

Integrated Postprandial Responses of the Diamondback Water Snake, *Nerodia rhombifer*

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ABSTRACT

Among snakes, the magnitude to which intestinal performance is regulated with feeding and fasting is adaptively linked to their natural feeding frequency. For infrequently feeding boas and pythons, gastrointestinal form and function are widely regulated with each feeding bout. In contrast, snakes that naturally feed more frequently modestly regulate intestinal function with each meal. To further explore the postprandial responses of a frequently feeding snake and assess whether such responses are matched in magnitude, we examined the postprandial metabolic, morphologic, and functional responses of the diamondback water snake (*Nerodia rhombifer*) following the consumption of catfish meals equaling 25% of their body mass. After feeding, *N. rhombifer* experienced 5.4-fold increases in metabolic rate and a specific dynamic action of 101 kJ that equaled 25.3% of the ingested energy. *Nerodia rhombifer* that were fed did not undergo any change in stomach tissue mass but did experience a rapid drop in gastric pH and a decline in tissue stores of pepsinogen. Feeding triggered an increase in pancreatic mass and a temporary loss of trypsin activity. The small intestine of *N. rhombifer* responded to feeding with a 70% increase in mass and a 27% increase in enterocyte length but no change in microvillus length. Intestinal nutrient uptake rates did not increase with feeding, whereas intestinal aminopeptidase-N activity increased by fivefold. The postprandial increases in metabolism and gastrointestinal morphology and function of *N. rhombifer* are of a lower magnitude than is characteristic of infrequently feeding snakes and are more similar to the responses observed for other frequently feeding species. In support of an adaptive interplay between feeding habits and digestive physiology, this study demonstrates that the regulation of gastrointestinal structure and function for the frequently

feeding *N. rhombifer* is generally modest and matched in magnitude.

Introduction

Vertebrates exhibit both a diversity of feeding habits (e.g., herbivory, omnivory, carnivory), as well as a broad continuum of feeding frequencies (Stevens and Hume 1995; Karasov and Martínez del Río 2007). The latter is exemplified by small endotherms (e.g., hummingbirds and shrews) at one end of the spectrum that must feed every few hours and large ectotherms (e.g., crocodylians, boas, and pythons) at the other end that naturally fast for many months (Crowcroft 1955; Pope 1961; Diamond et al. 1986; Christian et al. 1996). Over the past century, studies have examined the anatomical and physiological correlates of the gastrointestinal system to feeding habits (reviewed in Karasov and Diamond 1988; Stevens and Hume 1995; Karasov and Hume 1997). In general, herbivores possess larger gastrointestinal tracts that include organs (rumen, cecum, large intestine) that are more specialized for the fermentation of their plant diet and are able to digest and absorb carbohydrates more efficiently than carnivores (Karasov and Diamond 1988; Stevens and Hume 1995). In contrast, carnivores characteristically possess shorter gastrointestinal tracts with greater capacities to break down proteins and absorb amino acids (Karasov and Diamond 1988; Stevens and Hume 1995; Hume 2002).

An adaptive interplay between feeding habits and digestive form and function is hypothesized to extend beyond food habits to also include feeding frequency (Secor 2005a). The strongest evidence for such a relationship between the gastrointestinal tract and feeding frequency comes from studies on the post-feeding responses of snakes (Secor and Diamond 2000). Although snakes are obligate carnivores, they exhibit a diversity of feeding ecologies (Greene 1997). For example, the fossorial flat-headed snake, *Tantilla gracilis*, feeds very often on arthropod prey as evidenced by the large percentage of collected individuals (73%–94%) with food items within their gut (Cobb 2004). In contrast, “sit-and-wait” foraging boas, pythons, vipers, and rattlesnakes feed infrequently on relatively large vertebrate prey (Greene 1997; Murphy and Henderson 1997; Secor and Nagy 1994; Shine et al. 1998). From a digestive perspective, snakes range from those species or populations that are almost constantly digesting to those for which meal digestion is a relative rare event.

Studies on the digestive responses of snakes have identified an apparent adaptive correlate between feeding frequency and

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the magnitude by which the gastrointestinal tract regulates morphology and function (Secor and Diamond 2000; Secor 2005a). Some species of pythons, boas, and rattlesnakes that in nature experience long episodes of fasting between meals upregulate intestinal performance with feeding and subsequently downregulate the intestine on the completion of digestion (Secor et al. 1994; Secor and Diamond 2000; Ott and Secor 2007). In contrast, actively foraging snake species that feed more often in the wild experience modest changes in intestinal structure and function with the start and completion of digestion (Secor and Diamond 2000). An adaptive explanation for this dichotomy in digestive response centers on the energy conserved by downregulating digestive tissues during predicted long episodes of fasting for infrequently feeding snakes and no apparent energetic benefit from doing so if meals are more frequent, as is the case for frequently feeding animals (Secor and Diamond 2000; Secor 2005a).

This hypothetical evolutionary scenario would predict that postfeeding responses, whether morphological or physiological, are matched in magnitude regardless of feeding habits. As a case in point, on the ingestion of meal equal in mass to 25% of the snake's body mass, the infrequently feeding Burmese python experiences as much as a 15-fold increase in metabolic rate, a drop in gastric pH from 7 to 1.5, a near doubling in mass of the liver, pancreas, kidneys, and small intestine, a five-fold increase in microvillus length, and a three- to 10-fold increase in intestinal nutrient uptake and gastric, pancreatic, and intestinal hydrolase activities (Secor 2008). The evidence for frequently feeding snakes is in agreement; much more modest postprandial increase in metabolic rate (five- to eightfold), only a 50% increase in small intestinal mass, and no significant modulation of intestinal nutrient uptake rates with feeding (Secor and Diamond 2000). Unknown from that study is the extent that frequently feeding snakes regulate with feeding gastric pH, intestinal microvillus length, and gastric, pancreatic, and intestinal hydrolase activities.

To address the prediction that all postprandial responses of frequently feeding snakes are collectively of a small magnitude, we examined the postprandial metabolic, gastric, pancreatic, and intestinal responses of the frequently feeding diamondback water snake, *Nerodia rhombifer*. *Nerodia rhombifer* is an active foraging snake that feeds predominately on fish and is often found with food within its digestive tract (Mushinsky and Hebrard 1977; Aldridge et al. 2003). From fasted and fed snakes we measured (1) metabolic rate to illustrate their postprandial metabolic response and to quantify their specific dynamic action (SDA); (2) organ mass and intestinal morphology to determine the extent that tissue structure is modulated with feeding and fasting; (3) pH within the lumen of the esophagus, stomach, small intestine, and large intestine to identify temporal and spatial patterns of gut pH; (4) gastric, pancreatic, and intestinal hydrolase activity to assess the matched regulation of enzyme activities among different organs; and (5) intestinal nutrient uptake rates and capacities to examine the regulatory scope of intestinal performance. As predicted based on their frequently feeding habits, *N. rhombifer* experience

modest levels of change for most parameters of gastrointestinal form and function with feeding.

Material and Methods

Snake Feeding Ecology, Acquisition, and Care

The diamondback water snake (*Nerodia rhombifer*), one of the largest species of water snakes, inhabits a variety of aquatic habits in south-central United States and eastern Mexico (Gibbons and Dorcas 2004). The diet of this snake is predominately fish, which they capture by actively foraging within shallow and deeper waters of streams, rivers, ponds, and lakes (Mushinsky and Hebrard 1977; Gibbons and Dorcas 2004). Four ecological studies found that 24%–41% of *N. rhombifer* captured in the wild contained food items within their stomachs (Mushinsky and Hebrard 1977; Koffron 1978; Manjarrez and Macias Garcia 1991; Aldridge et al. 2003). Given that *N. rhombifer* clear their stomachs 4–5 d after feeding (as shall be demonstrated) and combined with the above percentages of snakes found digesting, we predict that *N. rhombifer* feed once every 10–18 d (Diana 1979). Hence, we categorized *N. rhombifer* as a frequently feeding snake that is actively involved in meal digestion for most of their activity season (Secor and Diamond 2000).

