Modest Regulation of Digestive Performance Is Maintained through Early Ontogeny for the American Alligator, *Alligator mississippiensis*

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ABSTRACT

The American alligator, *Alligator mississippiensis*, is an opportunistic carnivore that experiences an ontogenetic shift in food and feeding habits with an increase in body size. Alligators frequently feed on invertebrates and small fish as neonates and transition to feeding less frequently on larger vertebrates as they grow. We hypothesized that alligators experience an ontogenetic shift in the regulation of intestinal performance—modest regulation with frequent feeding early in life and wider regulation with less frequent feeding as they increase in body size. We tested this hypothesis by comparing postprandial responses in metabolic rate, organ masses, intestinal histology, digestive hydrolase activities, and intestinal nutrient uptake rates among neonate, juvenile, and subadult alligators. With feeding, alligators of all three age classes experienced a rapid increase in metabolic rate that peaked within 2 d and thereafter declined more slowly to prefeeding rates. Specific dynamic action increased with body mass and was equivalent to 32% of meal energy. For each age class, the majority of organs did not change in wet and dry mass with feeding. For subadult alligators, luminal gut pH varied regionally due to the acidic stomach, which continued to remain acidic with fasting. With feeding, epithelial enterocytes are remodeled from a pseudostratified to a stratified architecture and become infiltrated with lipid droplets. Feeding did not generate any significant change in the thickness of intestinal tissues, though it did induce an increase in enterocyte width and volume for subadults. For each age class, feeding generally did not result in significant changes in pancreatic trypsin, intestinal aminopeptidase, and intestinal nutrient uptake activities and capacities. Mass-specific nutrient uptake rates varied among age classes due to the higher rates exhibited by neonates. Among age classes, intestinal uptake capacities scaled allometrically (mass exponents <1) with body mass. Across these three age classes, the modest regulation of digestive performance with feeding and fasting for alligators appears to be ontogenetically conserved.

Keywords: *Alligator*, allometry, digestive enzymes, intestinal histology, intestinal performance, metabolism, nutrient uptake, organ mass, specific dynamic action.

Introduction

The adaptive interplay between feeding habits and digestive physiology is evident in two realms of feeding ecology. First, we have long been aware of the adaptive links between diet and the structure and function of the gut (Karasov and Diamond 1988). The second, a more recent discovery, is the adaptive relationship between feeding frequency and an animal's capacity to regulate digestive performance (Secor 2005*a*). The former, demonstrated across all major vertebrate taxa, is highlighted by the generalized finding that herbivores possess relatively enlarged digestive tracts, increased capacities for intestinal breakdown and absorption of carbohydrates, and specialized fermentation chambers (Stevens and Hume 1995; Karasov and Martínez del Rio 2007). Carnivores tend to possess smaller guts, emphasize intestinal breakdown and absorption of proteins and amino acids, and generally lack enlarged regions for fermentation (Stevens and Hume 1995; Karasov and Martínez del Rio 2007). The latter discovery, initially identified for snakes, stems from the observations that animals that experience long episodes of fasting due to infrequent feeding behaviors or extended periods of dormancy (e.g., estivation) possess the capacity to widely regulate digestive performance that involves the downregulation of their digestive system with fasting and the rapid upregulation of their gut with feeding (Secor 2005*a*, 2005*b*; Ott and Secor 2007*a*). In contrast, species that feed more frequently tend to experience modest regulatory responses (i.e., nonsignificant changes) in gut form and function when transitioning between fasting and feeding (Secor and Diamond 2000; Secor 2005*a*, 2005*b*; Day et al. 2014).

These adaptive phenomena may exist singularly or in tandem for species that experience ontogenetic shifts in diet, feeding habits, and/or energy requirements. The stichaeid fish *Cebidichthys violaceus* shifts from a strictly carnivorous larva to a chiefly herbivorous adult while at the same time experiencing an increase in activities of intestinal poly- and disaccharidases (α-amylase, maltase, and isomaltase) and decreased activity of the

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protease pepsin (German et al. 2004). The intestinal tract of herbivorous larval bullfrogs (*Rana catesbeiana*) undergoes a 30% reduction in mass and length relative to body size, an increase in luminal surface area per length, and a decrease in the active uptake of D-glucose following metamorphosis to carnivorous adults (Toloza and Diamond 1990). For mammals, weaning ceases neonatal milk consumption that is accompanied by declines in intestinal lactase and β-galactosidase activities and glucose and galactose uptake rates (Doell and Kretchmer 1962; Buddington and Diamond 1989, 1992; Toloza and Diamond 1992).

For gape-limited predators, an increase in body size and hence maximum meal size is often coupled with an ontogenetic decrease in prey number and/or feeding frequency (Houston and Shine 1993; Armstrong et al. 1996; Hirai 2002). Among fishes, amphibians, and reptiles, ontogenetic shifts in prey size may also be characterized by a gradual transition from an invertebrate-dominated diet for neonates and juveniles to one that also includes larger vertebrate prey for subadults and adults (Fitch 1960; Duellman and Lizana 1994; Armstrong et al. 1996; Wu et al. 2005). For the blacktail comber (*Serranus atricauda*), a doubling in body length occurs with a tripling of maximum prey width, a doubling in the percentage of fishes with empty stomachs, and a change in diet from 70% invertebrates to 70% fish (Morato et al. 2000). Ontogenetic shifts in food and feeding habits would predictably invoke an adaptive remodeling of intestinal form and function to enhance the assimilation of the new diet (over the old diet) and/or alter the regulatory spans of intestinal plasticity (e.g., increasing the magnitude with a decreased frequency of feeding).

The American alligator (*Alligator mississippiensis*) is an opportunistic carnivore that—as a product of its growth and size—experiences ontogenetic shifts in diet and feeding habits. Hatchling female and male alligators can potentially increase their body mass by 300- and 1,000-fold and their body length by 10- and 15-fold, respectively, as they mature and reach full size (Rootes et al. 1991; Woodward et al. 1995). Hatchling alligators frequently feed predominately on invertebrate prey, shifting to include fish and other small aquatic vertebrates as juveniles and transitioning with increasing size to feeding on larger prey (large fish, reptiles, birds, and mammals) less often (Wolfe et al. 1987; Delany 1990; Shoop and Ruckdeschel 1990; Delany et al. 1999; Saalfeld et al. 2011). Given the frequent nature of their feeding, young alligators would be expected to modestly regulate intestinal performance from meal to meal (Secor 2005*a*). However, as their food habits shift from an invertebrate-rich diet consumed frequently to a diet of larger vertebrate prey consumed less often, intestinal function may change to reflect the dietary shifts and performance could become more widely regulated.

We addressed these predictions by comparing the physiological responses to feeding among three age classes (neonate, juvenile, and subadult) of the American alligator. To encapsulate the integrative nature of their postprandial responses, we quantified for each age class metabolic rates, organ masses, intestinal histology, activities of digestive enzymes, and intestinal nutrient transport rates of fasted and fed individuals. Our aim was to determine whether young alligators modestly regulate intestinal performance with feeding and fasting and whether, with an increase in body size, they experience a broadening in the magnitude of their digestive responses. An alternative scenario is that alligators do not experience an ontogenetic shift in intestinal function or in the degree of regulation with increased body size; rather, modest magnitudes of postprandial responses are conserved across age classes.

Material and Methods

Animals and Their Maintenance

The American alligator is native to the southeastern United States and frequents coastal and inland wetland habitats (Joanen and McNease 1972; Ryberg et al. 2002; see fig. 1 in Joanen and McNease 1987). Male alligators mature in 6–10 yr (~25 kg), have a life expectancy of 80 yr, and can exceed 4 m in total length and a mass of 450 kg. Female alligators grow more slowly—maturing in 8–13 yr (~25 kg)—have a life expectancy of 45 yr, and can reach 3 m in total length and a mass of 120 kg (Chabreck and Joanen 1979; Rootes et al. 1991; Woodward et al. 1995). The alligators used in this study were provided by the Rockefeller Wildlife Refuge, Cameron Parish, Louisiana. Three age classes of alligators were used in this study: neonates (2–3 mo old; mean mass $\pm$ SEM $= 50.7 \pm 1.7$ g, $n = 14$), juveniles (1–2 yr old; 894 ± 26 g, $n = 9$), and subadults (2–3 yr old; $3{,}145 \pm 445$ g, $n = 6$). We housed neonates in 132-L aquaria (four per aquarium) and juveniles and subadults communally (separated by age class) in 3,200-L fiberglass tanks at the University of Alabama. All enclosures contained basking sites with overhead lamps and were filled with enough water to allow alligators to become fully submerged. Ambient temperatures were maintained at $28° \pm 2°$C, and the light cycle was set at 12L : 12D. Before experimentation, alligators were maintained on a weekly diet of prekilled minnows and newborn mice (neonates), adult mice (juveniles), and chicks, adult mice, and small rats (subadults). We fasted neonates for 14 d and juvenile and subadult alligators for 1 mo before experimentation to ensure that all digestive and postabsorptive activities had concluded. All animal care and experimentation were conducted with the approval of the University of Alabama Institutional Animal Care and Use Committee.

Standard Metabolic Rate, Postprandial Metabolism, and Specific Dynamic Action

For each age class, we used closed-system respirometry to quantify standard metabolic rates (SMRs) and to characterize the profiles of the postprandial metabolic response (Vleck 1987; Secor and Diamond 1997; Secor 2003). Alligators were individually placed into respirometry chambers (1.5–39 L) that were fitted with incurrent and excurrent air ports, each connected to a three-way stopcock. Respirometry chambers were maintained at constant temperature ($30° \pm 0.5°$C) within an environmental chamber (DS54SD; Powers Scientific, Pipersville, PA). Ambient air was constantly pumped through the respirometry chambers during nontesting periods. For each metabolic trial, an initial 45-mL air sample was pulled from the excurrent port and both

incurrent and excurrent ports were closed. One hour later, the excurrent port was opened and a second 45-mL sample was drawn. Air samples were pumped (75 mL min^{-1}) through a column of water absorbent (Drierite; W. A. Hammond Drierite, Xenia, OH) into a CO_2 analyzer (CD-3A; AEI Technologies, Pittsburgh, PA) and through a column of Drierite and CO_2 absorbent (Ascarite; Acros Organics, Fair Lawn, NJ) into an O_2 analyzer (S-3A/II; AEI Technologies). We calculated whole-animal (mL h^{-1}) and mass-specific (mL g^{-1} h^{-1}) rates of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) corrected for standard pressure and temperature using a modification of equation (9) in Vleck (1987).

We determined each alligator's SMR from measurements of $\dot{V}O_2$ of fasted animals at rest. For SMR trials, gas exchange was measured in the morning (~0700 hours) and evening (~1900 hours) for four consecutive days. For each individual, we calculated its SMR as the mean of its two lowest measured $\dot{V}O_2$ (Bessler et al. 2010). For postprandial trials, fasted alligators were fed rodent meals equal in mass to 5% of their body mass. Neonate ($n = 8$), juvenile ($n = 7$), and subadult ($n = 5$) alligators respectively consumed prekilled neonate (1.6–3.0 g), adult (18–25 g), and large adult (35–40 g) mice (RodentPro.com, Inglefield, IN). After being fed, individuals were placed back into their respirometry chambers and measurements of gas exchange were resumed at 12-h intervals for 3 d and thereafter at 24-h intervals for the following 5 d.

We quantified the following variables as described in Secor (2009): SMR (mean of the two lowest $\dot{V}O_2$ before feeding), peak $\dot{V}O_2$ (highest recorded $\dot{V}O_2$ following feeding), factorial scope of peak $\dot{V}O_2$ (calculated as peak $\dot{V}O_2$ divided by SMR), respiratory exchange ratio (RER; quantified as $\dot{V}CO_2/\dot{V}O_2$ at peak $\dot{V}O_2$), duration (time from feeding until $\dot{V}O_2$ was no longer significantly greater than SMR), specific dynamic action (SDA; total energy expended above SMR over the duration of elevated $\dot{V}O_2$), and SDA coefficient (SDA as a percentage of meal energy). We quantified SDA (kJ and kJ kg^{-1}) by multiplying the summed extra O_2 consumed (mL) above SMR over the duration by 19.8 J, assuming that the rodent meal catabolized (dry matter) was 70% protein, 25% fat, and 5% carbohydrate and generates a respiratory quotient of 0.73 (Gessaman and Nagy 1988).

