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Physiological responses to feeding, fasting and estivation for anurans

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Summary

Anuran estivation is characterized by long episodes of aphagia. To investigate whether estivating anurans downregulate intestinal performance as an adaptive mechanism to reduce energy expenditure, I compared the metabolic and intestinal responses to feeding, fasting and estivation among non-estivating and estivating species of the anuran families Bufonidae, Leptodactylidae and Ranidae. Standard metabolic rates of the estivating *Bufo alvarius*, *Ceratophrys ornata* and *Pyxicephalus adspersus* were significantly less than those of the non-estivating *Bufo marinus*, *Leptodactylus pentadactylus* and *Rana catesbeiana*. Whereas the digestion of rodent meals equaling 15% of anuran body mass generated significant metabolic responses for all species, specific dynamic action was significantly greater for the estivating species. For estivating species, feeding triggered more than a doubling of small intestinal mass and significant upregulation of intestinal nutrient transport rates, resulting in six- to tenfold increases in total intestinal nutrient uptake capacity. The postprandial intestinal responses of the non-estivating species were much more modest, averaging a 50% increase in small intestinal mass and 69% increase in uptake capacities. Following 1 month of laboratory-induced estivation, *C. ornata* and *P. adspersus* had further depressed metabolic rates by 20%, intestinal masses by 44%, and total intestinal uptake capacities by 60%. In a fashion similar to infrequently feeding, sit-and-wait foraging snakes, estivating anurans possess the capacity to severely downregulate intestinal performance with fasting and estivation, and subsequently upregulate the gut with feeding. The depression in gut performance during estivation aids in reducing energy expenditure, thereby increasing the duration that the animal can remain dormant while relying solely upon stored energy.

Key words: anuran, *Bufo*, *Ceratophrys*, *Leptodactylus*, *Pyxicephalus*, *Rana*, estivation, fasting, intestinal nutrient transport, specific dynamic action.

Introduction

Long episodes of fasting characterize the natural history traits of sit-and-wait foraging, hibernation, and estivation. Snakes that employ the sit-and-wait mode of foraging commonly fast for a month or more between their infrequent meals (Pope, 1961; Secor and Nagy, 1994; Slip and Shine, 1988). In cold-temperate environments, many animals hibernate for 5–6 months either underground or underwater, during which time they do not feed (Gregory, 1982; Pinder et al., 1992). In regions that experience annual dry seasons of high temperatures and low water and food availability, resident amphibians and reptiles will estivate in underground refugia, dormant and aphagic for the duration of the dry season (Abe, 1995; Loveridge, 1976; Seymour and Lee, 1974). During these extended periods of fasting, animals must rely upon stored energy for their metabolic needs, as well as provision enough energy to fuel post-emergence activities (for hibernators and estivators) and the digestion of the next meal. Hence, natural selection would favor mechanisms that reduce energy expenditure during such periods of aphagia.

An apparent adaptive mechanism employed by sit-and-wait foraging, infrequently feeding snakes to reduce energy expenditure while fasting includes the downregulation of their gastrointestinal (GI) tract upon the completion of digestion. The link between the fasting-related reduction in gut function and concurrent decrease in energy expenditure is suggested by the lower standard metabolic rate (SMR) of sit-and-wait foragers compared to that of active foraging snakes that feed more frequently (Secor and Diamond, 2000). In contrast to the sit-and-wait foragers, frequently feeding snakes do not downregulate intestinal function with fasting, but rather maintain elevated function until the next meal. For sit-and-wait foraging snakes, feeding is consequently met with a rapid upregulation of GI function, highlighted by 10- to 20-fold

increases in intestinal nutrient uptake (Secor and Diamond, 2000).

Selective mechanisms to reduce tissue metabolism would predictably also be present for both hibernating and estivating species. Evidence of such mechanisms for hibernating amphibians, reptiles and mammals include the overwintering reduction in intestinal mass or function (Carey, 1992; Csáky and Galluci, 1977; Qadri et al., 1970). Understandably, energy-conserving mechanisms would be more important for estivating species given that their body temperatures, and consequentially their metabolic rates, are substantially higher than hibernating species (Pinder et al., 1992). For several amphibian species, metabolic rates are reduced by 50–85% during estivation, reportedly a function of decreases in organ metabolism (Fuery et al., 1998; Guppy and Withers, 1999). Given the fasting responses of the GI tract of infrequently feeding snakes, the benefits of gut downregulation to conserve energy, and the adaptive plasticity of the intestine (Piersma and Lindström, 1997), I hypothesized that anuran species which estivate during their dry season severely downregulate intestinal performance with fasting and estivation, and consequently upregulate intestinal performance rapidly after feeding.

A test of this hypothesis would be best served by first demonstrating the wide regulation of intestinal performance in response to feeding and fasting among distantly related anurans that estivate, and second the narrow regulation of intestinal performance for species closely related to the estivating species but which do not estivate. The experimental design that I implemented to test this hypothesis compares several physiological responses to fasting and feeding between an estivating and a non-estivating species for three anuran families, Bufonidae, Leptodactylidae and Ranidae. For each species, I measured metabolic rates, organ masses, and intestinal morphology and nutrient uptake of individuals fasted and digesting. In addition, I took similar sets of measurements of *C. ornata* and *P. adspersus* following 1 month of laboratory-induced estivation. My objectives in this study were to demonstrate: (1) that the wide regulation of intestinal performance has evolved independently among anuran estivators, (2) that the magnitude by which anurans regulate intestinal performance is linked to their feeding ecology, (3) that intestinal performance is further reduced during estivation, and (4) that the up- and downregulation of intestinal performance is reflected, respectively, in elevated and depressed metabolic rates.