Snakes used in this study were captured by hand in midspring from commercial catfish ponds in Leflore County, Mississippi. At these ponds, *N. rhombifer* have continuous access to food (channel catfish, *Ictalurus punctatus*) and were frequently observed feeding. We maintained snakes together in a 3,000-L circular fiberglass tank at 24°–28°C under a 14L : 10D photoperiod. Snakes had continuous access to water and were fed pieces of catfish fillet weekly. Snakes were fasted for 30 d before study to ensure that they were postabsorptive. Care of and experimentation on snakes was approved by the University of Alabama Institutional Animal Care and Use Committee.

Measurements of Postprandial Metabolic Response

Using closed-system respirometry, we quantified the postprandial metabolic response of 12 adult male *N. rhombifer* (mean \pm SEM = 355 \pm 10 g) by measuring rates of oxygen consumption ($\dot{V}O_2$) from snakes that were fed after a 30-d fast (Secor and Diamond 1997). Fasted *N. rhombifer* were placed into individual respirometry chambers (4.5-L plastic containers fitted with inflow and outflow stopcocks) and maintained within a temperature-controlled environmental chamber at 30°C. For each sampling period, a 50-mL gas sample was drawn from each chamber, the chambers were sealed, and a second gas sample was drawn 1–2 h later. Gas samples were injected into an O₂ analyzer (S-3A/II, AEI Technologies, Naperville, IL) after passing through a column of water absorbent (Drierite) and CO₂ absorbent (Ascarite). We calculated $\dot{V}O_2$ (mL h⁻¹) after correcting for standard pressure and temperature as described by Vleck (1987). For each snake fasted, we measured $\dot{V}O_2$ twice a day (morning and evening) for 4 d and assigned the average of the lowest two recorded $\dot{V}O_2$ levels as its standard metabolic rate (SMR). Each snake was then fed several pieces of catfish

fillet with a combined weight that equaled $25.1\% \pm 0.1\%$ of the snake's body mass. Metabolic measurements were then resumed at 12-h intervals (0800 and 2000 hours) for 3 d and at 24-h intervals (0800 hours) thereafter for 7 more days. On the fifth day after feeding and after that morning's measurements, snakes were removed from their chambers, provided with water, and then returned to their chambers.

We characterized the postprandial metabolic response of each snake by quantifying the following variables; peak $\dot{V}O_2$ (highest recorded $\dot{V}O_2$ following feeding), factorial scope of peak $\dot{V}O_2$ (peak $\dot{V}O_2$ divided by SMR), SDA, and SDA coefficient (SDA as a percentage of meal energy). We quantified SDA (kJ) by summing the extra O_2 consumed above SMR during the period of significantly elevated $\dot{V}O_2$ and multiplying that value by 19.8 J per mL O_2 consumed, assuming that the dry matter of the catabolized catfish is 70% protein, 25% fat, and 5% carbohydrates and generates a respiratory quotient of 0.73 (Gessaman and Nagy 1988). We calculated the energy content of catfish meals by multiplying mass of the catfish meal by its energy equivalent (kJ g^{-1} wet mass) determined by bomb calorimetry. Five samples of catfish fillet (22.2 ± 1.0 g) were weighed (wet mass), dried, reweighed (dry mass), ground to a fine powder, and pressed into pellets. Three pellets from each sample were ignited in a bomb calorimeter (1266, Parr Instruments, Moline, IL) to determine energy content (kJ g^{-1}). For each sample, we determined wet-mass energy equivalent as the product of dry mass energy content (25.9 ± 0.3 kJ g^{-1}) and the catfish file dry mass percentage ($17.8\% \pm 0.7\%$). For the five samples, wet mass energy content averaged 4.62 ± 0.23 kJ g^{-1} .

Gut Contents, Organ Mass, and Tissue Preparation

We used 23 *N. rhombifer* (330 ± 20 g) to assess postprandial changes in organ mass and gastrointestinal morphology and function. Snakes were studied either after a 30-d fast ($n = 4$) or at 0.5 ($n = 4$), 1 ($n = 4$), 2 ($n = 4$), 4 ($n = 4$), or 10 ($n = 3$) d following the consumption of a catfish file meal that was equal in mass to $25.0\% \pm 0.1\%$ of the snake's body mass. There was no significant difference ($P = 0.99$) in mean body masses among the fasted and fed treatments. Fasted and fed snakes were killed by severing the spinal cord immediately behind the head. Following a midventral incision and recording of luminal pH (described below), we removed and weighed the mass of the esophagus, stomach, pancreas, gall bladder, liver, lungs, heart, small intestine, large intestine, coelomic fat bodies, and kidneys. For fed snakes, the stomach, small intestine, and large intestine were emptied of their contents and reweighed. The differences between full and empty mass of the stomach and intestines provide a measurement of the mass of their contents. A segment of stomach was scraped of its mucosa, and the mucosa was snap-frozen in liquid N_2 . A second segment of stomach was weighed and scraped of its mucosa, and both the mucosa and underlying muscularis/serosa layer were weighed, dried (at 60°C), and reweighed. A third segment was weighed, dried, and reweighed. We divided the small intestine into equal thirds, designated as the proximal, middle, and distal

segments. From each segment, a 1-cm section was scraped of its mucosa and the mucosa snap-frozen in liquid N_2 , and a second section was weighed and scraped of its mucosa, and the mucosa and underlying muscularis/serosa layer were weighed, dried, and reweighed. From the middle third of the small intestine, a 1-cm section was saved for histological examination. A segment of pancreas was snap-frozen in liquid N_2 , and another segment was weighed, dried, and reweighed. The remaining organs—esophagus, gall bladder, liver, lungs, heart, large intestine, and kidneys—were dried and reweighed.

Intestinal Morphology

We used light microscopy to identify postfeeding changes in the dimensions of the intestinal layers and enterocytes. Sections from the middle region of the small intestine were fixed in 10% neutral-buffered formalin solution, embedded in paraffin, cross sectioned ($6 \mu\text{m}$), and stained with hematoxylin and eosin on a glass slide. From each cross section, we measured mucosal thickness, muscularis/serosa thickness, mucosal circumference, and enterocyte height and width using a light microscopy and image-analysis software (Motic Image Plus, Richmond, British Columbia). We calculated the average of 10 measures of mucosal thickness, muscularis/serosa thickness, enterocyte height, and enterocyte width for each cross section. We calculated enterocyte volume using the equation for a cylinder; volume = $0.5 \text{ width}^2 \times \pi \times \text{height}$.

We used transmission electron microscopy to measure the height and diameter of intestinal microvilli for two fasted and three fed (2 d postfeeding) snakes. Small pieces of intestinal mucosa were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Spurr resin. Ultrathin sections (≈ 90 nm) were placed on copper grids, stained with uranyl acetate and lead citrate, and examined on a Hitachi electron microscope. For each sample, we measured the height and diameter of 100 microvilli and calculated microvillus surface area using the equation ($\text{diameter} \times \pi \times \text{height}$) + $(0.5 \times \text{diameter}^2 \times \pi)$.

Gastrointestinal pH

To assess regional and temporal differences in gastrointestinal pH, we measured luminal pH at 12 sites within the gastrointestinal tract. Following exposure of the gastrointestinal tract, we inserted the end of a pH probe constructed with a long slender shaft (Accument 13-620-95, Fisher Scientific, Pittsburgh, PA) into small incisions in the gastrointestinal tract and recorded pH at three locations (proximal, middle, and distal regions) within the esophagus, stomach, small intestine, and large intestine. The pH probe was calibrated with certified pH buffers (Fisher Scientific) before use for each snake.

Gastric Pepsin Activity

We measured for each snake the activity of the gastric peptidase pepsin (E.C. 3.4.23.1) following the procedure of Anson (1938).