Energy content of each meal was determined by bomb calorimetry. Five sets of three neonate mice and five individuals of adult and large adult mice were weighed (wet mass), dried to a constant mass at 60°C, reweighed (dry mass), ground to a fine powder, and pressed into pellets. Three pellets from each neonate set or individual adult or large adult mouse were ignited in a bomb calorimeter (1266; Parr Instruments, Moline, IL) to determine dry mass energy content. For each mouse size, we determined wet mass energy equivalent (kJ g^{-1}) as the product of dry mass energy content and the meal's dry mass percentage. An individual's meal energy content was calculated as the product of meal wet mass and wet mass energy equivalent. Specific and total energy content were respectively 5.92 ± 0.10 kJ g^{-1} and 15.8 ± 0.6 kJ for neonate mice, 6.82 ± 0.13 kJ g^{-1} and 153 ± 6 kJ for adult mice, and 7.91 ± 0.27 kJ g^{-1} and 281 ± 11 kJ for large adult mice.

Experimental Procedure

To examine the postprandial response in tissue structure and function, we compared organ mass, luminal pH, intestinal histology, pancreatic and intestinal enzyme activities, and intestinal nutrient uptake rates between fasted ($n = 3$) and fed ($n = 3$) individuals of each age class, as well as among age classes. Alligators were fasted for either 14 d (neonates) or 30 d (juveniles and subadults) and were either studied in the fasted state or fed rodent meals equaling 10% (neonates and juveniles) or 5% (subadults) of their body mass. Fasted and fed alligators were maintained at a constant temperature of 30°C within an environmental chamber for 2 d before study. For study, fasted and 2-d-postfed (2DPF) alligators were humanely euthanized by severing their spinal cord immediately posterior to the head, followed by pithing of the brain cavity. Individuals were then weighed and measured (snout-vent length and total length), and a midventral incision was made from the cloaca to the gular region to expose internal organs.

To assess regional and feeding effects on luminal pH of the gastrointestinal tract for subadult alligators, we used a slender, flexible pH probe (Accumet 13-620-95; Fisher Scientific, Pittsburgh, PA) to measure luminal pH at 12 locations extending from the proximal esophagus to the distal large intestine. We also measured stomach pH for both fasted and fed neonates. The pH probe was calibrated with certified pH buffer (Fisher Scientific) before use for each alligator. For all alligators, each organ was removed, weighed, emptied of any contents, and reweighed if necessary. Organs (or portions of) were dried to a constant mass at 60°C and reweighed to determine organ dry mass.

Tissue Histology

Segments from the proximal small intestine of neonates and from the proximal, middle, and distal one-thirds of the small intestine of subadults were fixed in reptilian Ringer's buffered formalin solution (see Secor et al. 1994 for chemical composition of reptilian Ringer's solution). Fixed samples were commercially embedded in paraffin, sectioned (6 μm), placed on glass slides, and stained with hematoxylin and eosin (Mass Histology; Worcester, MA). Using stained cross sections of each sample, we measured from 10 different locations the thickness of the mucosa/submucosa and muscularis/serosa layers and the height and width of an enterocyte using a digital compound microscope (57900; Boreal Science, St. Catharines, Ontario) linked to a computer with image analysis software (Motic Image Plus, Richmond, British Columbia). We calculated for each of the 10 enterocytes its volume based on a formula for a cylinder (volume = 0.5 $width^2 \times \pi \times$ height). We used the mean of the 10 measurements of tissue thicknesses and enterocyte size from each individual for analyses.

We used transmission electron microscopy (TEM) to compare and identify any postprandial changes in intestinal microvillus length and width for all six neonates and five of the six subadult alligators. Small samples of proximal small

intestine were fixed in 2.5% reptile Ringer's buffered gluteraldehyde, postfixed in 1% osmium tetroxide, dehydrated in a graded series of ethanol, and embedded in Spurr resin (Electron Microscopy Sciences, Hatfield, PA). Ultrathin sections (~80 nm) were placed on copper mesh grids and examined using a transmission electron microscope (Hitachi H-7650). Using images of the microvillus border, we measured the length and width of 30–100 intact microvilli using ImageJ (US National Institutes of Health, Bethesda, MD). For each individual, mean values of microvilli length and width were used for statistical analysis.

Pancreatic and Intestinal Hydrolase Activities

We measured the activity of the pancreatic protease, trypsin, following the procedures of Preiser et al. (1975). Segments of pancreas were homogenized in phosphate-buffered saline (PBS; 1:50 dilution) on ice, with trypsin activated by 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. We terminated the reaction after 30 min with 30% acetic acid. Sample absorbances (in duplicates) were measured spectrophotometrically at 410 nm, and trypsin activity (μmol min^{-1} mg^{-1}) was calculated using a p-nitroanilide standard curve. Total capacity of pancreatic trypsin activity was calculated as a product of mass-specific trypsin activity and pancreas wet mass.

Small intestine aminopeptidase-N (APN) activity was measured following the procedures of Wojnarowska and Gray (1975). Intact 2-cm samples of proximal small intestine for neonates and juveniles and mucosa scraped from 2-cm portions of proximal, middle, and distal segments for subadults were homogenized in PBS (1:250 dilution) on ice. The tissue homogenate was incubated for 30 min at 37°C with 0.34 mM leucyl-β-naphthylamide as a substrate and p-hydroxymercuricbenzoic acid to inhibit nonspecific cytosol peptidases. Following incubation, sample absorbance was measured spectrophotometrically at 560 nm and APN activity (μmol min^{-1} mg^{-1}) was calculated from a standard curve using β-naphthylamine. For subadult alligators, total small intestinal capacity for APN activity was calculated by summing for the three segments the product of segment wet mass and segmental mass-specific APN activity. Since APN activity for subadults was measured from scraped mucosa, activity for each segment was corrected to 1 g of intact intestine based on relative mucosal mass (mucosal mass/intact intestine mass) determined for each 2-cm sample.

Intestinal Nutrient Uptake

We calculated uptake rates 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; Secor and Diamond 2000). For each intestinal one-third, 1-cm segments of everted intestine were incubated in reptile Ringer's solution for 5 min at 30°C, followed by a 2-min incubation in reptile Ringer's solution containing both an unlabeled and a radiolabeled nutrient (^{3}H-L-leucine, ^{3}H-L-proline, or ^{14}C-D-glucose) and a radiolabeled adherent fluid marker (^{14}C-polyethylene glycol for amino acids or ^{3}H-L-glucose for D-glucose). We measured for each intestinal one-third total uptake (carrier mediated and passive) of the amino acids L-leucine and L-proline and the carrier-mediated uptake of D-glucose as nmol min^{-1} mg^{-1}. We likewise calculated total small intestinal uptake capacities for each solute as the summed products of mass-specific uptake rates and mass for the three intestinal segments.

Statistical Methods

For each age class, we used a repeated-measures ANOVA to test for significant effects of time on postprandial $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ (as mL h^{-1} and mL g^{-1} h^{-1}, respectively). We employed pairwise mean comparison (Tukey-Kramer test) to determine the time point that postprandial $\dot{V}_{O_2}$ was no longer significantly greater than SMR (i.e., duration of the SDA response). We similarly used a repeated-measures ANOVA to examine the effects of gut position on luminal pH for fasted and fed subadult animals and identified differences between gut positions with pairwise mean comparison (Tukey-Kramer). For each gut position, we used a one-way ANOVA to determine significant differences in luminal pH between fasted and fed subadults, in gastric pH between neonate and subadult alligators, and in blood pH between fasted and fed subadults. Separately for fasted and fed individuals, we employed a repeated-measures ANOVA to test the effects of intestinal position (proximal, middle, and distal) on the thickness of the intestinal mucosa/submucosa and muscularis/serosa layers for subadults, on intestinal APN activity for subadults, and on nutrient uptake rates for all age classes. For statistically significant outcomes of ANOVAs, pairwise mean comparison (Tukey-Kramer) identified differences between intestinal positions. A two-way ANOVA was used to test for treatment (fasting vs. 2DPF) and age class effects (neonate, juvenile, and subadult) on mass-specific enzyme activities and intestinal nutrient uptake rates. We tested treatment and age class effects on wet and dry organ masses; intestinal tissue thickness; enterocyte length, width, and volume; microvillus length and width; pancreatic trypsin capacity; and intestinal uptake capacities using a two-way ANCOVA with body mass as the covariate. We tested singularly for treatment effects on enterocyte length, width, and volume for the middle and distal small intestine and on APN capacity for subadult alligators using a one-way ANCOVA with body mass as the covariate. For each two-way ANOVA or ANCOVA that generated a significant interaction between age class and treatment effects, we followed with a pairwise mean comparison (Tukey-Kramer) to identify significant differences among age classes and treatment. To test for homogeneity of variance in the ANOVA, we performed Levene's test followed by Welch's ANOVA if necessary. For ANCOVAs, we visually checked the distribution of the residuals to ensure the normality of the data. To identify the relationships between body mass and metabolic rates, SDA, organ masses, and intestinal nutrient uptake capacity, we performed linear regression analyses using

log-transformed data. For regression analyses of organ mass and uptake capacities, we combined data from fasted and fed individuals. All statistical analyses were performed using the GLM, MIXED, REG, or ANOVA procedure in SAS 9.1.3 (SAS Institute, Cary, NC). We designated the level of significance as $P \leq 0.05$ and report mean values as mean ± 1 SEM, and we report mass exponents from regression analyses with a $\pm 95\%$ confidence interval.

Results

Metabolic Responses to Feeding

Alligators of all age classes experienced significant variation in postprandial rates of gas exchanged ($F_{11,44-77} > 11.3$; all $P < 0.0001$), with noted matched increases in $\dot{V}O_2$ and $\dot{V}CO_2$, peaking at 1.5 d postfeeding for neonates and juveniles and at day 2 for subadults (fig. 1). Following these peaks, metabolic rates declined and returned to levels not significantly greater than SMR at 4, 6, and 6 d, respectively, for neonates, juveniles, and subadults (table 1). Whole-animal (mL h^{-1}) and mass-specific (mL h^{-1} g^{-1}) SMR and peak $\dot{V}O_2$ varied significantly (all $P < 0.0001$) among age classes chiefly because of the 60-fold range in body mass among individuals (table 1). It is notable that neonates exhibited significantly (all $P < 0.0003$) greater mass-specific rates of SMR and peak $\dot{V}O_2$ than either juveniles or subadults (table 1). The scope of peak $\dot{V}O_2$ also varied significantly ($P < 0.021$) as subadult alligators experienced a significantly ($P < 0.023$) greater scope (3.83 ± 0.23) compared with neonates (2.81 ± 0.19) but not compared with juveniles (2.92 ± 0.27; table 1). RERs, measured at the time point of peak $\dot{V}O_2$ for each individual, did not vary among age classes and averaged 0.760 ± 0.005 among all individuals. The 65-fold variation in SDA (kJ) is likewise due to the age-specific range in body mass (table 1). Standardized to body mass, SDA (kJ kg^{-1}) continued to vary significantly ($P < 0.013$) as subadult values were greater (all $P < 0.042$) than those of neonates and juveniles (table 1). The SDA coefficient (SDA as a percentage of meal energy) did not vary among age classes, averaging $32.1\% \pm 1.9\%$ across the three age classes (table 1).

