*Copeia*, 1999(1), pp. 75–84

# Maintenance of Digestive Performance in the Turtles *Chelydra serpentina*, *Sternotherus odoratus*, and *Trachemys scripta*

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In our continued investigation of the adaptive interplay between feeding ecology and digestive physiology, we measured postfeeding responses of juvenile *Chelydra serpentina*, adult *Sternotherus odoratus*, and subadult *Trachemys scripta*, three aquatic turtle species that feed at frequent intervals and consume a catholic diet of plants and animals. In this study, we measured $O_2$ consumption rates from fasting and digesting individuals and compared intestinal nutrient uptake rates and organ masses of turtles fasted for one month with turtles sacrificed one day after the ingestion of a meal equivalent to 5–11% of body mass. $O_2$ consumption during digestion peaked at rates 3.4, 2.1, and 2.7 times fasting values, respectively, for *C. serpentina*, *S. odoratus*, and *T. scripta*—factors much smaller than those documented previously for reptile species that normally consume large meals at long intervals. None of the turtle species experienced significant postfeeding changes in intestinal uptake of amino acids or D-glucose. Ratios of amino acid uptake rates to D-glucose uptake rates were much greater than 1.0 for each species, either fasted or fed, a finding characteristic of other carnivores as well. Total intestinal capacity to transport the amino acids L-leucine and L-proline and the sugar D-glucose did not change with feeding for any of the turtle species. None of the species experienced significant differences in intestinal mass or enterocyte morphology between fasted and fed individuals. *Chelydra serpentina* and *T. scripta* experienced no significant postfeeding changes in organ masses, and the only changes for *S. odoratus* were 59% and 42% increases in stomach wet and dry masses, respectively, upon feeding. Thus, juvenile *C. serpentina*, adult *S. odoratus*, and subadult *T. scripta* maintain the functional and morphological integrity of their guts during fasting and exhibit only modest metabolic responses to feeding. We hypothesize that these are adaptive traits characteristic of species that frequently consume and digest small meals.

FEEDING habits, including diet composition, meal size, and meal frequency, have had profound effects on the evolved characteristics of the digestive system. For example, the small intestines of herbivores transport simple sugars at much faster rates than they transport amino acids, whereas the opposite trend occurs for carnivores (Karasov and Diamond, 1988). Recently, it has been discovered for Burmese pythons (*Python molurus*) that larger meals elicit greater activities of intestinal nutrient transporters and larger metabolic responses (Secor and Diamond, 1997a, 1997b).

Meal frequency in the wild, at least for snakes, is correlated with the magnitudes of functional and morphological responses of the gut to ingestion of food (Secor and Diamond, 1994; Secor et al., 1994; Secor and Diamond, 1995). We have found, for several snake species that feed relatively frequently in the wild, that changes in gut performance after feeding are modest or nonexistent (Secor and Diamond, 1994). That is, after completing digestion, they maintain the functional capacities of their guts, an appropriate response because their next meal is likely to arrive soon (Secor and Diamond, 1994). In contrast, species of snakes that feed relatively infrequently in the wild up- and down-regulate intestinal function and morphology with the initiation and completion of digestion (Secor et al., 1994; Secor and Diamond, 1995). For these snakes, the down-regulation of gut performance that occurs with the completion of digestion serves to conserve energy during the usually long fasting periods between meals. Hence rapid up-regulation of gut performance upon feeding is necessary for successful digestion.

We hypothesize that these adaptive scenarios linking feeding frequency and the regulation of digestive performance apply to other taxa as well. In support, we have preliminary evidence that anuran species, which estivate and fast for months during the dry season, regulate gut performance as do infrequently feeding snakes, whereas frequently feeding anuran species maintain intestinal function for weeks after digestion has completed (Secor and Diamond, 1996; unpubl. obs.). To test the generality of our adaptive hypothesis regarding digestive responses requires the study of other taxa besides

snakes and anurans. Turtles afford a good starting point. The vast majority of aquatic turtle species are omnivorous opportunists in their feeding habits, feeding frequently on both plant and animal matter (Ernst and Barbour, 1989; Ernst et al., 1994). We would therefore predict that aquatic turtles, like frequently feeding snakes and anurans, maintain intestinal functional integrity even after an extended fasting bout.

We tested this prediction by examining the metabolic, functional, and morphologic responses to feeding of juvenile snapping turtles (*Chelydra serpentina*; family Chelydridae), adult common musk turtles (*Sternotherus odoratus*; family Kinosternonidae), and subadult red-eared sliders (*Trachemys scripta*; family Emydidae). We selected these three turtles because they feed frequently in the wild (Punzo, 1975; Ernst, 1986; Ernst et al., 1994) and because they represent three separate turtle families. The primary objective of our study was to compare $O_2$ consumption rates, intestinal nutrient uptake rates, and intestinal and organ morphology between fasted and digesting turtles. Our second objective was to compare the results from these three turtle species with measurements taken from other taxa to evaluate the generality of our adaptive hypothesis about digestive responses.

## Materials and Methods

*Animals and maintenance.*—*Chelydra serpentina, S. odoratus,* and *T. scripta* exist in many types of aquatic habitats throughout the eastern and central United States, with the former two species ranging into southern Canada. These turtle species as juveniles and adults possess a very catholic diet, feeding opportunistically on vertebrates, invertebrates, carrion, and plants (Ernst et al., 1994). *Chelydra serpentina,* one of the largest species of freshwater turtles (carapace length and body mass up to 49 cm and 34 kg, respectively), actively forages when young but as adults will often lie in ambush to seize prey (Ernst et al., 1994). *Trachemys scripta* is a medium-sized turtle (maximum carapace length and body mass of 28 cm and 4 kg, respectively) that forages during the day in shallow water for aquatic plants and small animals (Ernst et al., 1994). The smaller *S. odoratus* (maximum carapace length and body mass 14 cm and 200 g, respectively) actively forages on the bottom, searching and seizing invertebrates, small vertebrates, and vegetation (Ernst et al., 1994).