Scraped mucosa from the midregion of the stomach was homogenized in PBS buffer (1 : 50 dilution) on ice. Pepsin was activated at 37°C using 0.031 mM hemoglobin as a substrate and adjusting the pH to 2.0 with 300 mM hydrochloric acid. The reaction was terminated after 30 min with 5% trichloroacetic acid, and the sample absorbance was measured spectrophotometrically (DU 530, Beckman Coulter, Fullerton, CA) at 280 nm and compared to a L-tyrosine standard curve. Pepsin activity was quantified as micromoles of substrate liberated per minute of incubation per gram of mucosa and per milligram of protein. Protein content of all homogenates was determined using the Bio-Rad protein assay kit, based on the method of Bradford (1976).

Pancreatic Trypsin Activity

Pancreatic trypsin activity was determined using the method of Preiser et al. (1975). We homogenized a segment of each pancreas in PBS buffer (1 : 50 dilution) on ice and activated trypsin using a 1% enterokinase solution. The homogenate was incubated at 37°C with 0.91 mM N- α -benzoyl-L-arginine p-nitroanilide hydrochloride which trypsin cleaves to form p-nitroanilide. The reaction was terminated at 30 min with 30% acetic acid. Sample absorbance was measured spectrophotometrically at 410 nm and trypsin activity ($\mu\text{mole min}^{-1} \text{mg}^{-1}$ protein) was calculated using a p-nitroanilide standard curve and sample protein content.

Intestinal Aminopeptidase-N Activity

For the proximal, middle, and distal thirds of the small intestine we measured the activity of aminopeptidase-N (APN) following the procedure of Wojnarowska and Gray (1975). Mucosa scraped from each intestinal segment was homogenized in PBS buffer (1 : 10 dilutions) on ice and later incubated at 37°C for 30 min with 0.34 mM leucyl- β -naphthylamide as the substrate and p-hydroxymercuribenzoic acid to inhibit nonspecific cytosol peptidases. Following incubation, sample absorbance was measured spectrophotometrically at 560 nm and APN activity ($\mu\text{mole min}^{-1} \text{mg}^{-1}$ protein) was calculated from a standard curve developed with β -naphthylamine and sample protein content.

Intestinal Nutrient Uptake

We calculated rates of uptake across the intestinal brush-border membrane for the amino acids L-leucine and L-proline and the sugar D-glucose using the everted sleeve technique (Karasov and Diamond 1983; Secor et al. 1994). For each intestinal third, a 1-cm sleeve of everted intestine was preincubated at 30°C for 5 min in reptile Ringer's solution and then incubated at 30°C for 2 min in reptile Ringer's solution containing both an unlabeled and radiolabeled nutrient (^3H -L-leucine, ^3H -L-proline, or ^{14}C -D-glucose) and a radiolabeled adherent fluid marker (^{14}C -polyethylene glycol for amino acids or ^3H -L-glucose for D-glucose). We measured from each intestinal third the total

uptake (passive and carrier-mediated) of each amino acid and the carrier-mediated uptake of D-glucose as $\text{nmole min}^{-1} \text{mg}^{-1}$.

Organ Functional Capacity

We quantified for each snake the total capacity of the stomach, pancreas, and small intestine to hydrolyze substrate and for the small intestine to absorb nutrients as described in Cox and Secor (2008). For the stomach, the capacity of pepsin activity was calculated as the product of estimated stomach mucosal mass (determined from mass of scraped mucosa), and pepsin activity was quantified per gram of mucosa ($\mu\text{mole min}^{-1} \text{g}^{-1}$). Total pancreatic trypsin capacity for each snake was calculated by multiplying pancreas wet mass by trypsin activity quantified per gram of tissue ($\mu\text{mole min}^{-1} \text{g}^{-1}$). Total intestinal capacity for APN activity was calculated as the sum of capacities (activity per gram of mucosa times estimated mucosal mass) for each intestinal segment. Similarly, intestinal uptake capacity for each nutrient was quantified as the sum of the calculated capacities (mass-specific uptake rate times segment mass) for each intestinal segment.

Statistical Methods

We used a repeated-measures ANOVA to test for an effect of time (d postfeeding) on $\dot{V}\text{O}_2$. A one-way ANOVA was used to test for significant treatment effects (fasting and fed time points) on mass-specific enzyme activities and intestinal nutrient uptake rates. We tested for similar treatment effects on organ masses, intestinal mucosa and muscularis/serosa thickness, enterocyte and microvillus dimensions, and enzyme and intestinal uptake capacities, using one-way ANCOVA with body mass as the covariate. We tested for significant differences for intestinal position using repeated-measures ANOVA. For each ANOVA and ANCOVA resulting in a significant difference, we employed a pairwise mean comparison (Tukey-Kramer procedure) to test for significant differences between time points or position. All statistical tests were carried out in Systat 10 (Systat Software 2002). We designate the level of significance as $P \leq 0.05$ and report mean values as means \pm 1 SEM throughout the manuscript.

Results

Postprandial Metabolic Response

SMRs of the 12 adult male *Nerodia rhombifer* at 30°C averaged $15.1 \pm 0.9 \text{ mL O}_2 \text{ h}^{-1}$. These snakes responded to their catfish meal by rapidly increasing $\dot{V}\text{O}_2$ such that within 12 h after feeding, their $\dot{V}\text{O}_2$ had tripled (Fig. 1). Metabolic rates peaked at 2.5 d postfeeding and declined more slowly thereafter (Fig. 1). The postprandial peak in $\dot{V}\text{O}_2$ averaged $81.1 \pm 3.3 \text{ mL h}^{-1}$, and the factorial scope of peak $\dot{V}\text{O}_2$ averaged 5.39 ± 0.20 . Postprandial $\dot{V}\text{O}_2$ returned to levels that were not significantly different from SMR by day 7. Over the 7-d duration of significantly elevated $\dot{V}\text{O}_2$, SDA averaged $101 \pm 5 \text{ kJ}$. The SDA coefficient

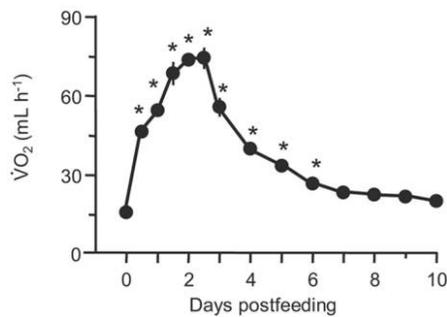


Figure 1. Postprandial oxygen consumption ($\dot{V}O_2$ as mL O_2 consumed h^{-1}) as a function of days postfeeding for 12 adult male *Nerodia rhombifer* (mean body mass = 355 ± 10 g) at $30^\circ C$. $\dot{V}O_2$ had increased to 5.1-fold fasted values by 2.5 d after feeding and returned to levels not significantly different from prefeeding $\dot{V}O_2$ by day 7. In this and other figures, error bars indicate ± 1 SEM and are omitted if the SEM is smaller than the width of the symbol used for the mean value, and an asterisk is inserted above a mean value to indicate that the mean is significantly ($P < 0.05$) different from the prefeeding mean.

(SDA as a percentage of meal energy) stemming from the catfish meals averaged $25.3\% \pm 1.1\%$.

Gastrointestinal Contents and pH

The mass of stomach contents declined rapidly after feeding, such that within 2 d after ingestion, $50.2\% \pm 3.5\%$ of the meal had passed into the small intestine (Fig. 2). By day 4 of digestion, stomachs were almost completely empty, and at day 10 they were empty (Fig. 2). Portions of the meal were already present within the small intestine by 12 h after feeding and continued to be present within the small intestine through day 4 (Fig. 2). We found fecal matter in the large intestine of fasted snakes and fed snakes, though very little material (0.54 ± 0.09 g) was present at day 10.

Luminal pH did not vary significantly among five time points for the proximal esophagus and proximal, middle, and distal regions of the small and large intestine (Fig. 3). For the middle and distal regions of the esophagus, there was a significant decline in pH at day 1 and 2 and a return to fasting levels by day 4 (Fig. 3). For the proximal, middle, and distal portions of the stomach, luminal pH differ ($P_s < 0.017$) among sampling periods, as pH for the three regions had significantly declined to 1.8–2.5 within 24 h after feeding. Luminal gastric pH had returned to levels not significantly different from fasting by day 4 for the proximal portion, and by day 10 for the middle and distal portions (Fig. 3).