For the alligators in this study (47.7–2,937 g), SMR and peak $\dot{V}O_2$ scaled allometrically with body mass (all $r^2 > 0.974$, all $P < 0.0001$), exhibiting mass exponents of 0.760 ± 0.060 and 0.819 ± 0.063, respectively (fig. 2*A*). Because of this difference in allometric scaling, there was an inherent increase in scope between SMR and peak $\dot{V}O_2$ with increased body mass. For these individuals, SDA (kJ) scaled allometrically ($r^2 = 0.984$, $P < 0.0001$) with a mass exponent of 1.075 ± 0.066 (fig. 2*B*). We found the SDA coefficient not to vary significantly as a function of body mass (fig. 2*C*).

Organ Masses

For each organ, wet or dry mass, there was a significant main effect of age class ($F_{2,13} = 4.72–48.37$, $P < 0.034$), with the exception of the spleen (wet and dry mass). The main effect of feeding treatment was significant ($F_{1,13} = 9.07–22.62$, $P < 0.035$) only for wet and dry esophagus, wet and dry lung, and wet stomach mass. However, for each of these there was a significant ($F_{2,13} = 5.32–9.93$, $P < 0.05$) interaction between age class and feeding treatment. When each age class was examined individually, the only significant ($F_{1,5} = 12.1–1,086$, all $P < 0.041$) effects of feeding treatment were heavier esophagus (wet and dry) for fed juveniles and subadults, heavier stomach (wet) for fed neonates and juveniles, heavier large intestine (wet and dry) for fed neonates, and heavier spleen (dry) for fasted juveniles (table 2).

There was an obvious large effect of age class on organ wet and dry masses given the 125-fold range in body mass across individuals. However, when viewed as a function of body mass (divided by body mass), organ wet masses for neonates are twice

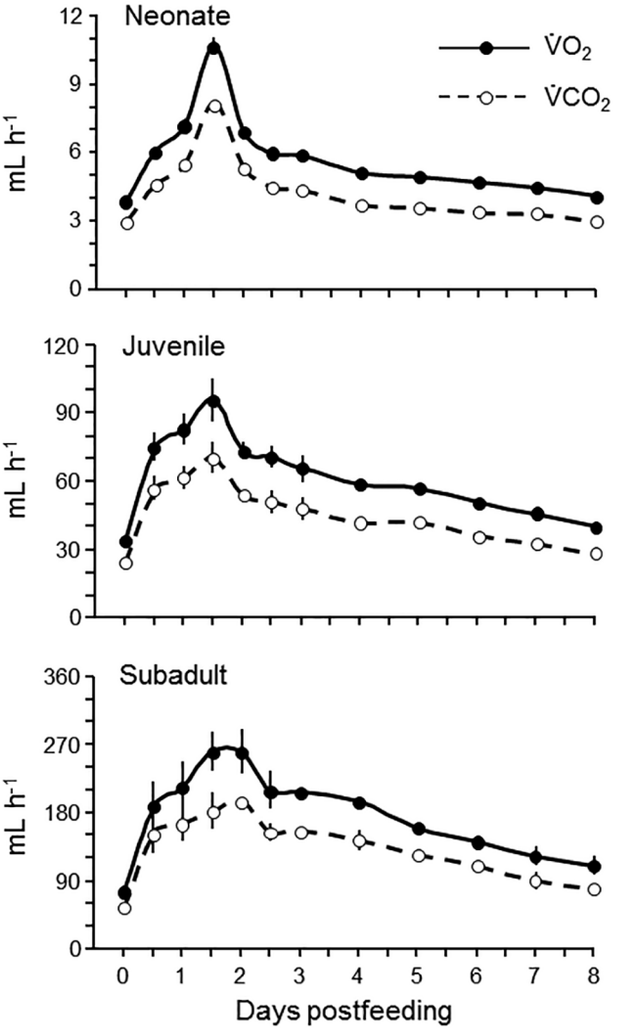

Figure 1. Postprandial profile of oxygen consumption ($\dot{V}O_2$) and CO_2 production ($\dot{V}CO_2$) as a function of days postfeeding for neonate ($n = 8$), juvenile ($n = 7$), and subadult ($n = 5$) American alligators, *Alligator mississippiensis*. Rates of gas exchanged peaked at 1.5–2 d postfeeding following a 2.8-, 2.9-, and 3.8-fold increase in $\dot{V}O_2$ for neonate, juvenile, and subadult alligators, respectively. In this and subsequent figures, error bars indicate $\pm$SEM and are omitted if the SEM is smaller than the width of the symbol used for the mean value.

Table 1: Body mass, meal mass, standard metabolic rate (SMR), and six variables of the postprandial metabolic response for three age classes of the American alligator, *Alligator mississippiensis*, at 30°C

Variable	Neonate	Juvenile	Subadult	*F*	*P*
N	8	7	5	...	...
Body mass (g)	54.4 ± 1.6^{A}	907 ± 32^{B}	2,530 ± 149^{C}	329	<.0001
Meal mass (g)	2.72 ± .08^{A}	45.3 ± 1.6^{B}	127 ± 8^{C}	26.2	<.0001
Meal mass (% body mass)	5.00 ± .01	4.99 ± .01	5.00 ± .01	.660	.536
SMR (mL O_2 h^{-1})	3.88 ± .22^{A}	34.2 ± 3.5^{B}	71.1 ± 5.2^{C}	3.75	.046
SMR (mL O_2 g^{-1} h^{-1})	.071 ± .004^{B}	.038 ± .004^{A}	.028 ± .003^{A}	37.2	<.0001
Peak $\dot{V}_{O_2}$ (mL h^{-1})	10.7 ± .4^{A}	97.1 ± 9.1^{B}	274 ± 30^{C}	4.82	.023
Peak $\dot{V}_{O_2}$ (mL g^{-1} h^{-1})	.196 ± .008^{B}	.108 ± .011^{A}	.110 ± .015^{A}	24.5	<.0001
Scope (peak $\dot{V}_{O_2}$/SMR)	2.81 ± .19^{A}	2.92 ± .27^{A,B}	3.83 ± .23^{B}	4.89	.021
RER ($\dot{V}_{CO_2}/\dot{V}_{O_2}$)	.767 ± .011	.749 ± .029	.763 ± .044	.136	.873
Duration of SDA (d)	4	6	6	...	...
SDA (kJ)	5.10 ± .35^{A}	91.4 ± 8.0^{B}	352 ± 32^{C}	7.64	.005
SDA (kJ kg^{-1})	93.9 ± 5.7^{A}	101.4 ± 9.3^{A}	141.6 ± 17.0^{B}	5.67	.013
SDA coefficient (% meal kJ)	31.7 ± 1.9	29.8 ± 2.8	35.8 ± 4.3	1.01	.386

Note. Values are presented as means ±1 SE. Values of *F* and *P* are from ANOVAs (age class effects) for body mass, meal mass, SMR (mL O_2 g^{-1} h^{-1}), peak $\dot{V}_{O_2}$ (mL g^{-1} h^{-1}), scope, respiratory exchange ratio (RER), specific dynamic action (SDA; kJ kg^{-1}), and SDA coefficient and from ANCOVAs (body mass as the covariate) for SMR (mL O_2 h^{-1}), peak $\dot{V}_{O_2}$ (mL h^{-1}), and SDA (kJ). Different superscript uppercase letters next to data denote significant ($P < .05$) differences between means among the three age classes as determined from post hoc pairwise comparisons (Tukey's honestly significant difference test).

that for subadults. Using additional data, we explore the allometric relationships of organ masses in "Discussion."

Gut Contents and Gastrointestinal pH

Following 2 d of digestion for the nine fed alligators in this study, the mass of stomach and intestinal contents were equivalent to 82.4% ± 2.5% and 4.5% ± 1.1% of meal mass, respectively. There were no significant differences among age classes in the percentage of the meals remaining within either the stomach or the small intestine.

Fasted and fed subadult alligators experienced significant variation (fasted: $F_{11,22} = 27.1$; fed: $F_{11,22} = 72.2$; all $P < 0.001$) in luminal pH throughout their gut (fig. 3). Most notable was the significantly (all $P < 0.001$) lower pH (2.82 ± 1.27) within fasted and fed stomachs (because of its production of HCl) compared with the other regions of the gastrointestinal tract. In addition, esophageal and most proximal small intestinal pH of fed individuals were significantly (all $P < 0.022$) lower than for the rest of the small intestine and the large intestine (fig. 3). For much of the subadult gut, there were no significant differences in luminal pH between fasted and fed individuals. The exceptions included a significantly lower pH ($F_{1,5} = 10.4$, all $P < 0.037$) for the middle esophagus and higher pH ($F_{1,5} = 10.4$, $P < 0.040$) for the third measured site of the small intestine for fed individuals (fig. 3). Gastric pH for fasted neonates (2.88 ± 0.73) did not differ from that of fasted subadults (middle region: 2.66 ± 0.41); however, fed neonates (1.88 ± 0.07) possessed a significantly ($F_{1,5} = 19.0$, $P < 0.012$) more acidic gastric lumen than fed subadults (middle region: 2.72 ± 0.18). For subadults, whole-blood pH for fed animals (7.36 ± 0.03) was significantly ($F_{1,5} = 30.9$, $P < 0.006$) greater than for fasted individuals (7.18 ± 0.01).

Small Intestinal Morphology

For fasted neonate and subadult alligators (intestinal histology was not conducted on juveniles), enterocytes of the mucosal epithelium were arranged in a pseudostratified fashion with noticeable partial overlap of adjoining cells and stacking of nuclei (fig. 4*A*). Lymphocytes were scattered within the epithelium as well as distinct paracellular spaces between enterocytes. Interspersed among the enterocytes were goblet cells that increased in number distally to occupying up to one-quarter of the epithelial surface for the distal small intestine (fig. 4*A*). The microvilli, when viewed with TEM, were found to be tightly arranged and extending perpendicular from the apical surface of enterocytes (fig. 4*C*, 4*E*). Feeding generated a remodeling of the intestinal epithelium; enterocytes took on the distinct columnar shape, arranged in a single layer, with fairly straight borders between adjoining cells (fig. 4*B*). The nuclei were frequently aligned in a single row, close to the basal edge of the cell. Goblet cells and lymphocytes continued to be present within the epithelium, the former continuing to increase in density distally along the small intestine. The apical brush border continued to be prominent when viewed with light microscopy, and when observed with TEM the microvilli still maintained their characteristic straight and parallel formation (fig. 4*B*, 4*D*, 4*F*). An additional distinction between fasted and fed epithelium was the complete lack of lipid droplets within enterocytes of fasted individuals and their presence (1–3 μm in diameter) within the enterocytes of fed animals (fig. 4*G*, 4*H*). For fed intestine, the number and accumulative area of the droplets within enterocytes varied from their complete absence to filling much of the nonnuclear space.