Turtles used in this study were obtained from several sources: four *C. serpentina* and three *S. odoratus* were provided by the Savanna River Ecology Laboratory (Aiken, SC); and two *C. serpentina,* four *S. odoratus,* and all *T. scripta* were purchased commercially. We housed turtles individually in plastic containers (37 × 25 × 14 cm) half-filled with water at 25–30 C under a light:dark photoperiod of 14:10 h. Turtles were fed small pieces of beef and fish (sprinkled with calcium and vitamin powder) twice each week.

Prior to experiments, turtles were fasted for one month to ensure that they were postabsorptive and to allow ample time for any potential down-regulation of gut performance. When turtles were fed, each was allowed to consume small pieces of beef (≈ 1 g) until satiety. We conducted two sets of experiments. First, we measured postfeeding metabolic response ($O_2$ consumption) from three *C. serpentina* (mean mass ± 1 SE = 101 ± 6 g), four *S. odoratus* (61 ± 6 g), and four *T. scripta* (356 ± 72 g) that had consumed meals equaling 11.3 ± 1.6%, 5.0 ± 0.5%, and 5.2 ± 0.4% of their body mass, respectively. Second, we used six *C. serpentina* (79 ± 13 g), seven *S. odoratus* (65 ± 2 g), and six *T. scripta* (325 ± 52 g) to document functional and morphological responses following feeding. For each species, we measured intestinal nutrient uptake, intestinal mass and morphology, and the mass of other organs from three or four (*S. odoratus* only) individuals killed following the 30-day fast and from three others killed one day after feeding. For these measurements, meal sizes equaled 10.0 ± 2.0%, 5.5 ± 0.7%, and 5.9 ± 1.2 % of body mass for *C. serpentina, S. odoratus,* and *T. scripta,* respectively.

*Metabolic response.*—We used closed-system respirometry [methods described in detail in Vleck (1987) and Secor et al. (1994)] to measure aerial oxygen consumption rates ($\dot{V}O_2$ in units of mL $O_2 \cdot g^{-1} \cdot h^{-1}$) of turtles at 30 C prior to and after feeding. Fasted turtles were placed on moist towels (to reduce desiccation) in individual respirometry chambers (0.5–1.5 L) for two days prior to measurements to accustom them to the chambers. We then measured each turtle's $\dot{V}O_2$ (corrected for standard temperature and pressure) at 12- to 24-h intervals (at 0600 and 1800 PST) for four days prior to and up to eight days following feeding. At three-day intervals, turtles were returned to their water-filled chambers for several hours to rehydrate.

*Nutrient uptake measurements.*—We measured rates of nutrient uptake across the intestinal brush-border membrane of the small and large intestine [for details of methodology, see Karasov and Diamond (1983) and Secor et al.

(1994)]. Briefly, after severing their spinal cord just posterior to the skull, the plastron was removed, and the intestine excised, rinsed in ice-cold Ringer's solution, everted, and sectioned into 1-cm sleeves. Everted sleeves were mounted on grooved metal rods and preincubated for 5 min in Ringer's solution at 30 C and then incubated for 2 min at 30 C in Ringer's solution containing a radiolabeled nutrient and an adherent fluid marker labeled with a different radioisotope. We measured intestinal uptake rates of the amino acids L-leucine, L-lysine, and L-proline (each at a concentration of 50 mM and labeled with $^3H$) and carrier-mediated transport of the sugar D-glucose (at 20 mM and labeled with $^{14}C$). To correct for labeled nutrients adherent to the tissue surface, we used polyethylene glycol (labeled with $^{14}C$) for the amino acids, and L-glucose (labeled with $^3H$, thereby also correcting for passive D-glucose uptake) for D-glucose. In each species, we measured uptake rates (expressed as nmoles $\cdot$ $min^{-1}$ $\cdot$ $mg^{-1}$ of sleeve wet mass) of L-leucine, L-proline, and D-glucose from each of the three regions (proximal, middle, and distal thirds) of the small intestine. In addition, for *C. serpentina* and *S. odoratus*, uptake rates of L-lysine were measured from the proximal and middle small intestinal regions, and uptake rates of L-proline and D-glucose were measured from the large intestine. As a comparative index of intestinal adaptation to natural meal composition (Karasov and Diamond, 1988), we calculated ratios of amino acid uptake to D-glucose uptake. As described previously in Secor (1995), we derived the total uptake capacity ($\mu$moles/min) of the whole length of the small intestine for L-leucine, L-proline, and D-glucose by summing the uptake capacities of each intestinal region [a product of uptake rate (nmoles $\cdot$ $min^{-1}$ $\cdot$ $mg^{-1}$) times mass (mg)].

*Morphological response.*—We measured the wet mass of the total small intestine and calculated total intestinal dry mass and wet and dry masses of the mucosal and serosal layers. Our calculations were made from 1-cm segments from the proximal, middle, and distal regions: we scraped the mucosal layer from the serosa, weighed the wet masses of these samples, dried them to a constant mass (at 60 C), and reweighed them. For fasted and fed *C. serpentina* and *S. odoratus*, we also used light microscopy to measured, from formalin-fixed and paraffin-embedded intestinal tissues, the height and diameter of five enterocytes. We used these measurements to calculate enterocyte volume using the formula for a cylinder [$\pi(d/2)^2h$]. Because postfeeding mass changes have been observed for organs other than the small intestine in other species (Toloza et al., 1991; Secor and Diamond, 1995), we measured the wet and dry masses of the heart, paired lungs, liver, stomach, pancreas, large intestine, and paired kidneys from fasted and fed individuals of the three turtle species.