Materials and methods

Anuran ecology and their maintenance

The estivating species I selected to study from the Bufonidae, Leptodactylidae and Ranidae are, respectively, *Bufo alvarius* Girard, *Ceratophrys ornata* Bell and *Pyxicephalus adspersus* Tschudi. The non-estivating species are, respectively, *Bufo marinus* L., *Leptodactylus pentadactylus* Laurenti and *Rana catesbeiana* Shaw. I selected these six anuran species because each is relatively large as adults (>100 g), thereby providing enough tissues for nutrient transport measurements, and each will feed upon small rodents, so that meal type could be standardized among experiments. Critical to the design of the study is that each species pair within a family includes a species known to estivate yearly for 6–10 months and a species that remains active and feeding for most of the year. For Bufonidae, *B. alvarius*, the Colorado River toad, inhabits the Sonoran Desert of southern Arizona and the northwest corner of Mexico and emerges from a 9–10 month estivation with the onset of the summer monsoon season to feed and reproduce (Sullivan and Fernandez, 1999). *Bufo marinus*, the marine toad, is currently established in many tropical and subtropical regions of the world, and is generally active and feeding year-around (Easteal, 1981; Zug and Zug, 1979). The leptodactylid *C. ornata*, the ornate horned frog, of Argentina, Paraguay, Uruguay, and southern Brazil buries itself underground at the start of the dry season and becomes covered in a cocoon of dried epidermis that serves to reduce water loss, and reemerges in the wet season (McClanahan et al., 1976). In contrast, the leptodactylid *Leptodactylus pentadactylus*, the South American bullfrog, is largely active all year round in its distribution through Central America and northern South America (Savage, 2002). The ranid *P. adspersus*, the African bullfrog, inhabits primarily the open savannas of sub-Saharan Africa and estivates underground for most of the year, also cocooning itself with compact layers of dried skin to reduce evaporative water loss (Loveridge, 1976). In North America, the ranid *R. catesbeiana*, the American bullfrog, is active and feeds from spring to fall, while hibernating where necessary during winter months (Dundee and Rossman, 1989; Conant and Collins, 1991).

Bufo marinus, *C. ornata*, *L. pentadactylus* and *R. catesbeiana* were purchased commercially and originated, respectively, from southern Florida, a captive-propagated colony in southern California, Suriname, and the southeastern United States. *Bufo alvarius* were collected under a state collecting permit in southern Arizona, and *P. adspersus* were captured in Zimbabwe. Individuals of each species were housed either individually in plastic storage boxes (20 liters) or together in large plastic or fiberglass containers at 24–27°C under a photoperiod of 14 h:10 h L:D. Anurans had access to water in each container and were fed a diet of crickets or small rodents at 3–7 day intervals. Prior to metabolic and nutrient transport trials, individuals were fasted for 2 weeks to ensure that their guts were emptied and that all digestive activities had ceased. Because anurans commonly store water in their bladder, I manually emptied their bladder by inserting a glass rod into their cloaca and gently squeezing their hind section before measuring body mass. For this study, I used 10 *B. alvarius* [142.2±8.3 g (mean ± 1 S.E.M.)], 12 *B. marinus* (139.3±7.7 g), 11 *C. ornata* (185.9±21.6 g), 9 *L. pentadactylus* (157.8±14.9 g), 10 *P. adspersus* (227.6±10.9 g) and 11 *R. catesbeiana* (273.3±22.5 g). Animal care and experimentation were conducted under the approval of the UCLA Animal

Research Committee and the University of Alabama Institutional Animal Care and Use Committee.

Metabolic response to feeding

I quantified metabolic rates as rates of oxygen consumption ($\dot{V}_{O_2}$) measured using closed-system respirometry as described by Vleck (1987) and Secor and Diamond (1997). Anurans were placed individually into respirometry chambers (2–3.5 liter) and maintained at 30°C within an environmental chamber. Each respirometry chamber was fitted with an incurrent and excurrent air port, each attached to a three-way stopcock. Air was pumped into the chambers through the incurrent air port. A small amount of water was placed in each chamber to prevent anurans from desiccating from the constant air flow. For each $\dot{V}_{O_2}$ measurement, a 20 ml air sample was withdrawn from the excurrent air port, and both incurrent and excurrent ports were then closed to seal the chamber. 0.5–1 h later, the excurrent air port was opened and a second 20 ml air sample was withdrawn. Air samples were pumped (125 ml min^{-1}) through a column of water absorbent (Drierite; W. A. Hammond Drierite Co., Xenia, OH, USA) and CO_2 absorbent (Ascarite II; Thomas Scientific, Swedesboro, NJ, USA) into an O_2 analyzer (S-3A/II; AEI Technologies, Pittsburgh, PA, USA). I calculated whole-animal (ml h^{-1}) and mass-specific (ml g^{-1} h^{-1}) rates of oxygen consumption corrected for standard pressure and temperature as described by Vleck (1987).

Each metabolic trial began with the measurement in fasted anurans of $\dot{V}_{O_2}$ twice a day (at ~08:00 h and ~20:00 h) for 3 days. For each anuran, I assigned its SMR as the lowest $\dot{V}_{O_2}$ measured over those 3 days. After SMR measurements, anurans were fed rodent (neonate rats) meals equal in mass to 15% of anuran body mass. Anurans were then returned to their respirometry chambers and metabolic measurements were continued at 12 h intervals (at ~08:00 h and ~20:00 h) for 3 days and thereafter at 1 day intervals (at ~08:00 h) for 5 more days. For each metabolic trial, I quantified the following seven variables: SMR, 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), duration (time from feeding that $\dot{V}_{O_2}$ was no longer significantly greater than SMR), total energy expended above SMR over the duration of significantly elevated $\dot{V}_{O_2}$, [specific dynamic action (SDA) quantified as kJ and kJ g^{-1}], and SDA coefficient (SDA quantified as a percentage of the energy content of the meal). I quantified SDA (kJ) by summing the extra O_2 consumed above SMR during the duration of the significant metabolic response and multiplying that value by 18.3 J ml^{-1} O_2 consumed, assuming the catabolism of a diet that is 70% protein, 25% fat and 5% carbohydrate, and an RQ of 0.75 (Gessaman and Nagy, 1988). The energy content of the rodent meals was calculated based on an energy equivalent of 5.98 kJ g^{-1} wet mass, as determined from bomb calorimetry. Briefly, five neonate rats (20.4±0.6 g) were each weighed, dried, reweighed and ground to a fine powder which was pressed into pellets. Three pellets from each rodent were ignited in a bomb calorimeter (1266; Parr Instrument Co., Moline, Illinois, USA) to determine energy content (kJ g^{-1}). Wet mass energy content of the rodent meals (5.98 kJ g^{-1}) represents the average energy content of the five rodents, each calculated from the mean energy of the three pellets (24.43±0.19 kJ g^{-1} dry mass) and the rodent's relative body water content (75.6±0.4% of body mass).