For fasted and fed subadult alligators, we found no significant effect of intestinal position (proximal, middle, or distal) on the thickness of the mucosa/submucosa and muscularis/serosa layers

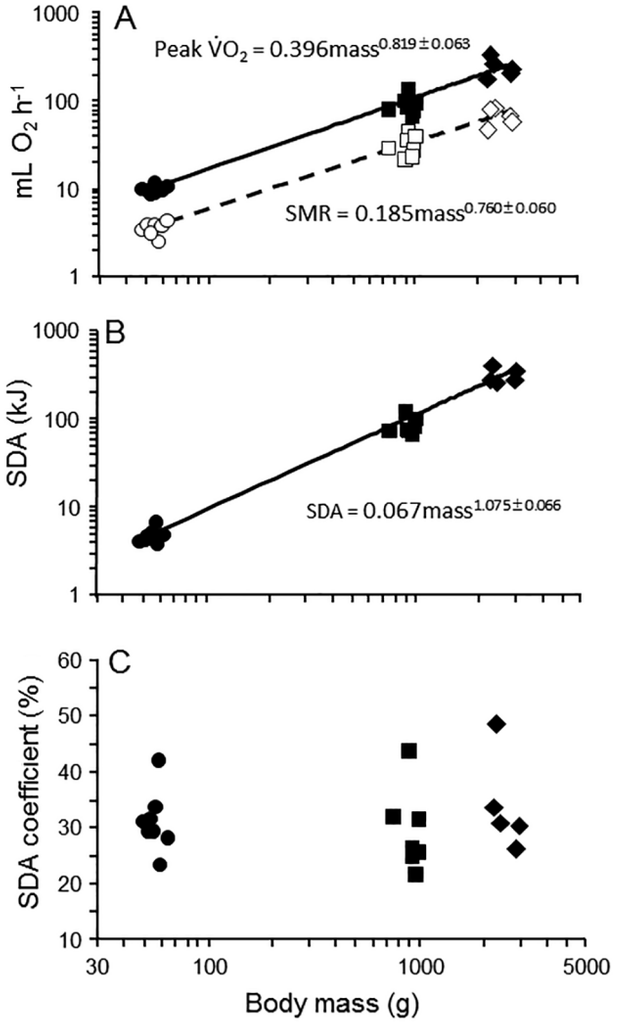

Figure 2. Allometric scaling of standard metabolic rate (SMR) and peak postprandial $\dot{V}O_2$ (*A*) and specific dynamic action (SDA; *B*), and body size effects on SDA coefficient (*C*) for neonate (circle), juvenile (square), and subadult (diamond) American alligators, *Alligator mississippiensis*. Body mass, SMR, and peak $\dot{V}O_2$ were log transformed before generating allometric equations for SMR, peak $\dot{V}O_2$, and SDA. Mass exponents in this figure and subsequent figures are presented with a ±95% confidence interval.

or on enterocyte length, width, or volume (table 3). Age class (neonate vs. subadults) alone (not treatment) had a significant impact on both proximal mucosal/submucosal ($F_{1,10} = 8.15$, $P < 0.025$) and muscularis/serosal ($F_{1,10} = 152$, $P < 0.0001$) thickness; in both cases, tissue layers were thicker for subadult alligators (table 3). In contrast, it was treatment and not age class that significantly impacted proximal enterocyte width ($F_{1,10} = 13.0, P < 0.009$) and volume ($F_{1,10} = 16.0, P < 0.0055$), greater in both cases for fed subadults (table 3). For subadults, fed individuals possessed (all $P < 0.049$) taller, wider, and larger-volume enterocytes than fasted individuals for the middle small intestine and larger-volume enterocytes for the distal small intestine (table 3). We observed no treatment effect on microvillus length or width (table 3). However, there was a significant age class effect on microvillus length ($F_{1,10} = 8.19, P < 0.03$; subadult > neonate).

Digestive Enzyme Activities

Trypsin. A two-way ANOVA identified significant age class effects ($F_{2,13} = 8.97$, $P < 0.0042$), as well as a significant interaction between age class and feeding treatment ($F_{2,13} = 5.63$, $P < 0.019$) on pancreatic trypsin activity. Trypsin activity of fed juveniles was significantly (all $P < 0.035$) greater than that for fasted juveniles and fasted and fed subadults (fig. 5*A*). There was a significant main effect of age class ($F_{2,13} = 10.4$, $P < 0.003$) on total pancreatic trypsin capacity (neonate < juvenile, subadult). We found feeding to have no impact on trypsin capacity regardless of age class (fig. 5*B*).

Aminopeptidase-N. For fasted (though not for fed) subadult alligators, APN activity varied significantly ($F_{2,7} = 8.22$, $P < 0.039$) among the three regions of the small intestine, as proximal activity was significantly ($P < 0.036$) greater than distal activity (fig. 6*A*). For each intestinal position, activity did not differ statistically (although marginally, $0.052 <$ all $P < 0.079$) between fasted and fed subadults (fig. 5*A*). There was a significant age class effect ($F_{2,13} = 68.9, P < 0.0001$) on proximal intestinal APN activity, as activity differed significantly between each age class (all $P < 0.010$; subadults < neonates < juveniles; fig. 6*B*). Although there was no treatment effect on APN activity among age classes, total APN capacity was significantly ($F_{1,5} = 32.3$, all $P < 0.011$) greater for fed compared with fasted subadults (fig. 6*C*).

Intestinal Nutrient Uptake

Positional Effects. Uptake rates of L-leucine varied significantly ($F_{2,7} = 45.5, P < 0.002$) only among intestinal positions for fed juveniles (fig. 7). Uptake rates of L-proline varied significantly among positions in three cases: fasted neonates ($F_{2,7} = 12.9$, $P < 0.018$), fed juveniles ($F_{2,7} = 23.8$, $P < 0.006$), and fasted subadults ($F_{2,7} = 106, P < 0.0003$; fig. 7). For D-glucose, uptake rates varied significantly ($F_{2,7} = 17.4-72.9$, all $P < 0.011$) among the three regions in all cases, with the exception of fasted subadults (fig. 7). In general, there was a decreasing gradient in uptake rates from proximal to distal regions; for example, D-glucose uptake rates by the distal region averaged 7% ± 3% of rates measured for the proximal region.

Age Class and Feeding Effects. We conducted a two-way ANOVA to determine the individual effects and interactions of age class and fasting/feeding treatments on nutrient uptake for each region of the small intestine. A significant interaction between the main effects was detected for L-leucine ($F_{2,13} = 4.13$, $P < 0.044$) and D-glucose ($F_{2,13} = 6.05, P < 0.016$) uptake for the middle intestinal region. A significant ($F_{2,13} = 11.1-92.0$, all $P < 0.001$) age class effect was identified for proximal intestinal uptake for the three nutrients, L-proline uptake by the middle intestine, and distal uptake for both L-leucine and L-proline.

Table 2: Body mass, body size, and empty wet and dry organ masses of neonate, juvenile, and subadult American alligators, *Alligator mississippiensis*, fasted and at 2 d postfeeding (2DPF)

	Neonate		Juvenile		Subadult	
	Fasted	2DPF	Fasted	2DPF	Fasted	2DPF
N	3	3	3	3	3	3
Body mass (g)	44.3 ± 3.6	47.0 ± 2.8	904 ± 39	905 ± 20	3,333 ± 954	2,958 ± 216
Snout-vent length (cm)	13.4 ± .7	13.7 ± .3	35.7 ± .4	33.0 ± 1.0	53.2 ± 4.7	51.2 ± 1.4
Total length (cm)	27.7 ± .7	27.9 ± .4	73.8 ± .9	70.8 ± 1.7	109 ± 10	107 ± 2
Wet mass (g):						
Esophagus[I]	.296 ± .046	.370 ± .068	4.31 ± .06	6.02 ± 1.66*	11.8 ± 3.0	14.4 ± .6***
Heart[A]	.242 ± .069	.192 ± .014	3.06 ± .34	3.39 ± .23	6.72 ± 2.68	4.80 ± .44
Lung[I]	.338 ± .080	.450 ± .011	5.36 ± .51	4.65 ± .25	22.1 ± 10.3	12.8 ± .7
Liver[A]	.678 ± .109	.864 ± .059	15.9 ± 2.8	15.0 ± 1.9	30.6 ± 10.5	25.0 ± 1.4
Stomach[I]	1.30 ± .12	1.87 ± .06*	15.5 ± .2	19.0 ± .5*	46.9 ± 13.4	47.9 ± 4.4
Pancreas[A]	.045 ± .004	.047 ± .003	.716 ± .195	.842 ± .169	1.86 ± .89	1.59 ± .21
Gall bladder[A]	.058 ± .030	.034 ± .010	.558 ± .133	.736 ± .022	.751 ± .275	.530 ± .008
Spleen	.030 ± .006	.039 ± .004	.828 ± .114	.640 ± .068	2.33 ± .76	1.88 ± .13
Small intestine[A]	.714 ± .045	.775 ± .032	16.9 ± 4.6	13.6 ± 2.3	37.6 ± 18.7	24.9 ± 1.6
Large intestine[A]	.233 ± .012	.352 ± .030*	3.87 ± .08	3.64 ± .26	15.4 ± 6.8	12.1 ± 1.4
Kidney[A]	.296 ± . 010	.332 ± .008	3.64 ± .36	4.01 ± .19	7.91 ± 2.87	7.71 ± .95
Dry mass (g):						
Esophagus[I]	.049 ± .007	.053 ± .007	.788 ± .002	.987 ± .250*	2.19 ± .49	2.64 ± .10**
Heart[A]	.032 ± .008	.027 ± .002	.469 ± .046	.538 ± .055	...	...
Lung[I]	.046 ± .011	.058 ± .003	.921 ± .076	.737 ± .059	3.28 ± 1.57	1.76 ± .11
Liver[A]	.195 ± .039	.242 ± .015	4.21 ± .66	3.29 ± .69	7.85 ± 2.42	5.81 ± .40
Stomach[A]	.197 ± .013	.233 ± .011	2.65 ± .02	2.81 ± .07	8.00 ± 2.51	6.72 ± .58
Pancreas[A]	.010 ± .001	.010 ± .001	.153 ± .041	.146 ± .047	.415 ± .210	.291 ± .054
Gall bladder[A]	.009 ± .003	.008 ± .003	.110 ± .026	.105 ± .035	.154 ± .055	.088 ± .007
Spleen[A]	.007 ± .002	.009 ± .001	.190 ± .038	.136 ± .015*	.521 ± .176	.418 ± .023
Large intestine[A]	.035 ± .001	.050 ± .003*	.647 ± .016	.589 ± .037	2.51 ± 1.10	1.74 ± .20
Kidney[A]	.059 ± .002	.066 ± .003	.716 ± .070	.726 ± .032	1.52 ± .54	1.33 ± .18

Note. Values are presented as means ±1 SE. Superscript A identifies organs for which there is a significant main effect of age and no significant interaction as determined from a two-way ANCOVA (with body mass as the covariate). Superscript I identifies organs for which there was a significant interaction between age and treatment. For each age class, a one-way ANCOVA (with body mass as the covariate) identified organ wet and dry masses that differed between fasted and fed (2DPF) alligators. Statistically significant differences between fasted and fed for the age classes are indicated with asterisks.

*$P < 0.05$.

**$P < 0.01$.

***$P < 0.001$.

For fasted and fed intestine, mass-specific nutrient uptake rates tended to decrease with an increase in age, as noted by the fact that intestinal nutrient uptake rates of subadults averaged 29% ± 3% of rates measured for neonates (fig. 7). In only one instance was there a significant ($F_{1, 13} = 4.98$, $P < 0.046$) effect of feeding treatment on uptake rates, with fed individuals possessing lower rates of L-leucine uptake by the distal intestine (fig. 7).

Uptake Capacities. There were no significant effects (or significant interaction) of age class or feeding treatment on the summed uptake capacity of the small intestine for each nutrient (fig. 8). Intestinal uptake capacities of L-leucine, L-proline, and D-glucose scaled allometrically (all $r^2 > 0.735$, all $P < 0.0001$) with body mass, with respective mass exponents of 0.631 ± 0.095, 0.518 ± 0.080, and 0.614 ± 0.187 (fig. 9). The decreases in capacity relative to body mass with age are a function of the allometric scaling of the small intestine (mass exponent = 0.887 ± 0.096) and the body size reduction of mass-specific nutrient uptake rates (fig. 7).