*Statistical analysis.*—We used a repeated-measures design analysis of variance (ANOVA) to test for significant effects of sampling time on $\dot{V}O_2$ and of intestinal position on nutrient uptake rates (Zar, 1974). In conjunction with each of these tests, we used a priori planned pairwise comparisons to identify significant differences between pairs of values. For each species, we used one-way ANOVA to test for significant postfeeding changes in intestinal uptake rates and capacities. Analysis of covariance (ANCOVA), with body mass as the covariate, tested for significant differences in small intestinal and other organ wet and dry masses and enterocyte volume between fasted and fed individuals. We present our results as means $\pm$ 1 SE. Results of statistical tests are reported in terms of their *F*- and *P*-values, and we designate the level of statistical significance as $P \leq 0.05$. We used Statistical Analysis Systems software (SAS, Inc., vers. 6.03, Cary, NC, 1988, unpubl.) to perform all statistical analyses.

## Results

*Metabolic response.*—$\dot{V}O_2$ differed significantly among sampling times (pre- and postfeeding) for *C. serpentina* ($F_{10,20} = 21.0$, $P \leq 0.0001$), *S. odoratus* ($F_{10,28} = 7.13$, $P \leq 0.0001$), and *T. scripta* ($F_{9,27} = 14.9$, $P \leq 0.0001$). During the four days prior to feeding, metabolic rates (at 30 C) of fasting *C. serpentina* (n = 3), *S. odoratus* (n = 4), and *T. scripta* (n = 4) averaged 0.054 $\pm$ 0.002 mL $O_2$ $\cdot$ $g^{-1}$ $\cdot$ $h^{-1}$, 0.045 $\pm$ 0.004 mL $O_2$ $\cdot$ $g^{-1}$ $\cdot$ $h^{-1}$, and 0.044 $\pm$ 0.004 mL $O_2$ $\cdot$ $g^{-1}$ $\cdot$ $h^{-1}$, respectively. By 24 h postfeeding, $\dot{V}O_2$ of *C. serpentina* had increased significantly ($P < 0.0001$) to a peak of 0.18 $\pm$ 0.01 mL $\cdot$ $g^{-1}$ $\cdot$ $h^{-1}$, 3.4 times the fasting average (Fig. 1). *Chelydra serpentina* continued to maintain significantly elevated $\dot{V}O_2$ until day 5; thereafter $\dot{V}O_2$ did not differ significantly from fasting values. $\dot{V}O_2$ of *S. odoratus* had increased significantly ($P < 0.0001$) within 24 h and peaked 36 h postfeeding at 0.093 $\pm$ 0.006 mL $\cdot$ $g^{-1}$ $\cdot$ $h^{-1}$, 2.1 times the fasting average. For *S. odoratus*, $\dot{V}O_2$ remained elevated until day 4, after which $\dot{V}O_2$ had declined to prefeeding levels (Fig. 1). $\dot{V}O_2$ of *T. scripta* also became significantly elevated by 24 h post-

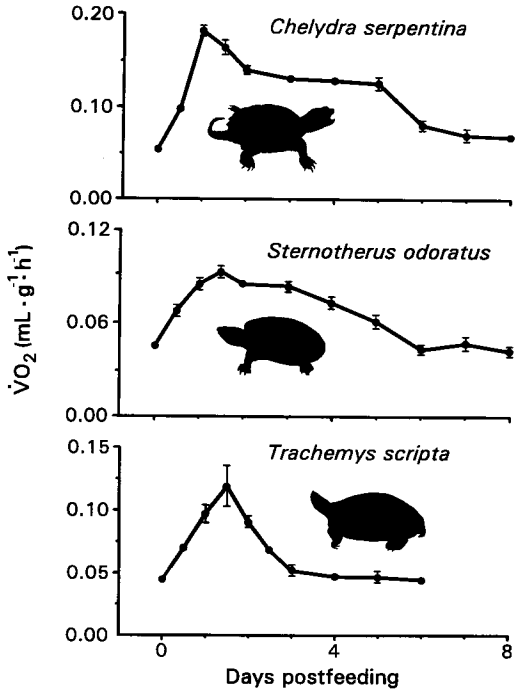


Fig. 1. Mean $O_2$ consumption rates ($\dot{V}O_2$ as mL $O_2$ · g$^{-1}$ · h$^{-1}$ at 30 C) of juvenile *Chelydra serpentina* (n = 3), adult *Sternotherus odoratus* (n = 4), and subadult *Trachemys scripta* (n = 4) prior to and up to eight days following the ingestion of meals equaling 11.3%, 5.0%, and 5.2% of body mass, respectively. $\dot{V}O_2$ at day 0 is the mean of fasting values measured over a four-day period prior to feeding. In this and subsequent figures, the vertical bars represent ± 1 SE but are omitted if the error bars are smaller than the symbol for the mean value. Note that $\dot{V}O_2$ during digestion peaks at values 3.4, 2.1, and 2.7 times fasting values, respectively, for *C. serpentina*, *S. odoratus*, and *T. scripta*.