Organ Mass and Gastric Pepsin Activity

We found no significant postprandial change in the wet and dry masses of *N. rhombifer* stomachs, or of its mucosa and muscularis/serosa components (Fig. 4). Likewise, wet and dry masses of the esophagus, heart, lung, liver, large intestine, kidneys, and coelomic fat bodies did not significantly vary among fasting and feeding time points (Fig. 4). Gall bladder wet and

dry mass did vary (both $P_s < 0.05$) among sampling periods, decreasing by 33.0% after feeding (Fig. 4).

Gastric pepsin activity varied significantly ($P = 0.041$) among sampling times, decreasing by 80% within 48 h after feeding before returning to fasting rates by day 4 (Fig. 5). Gastric pepsin capacity, the product of mucosal mass and pepsin activity, did not vary significantly ($P = 0.066$) among time treatments (Fig. 5).

Pancreatic Mass and Trypsin Activity

Pancreatic wet and dry masses also varied significantly (both $P_s < 0.05$) among time points, increasing by 54% and 74%, respectively, by day 2 of digestion (Fig. 4). Pancreatic trypsin activity also varied significantly ($P = 0.009$) among sampling periods, due largely to the 15% drop in activity 12 h after feeding (Fig. 6). By day 1 of digestion, trypsin activity returned to fasting levels and remained so through day 10. Total pancreatic capacity for trypsin activity varied significantly ($P = 0.027$) across fasted and fed samples, increasing by 57% by day 2 of digestion (Fig. 6).

Intestinal Mass and Histology

Nerodia rhombifer experienced as much as a 70% increase in small intestinal mass after feeding. Regional postprandial increases in small intestinal mass were observed for the proximal (by 86%) and middle (by 82%) segments, whereas the distal segment did not vary in mass with feeding (Fig. 7). Mass of the mucosa layer of the small intestine increased significantly

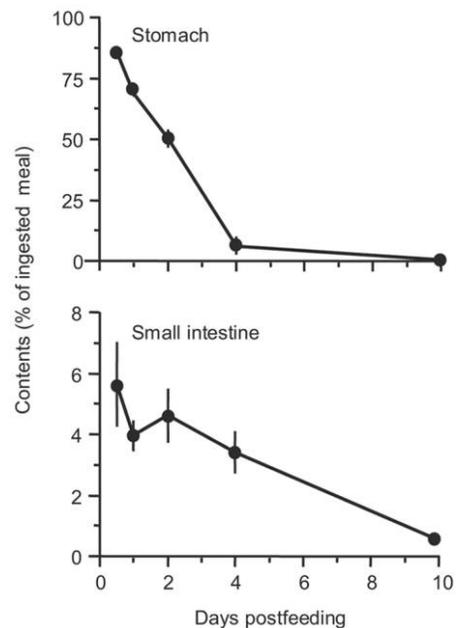


Figure 2. Percentage of ingested meal presence in the stomach and small intestine of *Nerodia rhombifer* following feeding. Within 2 d after feeding, 50% of the meal has already passed from the stomach into the small intestine.

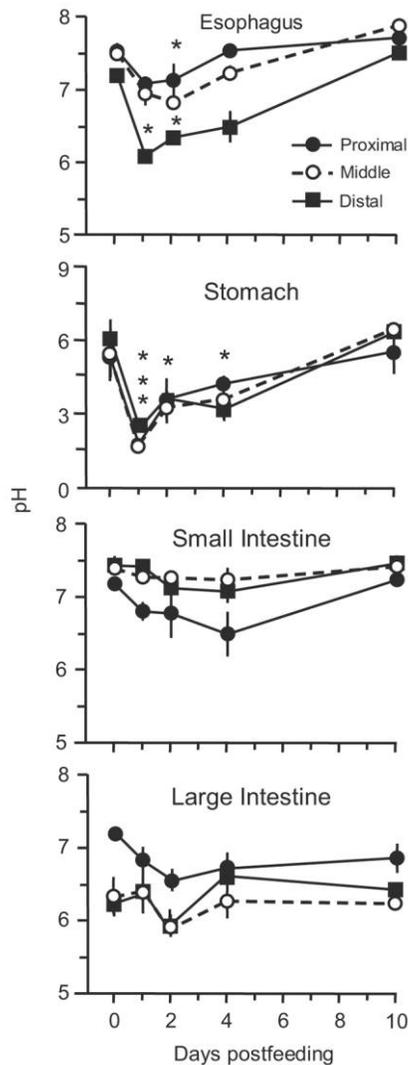


Figure 3. Luminal pH of the proximal, middle, and distal regions of the esophagus, stomach, small intestine, and large intestines as a function of days postfeeding for *Nerodia rhombifer*. Note that the middle (at day 2) and distal regions of the esophagus and all portions of the stomach at day 1 and distal stomach at day 2 and 4 experience a significant decrease in pH after feeding.

(both $P_s < 0.05$) with feeding for the proximal and distal segments, thereby resulting in an overall increase in mucosa mass (by 114%) for the complete small intestine. We observed no significant postprandial changes in the wet mass of the muscularis/serosa layer for each of the three intestinal segments.

Small intestinal mucosa thickness varied significantly ($P = 0.009$) among sampling time points, increasing by 53% within 12 hours after feeding and by 62% by day 4 of digestion (Fig. 8). In contrast, width of the muscularis/serosa layer did not vary significantly among fasted and fed time points (Fig. 8). Enterocytes significantly ($P = 0.003$) varied in length after feeding, increasing by 27%, but did not change in width (Fig. 8). This response generated a significant postprandial increase in enterocyte volume ($P = 0.049$) by 80% at day 4 of digestion

(Fig. 8). *Nerodia rhombifer* experience no significant postfeeding change in the length, width, or surface area of their intestinal microvilli (Fig. 9).

Intestinal Aminopeptidase-N and Nutrient Uptake Activity

We observed significant ($P_s < 0.05$) variation in APN activity among sampling times for each of the three segments of the small intestine (Fig. 10). For each segment, feeding triggered more than a fivefold increase in APN activity within a day or two. There was also a significant ($P = 0.005$) positional effect on APN activity. Peak APN activity of the distal segment averaged 52.4% less than activities of the proximal and middle segments (Fig. 10). As a function of the postprandial increases in intestinal mass and APN activity, the small intestines total capacity for APN activity rose significantly ($P = 0.017$) with feeding to peak at sevenfold of fasting levels by day 2 (Fig. 11).

Feeding failed to induce any significant change in uptake rates of L-leucine, L-proline, or D-glucose for each of the three regions of the snake's small intestine (Fig. 12). We observed

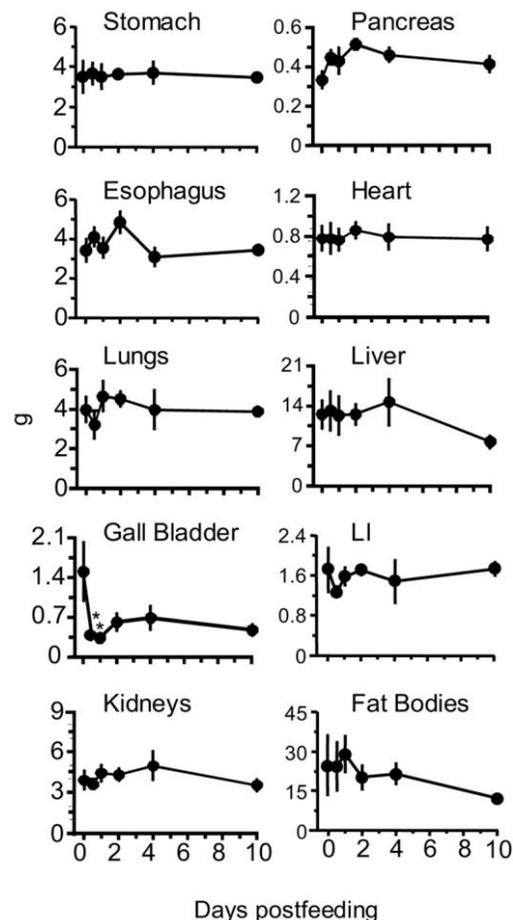


Figure 4. Wet mass of the stomach, pancreas, esophagus, heart, lungs, liver, gall bladder, large intestine, kidneys, and coelomic fat bodies as a function of days postfeeding for *Nerodia rhombifer* of these organs. We found only the pancreas and gall bladder to change significantly in mass following feeding.