Intestinal nutrient uptake

From three fasted and three fed individuals of each anuran species, I quantified mass-specific rates of nutrient transport across the intestinal brush border membrane using the everted sleeve technique (Karasov and Diamond, 1983; Secor et al., 1994). Anurans were killed by severing their spinal cord immediately posterior to the skull either following a 2-week fast ('fasted') or after the 2-week fast, 1 day following the consumption of a rodent meal (neonate rats) equaling 15% of anuran body mass ('fed'). From each anuran, I removed and weighed the small intestine, flushed it of its contents, reweighed it, and divided it into thirds of equal length. Each third was weighed, everted and divided into 1 cm segments. Sleeves were mounted on metal rods, incubated first in amphibian Ringer's solution at 30°C for 5 min, and then incubated for 2 min at 30°C in stirred amphibian Ringer's solution containing an unlabeled and radiolabeled nutrient and an adherent fluid marker labeled with a different radioisotope. For each intestinal third, I measured the transport of the amino acids L-leucine and L-proline (each at 50 mmol l^{-1} and labeled with ^3H) and of the sugar D-glucose (at 20 mmol l^{-1} and labeled with ^{14}C). The adherent fluid markers polyethylene glycol (labeled with ^{14}C) and L-glucose (labeled with ^3H) corrected for the amount of radiolabeled amino acids and D-glucose, respectively, adherent to the everted intestinal sleeve. L-glucose measures also serve to correct for the passive diffusion of D-glucose. With these corrections, total amino acid uptake (passive and carrier-mediated) and carrier-mediated D-glucose uptake rates are quantified as nanomoles of nutrient transported per minute of incubation per milligram of sleeve wet mass. As previously observed for snakes, nutrient uptake rates of the proximal and middle intestinal regions were consistently similar and, in many cases, greater than those of the distal region (Secor and Diamond, 2000). Therefore, as in earlier studies, I report separately the averaged uptake rates of the proximal and middle segments (reported as the anterior intestine), and of the distal third of the small intestine. I assigned as a measure of overall intestinal performance the small intestine's total capacity to transport nutrients. Intestinal nutrient uptake capacity (μmol min^{-1}) was calculated as the summed products of nutrient uptake rates (nmol min^{-1} mg^{-1}) times intestinal mass (mg) for each intestinal third (Secor and Diamond, 1995).

Two studies have demonstrated that the everted sleeve technique severely damages the mucosa of avian intestines, thereby negating its use for accurately measuring nutrient uptake for those species of birds (Starck et al., 2000; Stein and Williams, 2003). To assess the potential damage caused by the everted sleeve method on anuran intestines, I compared two

sets of intestinal samples removed from the anterior portion of the small intestine of fasted and fed *B. marinus* and *R. catesbeiana* at three stages of the procedure: prior to eversion, immediately following eversion, and after everted tissues had been incubated at 30°C in unstirred amphibian Ringers for 5 min and in stirred amphibian Ringers for 2 min. Samples were prepared for light microscopy (as explained below) and examined for damages to the mucosal layer. Following eversion and incubation, I found no discernable damage to the intestinal mucosa, nor any significant change in villus length (measured from the edge of the smooth muscle layer to the tip of the villus, N=20 per treatment per individual) for either species, fasted or fed (Fig. 1). As observed for reptiles (Secor, 2005; Tracy and Diamond, 2005), the everted sleeve method apparently does not damage the intestinal mucosa of anurans, therefore the method can provide a reliable measure of intestinal function.

Intestinal morphology and organ masses

The postprandial response of intestinal morphology was assessed as changes in (1) intestinal mass, (2) intestinal length, (3) mucosa and muscularis/serosa thickness and (4) enterocyte dimensions. The first two sets of measurements were made immediately after the intestine was removed from the individual anuran and rinsed of any contents. The second pair of measurements was made from light microscopic preparations of intestinal segments taken from the proximal third of the small intestine. Single 1 cm sleeves of intestine were fixed in 10% neutral-buffered formalin solution, embedded in paraffin, sectioned at 6 μm, and stained with Hematoxylin and Eosin. For each anuran, mucosa and muscularis/serosa thickness was calculated as the mean of 5 measurements of each component using a light microscope and video camera linked to a computer and image-analysis software (Motic Image Plus, BC, Canada). From these same images I measured the height and width of ten enterocytes, from which I calculated enterocyte volume (based on a formula for a cube). To assess the effects of feeding on other organs besides the small intestine, I weighed the wet mass of the heart, lungs, liver, empty stomach, spleen, pancreas, gall bladder, empty large intestine and kidneys immediately after their removal from the animal, dried each in an oven (60°C), and weighed their dry mass. Both the stomach and large intestine were weighed with contents and after contents were removed.

Physiological responses to estivation

To identify the metabolic and tissue responses to estivation, I measured metabolic rate, organ masses, and intestinal function of *C. ornata* and *P. adspersus* following 1 month of laboratory-induced estivation. Individual frogs were induced into a state of estivation by simply removing their access to water. Within days, frogs became stationary and assumed a hunched-over posture as their outer layer of skin began to dry and harden (Loveridge and Withers, 1981). For the remaining weeks, frogs did not move within their 'cocoon' of dried skin.

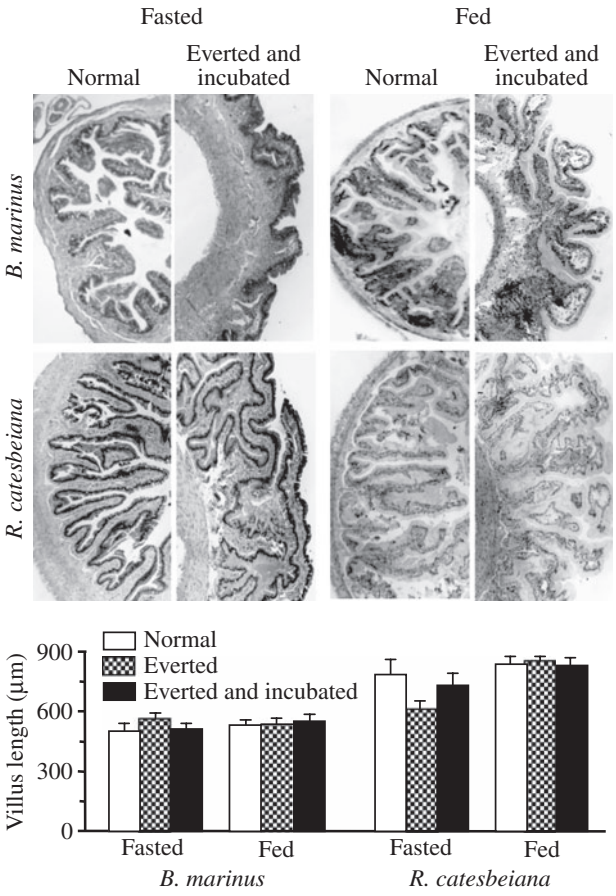

Fig. 1. Light microscopy photographs of the intestinal mucosa prior to (normal) and after intestinal segments have been everted and incubated for fasted and fed *Bufo marinus* and *Rana catesbeiana*. Also illustrated are means of villus length of normal non-everted intestinal segments, everted intestinal segments, and everted intestinal segments that have been incubated in unstirred and stirred Ringers solution for fasted and fed *B. marinus* and *R. catesbeiana*. Note that villus length is not altered following everting and incubation. In this and all following figures, error bars represent ±1 S.E.M. and are omitted if the S.E.M. is smaller than the width of the symbol for mean value.