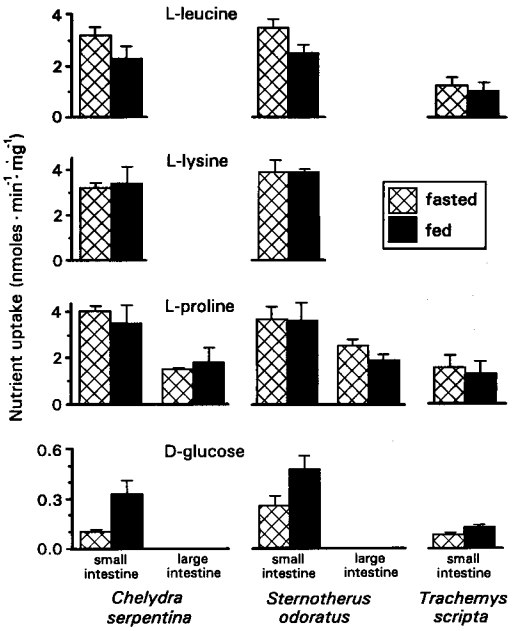


Fig. 2. Intestinal uptake rates (nmoles · min$^{-1}$ · mg$^{-1}$ at 30 C) of the amino acids L-leucine, L-lysine, L-proline, and the sugar D-glucose by fasted and fed (one day postfeeding) juvenile *Chelydra serpentina*, adult *Sternotherus odoratus*, and subadult *Trachemys scripta*. For this and the next two figures, solute uptake was measured from 1-cm tissue sleeves (two to three sleeves per turtle per solute) taken from three fasted *C. serpentina*, four fasted *S. odoratus*, three fasted *T. scripta*, and three fed turtles of each species. All solutes were measured in the small intestine, whereas L-proline and D-glucose were also measured in the large intestine of *C. serpentina* and *S. odoratus*. Note that there is no statistically significant difference between fed and fasted values for any solute in any species.

feeding and peaked at 36 h at 0.12 ± 0.02 mL · g$^{-1}$ · h$^{-1}$, 2.7 times the fasting average. $\dot{V}O_2$ for this turtle remained elevated until day 3; thereafter rates did not differ from fasting values.

The extra energy expended for digestion is termed the specific dynamic action (SDA) of the meal (Kleiber, 1975). The SDA from these meals (calculated as described in fig. 1 of Secor and Diamond, 1997b) equaled 18 ± 1 kJ (22 ± 2% of ingested energy), 3.7 ± 0.4 kJ (17 ± 2% of ingested energy), and 27 ± 3 kJ (21 ± 2% of ingested energy) respectively, for *C. serpentina*, *S. odoratus*, and *T. scripta* [assuming 19.5 J of energy expended per mL $O_2$ consumed and an energy equivalent of 7.3 kJ per gram wet mass for the beef meals (Gessaman and Nagy, 1988; Holland et al., 1991)].

*Functional response.*—For fasted and fed turtles of each species, nutrient uptake rates did not differ significantly among the proximal, middle, and distal segments of the small intestine, with the exception of L-lysine and D-glucose at day 1 postfeeding for *S. odoratus* ($P$ = 0.02) and *T. scripta* ($P$ = 0.0008), respectively. For *C. serpentina* and *S. odoratus* (we did not measure nutrient uptake in *T. scripta* large intestine), uptake rates of L-proline by the large intestine were half of rates by the small intestine, and there was no measurable active uptake of D-glucose by the large intestine. Hence, we averaged, for each individual and each solute, the uptake values for the three small intestinal segments, and we report separate analyses for the total small intestine and the large intestine.

Upon feeding, *C. serpentina* small intestine experienced no significant increase in uptake rate of any nutrient (Fig. 2). This turtle's large intestine also did not experience postfeeding

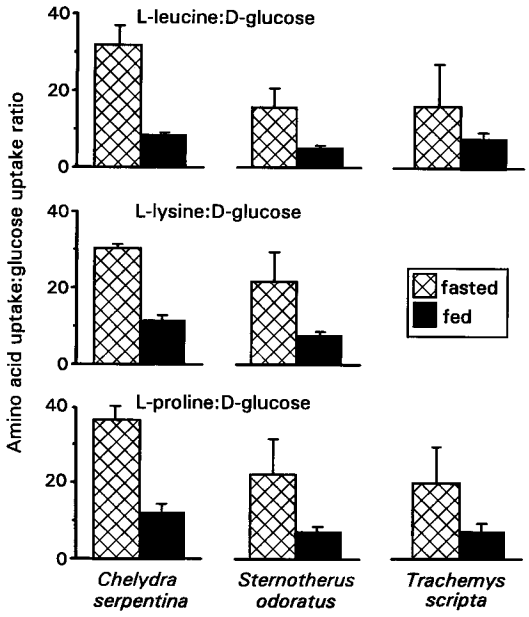


Fig. 3. Ratios of amino acid uptake to D-glucose uptake of the small intestine for fasted and fed juvenile *Chelydra serpentina*, adult *Sternotherus odoratus*, and subadult *Trachemys scripta*. Note that ratios are all greater than 1.0, characteristics of omnivores and carnivores.

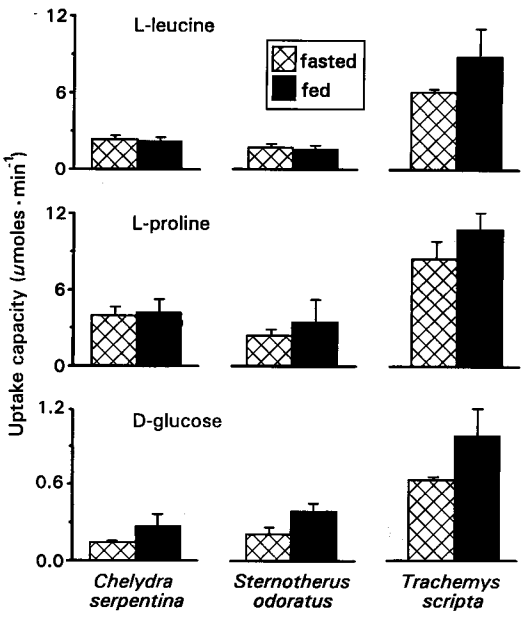


Fig. 4. Uptake capacities (μmoles/min) of the complete small intestine for the amino acids L-leucine and L-proline and the sugar D-glucose, for fasted and fed juvenile *Chelydra serpentina*, adult *Sternotherus odoratus*, and subadult *Trachemys scripta*. Note that intestinal uptake capacities did not change significantly with feeding for any solute in any species.

changes in its uptake of L-proline or D-glucose. There was no active transport of D-glucose by the large intestine for either fasted or fed individuals. For the small intestine, the ratios of L-leucine, L-lysine, and L-proline uptake to D-glucose uptake were significantly greater than 1.0 for both fasted and fed individuals (Fig. 3). For *C. serpentina*, total uptake capacity of the complete small intestine (summed products of regional uptake rates times regional masses) for L-leucine, L-proline, and D-glucose did not differ significantly between fasting and digesting individuals (Fig. 4).