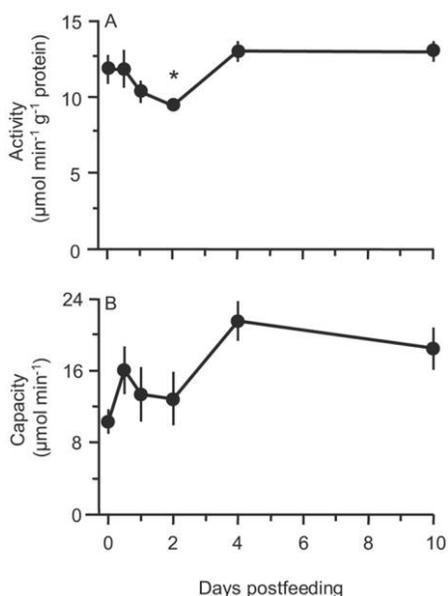


Figure 5. Gastric pepsin (A) activity and (B) capacity as a function of days postfeeding for *Nerodia rhombifer*. Postprandial pepsin activity of the gastric mucosa significantly decreased, whereas pepsin capacity did not significantly vary among fasted and fed samples.

no positional effects on L-leucine or L-proline uptake rates; however, D-glucose uptake varied significantly ($P < 0.0001$) among segments, averaging 6.7-fold greater for the proximal segment compared to the distal segment (Fig. 12). Intestinal uptake capacity for both L-leucine and L-proline varied significantly among sampling times, increasing by 80%–90% after feeding and then returning to fasting levels by day 10 (Fig. 11). In contrast, *N. rhombifer* intestines did not vary with feeding in its capacity to actively transport D-glucose (Fig. 11).

Discussion

For vertebrates, the ingestion of food triggers a cascade of physiological responses that results in the digestion and assimilation of the meal. These responses may be very dramatic in nature, as observed for infrequently feeding species of snakes (e.g., boas and pythons). More often such responses are more subtle in magnitude to reflect the more frequent feeding habits of most vertebrates. It is this latter scenario of postprandial responses that apparently characterizes the digestive physiology of the frequently feeding *Nerodia rhombifer*. From fasting to feeding, *N. rhombifer* experience no significant change in intestinal nutrient uptake and microvillus length. Postprandial increases in metabolic rate, intestinal mass, or intestinal uptake capacity are modest compared with documented changes exhibited by infrequently feeding snakes. We shall discuss for the *N. rhombifer* their metabolic and organ-specific postprandial responses, how these responses are integrated, and the adaptive interplay between their feeding habits and digestive physiology.

Postprandial Metabolic Response

Nerodia rhombifer digesting a fish meal equaling 25% of their body mass experience as much as a 5.4-fold increase in $\dot{V}O_2$ and maintain an elevated level of $\dot{V}O_2$ for 7 d. We suspect that much of this postprandial response is driven by the gastric breakdown of the meal, growth of the pancreas and intestinal mucosa, and the assimilation of absorbed nutrients. *Nerodia rhombifer*'s postprandial increase in metabolic rate and calculated SDA are within the range of values documented for other frequently feeding species of snakes digesting similarly sized meals (Table 1; Secor 2009). In comparison, infrequently feeding snakes experience factorial scopes of peak $\dot{V}O_2$ that are twice that and SDAs that are 65% greater than that observed for frequently feeding snakes (Table 1; Secor 2009). The difference in the magnitude of the postprandial metabolic response between frequently and infrequently feeding snakes is due in part to the lower SMR of infrequently feeding species, a potential product of their downregulated gut while fasting (Ott and Secor 2007). With a SMR that is 50% greater than that predicted for the same sized infrequently feeding snake and a peak $\dot{V}O_2$ that is low even for frequently feeding species, the *N. rhombifer* of this study experience a relatively modest metabolic response and SDA (Secor and Diamond 2000; Ott and Secor 2007). On the other hand, the calculated SDA coefficient (25.3 ± 1.1) for

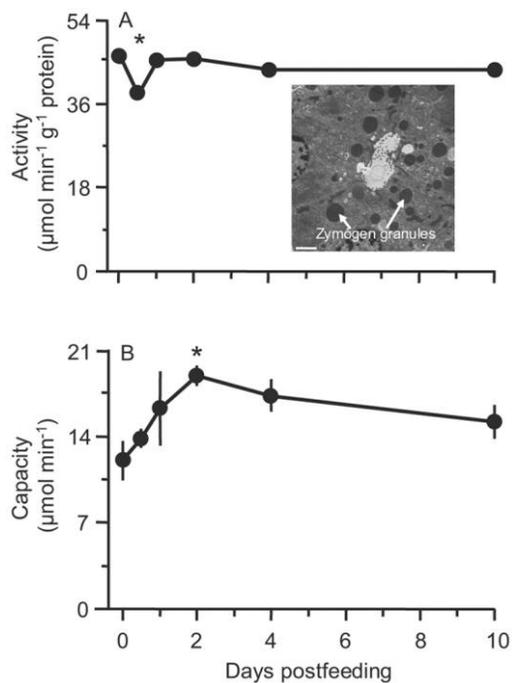


Figure 6. Pancreatic trypsin (A) activity and (B) capacity as a function of days postfeeding for *Nerodia rhombifer*. Trypsin activity dropped immediately following feeding and then soon returned to prefeeding levels. Trypsin capacity increased by day 2 due to the increase in pancreatic mass. The insert in A is a transmission electron micrograph of pancreatic acinar cells of *N. rhombifer* (10 d postfeeding) to illustrate the zymogen granules containing inactive and active enzymes. Scale bar = 2 µm.

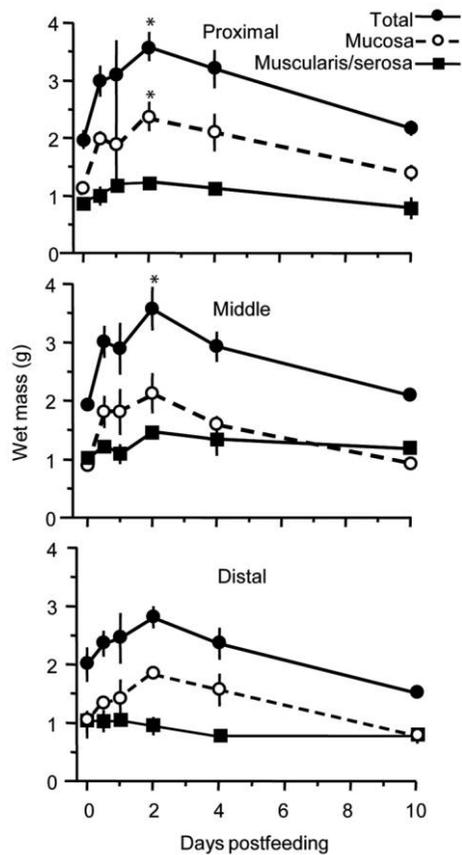


Figure 7. Wet mass of the small intestine and its mucosa and muscularis/serosa components for the proximal, middle, and distal portions of the *Nerodia rhombifer* small intestine as a function of time postfeeding. Significant postprandial increase in mucosal mass and total mass was observed for the proximal and middle intestinal segments.

the snakes of this study is greater than that documented for other frequently feeding snakes and within the range of coefficients recorded for infrequently feeding snakes digesting rodent meals (Secor and Diamond 2000; Ott and Secor 2007; Secor 2009). The higher SDA coefficient for these water snakes stems from the lower energy content of their catfish meals ($4.62 \pm 0.23 \text{ kJ g}^{-1}$) compared with rodent meals ($6.5\text{--}8.0 \text{ kJ g}^{-1}$) used in other studies; therefore, their SDA represents a greater percentage of the meal's energy.

