they did not have sufficient digesta in every gut region for analysis. For those regions where there was sufficient digesta, these turtles had SCFA concentrations within the range found in other turtles fed the same diet. Turtles fed mixed diets were not included in this analysis because only two turtles in each treatment had sufficient digesta in every gut region for SCFA analysis. However, SCFA concentrations for turtles on mixed diets are presented for comparison.

3. Results

The wide turtle size range in the first trials was due to two small turtles in the 100% duckweed treatment (size range without those animals = 902.25–1451.11). Because of this, all analyses were done twice including and not including these animals. The results did not differ, so the analyses including those animals are presented here.

Mass-specific intake did not vary significantly among turtles fed the four diets (dry matter: $p = 0.089$; organic matter: $p = 0.062$; Table 3). However, digestibility of every dietary component did vary significantly ($p < 0.01$; Table 3) as did digestible intake of dry matter ($p = 0.014$) and energy and nitrogen ($p < 0.005$). Transit time did not vary significantly among diets ($p = 0.221$).

On the 67% duckweed diet, digestibility of all dietary components was significantly less than predicted (i.e., 95% confidence intervals did not overlap zero; Fig. 1) except for nitrogen which was significantly greater. This effect was most dramatic for the ADF portion of the diet, which was only 55% as digestible as predicted (32.2% vs. 71.5%). Digestible dry matter intake was also significantly less than expected (0.8 vs. 1.0 mg g turtle^{-1}day^{-1}) as was digestible energy intake (15.4 vs. 17.4 kJ g turtle^{-1} day^{-1}). There was no significant difference for digestible nitrogen intake.

On the 14% duckweed diet, NDF and ADF digestibilities were significantly depressed relative to expected values (NDF: 81.3% vs. 91.8%, ADF: 74.5% vs. 84.6%; Fig. 1). All other digestibilities were significantly higher than predicted (Fig. 1) with the most dramatic effect for the lipid component of the diet

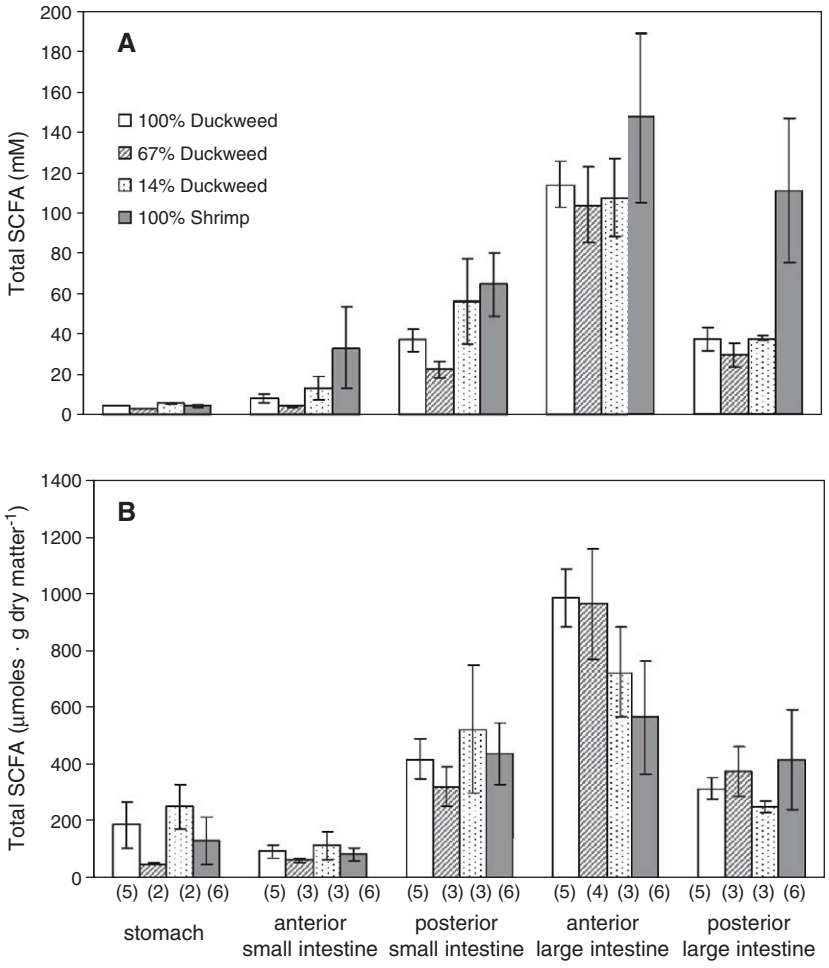

Fig. 2. Concentrations of SCFAs in the digestive tracts of *T. scripta* fed duckweed, shrimp, and mixed diets. Bars represent standard errors. Samples sizes are indicated in parentheses under each column. A) Concentrations are presented on molar basis. B) Concentrations are presented on dry matter basis.