*Sternotherus odoratus* also did not exhibit significant differences in small intestinal uptake rates of measured amino acids or D-glucose between fasted and fed treatments (Fig. 2). The large intestine of these turtles likewise experienced no significant difference between treatments in its uptake of L-proline or D-glucose (this turtle species' large intestine, either fasted or fed, also did not actively transport D-glucose). Ratios of L-leucine, L-lysine, and L-proline uptake to D-glucose uptake were also significantly greater than 1.0 for fasted and fed *S. odoratus* (Fig. 3). The small intestine of fasted and fed *S. odoratus* likewise experienced no significant differences in its uptake capacity for L-leucine, L-proline, or D-glucose (Fig. 4).

Fasted and fed *T. scripta* did not exhibit significant differences in small intestinal uptake rates of any measured nutrient (Fig. 2). Ratios of L-leucine and L-proline uptake to D-glucose uptake were also greater than one for fasted and fed turtles; ranging from 7.1 ± 1.8 to 20.2 ± 9.6 (Fig. 3). We detected no significant difference between fasted and fed *T. scripta* in total intestinal capacity for the transport of L-leucine, L-proline, or D-glucose (Fig. 4).

*Morphologic responses.*—Enterocyte volume did not differ significantly between fasted (227 ± 43 $\mu m^3$) and fed (269 ± 32 $\mu m^3$) *C. serpentina*. These turtles also did not exhibit any significant differences in small intestinal wet or dry mass (Table 1) nor any differences in the wet and dry masses of the intestinal mucosa and serosa. We found no significant difference between fasted and fed *C. serpentina* in wet and dry masses of the heart, lungs, liver, stomach, pancreas, large intestine, and kidneys (Table 1).

Feeding also failed to induce significant changes (158 ± 42 $\mu m^3$ fasted vs 155 ± 43 $\mu m^3$ fed) in enterocyte volume for *S. odoratus*. We

TABLE 1. BODY MASS AND WET AND DRY MASSES OF ORGANS OF FASTED AND FED (ONE DAY POSTFEEDING) JUVENILE *Chelydra serpentina*, ADULT *Sternotherus odoratus*, AND SUBADULT *Trachemys scripta*. Values are presented as mean ± 1 SE, and statistical comparisons are made with one-way ANCOVA with body mass as the covariate. None of the species experienced a significant difference between fasted and fed individuals in wet or dry organ masses, with the exception of wet and dry masses of *S. odoratus* stomach.

| | Wet mass | | | Dry mass | | |
|---|---|---|---|---|---|---|
| | Fasted | Fed | ANCOVA *P*-values | Fasted | Fed | ANCOVA *P*-values |
| *Chelydra serpentina* | n = 3 | n = 3 | | n = 3 | n = 3 | |
| Body mass | 77.7 ± 16.4 | 80.4 ± 23.1 | 0.92 | | | |
| Heart | 0.30 ± 0.07 | 0.40 ± 0.10 | 0.08 | 0.04 ± 0.01 | 0.05 ± 0.02 | 0.50 |
| Lung | 0.61 ± 0.13 | 0.70 ± 0.18 | 0.60 | 0.08 ± 0.02 | 0.10 ± 0.01 | 0.64 |
| Liver | 2.01 ± 0.38 | 2.51 ± 1.08 | 0.55 | 0.50 ± 0.06 | 0.59 ± 0.23 | 0.70 |
| Stomach | 1.09 ± 0.14 | 1.23 ± 0.32 | 0.36 | 0.17 ± 0.02 | 0.17 ± 0.04 | 0.51 |
| Pancreas | 0.06 ± 0.02 | 0.08 ± 0.03 | 0.63 | 0.014 ± 0.004 | 0.015 ± 0.005 | 0.92 |
| Small intestine | 0.94 ± 0.18 | 1.66 ± 0.62 | 0.10 | 0.18 ± 0.03 | 0.23 ± 0.08 | 0.28 |
| Large intestine | 0.41 ± 0.06 | 0.72 ± 0.38 | 0.29 | 0.049 ± 0.004 | 0.084 ± 0.046 | 0.35 |
| Kidneys | 0.21 ± 0.04 | 0.23 ± 0.07 | 0.53 | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.65 |
| *Sternotherus odoratus* | n = 4 | n = 3 | | n = 4 | n = 3 | |
| Body mass | 63.7 ± 3.9 | 65.8 ± 1.7 | 0.91 | | | |
| Heart | 0.27 ± 0.04 | 0.25 ± 0.02 | 0.42 | 0.04 ± 0.01 | 0.04 ± 0.01 | 0.54 |
| Lung | 0.75 ± 0.07 | 0.75 ± 0.03 | 0.85 | 0.10 ± 0.02 | 0.10 ± 0.02 | 0.92 |
| Liver | 2.90 ± 0.87 | 2.47 ± 0.62 | 0.73 | 0.95 ± 0.35 | 0.60 ± 0.22 | 0.48 |
| Stomach | 0.26 ± 0.03 | 0.41 ± 0.01 | 0.01 | 0.039 ± 0.005 | 0.053 ± 0.003 | 0.03 |
| Pancreas | 0.06 ± 0.01 | 0.04 ± 0.01 | 0.07 | 0.012 ± 0.002 | 0.007 ± 0.002 | 0.08 |
| Small intestine | 0.74 ± 0.13 | 0.89 ± 0.14 | 0.45 | 0.14 ± 0.02 | 0.19 ± 0.05 | 0.61 |
| Large intestine | 0.17 ± 0.02 | 0.28 ± 0.08 | 0.27 | 0.027 ± 0.003 | 0.036 ± 0.009 | 0.42 |
| Kidneys | 0.27 ± 0.05 | 0.36 ± 0.04 | 0.21 | 0.05 ± 0.01 | 0.06 ± 0.01 | 0.29 |
| *Trachemys scripta* | n = 3 | n = 3 | | n = 3 | n = 3 | |
| Body mass | 330 ± 93 | 320 ± 70 | 0.94 | | | |
| Heart | 0.94 ± 0.28 | 0.83 ± 0.14 | 0.59 | 0.15 ± 0.04 | 0.13 ± 0.02 | 0.65 |
| Lung | 4.45 ± 1.87 | 3.64 ± 1.10 | 0.46 | 0.44 ± 0.19 | 0.40 ± 0.11 | 0.80 |
| Liver | 11.11 ± 3.16 | 9.96 ± 2.21 | 0.25 | 3.08 ± 1.07 | 2.49 ± 0.76 | 0.33 |
| Stomach | 4.51 ± 2.22 | 4.47 ± 0.81 | 0.96 | 0.70 ± 0.33 | 0.72 ± 0.15 | 0.89 |
| Pancreas | 0.39 ± 0.11 | 0.38 ± 0.08 | 0.97 | 0.066 ± 0.023 | 0.068 ± 0.014 | 0.78 |
| Small intestine | 6.31 ± 2.37 | 10.38 ± 2.03 | 0.09 | 1.30 ± 0.57 | 2.01 ± 0.34 | 0.15 |
| Large intestine | 2.34 ± 0.93 | 2.56 ± 0.55 | 0.65 | 0.31 ± 0.13 | 0.34 ± 0.08 | 0.61 |
| Kidneys | 0.85 ± 0.21 | 0.86 ± 0.16 | 0.83 | 0.41 ± 0.04 | 0.14 ± 0.02 | 0.92 |