Gastric Response

Following ingestion, *N. rhombifer* rapidly begin the gastric breakdown and passage of their meal as noted by the exiting of 15% of the meal from the stomach within 12 h after feeding. Within 24 h of feeding, the relative amount of the meal that has passed from the stomach for *N. rhombifer* (31%) is similar to that of other frequently feeding snakes that are digesting intact rodents and twice that observed for infrequently feeding species digesting intact rodents (Table 1). Whereas *N. rhombifer* do not regulate intestinal nutrient transport with fasting and feeding, they do regulate gastric acid production. With feeding,

luminal gastric pH dropped from 5.5 to 2.0 within 24 h, and upon the completion of digestion returned back to prefeeding levels. The Burmese python similarly experiences the turning on and shutting off of gastric acid production with the start

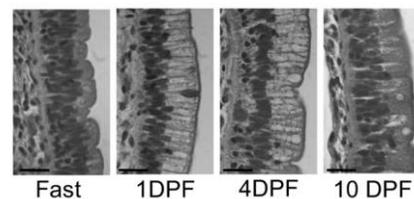
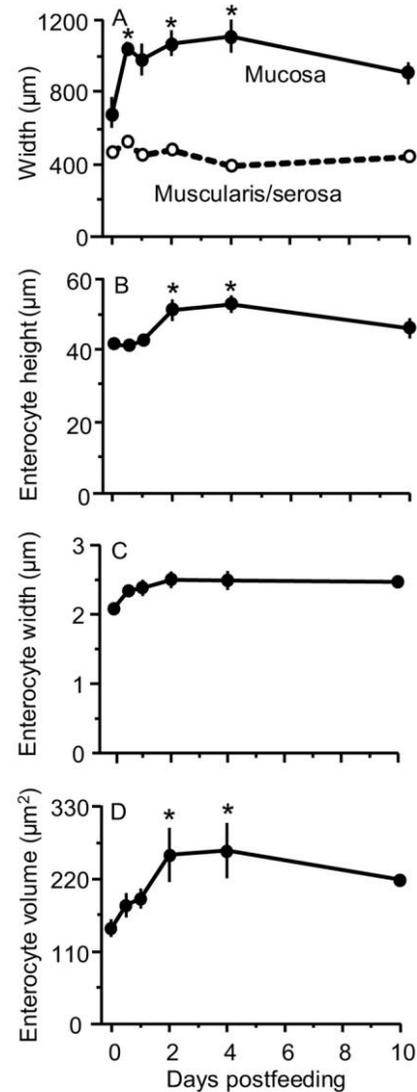


Figure 8. Mucosa and muscularis/serosa thickness, enterocyte height, enterocyte width, and enterocyte volume as a function of days postfeeding for the middle region of *Nerodia rhombifer* small intestine. Both mucosal width and enterocyte height increased significantly after feeding. The light micrographs illustrate the morphological changes of the intestinal epithelium from fasting (fast) to 1, 4, and 10 d postfeeding (DPF). Scale bar = $20 \mu\text{m}$. A color version of this figure is available in the online edition of *Physiological and Biochemical Zoology*.

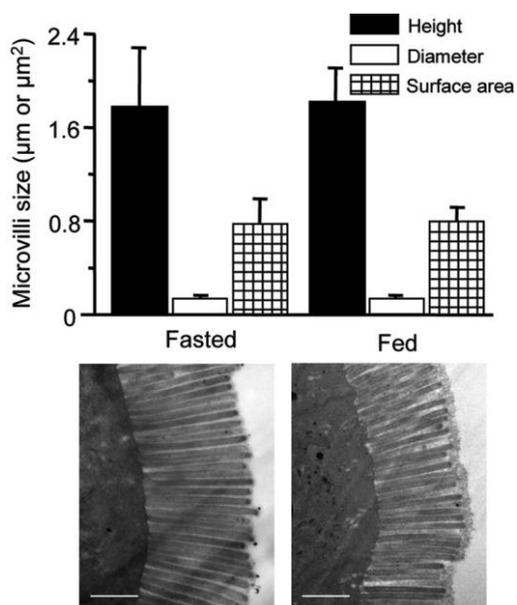


Figure 9. Mean (+SEM) intestinal microvillus height (μm), width (μm), and volume (μm^2) of two fasted and three fed (2 d postfeeding) *Nerodia rhombifer*. Below are transmission electron micrographs of intestinal microvilli of a fasted and fed *N. rhombifer* to illustrate the lack of a postprandial change in microvillus size. Scale bar = $1 \mu\text{m}$.

and completion of gastric digestion, but in contrast to water snakes, Burmese pythons also widely regulate intestinal nutrient transport (Secor and Diamond 1995; Secor 2003).

We observed no change in the mass of the stomach or of its mucosal or serosal components. Likewise, other frequently feeding snakes (*Coluber constrictor*, *Lampropeltis getula*, *Masticophis flagellum*, and *Pituophis melanoleucus*) and anurans (*Bufo marinus*, *Leptadactylus pentadactylus*, and *Rana catesbeiana*) experience no postprandial change in stomach tissue mass (Secor and Diamond 2000; Secor 2005b). The postprandial decrease in tissue pepsin activity and increase upon the completion of digestion may at first appear counterintuitive until considering that this pattern represents the immediate release of pepsinogen (which is activated to pepsin within the gastric lumen) from the oxyntopeptic cells, with feeding and the replenishing of tissues stores after the stomach has cleared. Cox and Secor (2008) observed for the Burmese python the same pattern of gastric pepsin activity and the postprandial depletion of pepsinogen-containing zymogen granules from the oxyntopeptic cells.

Pancreatic Response

The apparent postprandial increase in pancreatic mass (Fig. 4) may reflect a hypertrophic response to increase the functional capacity of the pancreas, specifically to elevate the production of proteases, lipases, and amylase, and NaHCO_3 solution to neutralize the acidic chyme exiting from the stomach. Interestingly, a postprandial increase in pancreatic mass has not been observed for other frequently feeding snakes but has been re-

ported for the infrequently feeding *Boa constrictor*, *Python brongersmai*, and *Python molurus* (Secor and Diamond 2000; Ott and Secor 2007; Cox and Secor 2008).

As observed for gastric pepsin activity, pancreatic trypsin activity of *N. rhombifer* decreased (though momentarily) after feeding, before returning to fasting levels within 24 h. This suggests that *N. rhombifer* store inactive trypsinogen (activated to trypsin within the intestine) within pancreatic acinar cells between meals. These stores are partly depleted with the initial release of enzymes immediately after feeding but then are soon replenished to continue serving meal digestion. When coupled with the increase in pancreatic mass, the total capacity of the pancreas for trypsin activity increases by 50% by the second day of digestion.

The postprandial pattern of trypsin activity (i.e., trypsinogen presence) for *N. rhombifer* is quite different from that observed for *P. molurus*. Pancreatic trypsin activity of fasted *P. molurus* is low and increases by 5.7-fold after feeding, a response that is concurrent with a 20-fold increase in pancreatic amylase activity (Cox and Secor 2008). Whereas *N. rhombifer* produces and stores trypsinogen within their pancreas between meals, *P.*

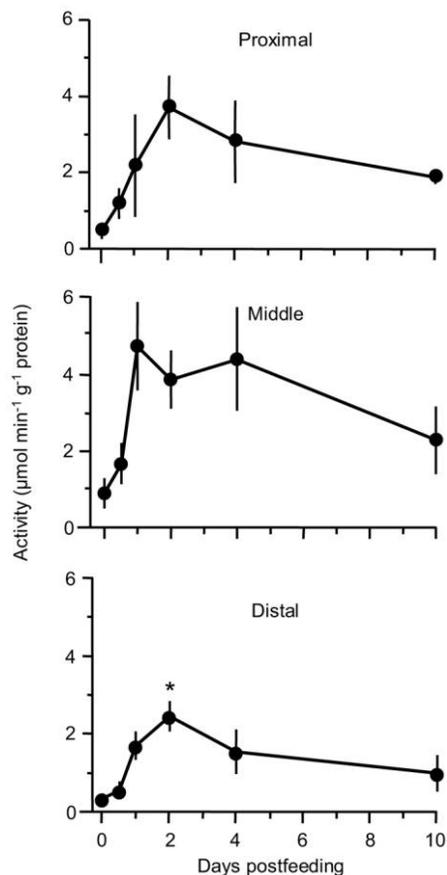


Figure 10. Aminopeptidase-N (APN) activity of the proximal, middle, and distal segments of the small intestine as a function of days postfeeding for *Nerodia rhombifer*. For all three segments of the small intestine, *N. rhombifer* experiences a postprandial increase in APN activity.