Table 4
Short-chain fatty acid composition in the anterior large intestines of *T. scripta* fed duckweed, shrimp, and mixed diets

		Short-chain fatty acid					
	n	Acetate	Propionate	Butyrate	Isobutyrate	Valerate	Isovalerate
100% duckweed	5	78.7±2.9	10.3±2.4	8.7±0.4	1.1±0.2	0.2±0.2	1.0±0.3
67% duckweed, 33% shrimp	4	74.0±6.4	11.3±3.9	8.9±3.5	2.1±0.4	0.6±0.3	3.0±0.9
14% duckweed, 86% shrimp	3	74.5±1.5	8.3±4.2	9.8±0.9	3.4±2.3	1.7±0.5	2.3±0.1
100% shrimp	6	59.8±5.1	15.6±3.3	14.0±2.2	2.7±0.7	2.6±1.2	5.2±1.7

Values are mean percentages of total SCFAs±standard errors.

(88.5% vs. 78.5%). Digestible dry matter intake was significantly higher than expected (1.9 vs. 1.7 mg g turtle^{-1} day^{-1}) as was digestible energy intake (39.9 vs. 38.2 kJ g turtle^{-1} day^{-1}). There was no significant difference for digestible nitrogen intake.

SCFA concentrations varied significantly between gut regions (Fig. 2; molar basis: $F_{4,36}=12.64$, $p<0.001$; dry mass basis: $F_{4,36}=11.33$, $p<0.001$). However, there was no difference between duckweed and shrimp diets (molar basis: $F_{1,9}=2.86$, $p=0.125$; dry mass basis: $F_{1,9}=0.74$, $p=0.411$), and there was no interaction between gut region and diet (molar basis: $F_{4,36}=1.08$, $p=0.361$; dry mass basis: $F_{4,36}=1.60$, $p=0.196$). The SCFA concentrations in the digestive tracts of turtles fed the mixed diets were comparable to those of turtles fed pure diets (Fig. 2).

The digestive tracts of turtles fed all four diets contained the following SCFAs: acetate, propionate, butyrate, isobutryate, valerate, and isovalerate (Table 4). In the anterior large intestine, where SCFAs peaked, acetate was the primary acid produced followed by propionate and butyrate. On the shrimp diet, acetate concentrations were 24% lower and propionate and butyrate concentrations 52–61% higher relative to concentrations measured in turtles fed duckweed.

4. Discussion

Digestible intake of energy and nitrogen are the best measures of diet value because these measures integrate both the quantity of food consumed and the ability of the animal to digest it (Bjorndal and Bolten, 1993). In terms of digestible energy intake, there were significant differences between the groups of turtles fed different diets, suggesting both negative and positive nonadditive effects. We believe this is the first evidence of two diet items fed in different ratios producing opposite effects. In terms of digestible nitrogen intake, turtles did not experience a nonadditive effect on either mixed diet.

The apparent negative nonadditive effect experienced by turtles on the 67% duckweed diet was most dramatic for ADF digestibility. This component of the diet was primarily derived from duckweed fiber (86%), which is usually fermented by gut symbionts. These results therefore suggest that the negative nonadditive effect arose from an alteration in microbial fermentation. SCFA concentrations in turtle large intestines indicate that fermentation played at least some role in the digestion of all four diets, including shrimp. Possible substrates for shrimp fermentation included proteins, lipids, and chitin. Chitin, as measured by ADF, was 83% digestible for the shrimp diet and was the most likely substrate for fermentation because most proteins and lipids were probably digested and absorbed in the stomach and small intestine. This is particularly true for this diet because exoskeletons were opened at the anterior and posterior ends exposing the underlying tissue. Chitin fermentation has been observed in minke whales, *Balaenoptera acutorostrata* (Olsen et al., 2000) and Adélie penguins, *Pygoscelis adeliae* (Stemmler et al., 1984).

A negative nonadditive effect on the 67% duckweed diet could have arisen if shrimp was more easily fermented than duckweed, and gut symbionts preferentially fermented shrimp over duckweed. This preference would decrease digestibility of the duckweed portion of the diet. However, this is not likely because the ratios of individual SCFAs produced during fermentation of the 66% duckweed diet more closely resembled ratios produced on the duckweed diet than on the shrimp diet. This suggests that duckweed, not shrimp, was the primary substrate for fermentation of the 66% duckweed diet.

An alternative explanation for a negative nonadditive effect may be related to differences in transit time between the diet items. Although there was no statistically significant difference in transit time between duckweed and shrimp, the median transit time of duckweed was more than twice as long as that of shrimp (7.1 vs. 3.0 days). The lack of statistical significance may stem from the crude method of measuring transit time and low statistical power associated with small sample size. If differences in transit time between duckweed and shrimp exist, a negative nonadditive effect could occur if adding shrimp to duckweed decreased transit time. Digesta would be exposed to gut symbionts for less time, reducing digestibility, particularly that of fiber.