During this time, frogs lost on average 35% of their body mass, presumably as water. I measured metabolic rates of *C ornata* (283±44 g, N=3) and *P. adspersus* (235±19 g, N=4) following 1 month of estivation as described above. Three individuals of each species were then killed for measurements of intestinal mass and nutrient uptake, and organ masses as previous described.

Statistical analyses

For each SDA trial, I used a repeated-measures design analysis of variance (ANOVA) to test for significant effects of time (before and after feeding) on $\dot{V}_{O_2}$ (as ml h^{-1} and ml g^{-1} h^{-1}). Each ANOVA was followed with a *post hoc* pairwise mean comparison (Tukey–Kramer procedure) to identify significant changes in $\dot{V}_{O_2}$ between sampling time points and when $\dot{V}_{O_2}$ did not differ from SMR. I used ANOVA

for mass-specific measures and ANCOVA (log body mass as a covariate) for whole-animal measures to test for species effects on metabolic variables. For each anuran family, I similarly tested for differences between the estivating and non-estivating species. Because of the significant variation in body mass among the six anuran species (Table 1), I recalculated whole-animal measures of SMR, peak $\dot{V}_{O_2}$ and SDA of each anuran assuming a body mass of 180 g (the approximate average mass of all 36 anurans). Recalculated values were determined from allometric equations presented in table 4 of Secor and Falkner (2002) for *B. marinus*, assuming mass exponents of 0.69, 0.85 and 1.02, respectively, for SMR, peak $\dot{V}_{O_2}$, and SDA. The postprandial responses were assessed by ANOVA for mass-specific rates of intestinal nutrient uptake and by ANCOVA (body mass as a covariate) for intestinal nutrient uptake capacity, intestinal mass and morphology, and the masses of other organs. Likewise, I tested for significant changes from fasting to estivation in metabolic rate, intestinal nutrient uptake and morphology. ANOVA and ANCOVA results are reported in terms of their *P* values, and I provide *P* values of selected pairwise mean comparisons. Statistical significance is designated as $P<0.05$ and mean values are reported as means ±1 S.E.M.

Results

Metabolic response

Pre-feeding SMR, quantified as ml O_2 g^{-1} h^{-1} or ml O_2 h^{-1} (absolute or mass-adjusted), varied significantly ($P<0.0001$) among the six anuran species (Table 1). For each family, the estivating species possessed significantly ($P<0.003$) lower SMR than the non-estivating species. On average, SMR of the estivating species was 62% of that of the species that do not estivate.

For all six species, feeding induced a significant metabolic response ($P<0.0001$). Within 12 h, $\dot{V}_{O_2}$ had risen by 100–300%, and by 24 h, rates were 200–1000% greater than SMR (Fig. 2). Peaks in $\dot{V}_{O_2}$ were attained between 1 and 2.5 days postfeeding, with little difference in the time of peak rates between estivating and non-estivating species. Postprandial peaks in $\dot{V}_{O_2}$, were significantly ($P<0.0001$) different among the six species, but only for Leptodactylidae was there a significant difference between the two species (*C. ornate* > *L. pentadactylus*; Table 1). The factorial scope of peak $\dot{V}_{O_2}$ differed significantly ($P<0.0001$) among the six species, ranging from 3.5 for *L. pentadactylus* to 11.6 for *C. ornata*. For each family, the estivating species possessed significantly ($P<0.0001$) greater scopes than the non-estivating species (Table 1). The duration of significantly elevated $\dot{V}_{O_2}$ ranged between 4 and 7 days, with duration of the SDA response averaging a day longer for estivating species (Table 1).

The energy expended on meal digestion and assimilation (SDA as kJ adjusted to body mass of 180 g) varied significantly ($P<0.0001$) among the six species, as

Table 1. *Body mass, meal size, standard metabolic rate (SMR) and postfeeding metabolic measures of peak oxygen consumption ($\dot{V}_{O_2}$), scope of peak $\dot{V}_{O_2}$, duration, specific dynamic action (SDA) and SDA coefficient of six species of anurans*

	Bufonidae			Leptodactylidae			Ranidae		
Variable	*B. alvarius*	*B. marinus*	*P*	*C. ornata*	*L. pentadactylus*	*P*	*P. adspersus*	*R. catesbeiana*	*P*
Body mass (g)	140±8[a]	137±4[a]	0.754	169±27[a,b]	173±13[a,b]	0.913	225±15[b]	238±33[b]	0.724
N	6	6		6	6		6	6	
Meal size (% of body mass)	15.0±0.0[a]	15.1±0.10[a]	0.495	15.0±0.1[a]	15.0±0.1[a]	0.817	14.9±0.1[a]	15.0±0.1[a]	0.551
SMR (ml O_2 h^{-1})	4.71±0.28[a]	6.91±0.35[a,b]	0.0008	5.97±0.98[a,b]	8.41±0.32[b]	0.045	5.93±0.55[a,b]	15.0±2.5[c]	0.0002
SMR (ml O_2 h^{-1}) adjusted to body mass of 180 g	5.60±0.20[a]	8.37±0.49[b]	0.0004	6.20±0.42[a,b]	8.74±0.27[b]	0.005	5.05±0.27[a]	12.2±1.2[c]	0.0002
SMR (ml O_2 g^{-1} h^{-1})	0.034±0.001[a,b]	0.051±0.001[c]	0.0004	0.036±0.002[b]	0.050±0.003[c]	0.003	0.026±0.001[a]	0.063±0.005[d]	0.0002
Peak $\dot{V}_{O_2}$ (ml h^{-1})	30.4±6.5[a]	27.0±2.1[a]	0.175	69.4±11.1[b]	29.5±2.3[a]	<0.0001	56.6±7.0[b]	60.9±12.9[b]	0.740
Peak $\dot{V}_{O_2}$ (ml h^{-1}) adjusted to body mass of 180 g	37.6±1.1[a,b]	33.9±2.5[a]	0.209	71.8±4.4[b]	30.6±1.1[a]	<0.0001	46.6±4.4[a]	46.9±7.1[a]	0.672
Peak $\dot{V}_{O_2}$ (ml g^{-1} h^{-1})	0.216±0.005[a]	0.196±0.004[a]	0.230	0.405±0.022[b]	0.171±0.006[a]	<0.0001	0.251±0.023[a]	0.250±0.036[a]	0.631
Scope of peak $\dot{V}_{O_2}$	6.46±0.13[b]	3.90±0.30[a]	<0.0001	11.6±14[c]	3.50±0.21[a]	<0.0001	9.65±0.91[c]	3.94±0.35[a]	<0.0001
Duration (days)	7	5		5	5		6	4	
SDA (kJ)	36.6±3.0[a,b,c]	23.8±1.5[a]	0.001	50.8±9.0[c,d]	30.5±2.6[a,b]	0.0001	57.3±4.3[d]	41.2±8.1[b,c]	0.040
SDA (kJ) adjusted to body mass of 180 g	47.0±2.5[b]	31.4±1.7[a]	0.0004	52.4±3.4[b]	31.8±1.4[a]	0.0002	45.8±2.65[b]	30.3±3.6[a]	0.0008
SDA (kJ kg^{-1})	260±11[b]	173±8[a]	0.0004	291±17[b]	179±8[a]	0.0002	256±15[b]	169±20[a]	0.0008
SDA coefficient	28.9±1.2[b]	19.2±1.0[a]	0.0005	32.3±1.9[b]	19.6±0.8[a]	0.0003	28.6±1.5[b]	18.9±2.3[a]	0.002