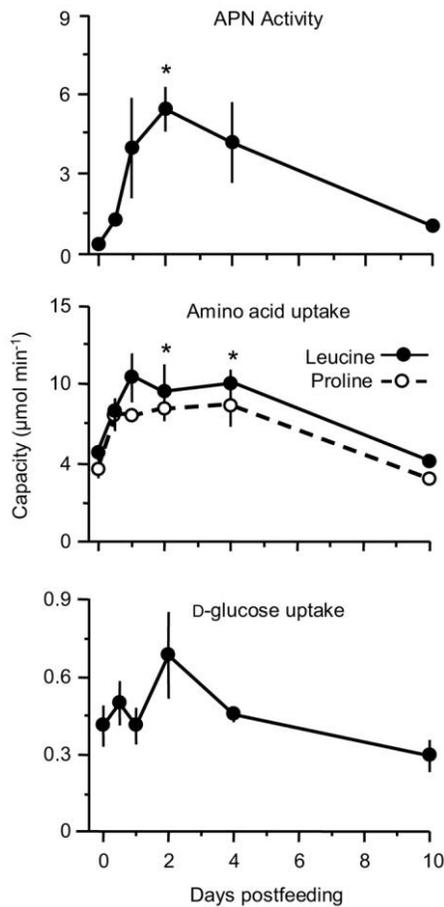


Figure 11. Intestinal capacities for aminopeptidase-N (APN) activity and L-leucine, L-proline, and D-glucose uptake as a function of days postfeeding for *Nerodia rhombifer*. Capacities increased with feeding for APN activity and the uptake of L-leucine and L-proline.

molurus apparently allow their pancreatic enzymes to become partly depleted after digestion is completed and then rapidly synthesizes these enzymes with feeding. The rate at which enzymes are produced after feeding is undetermined, since our assay values can reflect only how much is present—a sum of enzyme production, storage, and release. The assumption that frequently feeding snakes, such as *N. rhombifer*, do not strongly regulate pancreatic function with feeding and fasting is not supported by the findings for another frequently feeding natricine snake, *Natrix tessellata* which experiences with feeding 190% and 200% increases in the activity of the pancreatic enzyme, chymotrypsin and amylase, respectively (Zalkah and Bdolah 1987).

Intestinal Response

From a fasted state, feeding generates a characteristic increase in small intestinal mass (Dunel-Erb et al. 2001; Karasov et al. 2004; Secor 2005b). Among snakes, the magnitude of this increase varies with respect to feeding habits; frequently feeding species experience on average a 60% increase in small intestinal

mass, whereas for infrequently feeding species the increase in small intestinal mass averages 117% (Table 1). For *N. rhombifer* the 70% increase in small intestinal mass is largely due to the thickening of the mucosa layers for the proximal two-thirds of the intestine. Mucosal thickening can generally be explained by an increase in enterocyte volume, which for *N. rhombifer* had increased by 75% by day 4 of digestion. In addition to intracellular mechanisms of cellular growth, the absorption of lipids and formation of lipid droplets within enterocytes can be contributing to enterocyte hypertrophy. We observed a scattering of small lipid droplets within enterocytes of *N. rhombifer* that were smaller and less numerous than those observed for enterocytes of recently fed *P. molurus* (Starck and Beese 2002; Lignot et al. 2005). Like other frequently feeding snakes, *N. rhombifer* experiences no significant postprandial increase in microvillus length (Table 1). In contrast, the microvilli of infrequently feeding boas and pythons rapidly lengthen with feeding and then shorten after the completion of digestion (Lignot et al. 2005; Secor and Ott 2007; Table 1).

The functional response of the *N. rhombifer*'s small intestine is contradictory; nutrient uptake rates do not significantly in-

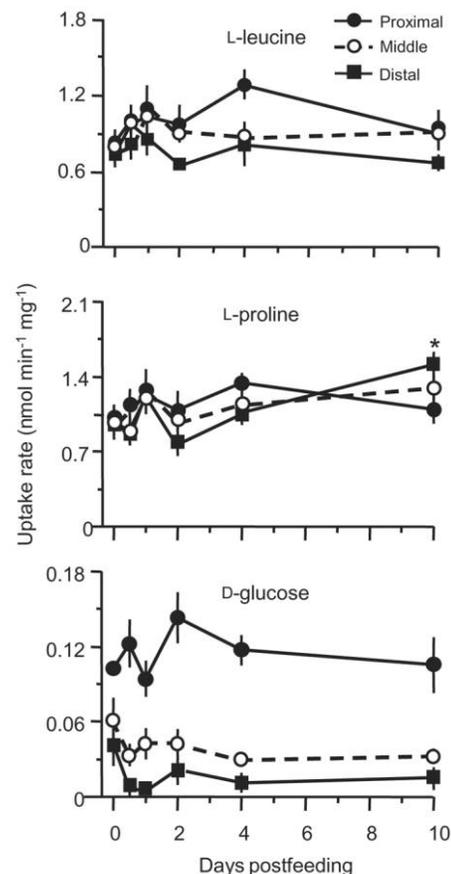


Figure 12. Uptake rates of L-leucine, L-proline, and D-glucose as a function of days postfeeding for the proximal, middle, and distal segments of the *Nerodia rhombifer* small intestine. This snake experienced no significant postprandial change in regional uptake rates for any of the three measured nutrients.

Table 1: Postprandial responses of frequently feeding and infrequently feeding species of snakes

Species	Passage at 1 d (%)	Peak Metabolic Scope	SDA (kJ/kg)	Small Intestinal Mass	Microvillus Length ^a (μm)	Intestinal Uptake Capacities			Source
						L-leucine	L-proline	D-glucose	
Frequently feeding:									
<i>Coluber constrictor</i>	26.4	5.4	309	1.37	1.06	1.2	1.3	3.3	Secor and Diamond 2000
<i>Corallus hortulanus</i>	...	5.8	274	2.00	1.41	2.2	2.0	3.1	Secor and Ott 2007
<i>Lampropeltis getula</i>	36.0	7.0	298	1.50	1.45	1.5	1.7	1.6	Secor and Diamond 2000
<i>Masticophis flagellum</i>	31.8	5.9	258	1.25	1.26	1.8	1.6	1.8	Secor and Diamond 2000
<i>Nerodia rhombifer</i>	31.5	5.4	293	1.70	1.03	1.9	1.8	1.7	This study
<i>Pituophis melanoleucus</i>	25.2	8.0	288	1.69	1.11	1.4	1.6	1.0	Secor and Diamond 2000
<i>Thamnophis marcianus</i>	50.4	5.7	307	1.71	1.02	1.6	1.8	2.7	S. Secor, unpublished data
<i>Thamnophis sirtalis</i>	66.3	6.9	257	1.57	1.31	1.0	1.4	2.0	S. Secor, unpublished data
Infrequently feeding:									
<i>Boa constrictor</i>	12.2	18.5	670	2.06	1.72	11.2	7.9	10.3	Secor and Diamond 2000
<i>Crotalus cerastes</i>	5.0	9.9	455	2.50	1.35	13.0	5.0	17.6	Secor et al. 1994
<i>Lichanura trivirgata</i>	21.4	15.9	357	2.52	2.51	6.2	7.6	10.3	Secor and Diamond 2000
<i>Python brongersmai</i>	...	11.3	422	2.28	7.59	2.4	2.0	35.5	Ott and Secor 2007
<i>Python molurus</i>	20.6	14.5	477	2.39	4.84	16.3	10.2	18.3	Lignot et al. 2005; Ott and Secor 2007
<i>Python regius</i>	...	9.9	447	1.64	6.17	4.6	6.1	49.3	Ott and Secor 2007
<i>Python reticulatus</i>	...	10.4	474	1.66	6.57	7.2	7.6	21.4	Ott and Secor 2007
<i>Python sebae</i>	...	11.7	496	2.30	5.62	7.3	6.7	31.6	Ott and Secor 2007