Discussion

We introduced this study with the hypothesis that an ontogenetic shift in diet and feeding habits for American alligators is coupled with an ontogenetic change in the extent that gastrointestinal performance is regulated. To support our hypothesis, we leveraged published information on alligator feeding habits and known descriptions of ontogenetic shifts in digestive structure and function associated with shifts in diet. A prediction of this hypothesis is that with an increase in meal size and a

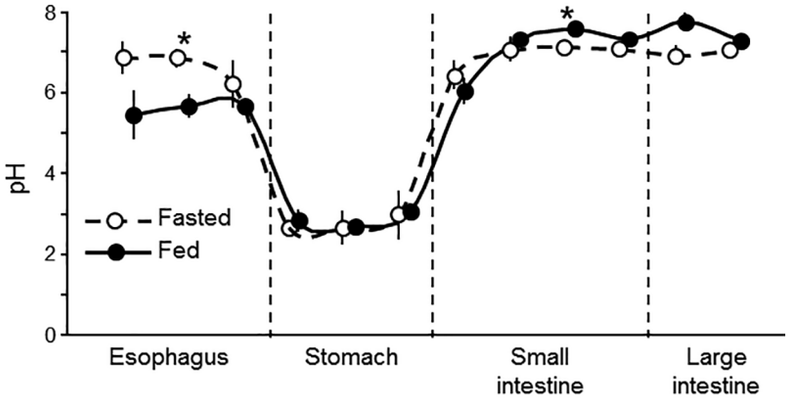

Figure 3. Profile of luminal pH measured at 12 sites within the gastrointestinal tract for fasted and fed (2 d postfeeding) subadult American alligators, *Alligator mississippiensis*. Note the acidic nature of the stomach for fasted and fed animals. Asterisks note regions of the gastrointestinal tract where pH differed significantly (one-way ANOVA, $P < 0.05$) between fasted and fed alligators.

decrease in feeding frequency, larger alligators experience a greater regulatory response to feeding and fasting. However, we failed to identify a collective ontogenetic change, or even a size-related trend, in the degree that alligators respond to feeding or the extent that intestinal performance is regulated. Across these three age classes of alligators, our alternative hypothesis appears the most sound—that the patterns and mechanisms of postprandial responses are conserved ontogenetically for alligators. In the ensuing discussion, we comment on the alligator's postprandial metabolic, morphological, and functional responses. We interpret from these findings the adaptive interplay between the alligator's feeding habits and their digestive physiology and note a potential caveat that may undermine the demonstration of an ontogenetic shift in their digestive regulation.

Metabolic Responses to Feeding

Alligators experience the characteristic profile of postprandial metabolism: a rapid increase in gas exchange that peaks within 2 d and then declines more slowly to baseline levels (Secor 2009). The magnitude and duration of the postprandial metabolic response and SDA are heavily influenced by meal size and composition (Secor 2009). Therefore, cross-study comparisons are best conducted when experimental meal size and type are similar. Alligators (~700 g) fed fish meals that were 5% of their body mass experienced a scope of peak $\dot{V}O_2$ of 2.95 (2.92 in this study for juveniles), a duration of 5.2 d (6 d in this study), and an SDA (43.7 kJ) that was equivalent to 17.8% of meal energy (Coulson and Hernadez 1979). The higher predicted SDA (~77 kJ for a 700-g alligator from fig. 2*B*) and SDA coefficient (fig. 2*C*) observed in this study may reflect the greater effort expended to digest intact rodents (fed in this study) compared with strips of filleted fish fed to alligators in Coulson and Hernandez (1979). Alligators responded to larger meals (7.5% and 10% of body mass) with postprandial scopes of peak $\dot{V}O_2$ that rose to 3.68 and 4.17, respectively (Coulson and Hernandez 1983; Busk et al. 2000). Neonates in this study, when fed rodent meals that were 10% of their body mass, experienced a peak $\dot{V}O_2$ of 3.54 (vs. 2.81 for 5% meals), a duration of elevated metabolism of 6 d (4 d for 5% meals), and an SDA of 12.98 kJ (5.33 kJ for 5% meals). Reptiles characteristically experience a little more than a doubling (averaging 120% increase) of SDA with a doubling of relative meal size, in part because of a greater postprandial peak and longer duration of elevated metabolism (Secor 2009).

For the saltwater crocodile (*Crocodylus porosus*) and broad-nosed caiman (*Caiman latirostris*), meals of chicken necks equaling 3% and 11.5% of body mass generated more modest increases in $\dot{V}O_2$ of 101% and 63%, respectively (Starck et al. 2007; Gienger et al. 2012). One explanation for the smaller increase in postprandial $\dot{V}O_2$ in these studies compared with our study is the smaller relative meal size (for the former study) and the fact that chicken necks may take less effort to digest compared with intact rodents (for both studies).

An alternative metric to quantifying the cost of digestion is to express SDA as a percentage of meal energy, termed the "SDA coefficient" (McCue 2006; Secor 2009). After combining the data of this study with those of Coulson and Hernandez (1979, 1983), we find SDA to increase linearly as a function of meal energy with a scope of 0.31 (fig. 10*A*). When digesting intact prey, alligators expend an equivalent of approximately 30% of the meals' energy to fuel that meal's digestion and assimilation. Across a broad taxonomic range of invertebrates and vertebrates, 30% is at the high end of documented SDA coefficients, similar to values calculated for a few fishes, anurans, and infrequently feeding snakes (Secor 2009).

How do the postprandial metabolic response and SDA of alligators compare with those of other reptiles? Similar to the Burmese python and corn snake (*Pantherophis guttatus*), the alligator's postprandial peak $\dot{V}O_2$ scaled at a greater mass exponent than SMR; thus, the scope of peak $\dot{V}O_2$ increases with body mass (Secor and Diamond 1997; Crocker-Buta and Secor 2014). Such scaling relationships have also been observed for amphibians and resemble the noted greater allometric scaling of exercise-induced $\dot{V}O_{2\,max}$ compared with SMR documented for lizards (Garland

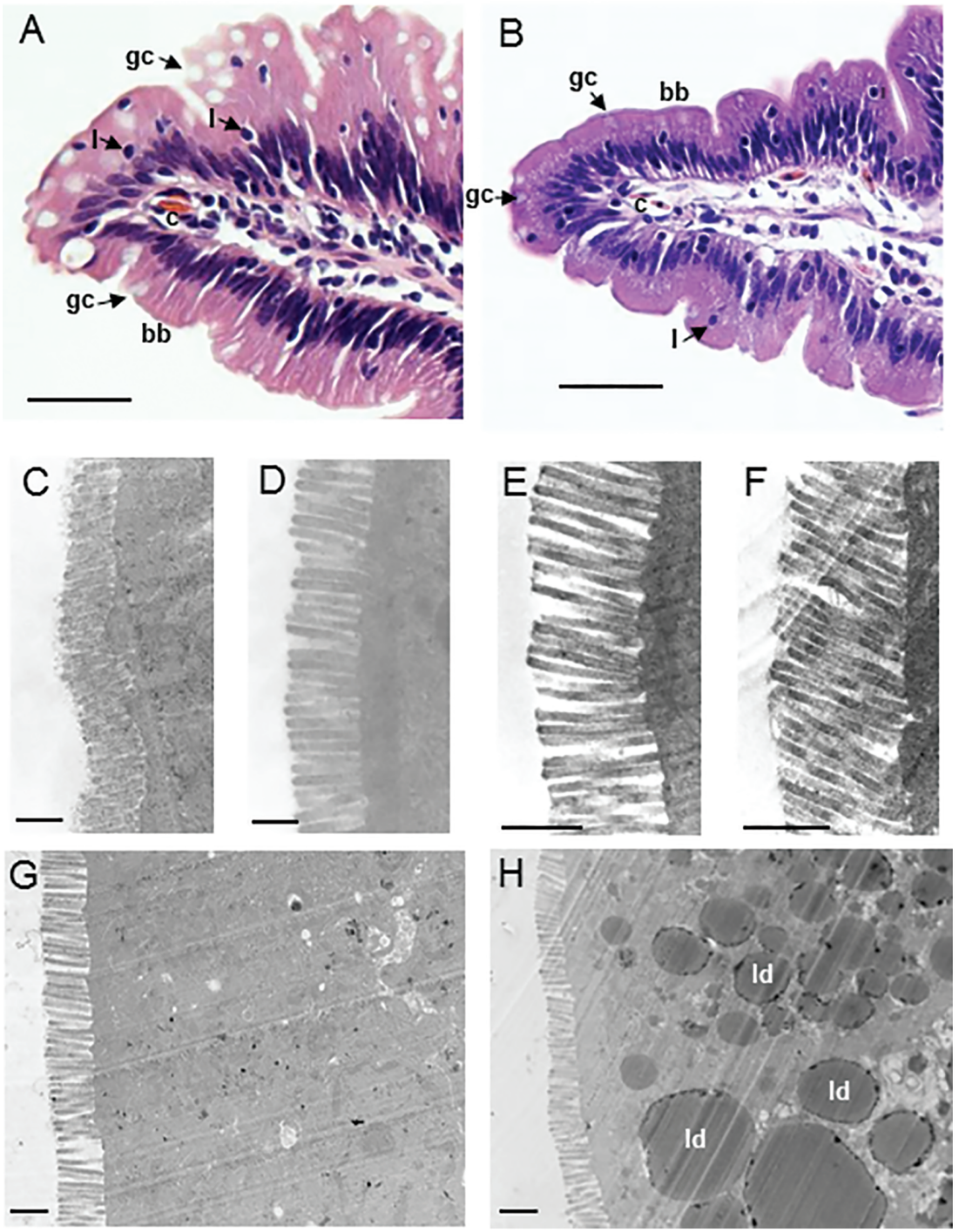

Figure 4. Light microscopy images of intestinal villi demonstrating the pseudostratified arrangement (overlapping, bunched nuclei) of enterocytes for fasted (*A*) and the stratified arrangement (parallel, single layer, nuclei) of enterocytes for fed (*B*) subadult American alligators, *Alligator mississippiensis*, and transmission electron microscopy images of intestinal microvilli of fasted (*C*) and fed (*D*) neonate and fasted (*E*) and fed (*F*) subadult alligators. Note the lack of a noticeable change in microvillus length with feeding. The apical ends of enterocytes are devoid of lipid droplets during fasting (*G*) and become filled with lipids during meal digestion (*H*). bb = brush border; c = capillary; gc = goblet cell; l = lymphocyte; ld = lipid droplet. Scale bars: *A*, *B* = 50 μm; *C*–*H* = 1 μm.

Table 3: Thickness of the mucosa/submucosa and muscularis/serosa layers; height, width, and volume of enterocytes; and height and width of microvilli for the proximal small intestine of a neonate American alligator, *Alligator mississippiensis*, and for the proximal, middle, and distal small intestine of a subadult American alligator, fasted and at 2 d postfeeding (2DPF)

	Neonate		Subadult	
	Fasted	2DPF	Fasted	2DPF
Proximal:				
Mucosa/submucosa thickness (μm)[A]	200 ± 27	235 ± 30	557 ± 39	595 ± 38
Muscularis/serosa thickness (μm)[A]	534 ± 52	589 ± 20	2,183 ± 302	1,969 ± 80
Enterocyte height (μm)	29.4 ± 3.7	30.3 ± 3.0	34.7 ± 3.4	42.2 ± 2.1
Enterocyte width (μm)[T]	4.99 ± .28	5.48 ± .22	4.50 ± .37	5.84 ± .13*
Enterocyte volume (μm^3)[T]	227 ± 15	265 ± 31	242 ± 8	391 ± 26*
Microvillus length (nm)[A]	920 ± 129	953 ± 139	1,333 ± 259	2,053 ± 214
Microvillus width (nm)	113 ± 1	124 ± 2*	109 ± 1	99 ± 10
Middle:				
Mucosa/submucosa thickness (μm)	...	...	558 ± 241	649 ± 51
Muscularis/serosa thickness (μm)	...	...	2,225 ± 683	1,618 ± 90
Enterocyte height (μm)	...	...	30.0 ± 1.9	39.0 ± .4*
Enterocyte width (μm)	...	...	4.30 ± .23	5.55 ± .13*
Enterocyte volume (μm^3)	...	...	202 ± 23	340 ± 10*
Distal:				
Mucosa/submucosa thickness (μm)	...	...	349 ± 58	530 ± 58
Muscularis/serosa thickness (μm)	...	...	2,122 ± 648	1,481 ± 63
Enterocyte height (μm)	...	...	34.2 ± 1.9	40.1 ± 2.3
Enterocyte width (μm)	...	...	4.97 ± .07	5.37 ± .11
Enterocyte volume (μm^3)	...	...	267 ± 13	337 ± 14*

Note. Mean body masses are presented in table 2. Values are presented as means ± 1 SE. Superscript A or T identifies organs for which there is a significant main effect of age (A) or treatment (T) and no significant interaction as determined from a two-way ANCOVA (with body mass as the covariate). For each age class, a one-way ANCOVA (with body mass as the covariate) identified those variables that differed between fasted and fed (2DPF) alligators. Statistically significant differences between fasted and fed for the age classes are indicated with an asterisk.