Differences in transit time between duckweed and shrimp could also generate a positive nonadditive effect on the 14% duckweed diet. Positive nonadditive effects were suggested for digestibility of all dietary components, except fiber. This effect was most dramatic for lipids, which were primarily derived from shrimp (89%). The assimilation of lipids requires emulsification with bile followed by micelle formation (Maynard et al., 1979). If the addition of duckweed to the shrimp diet increased transit time, it would provide more time for this process to occur. Differences in transit time could also explain why fiber digestibility of the 14% duckweed diet was significantly less than expected. If the mixed diet had a shorter transit time than the pure duckweed diet, there would be less time for microbial fermentation of cell well components.

Both positive nonadditive effects in ungulates and negative effects in tortoises have been attributed to differences in transit time between diet items (Table 1). The differential transit time

hypothesis is particularly compelling in this study because it can account for both the positive and negative effects observed between the same diet items at different ratios. However, because of the crude methodology and low samples sizes (particularly for the mixed diets), transit time differences between duckweed and shrimp were not accurately assessed. To test this hypothesis, transit time must be measured in more individuals using more accurate methods.

Although the differential transit time hypothesis explains opposite nonadditive effects with different ratios of plant and animal material, the hypothesis does not explain why on the 67% duckweed diet, nitrogen digestibility was elevated, whereas digestibility of all other dietary components was depressed. Because digestibilities measured in this study were apparent rather than true digestibilities, the elevated nitrogen digestibility could be attributed to a decrease in nitrogen loss from intestinal sloughing, mucus production, or bacterial protein, rather than a change in digestibility of the diet. Regardless, the most meaningful measure for nitrogen from the perspective of the animal remains digestible nitrogen intake that demonstrated no nonadditive effect.

The negative nonadditive effect suggested on the 67% duckweed diet contrasts with the positive effect previously measured in adult *T. scripta* fed a diet containing 77% duckweed, *S. polyrhiza*, and 23% mealworm larvae, *Tenebrio sp.* (Bjorndal, 1991). These conflicting results may be related to differences in how turtles processed the two duckweed species. Although the nutrient composition of *S. polyrhiza* (88.6% organic matter, 38.5% NDF, 20.2% ADF, 5.1% nitrogen, 5.1% lipids and 18.1 kJ g dry matter^{-1}) was similar to that of *L. valdiviana,* digestible energy intake from *S. polyrhiza* was 33% less than that from *L. valdiviana*. *S. polyrhiza* also had a higher intake (3.0 mg dry matter g turtle^{-1} day^{-1}), more rapid transit time (3.0 days) and lower digestibility, particularly with respect to fiber (NDF digestibility = 25%; ADF = 9%). Such differences suggest that microbial fermentation played a less important role in the digestion of *S. polyrhiza* than in the digestion of *L. valdiviana*, as was found in a similar comparison between *T. scripta* fed *S. polyrhiza* and another aquatic plant, *Hydrilla verticillata* (Bjorndal and Bolten, 1993). The positive nonadditive effect experienced on the *S. polyrhiza* mixed diet could have resulted from larval nutrients stimulating microbial population growth to a size that allowed fermentation to play a more substantive role in *S. polyrhiza* digestion. Such an input of nutrients was not required for effective fermentation of *L. valdiviana*.

Several differences in the physical structure and chemical composition of these duckweeds may explain why *T. scripta* relied on fermentation to varying degrees for their digestion. First, duckweed fronds are surrounded by a waxy cuticle that acts as a physical barrier to fermentation by gut symbionts (Bjorndal and Bolten, 1992); this cuticle is significantly thicker in *S. polyrhiza* than in *L. valdiviana* (Elias Landolt, pers. comm.). Second, *S. polyrhiza* is 165% higher in lignin content than *L. valdiviana* and contains two benzaldehydes, vanillin and syringaldehyde, that are not found in *L. valdiviana* (Blazey and McClure, 1968). *S. polyrhiza* also contains tannins, whereas *L. valdiviana* does not (Elias Landolt, pers. comm.). Both lignins and tannins can negatively influence digestive processing by herbivores (Robbins, 1993).

5. Conclusions

This study provides evidence that both positive and negative nonadditive effects can occur when animals consume the same diet items in different ratios. In natural populations of *T. scripta*, the ratio of plant to animal material in the diet can vary widely depending on the availability of resources (Hart, 1983; Parmenter and Avery, 1990). Because nonadditive effects can vary with different ratios of plant to animal material, turtles may benefit from positive nonadditive effects under some conditions, whereas they may incur costs from negative effects under other conditions.

Additionally, different plant and animal diet items can produce opposite associate effects even when fed in similar ratios. Both positive and negative effects for digestible energy intake have been demonstrated in *T. scripta* when animal material was added to a predominately plant diet (this study and Bjorndal, 1991). Additional research is needed with more diet items to determine how prevalent each effect is in wild *T. scripta* nutrition. If negative effects on digestible energy intake prevail, then turtles may experience no energetic advantage from including animal material in a plant diet. They may therefore include animal material for other dietary constituents, such as nitrogen.

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