also observed no significant differences in wet and dry masses of the small intestine of *S. odoratus* between fasted and fed individuals (Table 1). Similarly, wet and dry masses of the mucosal and serosal components of the small intestine experienced no significant responses to feeding. Wet and dry masses of the heart, lungs, liver, pancreas, large intestine, and kidneys did not significantly differ between fasted and fed *S. odoratus* (Table 1). We did observe a significant greater wet and dry mass (59% and 42% increase, respectively) of the stomach ($P$s < 0.035) for fed compared to fasted *S. odoratus*.

Fasted and fed *T. scripta* exhibited no significant difference in wet and dry masses of the small intestine nor in either the mucosal or serosal components. Wet and dry masses of the heart, lungs, liver, stomach, pancreas, large intestine, and kidneys likewise did not significantly differ between fasted and fed turtles (Table 1).

## DISCUSSION

In this study, *C. serpentina*, *S. odoratus*, and *T. scripta* exhibited no postfeeding changes in intestinal nutrient uptake rates or in the mass of most organs measured. Feeding did induce modest but statistically significant increases in oxygen consumption rates for all three species and for *S. odoratus* an increase in stomach mass. Drawing on other studies on digestive response, reptile metabolism, and turtle ecology, we shall discuss in turn the metabolic, functional, and

morphologic responses to feeding by juvenile *C. serpentina*, adult *S. odoratus*, and subadult *T. scripta*. We shall conclude by addressing three questions regarding the adaptive significance of turtle digestive physiology.

*Metabolic response.*—$\dot{V}O_2$ of fasting juvenile *C. serpentina*, adult *S. odoratus*, and subadult *T. scripta* did not differ significantly from each other and were, respectively, 42%, 61%, and 56% of predicted values based on a generalized regression equation for reptiles at 30 C [note that this equation of Bennett and Dawson (1976) includes only three turtle species, one being *T. scripta*, of 44 reptile species analyzed]. Following feeding, these turtles experienced the common phenomenon of an increase in metabolic rate (Jobling, 1981; Secor and Diamond, 1997b). Peak $\dot{V}O_2$ and SDA (normalized to body mass) were significantly ($Ps < 0.006$) greater for *C. serpentina* than for either *T. scripta* or *S. odoratus*, apparently a result of the relatively larger meals consumed by *C. serpentina* in our experiments (11.3% vs 5.2% and 5.0%). We have previously observed peak $\dot{V}O_2$ and SDA to increase with relative meal size for the python *Python molurus* (Secor and Diamond, 1997b).