Variables are defined in the text. Values are presented as means ± 1 S.E.M.

For each variable, superscript letters that differ denote significant ($P<0.05$) differences between means among the six species as determined from *post-hoc* pairwise comparison (Tukey's HSD test).

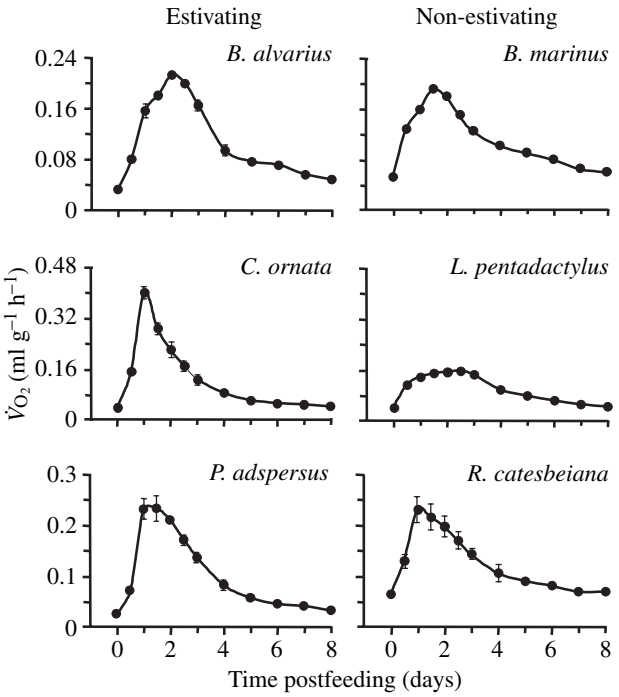

Fig. 2. Mean $\dot{V}_{O_2}$ at 30°C prior to (day 0) and up to 8 days following the ingestion of rodent meals 15% of anuran body mass for estivating *Bufo alvarius*, *Ceratophrys ornata* and *Pyxicephalus adspersus*, and non-estivating *Bufo marinus*, *Leptodactylus pentadactylus* and *Rana catesbeiana* (*N*=6 for each species).

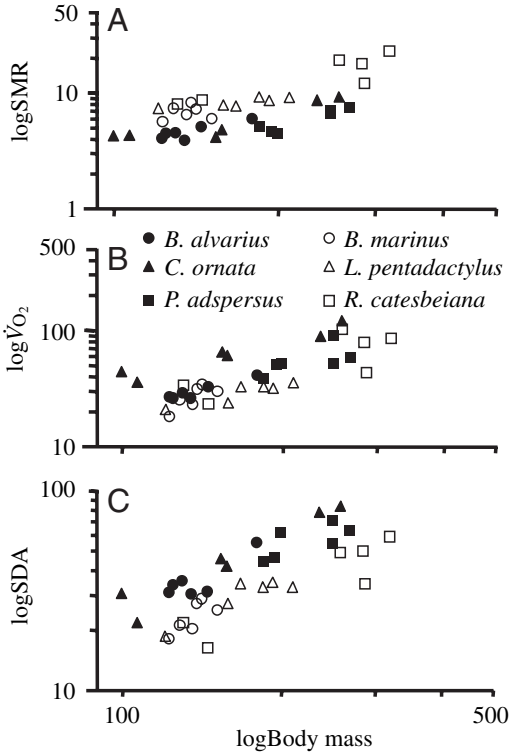

Fig. 3. Log SMR (A), peak postprandial $\dot{V}_{O_2}$ (B), and SDA quantified as kJ (C) plotted against log body mass for estivating *Bufo alvarius*, *Ceratophrys ornata* and *Pyxicephalus adspersus* and non-estivating *Bufo marinus*, *Leptodactylus pentadactylus* and *Rana catesbeiana*. Note the lower SMR and higher SDA of individuals of estivating species compared to individuals of non-estivating species.

the estivating species of each family possessed significantly higher SDA than the non-estivating species (Table 1). Likewise when SDA was quantified per animal mass or meal energy, it was greater for the estivating species. On average, SDA was equivalent to 30.1% and 19.2% of meal energy, respectively, for estivating and non-estivating species (Table 1).

Even within the narrow range of body masses (100–320 g) of anurans used in this study, SMR, peak $\dot{V}_{O_2}$ and SDA (kJ) scaled allometrically (P<0.0006, r^2=0.54–0.80) with body mass for estivating and non-estivating individuals (Fig. 3). Mass exponents of SMR differed significantly (P<0.05) between estivating (0.71) and non-estivating individuals (1.11), but did not differ for either peak $\dot{V}_{O_2}$ (1.04 for estivating and 1.24 for nonestivating species) or SDA (1.06 and 1.03, respectively). Illustrated as a function of body mass, individuals of estivating species possessed lower SMR, similar peak $\dot{V}_{O_2}$, and greater SDA than individuals of non-estivating species (Fig. 3).