Note. Percentages of ingested meal that has passed from the stomach after 1 d of digestion, the factorial scopes of the postprandial peak in metabolism, specific dynamic action (SDA), and the factorial increases with feeding in small intestinal mass, intestinal microvillus length, and intestinal uptake capacities for L-leucine, L-proline, and D-glucose. Meals consumed were catfish filets for *Nerodia rhombifer*, golden shiners (*Notemigonus crysoleucas*) for *Thamnophis marcianus* and *Thamnophis sirtalis*, and rodents (mice or rats) for all other species.

^a With the exception of *Python molurus*, all microvillus data are unpublished.

crease with feeding, although APN activity increases by as much as sevenfold. Although we report APN activity standardized to tissue protein content and nutrient uptake rate standardized to tissue mass, this pattern is maintained when both APN activity and nutrient uptake rates are standardized to tissue mass. In previous studies on frequently feeding snakes, nutrient uptake rates likewise did not increase postprandially (Secor and Diamond 2000; Secor and Ott 2007). In only one of those studies was APN activity also measured (for *Corallus hortulanus*) and found not to increase with feeding (Secor and Ott 2007). Matched increases in both intestinal nutrient uptake and APN activity have been observed for infrequently feeding *B. constrictor*, *Lichanura trivirgata*, *P. molurus*, *Python regius*, *Python reticulatus*, and *Python sebae* (Ott and Secor 2007; Secor and Ott 2007).

Largely as a product of the 70% increase in small intestinal mass with feeding, *N. rhombifer* experienced significant postprandial increases in the intestinal uptake capacity for the two studied amino acids, L-leucine and L-proline. The modest magnitude of these responses, as well as the lack of a significant postprandial increase in D-glucose uptake capacity, has likewise been observed for other frequently feeding snakes (Table 1). For these snakes, any postprandial increase in intestinal nutrient uptake capacity is largely due to a trophic response (increase in mucosal mass), whereas for infrequently feeding snakes, their large postprandial increase in intestinal nutrient uptake capacity is driven primarily by the upregulation of mass-specific function and secondarily by mucosal hypertrophy (Secor 2005a).

Integrated Digestive Response

Gastric breakdown, luminal passage, and intestinal absorption operate as a product of an integrated array of morphological and physiological responses that begin with the ingestion of a meal. For *N. rhombifer*, the arrival of the catfish meal into the stomach triggers the activation of dormant H^+/K^+ pumps of the oxyntopeptic cells that drive luminal pH from 5.5 to 2.0 within 24 h. At the same time, oxyntopeptic cells release stored pepsinogen that at a low pH (~ 4) is converted to the active protease, pepsin. The metabolic cost of upregulating gastric function is reflected in the 3.5-fold increase in $\dot{V}O_2$ within 24 h after feeding (Fig. 1). As the acidic gastric chyme is released into the small intestine, it is immediately met by secretions of the pancreas. Although not measured, we assume that $NaHCO_3$ solution is released to neutralize the chyme. This is evident by the rapid increase in luminal pH from 2.5 at the distal end of the stomach to 6.7 at the most proximal end of the small intestine, a distance of no more than 5 cm (Fig. 3). The chyme is also met by pancreatic proteases, amylase, and lipases. Released pancreatic trypsinogen is activated to the protease trypsin by the intestinal brush-border-bound enterokinase. We suspect that water snakes are also releasing the protease chymotrypsinogen and amylase, since both of these enzymes have been measured for other snakes (Zalkah and Bdollah 1987; Cox and Secor 2008).

Occurring with the entrance of the chyme into the small

intestine, the intestinal mucosa begins to thicken as enterocytes increase in size. For *N. rhombifer*, as observed for other snakes, this trophic response stems in part from the cellular accumulation of absorbed amino acids and lipids. Unlike many boas and pythons whose microvilli rapidly lengthen with feeding and then shorten following digestion, *N. rhombifer* maintain microvillus length between fasting and digesting bouts (Fig. 9). The lack of any significant postprandial change in microvillus length and surface area may explain why these snakes do not experience any significant increase in intestinal nutrient uptake rates. A suspected mechanism underlying the rapid upregulation of intestinal nutrient absorption for infrequently feeding snakes is the concurrent increase in microvillus length and hence surface area (Secor 2005b). If the density or activity kinetics of brush-border nutrient transporters does not significantly change with feeding, then with no change in available surface, transport activity should not change. This explanation however does not explain the large postprandial increase in APN activity for *N. rhombifer*. We can suggest only that a separate mechanism is in place that increases with feeding the number and/or activity of this brush-border-bound hydrolase. Similarly, an absence of a postprandial increase in intestinal amino acid uptake and at the same time a significant increase in APN activity was observed in the Gila monster (*Heloderma suspectum*; Christel et al. 2007).

For *N. rhombifer* that have consumed catfish fillets (no skin, bones, or internal organs) and maintained at a constant 30°C, gastric digestion was nearly completed by 4 d after feeding. We predict that the small intestine continues hydrolyzing and absorbing nutrients for at least another day or two and that the assimilation of absorbed nutrients is largely completed by the following day. Such a timetable of digestion and assimilation matches the postprandial profile of $\dot{V}O_2$, the peak of which occurs (2–2.5 d postfeeding) when the snake is concurrently digesting the meal, absorbing the nutrients, and assimilating their components. There is a significant decrease in $\dot{V}O_2$ at the time (4–5 d postfeeding) that the stomach is passing the last of its contents into the small intestine. The remaining 2–3 d of significantly elevated $\dot{V}O_2$ reflects the energy expended on completing nutrient uptake, transport, and assimilation (e.g., protein synthesis; Secor 2009).

Interplay between Digestive Physiology and Feeding Ecology

An aim of this study was to address the hypothesis that as a frequently feeding snake, *N. rhombifer* would experience more modest changes in gastrointestinal form and function between fasting and feeding bouts as compared to infrequently feeding snakes. The *N. rhombifer* that we studied had continuous access to food (catfish) before capture and possibly ate more frequently than earlier predicted. Secor and Diamond (2000) proposed an energetic model that illustrates the trade-offs between the frequency of feeding and the cost of gut regulation for snakes that feed frequently or infrequently. Feeding on average once every 2 wk or less, the model predicts that the preferred energetic strategy of *N. rhombifer* is to maintain gastrointestinal

performance between meals. Our morphological and physiological data supports this model and the adaptive link between frequent feeding and modest regulation of digestive performance for *N. rhombifer*. A similar adaptive scenario exists for other frequently feeding snakes (Table 1). On the other hand, in keeping with an adaptive response driven by feeding frequency, snakes that feed relatively infrequently experience much larger postprandial responses (Table 1). The upregulation of their gastrointestinal tract with feeding is reflected in a twofold greater postprandial metabolic response, 65% greater SDA, 100% greater increase in small intestinal mass, and nine- to 20-fold greater increases in intestinal microvillus length and nutrient uptake capacities when compared with frequently feeding species (Table 1). Although there is a distinct dichotomy for a number of postprandial responses with respect to feeding habits, *N. rhombifer* shares with infrequently feeding pythons the regulation with feeding and fasting of gastric acid production and intestinal APN activity. Further studies on frequently feeding species will ascertain whether the regulation of these components of digestion is an inherent trait of snakes or is driven by other selective mechanisms.

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