*$P < 0.05$.

1984; Garland and Else 1987). Whereas SMR and peak $\dot{V}_{O_2}$ scale allometrically for alligators, SDA scales isometrically, as it does for other species of reptiles and for amphibians (Secor and Diamond 1997; Secor and Faulkner 2002; Secor and Boehm 2006; Crocker-Buta and Secor 2014). To assess interspecific scaling of SDA for reptiles, we included data (published and unpublished) for 15 additional species (two turtles, three lizards, and 10 snake species) that had consumed pieces of meat (turtles) or rodent meals equaling 5% of their body mass. For these species (body mass = 54.5–2,530 g), SDA scaled with a mass exponent of 1.120 ± 0.122 (fig. 10*B*), which is not significantly different than 1.0 and similar to the interspecific isometric scaling described for snakes and amphibians (Secor and Boehm 2006; Secor et al. 2007; Secor 2009).

Postprandial Response of Organ Mass and Allometry

We observed only modest differences in the wet and dry masses of organs between fasted and fed individuals per age class. For those that did significantly differ, there was little consistency among age classes. Likewise, Slay (2015) observed no significant differences in the dry masses of the liver, small intestine, and large intestine between 60-d-fasted and 3-d-fed alligators (mean mass = 2.1 kg). However, fed individuals did possess significantly heavier (dry mass) stomachs and kidneys (Slay 2015). Significant postprandial changes in the wet mass of the liver and small intestine have been observed for the broad-nosed caiman (Starck et al. 2007). When measured repeatedly via ultrasonography for up to 24 d after feeding, the livers of these caimans did experience a 30% increase (although not significant) in size (Starck et al. 2007).

The relatively modest postprandial responses in organ masses experienced by the American alligator are comparable to those noted for fishes, amphibians, and other reptiles, including turtles, lizards, and frequently feeding snakes (Secor and Diamond 1999, 2000; Secor 2005*b*; Tracy and Diamond 2005; Christel et al. 2007; Cox and Secor 2010; Day et al. 2014). In contrast, the liver, pancreas, small intestine, and kidneys of infrequently feeding snakes increase significantly in mass (as much as 100%) with feeding and undergo atrophy once digestion is completed (Secor and Diamond 2000; Ott and Secor 2007*a*). It is hypothesized that the fasting-induced tissue atrophy and downregulation of organ function is an adaptive response to reduce metabolic expenditure during the predicted long episodes between meals experienced by infrequently feeding snakes (Secor and Diamond 2000; Secor 2001). For each of the studied age classes of American alligators, the lack of any discernible postprandial increases in organ mass (and, to be discussed later, in intestinal function) is suggestive that

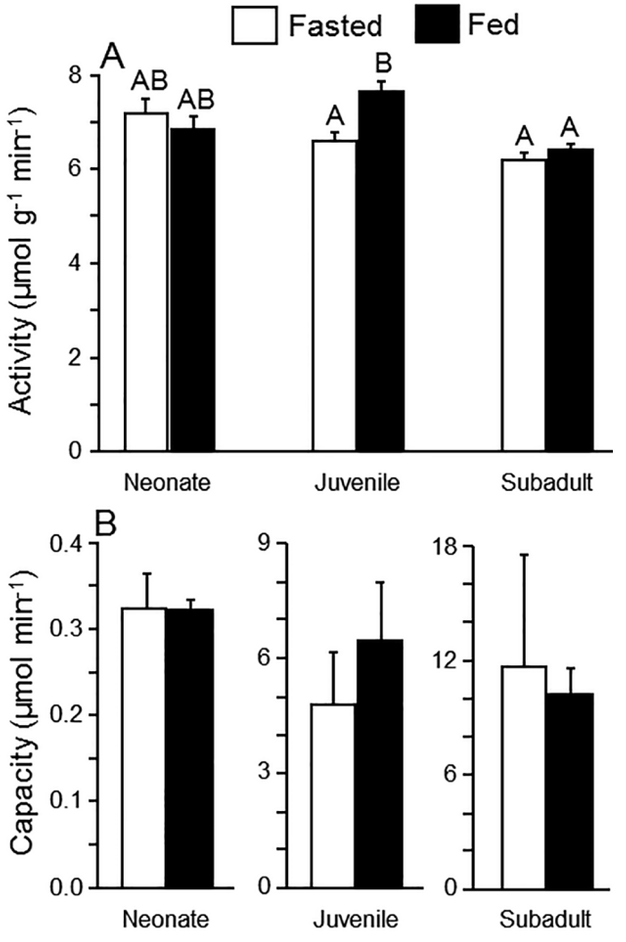

Figure 5. Pancreatic trypsin activity (*A*) and capacity (*B*) for fasted and fed (2 d postfeeding) neonate, juvenile, and subadult American alligators, *Alligator mississippiensis*. For both trypsin activity and capacity, a two-way ANOVA/ANCOVA identified the main effects of age class but not the main effects of treatment (fasted vs. fed). A significant interaction ($P < 0.019$) between age class and treatment effects for trypsin activity revealed that activity for fed juveniles was greater than that for fasted juveniles and fasted and fed subadults. Uppercase letters above activity bars denote significant ($P < 0.05$) differences among means for fasted and fed activities of the three age classes (Tukey's honestly significant difference test).

as alligators transition between fasting and digesting, organ performance remains fairly stable.

For the alligators of this study, the combined wet mass of most organs for fasted and fed individuals, with the exception of the spleen, scaled allometrically (all $r^2 > 0.830$, all $P < 0.0001$) with body mass, exhibiting mass exponents that ranged between 0.704 ± 0.168 (empty gall bladder) and 0.895 ± 0.060 (large intestine). However, given that our largest individual weighed 5,240 g—a fraction of the mass of a large adult male alligator—our scaling relationships may not be indicative of the entire size range of alligators. We supplemented this analysis with wet masses of the heart, liver, spleen, stomach, small intestine, and kidneys for nine individuals (0.13–99.0 kg) reported in Coulson and Hernandez (1983) and wet masses of the liver, spleen, and kidney for seven additional individuals (11.3–49.9 kg) wild caught in southwestern Louisiana in 1999 (R. Elsey, unpublished data). For this larger data set, the heart, liver, stomach, spleen, and kidney wet masses each scaled allometrically with body mass, whereas small intestinal wet mass scaled isometrically (fig. 11). Allometric scaling (with $b < 1.0$) in heart, lung, liver, and kidney wet masses has also been observed for hatchling female alligators, with a noted biphasic scaling of kidney and liver masses (Eme et al. 2019). For those two organs, there was a distinct break point, as individuals smaller than 120 g exhibited scaling exponents greater than 1.0, whereas larger individuals (120–500 g) exhibited scaling exponents less than 1.0. These findings draw attention to the need to be cognizant of organ-specific scaling relationships when analyzing size-dependent changes in organ performance.

Gastrointestinal pH

Luminal pH of the subadult alligator gut varied regionally due to the highly acidic nature of their stomach. For the esophagus, small intestine, and large intestine, luminal pH on average was nearly neutral (6.76 ± 0.11), whereas for both fasted and fed individuals, luminal pH of the proximal, middle, and distal regions of the stomach averaged 2.82 ± 0.13. Measurements taken at the distal end of the esophagus (within 2 cm of the stomach) and the proximal end of the small intestine (within 3 cm caudal to the pyloric sphincter) demonstrate a sharp gradient in luminal pH in the transitions from esophagus to stomach and from stomach to small intestine (fig. 3). Digesting Burmese pythons likewise experience such gradients in luminal pH at 2 d postfeeding, decreasing from 6.4 to 3.2 over a 3-cm span (1.5 cm anterior to 1.5 cm distal of the gastroesophageal junction) and increasing from 2.7 to 6.6 over a 5-cm span from the caudal end of the stomach to within 2 cm of the start of the small intestine (Bessler and Secor 2012; S. Secor, personal observation). Uriona et al. (2005) also observed the slight acidification of the alligator's distal esophagus, noting that for both fasted and fed individuals, luminal pH that was measured 3 cm proximal to the lower esophageal sphincter would occasionally drop below 5.5 (we observed this for two of our animals) and for a fed individual decreased to below 4.0. For the distal esophagus, protection against the damaging effects of acid reflux is provided at least in part because of mucous secreted by cells that populate the mucosal epithelium (Uriona et al. 2005).

Alligators do differ from snakes in maintaining an acidic stomach between meals. We observed no differences in luminal pH for each region of the stomach between fasted and fed individuals, a finding supported by Uriona et al. (2005). Snakes, on the other hand, shut down HCl production with the completion of gastric digestion and maintain a near neutral pH within the stomach until the next meal (Secor 2003; Bessler and Secor 2012; Secor et al. 2012). The production of HCl is expensive because of the hydrolyzation of a single ATP for each H^+ pumped into the gastric lumen (Reenstra and Forte 1981; Norberg and Mårdh 1990; Helander and Keeling 1993). Hence, the downregulation of acid production between meals observed for snakes, turtles, and teleost fishes is considered an energy-conserving adaptation that reduces basal metabolism, thereby increasing the duration that individuals can

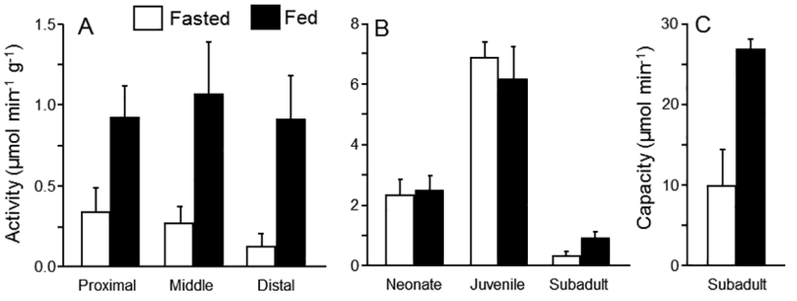

Figure 6. *A*, Aminopeptidase-N (APN) activity of the proximal, middle, and distal segments of the small intestine for fasted and fed (2 d postfeeding) subadult American alligators, *Alligator mississippiensis*. For fasted (though not for fed) subadult alligators, a decreasing gradient in APN activity is exhibited from proximal to distal regions (one-way ANOVA, $P < 0.036$; proximal > distal). *B*, APN activity of the proximal small intestine for fasted and fed neonate, juvenile, and subadult alligators. A two-way ANOVA revealed a significant age class effect, as each age class differed from the other two (Tukey, all $P < 0.010$; juvenile > neonate > subadult). However, there was no treatment effect or significant interaction. *C*, Fed subadult alligators experienced a significantly ($P < 0.011$) greater APN capacity than fasted individuals.

survive metabolizing only endogenous energy stores (e.g., fat; MacKay 1929; Fox and Musacchia 1959; Montgomery and Pollak 1988; Secor 2003). The alligator condition of maintaining HCl production between meals appears to be the dominant phenomenon among vertebrates, observed for fasting chondrichthyes, anurans, lizards, birds, and mammals (Ford 1974; Youngberg et al. 1985; Evans et al. 1988; Gauthier-Clerc et al. 2002; Papastamatiou and Lowe 2004; Papastamatiou et al. 2007; S. Secor, unpublished observations). Alligators and most other vertebrates habitually pay the cost of constant acid production for reasons that may