The 110%, 170%, and 240% postprandial increases in $\dot{V}O_2$ by *S. odoratus*, *T. scripta*, and *C. serpentina*, respectively, are within the range of increases experienced by digesting fish, amphibian, reptile, and bird species which feed relatively frequently (Jobling, 1981; Janes and Chappell, 1995; Secor and Diamond, 1996; see Fig. 5). Similarly, the turtle *Testudo denticulata* increases its metabolic rate by 100% to 280% following the consumption of bananas equaling 3.3–4.7% of body mass (Benedict, 1932); the metabolic rate of digesting juvenile painted turtles (*Chrysemys picta*) averages 220% higher than rates during fasting (Sievert et al., 1988); and the omnivorous turtle (*Kinixys spekii*) exhibited a 160%, 185%, and a 200% increase in $\dot{V}O_2$ during the digestion of millipedes, leaves, and fungi, respectively (Hailey, 1998). The magnitudes of responses of all six of these turtle species are small compared to the increase in $\dot{V}O_2$ during digestion by infrequently feeding amphibians and reptiles (Fig. 5). For example, sit-and-wait foraging *P. molurus* increases $\dot{V}O_2$ by 410% and 1000%, respectively, following the ingestion of meals 5% and 15% of body mass (Secor and Diamond, 1997b). Likewise, the monitor lizard *Varanus albigularis*, which fasts for many months in the wild, increases $\dot{V}O_2$ 900% during the digestion of meals 10% of body mass (Secor and Phillips, 1997).

SDA expressed as a percentage of ingested energy is a useful standard for comparing the cost of digestion among taxa (Jobling and Davies, 1980). These percentages for *S. odoratus* (17%), *T. scripta* (21%), and *C. serpentina* (22%), which do not statistically differ, are within or near the range of values (3–18%) for frequently feeding invertebrate and vertebrate species studied and lower than the values of 26–37% for several species of infrequently feeding snakes (Jobling, 1981; Secor and Diamond, 1997b).

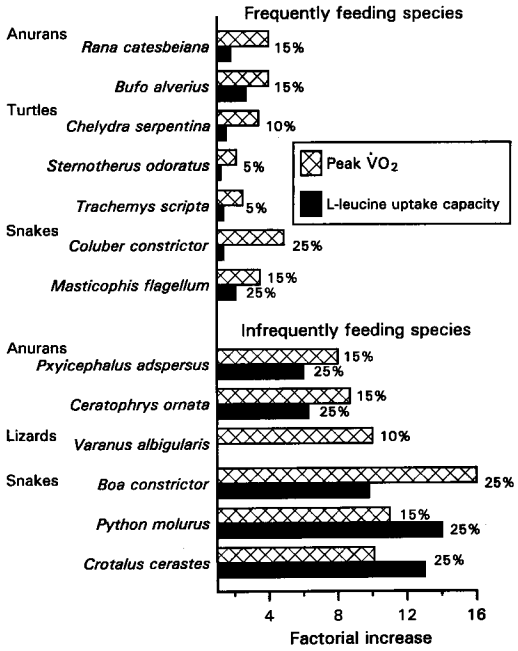


Fig. 5. Comparison between frequently feeding and infrequently feeding amphibians and reptiles in the factorial increase in $\dot{V}O_2$ and intestinal L-leucine uptake capacity following the digestion of meals equaling 5–25% of body mass (meal size is written next to each bar). Note the much larger postfeeding increases for infrequently feeding than frequently feeding species. Factorial increases are from this study and Secor et al. (1994), Secor and Diamond (1995, 1996, 1997b), Secor and Phillips (1997), and unpubl. obs.

*Functional response.*—For each nutrient measured, we found no interspecific differences in intestinal uptake rates for fasted or digesting turtles. In fact, our laboratory has measured similar intestinal uptake rates (nmoles · $min^{-1}$ · $mg^{-1}$) of amino acids and D-glucose from several species of fasted and fed snakes, lizards, frogs, salamanders, and the turtle *Terrapene carolina* (Karasov et al., 1985; Secor et al., 1994; Secor and Diamond, 1995; S. Secor, L. Kats, and J. Diamond, unpubl. obs.). The large intestine

of fasted and fed *C. serpentina* and *S. odoratus* did not actively transport D-glucose, whereas we have observed the active transport of D-glucose by the large intestine of fasted and fed snakes (*Python molurus* and *Boa constrictor*) and anurans (*Bufo alverius, Rana catesbeiana, Ceratophrys ornata,* and *Pyxicephalus adspersus*; Secor and Diamond, 1995, 1996; unpubl. obs.).

We found that, when we normalize uptake capacities to body mass (dividing by body mass) for each species, normalized values for fasted and fed turtles are within the range of normalized uptake capacities of other ectotherms, including trout (*Salmo gairdneri*), leopard frogs (*Rana pipiens*), bullfrogs (*R. catesbeiana*), and frequently feeding species of snakes including *Coluber constrictor, Masticophis flagellum,* and *Pituophis melanoleucus* (Buddington and Hilton, 1987; Buddington et al., 1991; unpubl. obs.). In contrast, normalized capacities are much higher for endotherms and for recently fed infrequently feeding snakes, such as sidewinders (*Crotalus cerastes*), Burmese pythons, and boa constrictors (*B. constrictor*; Secor et al., 1994; Secor and Diamond, 1995; unpubl. obs.). Normalized capacities for either L-leucine or L-proline uptake did not differ among the three species, whereas normalized D-glucose uptake capacities for *S. odoratus* were significantly (planned pairwise comparisons, $Ps < 0.043$) greater than those of the other two turtle species

Carnivores and omnivores transport amino acids at faster rates than they transport glucose across their intestinal brush-border membrane, whereas herbivores show the opposite relationship (Karasov and Diamond, 1988). The ratios of amino acid uptake rates to D-glucose uptake rates were much greater than 1.0 (5–38) for all three turtle species, regardless of digestive state (Fig. 3). In light of their omnivorous diet, the propensity of carnivores to transport amino acids at faster rates than glucose is presumably an adaptation to diets that are high in protein and low in carbohydrate.