Intestinal nutrient uptake

By 24 h postfeeding, stomach contents of fed anurans had been reduced by 14±3%, hence they were all experiencing intestinal digestion. For each anuran family studied, nutrient uptake rates were significantly upregulated with feeding for the estivating species, whereas the non-estivating species exhibited little postprandial change in intestinal function (Fig. 4). For Bufonidae, *B. alvarius* experienced significant (P<0.013) postprandial increases in anterior and distal L-leucine and L-proline uptake, and anterior D-glucose uptake. In contrast, only anterior D-glucose uptake exhibited a significant (P=0.002) postfeeding increase for *B. marinus*. Feeding also triggered significant (P<0.031) increases in intestinal uptake of all three nutrients for the anterior intestine, and for L-proline and D-glucose for the distal region for *C. ornata*, whereas there was no significant postprandial response in nutrient transport rates for *L. pentadactylus*. Likewise, *P. adspersus* significantly (P<0.048) increased with feeding anterior intestinal uptake of the three nutrients, as well as the distal uptake of D-glucose. *Rana catesbeiana* followed suit with the other non-estivating species and exhibited no significant change in intestinal nutrient uptake with feeding.

The postprandial response of intestinal nutrient uptake capacity, a product of small intestinal mass and mass-specific nutrient uptake rates, similarly differed between the estivating and non-estivating species (Fig. 5). With feeding, all three estivating species significantly (P<0.029) upregulated their intestine's capacity to transport the three nutrients. Uptake capacity of L-leucine, L-proline, and D-glucose increased on average by 6.3-, 6.7- and 10.3-fold among the three estivating species. For the non-estivators, only *B. marinus* experienced a

significant postprandial response in uptake capacity, D-glucose uptake capacity increasing (P=0.012) by threefold. For the non-estivating species, intestinal uptake capacity among the three nutrients averaged only 69% higher after feeding.

Organ morphology

For all six anurans, regardless of being fasted or fed, small intestinal mass declined distally as the proximal one-third of the intestine averaged 200% heavier than the distal one-third.

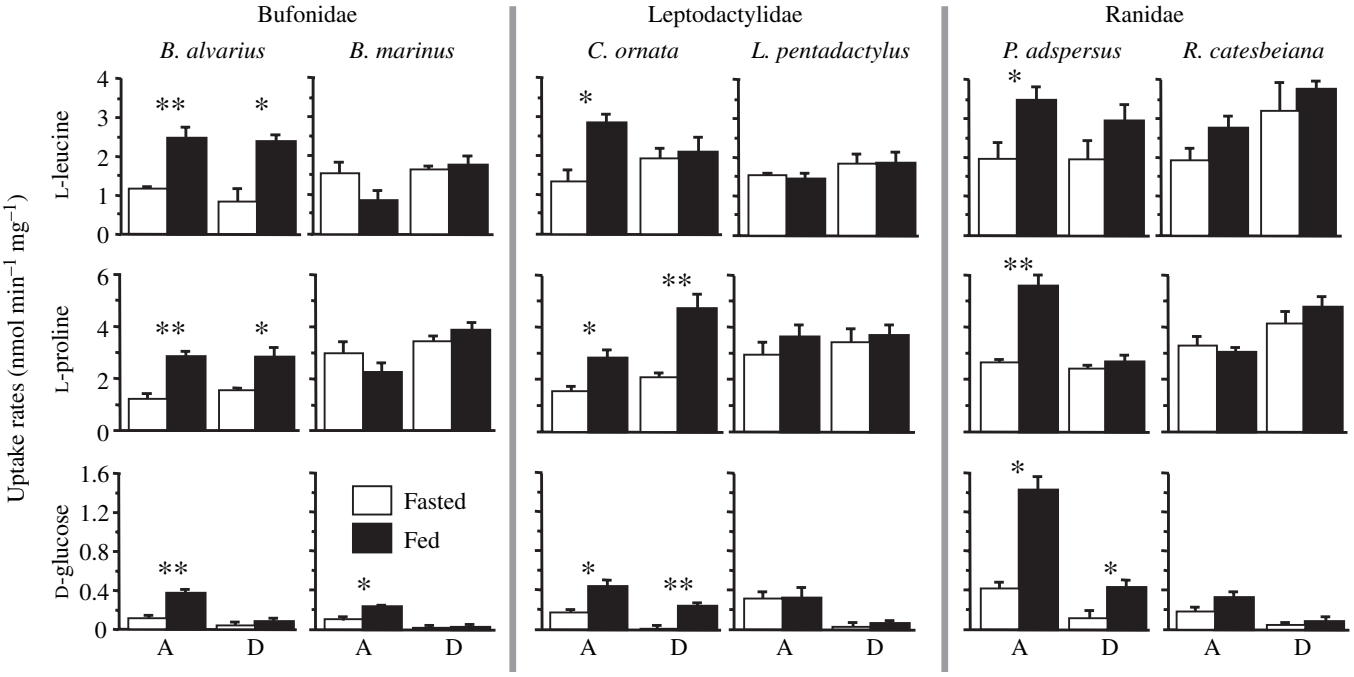

Fig. 4. Uptake rates of the amino acids L-leucine and L-proline and of the sugar D-glucose by the anterior (A) and distal (D) portions of the small intestine for each of six anuran species after a 2-week fast (fasted, open bars) and at 1 day postfeeding (fed, solid bars). Note that of the non-estivating species, only *Bufo marinus* experienced a postprandial increase in intestinal nutrient uptake (glucose uptake by the anterior small intestine). In contrast, all three estivating species experienced significant postprandial increases in the uptake rates of all three nutrients by the anterior small intestine and in half the cases by the distal small intestine. In this and subsequent figures, levels of significance (for change with feeding) are illustrated with asterisks; *P<0.05, **P<0.01, ***P<0.001.