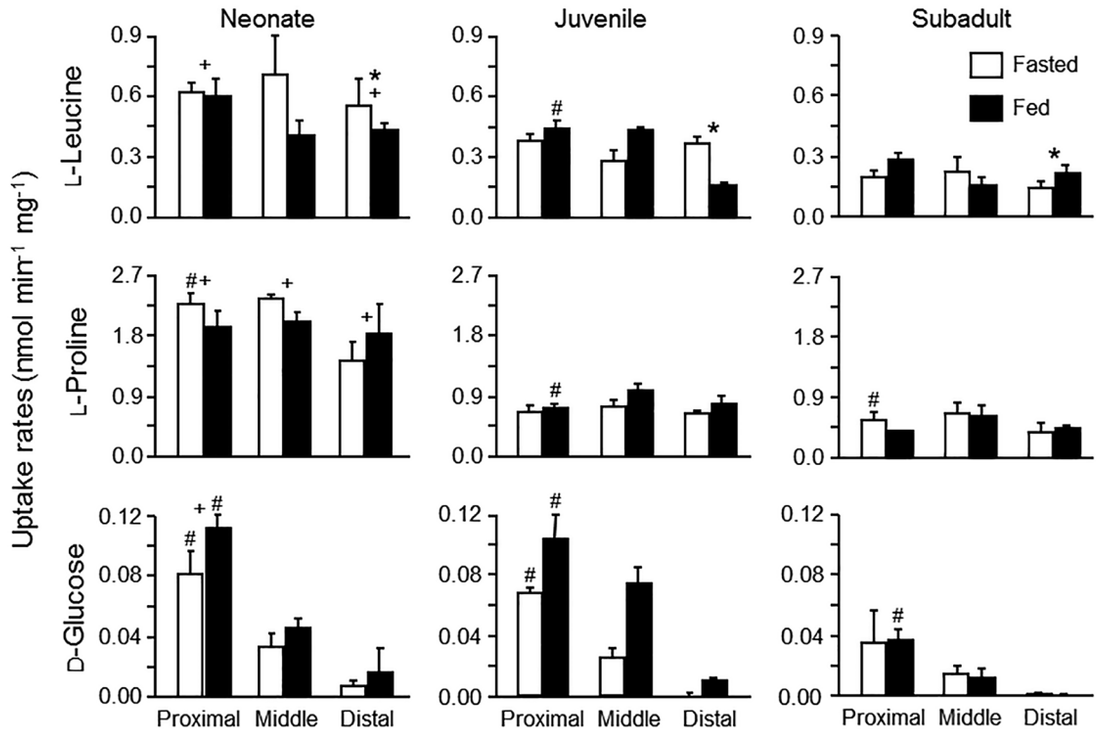

Figure 7. Uptake rates of L-leucine, L-proline, and D-glucose for each intestinal one-third for fasted and fed (2 d postfeeding) neonate, juvenile, and subadult American alligators, *Alligator mississippiensis*. Significant variation among intestinal position for each age class, nutrient, and treatment (fasted or fed) as determined from a repeated-measures ANOVA is designated with a number sign positioned above the bar for the proximal segment. Significant age class effects (two-way ANOVA with age class and treatment as main effects) for each nutrient and position are identified with a plus sign above the bars for neonates. Significant treatment effects for each nutrient and position are designated with an asterisk above the bars for each age class. Alligators in this study exhibited significant variation (nine of 18 nutrient/treatment/age class combinations) in nutrient uptake rates across intestinal position (especially for D-glucose) and significant age class effects (six of nine nutrient/position combinations). However, the only significant effect of treatment occurred for L-leucine uptake by the distal intestine.

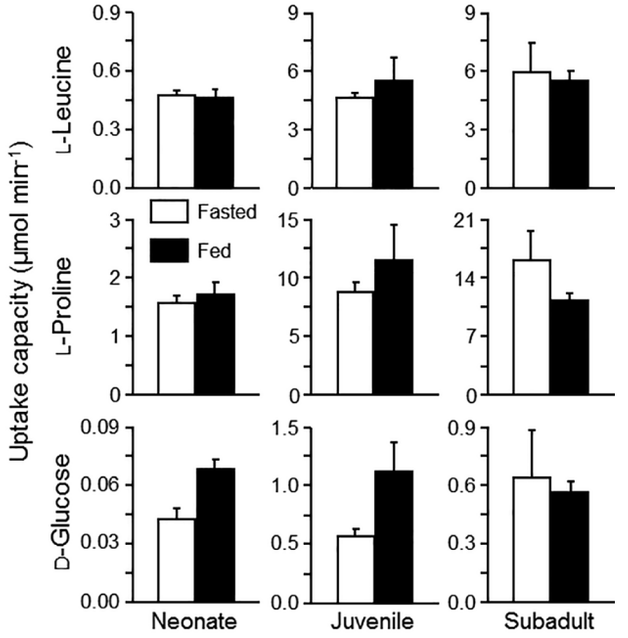

Figure 8. Intestinal L-leucine, L-proline, and D-glucose uptake capacities for fasted and fed (2 d postfeeding) neonate, juvenile, and subadult American alligators, *Alligator mississippiensis*. Alligators of this study did not exhibit either age class or treatment effects on intestinal uptake capacity for each of the three nutrients.

include their higher frequency of feeding (no need to constantly regulate) and as protection against the colonization of ingested bacteria.

Fed subadult alligators in this study did experience a significantly higher whole-blood pH compared with fasted individuals. For fed alligators, a higher blood pH, in conjunction with a rise in plasma HCO_3^-, has been identified as the acid-base phenomenon of an "alkaline tide" (Coulson et al. 1950). Although not measured in this study, feeding expectedly generated a reciprocal increase in HCO_3^- and decrease in Cl^- stemming from gastric acid production, as observed in other studies (Coulson and Hernandez 1983; Busk et al. 2000). We are cautious, however, in concluding that alligators experience a postprandial alkalinization of their blood given that our fasting blood pH averaged noticeably lower than previously documented for fasting individuals and that our fed values are characteristic of fed alligators (Coulson and Hernandez 1983; Busk et al. 2000). In short, we concur that while alligators do experience postprandial increases in circulating HCO_3^-, their blood pH remains relatively stable (Busk et al. 2000).

Intestinal Histology

The cellular histology of the intestinal epithelium has been well described in detail for both the American alligator and the broad-nosed caiman (Starck et al. 2007; Tracy et al. 2015). Our observations complement those two reports—specifically, the postprandial transition from a pseudostratified to a stratified arrangement of enterocytes, the filling at the apical ends of enterocytes with lipid droplets, and the positioning of nuclei along the basal margin of enterocytes. Identified in these studies is the fact that the mucosal epithelium of crocodilians possesses a scattering of intra- and extracellular lymphocytes with goblet cells interspersed between enterocytes.

The general histology and postprandial changes of the crocodilian intestinal epithelium is shared with other reptiles, as well as fishes and amphibians (Secor 2005*b*; Cox and Secor 2010; Day et al. 2014). A seemingly ubiquitous morphological phenomenon experienced by these vertebrates is the fasting-induced atrophy of the intestinal epithelium. As enterocytes shrink (usually reducing width), there is a tendency for cells to overlap the apical ends of adjoining cells, thereby generating a pseudostratified arrangement

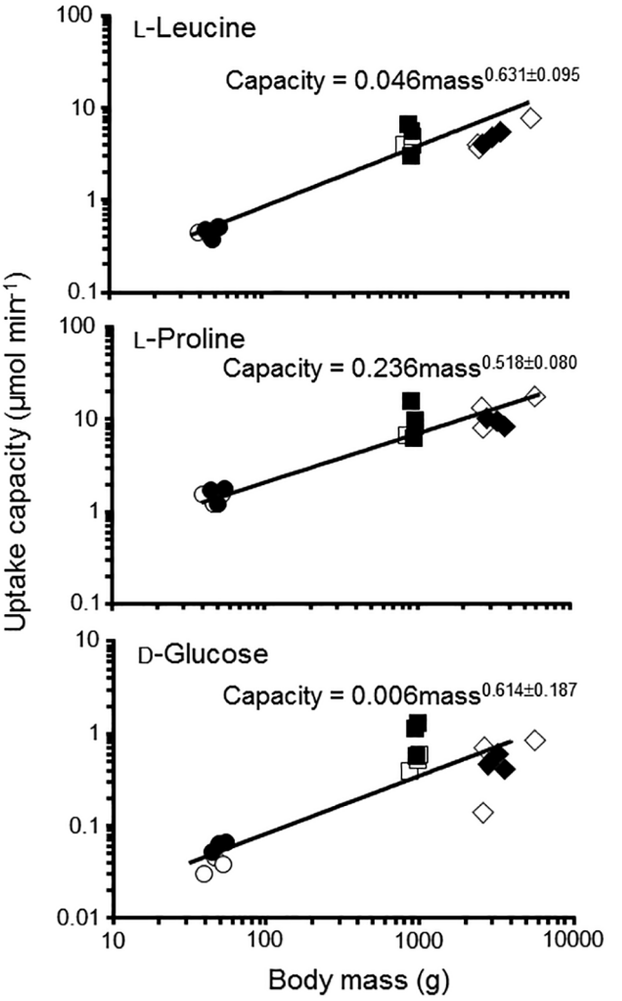

Figure 9. Allometric scaling of intestinal uptake capacities for L-leucine, L-proline, and D-glucose across three age classes of the American alligator, *Alligator mississippiensis*. Fasted and fed individuals are respectively noted with open and filled symbols for neonates (circles), juveniles (squares), and subadults (diamonds). Body mass and uptake capacities were log transformed prior to generating allometric equations. Alligators of this study exhibited negative allometry of uptake capacities, whereby an increase in body mass was not matched by reciprocal increases in small intestinal nutrient uptake capacities.

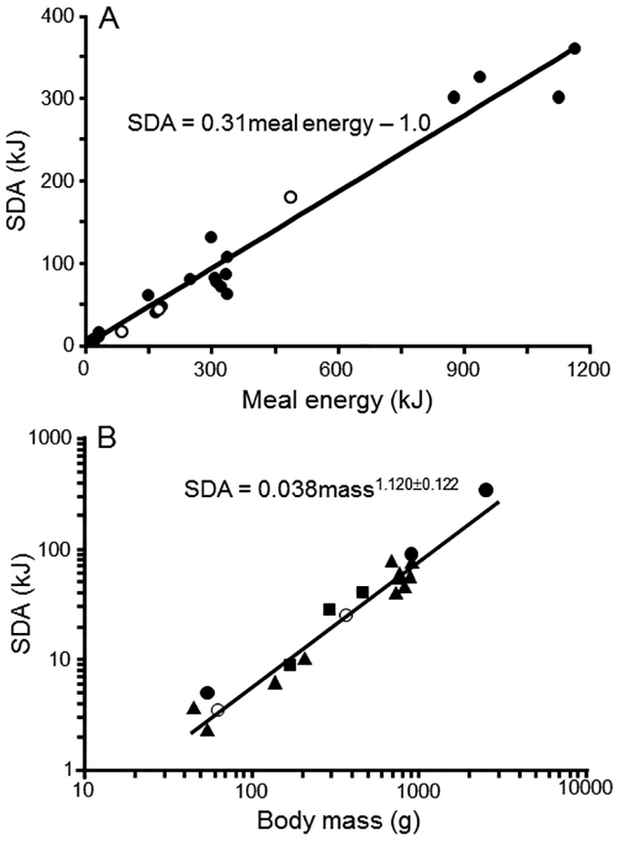

Figure 10. *A*, Specific dynamic action (SDA; kJ) plotted against meal energy for the American alligators (*Alligator mississippiensis*) of this study (filled circles) and data extracted from Coulson and Hernandez (1979, 1983; open circles). The slope of this line (0.31) identifies that alligators expend an equivalent of 31% of meal energy digesting and assimilating their meals. *B*, SDA (kJ) plotted against body mass of the three age classes of alligators in this study (filled circles), 10 species of snakes (filled triangles), three species of lizards (filled squares), and two species of turtles (open circles). All species consumed beef or rodent meals equaling 5% of their body mass. Among these 16 species of reptiles, SDA scales isometrically with body mass. Data were retrieved from Secor and Diamond (1997), Ott and Secor (2007*a*, 2007*b*), Bessler et al. (2010), Crocker-Buta and Secor (2014), and S. Secor (personal observation) for snakes; from Secor and Diamond (1999) for turtles; and from Christel (2007) and S. Secor (personal observation) for lizards.