*Morphologic response.*—None of the three turtle species experienced any significant increase in small intestinal wet mass following feeding. We have similarly observed a lack of significant postfeeding increase in small intestinal mass among frequently feeding snake and frog species (Secor and Diamond, 1994, 1996). In contrast, small intestinal mass increases two to fourfold following feeding for infrequently feeding snake and frog species (Secor and Diamond, 1994, 1996). The morphology of turtle enterocytes did not respond to feeding, whereas for infrequently feeding *C. cerastes* and *P. molurus* enterocyte dimensions increases significantly following feeding (Secor et al., 1994; Secor and Diamond, 1995). Feeding induced very limited mass change of other organs for our three turtle species; only *S. odoratus* stomach wet and dry masses were significantly greater in fed individuals. In an interspecific comparison of organ mass (normalized to body mass), we found in planned pairwise comparisons that *S. odoratus* possesses relatively larger kidneys ($Ps < 0.006$), smaller stomachs ($Ps < 0.0001$), and smaller large intestines ($Ps < 0.01$) than the other two species. *Trachemys scripta* was found to possess relatively larger small intestines ($Ps < 0.02$) and smaller hearts ($Ps < 0.007$) than the other two turtle species.

*Adaptive significance.*—Three questions about the adaptive significance of the postfeeding responses of *C. serpentina, S. odoratus, T. scripta,* and other turtle species suggest themselves. First, how do we interpret the overall postfeeding responses of these turtles with respect to their natural feeding habits? *Chelydra serpentina, S. odoratus,* and *T. scripta* feed frequently in the wild, as evidenced by the high percentage (70–100%) of wild-caught individuals with food within their guts and by the appearance of separate meals within the gut (Lagler, 1943; Punzo, 1975; Parmenter, 1980). All three species as juveniles and adults actively search the bottom of their aquatic habitat for food (plants, invertebrates, and vertebrates), a technique described by Ernst (1986) for *S. odoratus* as a "peer and probe method of food detection." In view of their high frequency of feeding and constant bouts of digestion, we predicted that these species would maintain intestinal performance following digestion and therefore exhibit no significant change in intestinal function or morphology after feeding. For most variables examined, our prediction held true; intestinal function and morphology and organ masses did not respond to feeding following an extended fast. Therefore we conclude that, like other frequent feeders, *C. serpentina, S. odoratus,* and *T. scripta* maintain the readiness of their digestive machinery even when not feeding.

Second, can we suggest cases in nature where gut performance is nevertheless likely to be regulated in turtle species? Temperate-zone populations of turtles generally hibernate during the winter in response to low environmental temperatures (Ernst et al., 1994). Associated with lower body temperatures and digestive quiescence, perhaps the functional capacity of turtle guts will be found to decrease during hibernation, as observed for lizards (Latif et al., 1967;

Karasov et al., 1985). Alternatively, their intestines may only atrophy while retaining nutrient transporter activities, as observed for hibernating ground squirrels (Carey, 1990). In either case, upon the turtle's arousal from hibernation and subsequent feeding, its gut may up-regulate function and/or morphology to levels maintained throughout the active season.

Although most turtle species appear to feed relatively frequently, a few species are documented to fast periodically without hibernating (Ernst et al., 1994). Most notable is the herbivorous desert tortoise (*Gopherus agassizii*) of North America that commonly fasts for a month during the active season, spends only 0.3% of the year feeding, and may experience 20-fold shifts in food consumption from one part of the active season to the next (Nagy and Medica, 1986). If up- and down-regulation of gut performance has evolved in response to large changes in digestive demand within turtles, *G. agassizii* is a likely candidate for such responses.

Third, are ontogenetic shifts in diet or meal size associated with corresponding shifts in gut function? Several turtle species (*Pseudemys concinna, Terrapene carolina, Emydura krefftii*), including *T. scripta*, are known to shift from a primarily carnivorous diet as juveniles to a largely herbivorous diet as adults (Georges, 1982; Ernst et al., 1994). For the marine fish *Cebidichthys violaceus*, which as young are carnivorous and become increasingly herbivorous with size, intestinal transport rates of the amino acid L-proline decline with body size in parallel with the decrease dietary protein intake (Buddington et al., 1987). A similar ontogenetic shift in intestinal nutrient transport may also occur for turtles that change diet with age.

In addition, *C. serpentina* undergoes an ontogenetic shift in foraging tactics and meal size (Ernst et al., 1994). Very young *C. serpentina* (as in this study) actively forage and feed on small prey, whereas adults will also lie in ambush to seize prey as large as ducks and muskrats (Lagler 1943; Ernst et al., 1994). Perhaps, very large *C. serpentina* will be found to demonstrate greater magnitudes of digestive regulation than small young individuals, as an evolutionary response to feeding less often on larger meals.

This study on *C. serpentina, S. odoratus*, and *T. scripta* has complemented our other studies on frequently feeding snake and anuran species, all of which have demonstrated a general lack of postfeeding responses (Fig. 5). In contrast, amphibian and reptile species that naturally experience long bouts of digestive quiescence exhibit significant postfeeding changes in gut function and morphology (Fig. 5). Hence we believe that this study supports our adaptive hypothesis on the correlated evolution of feeding ecology and digestive physiology, that frequently digesting organisms maintain gut performance whereas infrequently digesting species up- and down-regulate the gut in response to digestive demand (Secor et al., 1994; Secor and Diamond, 1995). Studies of other taxa, such as the infrequently feeding tortoise *Gopherus*, will provide further tests of this hypothesis.

## Acknowledgments

We wish to extend our appreciation to R. Abdelmalik, I. Chaudhary, J. Isaacs, K. Kim, R. Nagle, S. Sampogna, S. Szabo, R. Torres, F. Wayland, and especially C. Slotnick for their assistance in the experiments. Experimental protocols and animal care were approved by the University of California, Los Angeles Chancellor's Animal Research Committee. Support of this project was provided by National Institutes of Health NRSA fellowship DK 08878 and by National Institutes of Health grants GM 14772 and DK 42973 (to the UCLA Center for Ulcer Research and Education).

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