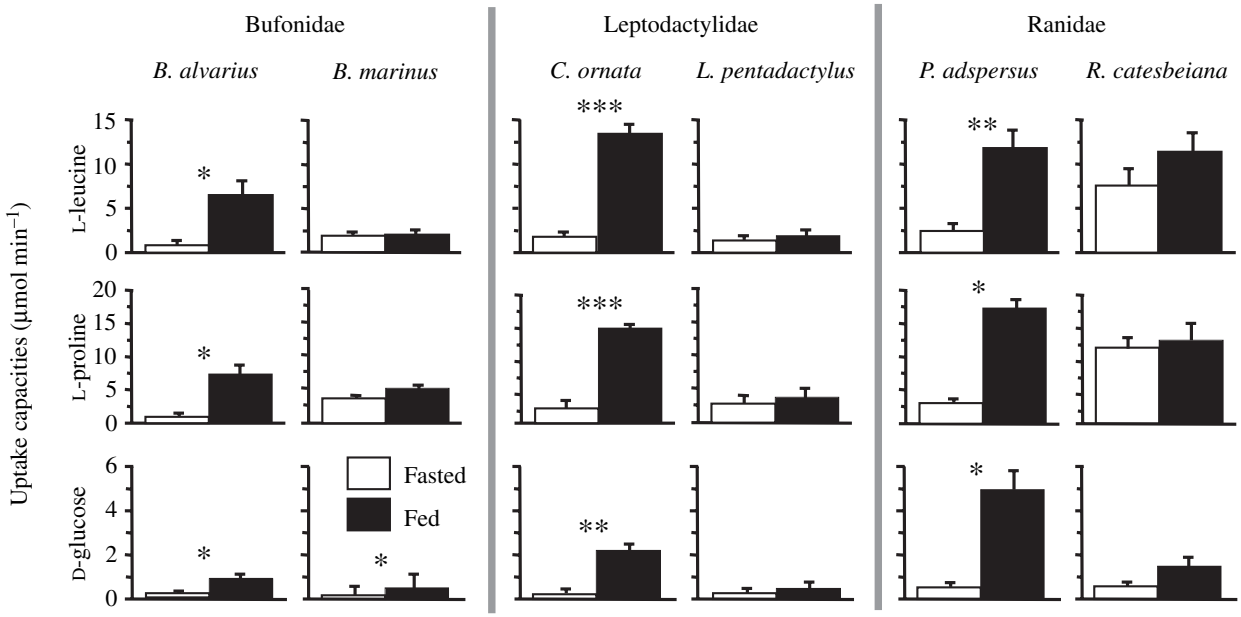

Fig. 5. Total intestinal uptake capacity for the amino acids L-leucine and L-proline and of the sugar D-glucose after a 2 week fast (fasted, open bars) and at 1 day postfeeding (fed, solid bars) for six anuran species. Note that the three non-estivating species experienced no significant postprandial increases in nutrient uptake capacity (with the exception of D-glucose for *Bufo marinus*), whereas all three estivating anuran species significantly upregulated intestinal uptake capacity of each nutrient with feeding.

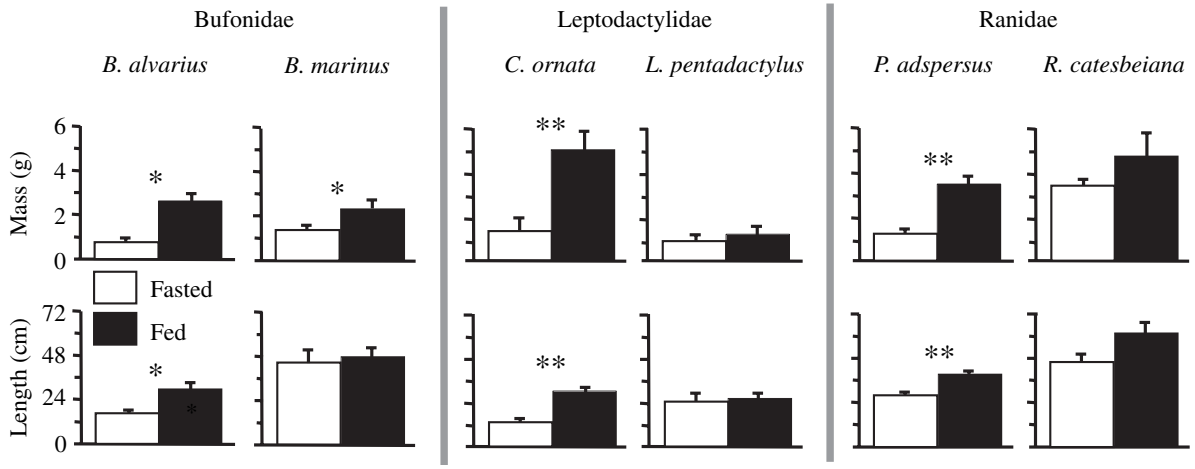

Fig. 6. Mass and length of the small intestine of two-week fasted (fasted, open bars) and fed (solid bars) anurans of six species. Each of three estivating species experienced significant postprandial increases in small intestinal mass and length, whereas only the non-estivating *Bufo marinus* experienced an increase in intestinal size.

For the three estivating species, feeding triggered a dramatic increase (averaging 230%) in small intestinal wet mass (Fig. 6). Among the non-estivating species, only *B. marinus* experienced an increase (P=0.016) in intestinal mass with feeding. For the three non-estivating species, average mass of the small intestine was only 50% greater after feeding. Small intestinal length also increased significantly (P<0.024) with feeding for the three estivating species, but did not for any of the non-estivating species (Fig. 6). Within 24 h after feeding, estivating species had experienced on average an 83% increase in small intestinal length.

For all six species, there was no significant difference in the thickness of the intestinal muscularis/serosa layer between fasted and fed individuals (Fig. 7). The thickness of the intestinal mucosa, largely a function of villus height, increased significantly (P<0.045) with feeding (by 75–130%) for the three estivating species, but did not vary for any of the non-estivating anurans (Fig. 7). Feeding had a significant (P<0.04) effect on enterocyte height for both species of bufonids, increasing the height of cells by 145% and 57%, respectively for *B. alvarius* and *B. marinus* (Fig. 7). In contrast, feeding did not impact enterocyte height for either species of Leptodactylidae or Ranidae. Feeding did induce significant (P<0.04) increases in enterocyte width for all six species, as enterocytes of estivating and non-estivating species increased by an average of 90% and 40%, respectively (Fig. 7). The postprandial increase in enterocyte length and/or width generated the concurrent increase (P<0.05) in enterocyte volume for five of the six species (Fig. 7). Enterocytes increased on average 150% in volume for non-estivators, and 440% in volume for the estivators.

The postprandial release of bile resulted in a significant (P<0.032) decline (65±6%) in the wet mass of the gallbladder for all six species (Tables 2 and 3). This was the only organ to exhibit a significant change in mass with feeding for the three non-estivating species (Table 3). In contrast, the three estivating species experienced significant postfeeding increases in the mass of the liver, stomach, spleen, pancreas, large intestine, and kidneys (Table 2). Most notable is the 65% and 45% respective increases in large intestinal and kidney mass for these three species and the doubling of stomach mass for *C. ornata* and *P. adspersus*.