(Starck and Beese 2001, 2002; Day et al. 2014). Feeding stimulates epithelial hypertrophy, and as enterocytes increase in width and volume, they straighten and become stacked side by side, forming a distinct stratified arrangement (Starck and Beese 2001, 2002; Lignot et al. 2005; Day et al. 2014). This postprandial transformation of enterocytes is the anatomical mechanism for the observed lengthening of the villi and thickening of the mucosal layer, with feeding documented for the broad-nosed caiman and other reptiles (Starck and Beese 2002; Lignot et al. 2005; Ott and Secor 2007*a*; Starck et al. 2007). The resulting increase in functional surface area provides an increased capacity for hydrolase activity and nutrient uptake.

Feeding for alligators undoubtedly triggers a cascade of events resulting in increases in gene expression and protein synthesis that contribute to enterocyte hypertrophy and remodeling, as documented for the Burmese python (Castoe et al. 2013; Andrew et al. 2015; Perry et al. 2019). An additional passive mechanism underlying enterocyte hypertrophy is the rapid accumulation of lipid droplets. For alligators and the broad-nosed caiman, intestinal enterocytes of fed individuals are filled with lipid droplets (Starck et al. 2007). Monoglycerides and fatty acids from a meal are transported across the brush border membrane and become reconstituted within the enterocytes as triglycerides, thereby forming droplets and thus increasing enterocyte volume. This same phenomenon has been observed for Burmese pythons and other snakes and may be a general characteristic among reptiles (Lignot et al. 2005).

Alligator enterocytes possess the characteristic apical brush border of tightly packed microvilli that serve to magnify membrane surface area. Starck et al. (2007) provided a detailed description of the brush border of the broad-nosed caiman and noted features of the microvilli that are in common with those of the American alligator. The intestinal microvilli of both species are relatively short (1–2 μm) compared with those observed in various species of snakes (3–4 μm; Lignot et al. 2005; Cox and Secor 2010; S. Secor, personal observations). Both crocodilians appear to experience modest changes in microvillus length with feeding, similar in magnitude to that observed for frequently feeding snakes, anurans, and the lizard *Heloderma suspectum* (Secor 2005*b*; Christel et al. 2007; Secor and Ott 2007; Cox and Secor 2010; Secor and Lignot 2010; S. Secor, personal observations). In contrast, infrequently feeding snakes and estivating anurans experience as much as a fivefold postprandial lengthening of their microvilli stemming from the dramatic shortening of their microvilli with fasting (Lignot et al. 2005; Secor and Ott 2007; Secor and Lignot 2010).

Digestive Performance

Given their high-protein diet, alligators undoubtedly rely heavily on the release of pancreatic proteases into the small intestine to hydrolyze polypeptides into small peptides and amino acids. For alligators of this study, feeding had a modest impact on pancreatic trypsin activity or capacity, significantly only for trypsin activity for juveniles. We therefore suspect that alligators do not widely regulate pancreatic enzyme activity (at least for trypsin) with feeding and fasting, thus maintaining a fully active exocrine pancreas between meals.

The products of pancreatic proteases (e.g., dipeptides, tripeptides) are further cleaved into individual amino acids by membrane-bound aminopeptidases before their transport across the brush border membrane. Alligators of all three age classes maintained consistent levels of APN activities between fasted and fed states. Likewise, Tracy et al. (2015) observed elevated activities of APN for fed American alligators and saltwater crocodiles.

The products of pancreatic and intestinal membrane-bound hydrolases are amino acids and monosaccharides that are transported across the brush border membrane via both carrier-mediated and passive mechanisms. In addition to these transmembrane mechanisms, crocodilians possess the capacity to transfer nutrients

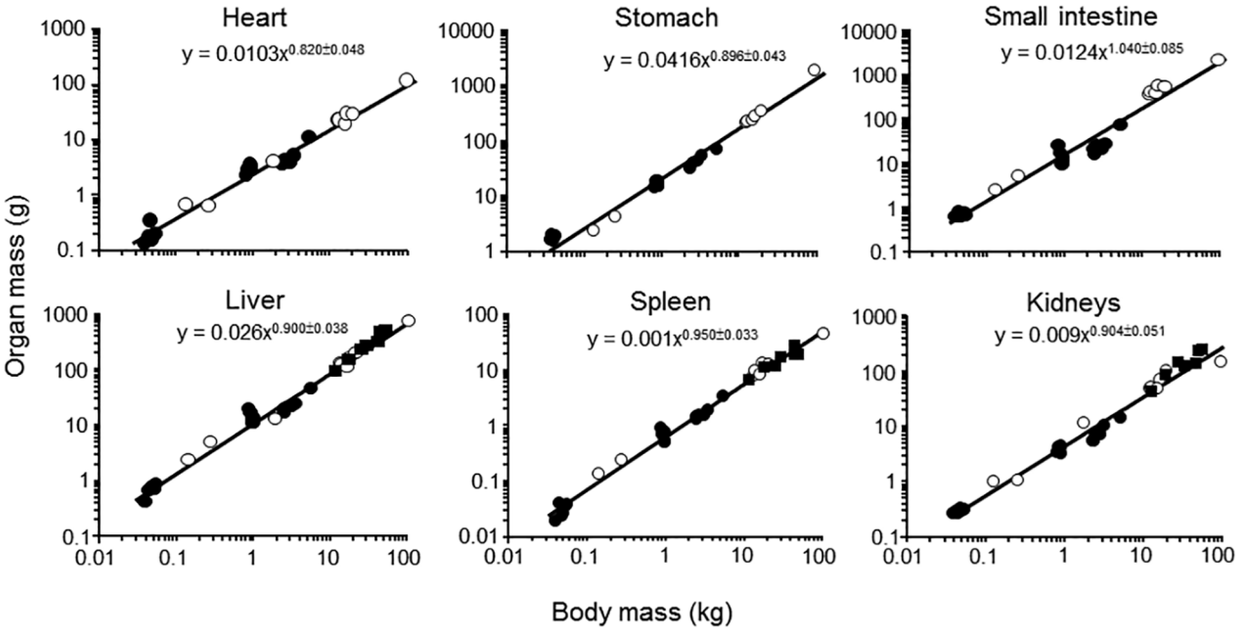

Figure 11. Wet mass (logged) of the heart, stomach, small intestine, liver, spleen, and kidneys plotted against body mass (logged) to illustrate the scaling relationship of organ masses for the American alligator, *Alligator mississippiensis*. Filled circles represent data from this study, open circles represent organ masses from Coulson and Hernandez (1983), and filled squares represent organ masses (only for liver, spleen, and kidneys) from wild-caught alligators (R. Elsey, unpublished data). For this combined data set, heart, liver, stomach, spleen, and kidney wet masses scale allometrically (mass exponents <1.0) with body mass, whereas small intestinal mass scales isometrically.

across the epithelium via paracellular absorption (Tracy et al. 2015). In this study, we quantified specifically carrier-mediated D-glucose transport and total transport (carrier mediated and passive) of L-leucine and L-proline across the apical brush border membrane. We noted among our age classes, fasted or fed, instances of significant positional effects (decreasing distally) on uptake rates, especially for D-glucose. Proximal to distal decreasing gradients in nutrient uptake rates, in particular for D-glucose, have also been documented for fishes, amphibians, other reptiles, birds, and mammals (Karasov et al. 1983, 1986; Dykstra and Karasov 1992; Secor and Diamond 1995; Secor 2005*b*; Christel et al. 2007; Ott and Secor 2007*a*; Cox and Secor 2010; Day et al. 2014). For crocodilians and other carnivores, this gradient in glucose uptake, as well as matched decreasing gradients in maltase activity, could reflect an adaptive de-emphasis along the small intestine of carbohydrate-processing machinery given the low abundance of carbohydrates in their diet (Karasov and Diamond 1988; Karasov and Martínez del Rio 2007).

Independent of intestinal position, mass-specific uptake rates for neonates tended to be greater than those for juvenile or subadult alligators. An anatomical explanation for this variation is the increased thickening of the intestinal wall with age—specifically, the layers of circular and longitudinal smooth muscle (muscularis; table 3). Therefore, as body size increases, the relative contribution of the mucosa to intestinal mass decreases and inherently so do mass-specific uptake rates. This decrease in uptake rates with alligator size combined with the allometric scaling of small intestinal mass (mass exponent $= 0.887 \pm 0.0964$) generates an allometric relationship (mass exponents ranging from 0.518 to 0.631) between body mass and total intestinal uptake capacity for each of the three nutrients. Similar scaling relationships of intestinal uptake capacities (L-leucine: 0.61; L-proline: 0.59; D-glucose: 0.80) had previously been described for the diamondback water snake, *Nerodia rhombifer* (Secor 2006).

Regulation and Ontogeny of Intestinal Function

Two salient points emerge from this study. First, alligators experience modest regulatory responses of their gastrointestinal tract to feeding and fasting. This is exemplified by the lack of any significance differences in intestinal nutrient uptake rates (with the exception of L-leucine uptake by the distal intestine) between fasted and fed individuals (fig. 7). Second, across the age classes in this study, alligators experienced no ontogenetic shift in the magnitude that digestive performance is regulated. The lack of any distinct changes in intestinal performance stems from the constant maintenance of the machinery (i.e., hydrolases, transporters) housed within the membrane of enterocytes, a function of the stability of membrane surface area, which itself is a product, in part, of maintaining microvillus length through fasted and fed states. In short, the American alligator (and possibly all other crocodilians) exhibits the form-function association by which the maintenance of the brush border surface area ensures the continuous operation of intestinal function. Retaining intestinal function between meals is hypothetically adapted to a more frequent feeding habit (Secor 2001, 2005*a*). By feeding frequently enough

such that the gut is in a near continuous state of digestion, the adaptive incentive for alligators is to maintain an idling gut between meals and thereby not wait for or pay the additional cost of gastrointestinal upregulation with each meal. Alligators (and potentially other crocodilians) are positioned at one end of a continuum (or one branch of a dichotomy) of an adaptive interplay between feeding habits and digestive response, characterized by frequent feeding and the modest regulation of gastrointestinal performance (Secor and Diamond 2000; Secor 2005*a*). At the other end of the continuum (or the other branch of a dichotomy) are those species (e.g., boas, pythons, estivating anurans) that have adaptively coupled long episodes between meals with the wide regulation of intestinal performance with each meal (Secor 2005*a*, 2008). For these animals, the cellular mechanism underlying the up- and downregulation of enterocyte function is the dramatic altering of surface area accomplished via the rapid lengthening of the microvilli with feeding and shortening once digestion is completed (Secor and Ott 2007; Secor 2008).

Across a 50-fold range in body mass, the young American alligators in this study continued to exhibit a modest degree of postprandial change in organ mass and digestive performance. Although we did not find support for an ontogenetic shift in the regulation of digestive performance, it may not be entirely out of the question that one does exist. Alligators have been shown to experience ontogenetic shifts in other aspects of their biology, including their habitat use, tooth shape, and bite force (Subalusky et al. 2009; Gignac and Erickson 2015). One limitation of this study and thus a caveat to our test of this hypothesis was the size of alligators that we were able to study. Because of housing logistics, institutional set limits, and safety concerns, we were unable to hold and study animals exceeding 10 kg. Given that an average-sized large adult male alligator can weigh 200–300 kg, there is a very large span of alligator body size and age that we were unable to include in this study. Therefore, our original hypothesis may gain some traction if we could demonstrate an ontogenetic shift in the regulation of digestive performance occurring later in life at a much larger body size.

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