Metabolic and organ responses to estivation

Ceratophrys ornata and *P. adspersus* experienced significant (P<0.045) reductions in $\dot{V}_{O_2}$ following 1 month of induced estivation within the laboratory (Fig. 8). Compared to frogs fasted for 1 month, but alert and hydrated, estivation reduced $\dot{V}_{O_2}$ by 25% and 16%, respectively, for *C. ornata* and *P. adspersus*. Anterior and distal uptake rates averaged lowered for estivating frogs compared to fasted individuals, but the difference was only significant (P=0.012) for D-glucose uptake by the anterior small intestine of *P. adspersus*. Concurrently, both species experienced significant estivation-induced reduction in small intestinal mass, declining on the order of 31% and 57%, respectively, for *C. ornata* and *P. adspersus* (Fig. 8). The loss of intestinal mass during estivation is largely responsible for the significant (P<0.046) lowering of intestinal uptake capacity of all three nutrients for both species (Fig. 8). Collectively, estivating frogs experienced a 60% reduction in intestinal uptake performance compared to fasted frogs, and a 93% reduction compared to fed frogs (Fig. 4). Whereas estivation resulted in a decrease in small intestinal mass, no other organ experienced a significant change in either wet or dry mass with estivation.

Discussion

An adaptive interplay between feeding habits and the regulation of digestive performance has previously been demonstrated for snakes (Secor and Diamond, 2000). In that study, the match between feeding habits and digestive

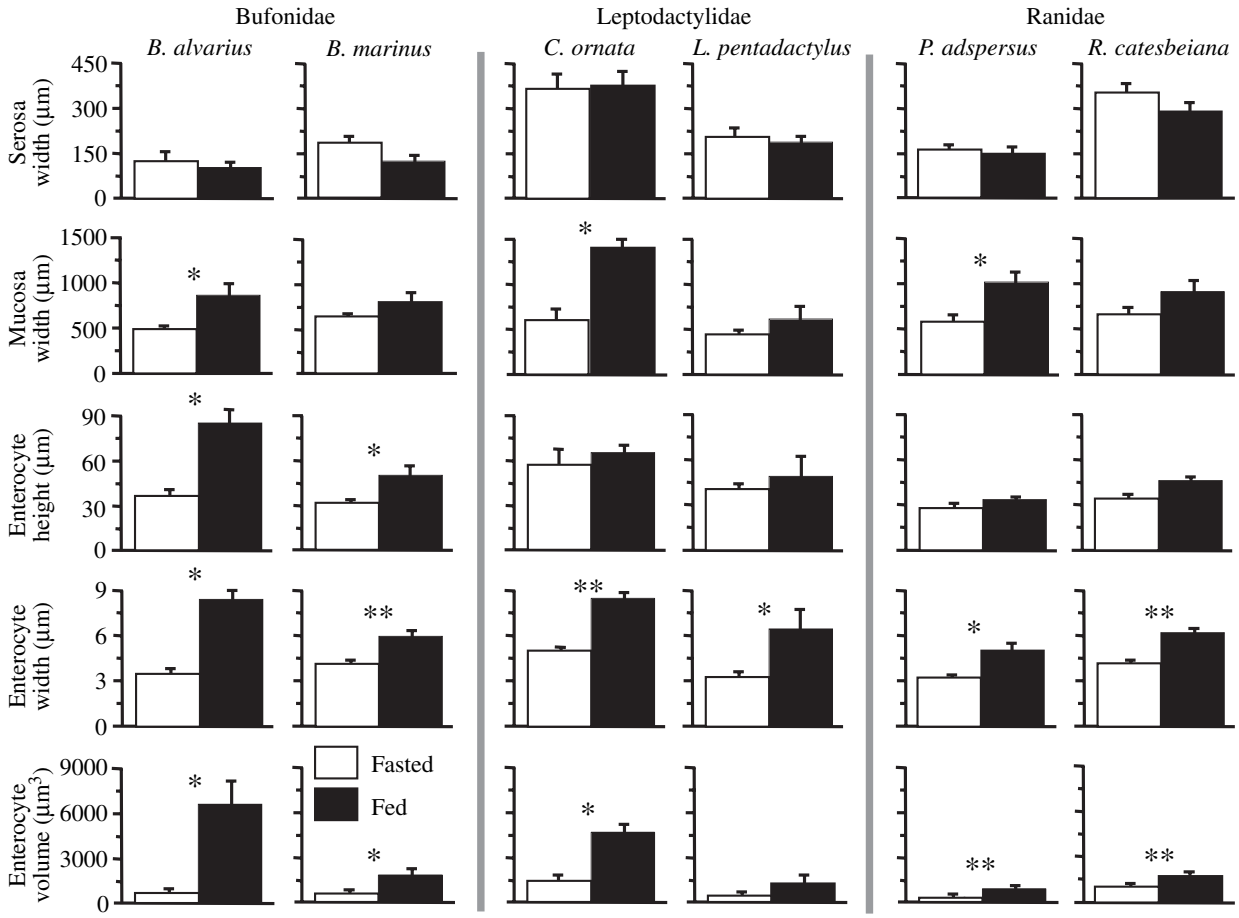

Fig. 7. Width of the intestinal serosa and muscularis/mucosa layers, and height, width and volume of intestinal enterocytes of six anuran species after a 2 week fast (fasted, open bars) and at 1 day postfeeding (fed, closed bars). Note that none of the six species experienced a significant change in serosa width, whereas mucosa width increased significantly postfeeding for the three estivating species. Enterocyte height increased postprandially for only the two species of bufonids, whereas enterocyte width and volume increased significant for all six species with feeding (with the exception of enterocyte volume for *Leptodactylus pentadactylus*).

physiology is potentially influenced by the fact that all of the frequently feeding species were of the same family, Colubridae. Although a phylogenetic independent contrast analysis revealed that the correlation between the magnitude of intestinal regulation and natural fasting interval is independent of phylogeny (Secor and Diamond, 2000), the potential impact of phylogeny still exists. One design objective of this study was to use species with contrasting ecologies from the same family and thereby reduce the confounding influence of phylogeny. For three anuran families, species that are dormant and aphagic for 6–10 months of the year regulate intestinal performance over a much greater span than species that are active and feed for most of the year**.** These findings support the hypothesis of an evolved match between feeding ecology and the capacity to regulate intestinal performance. In the ensuing discussion, I shall comment on how these anurans respond metabolically to feeding, the proximal mechanisms underlying the regulation of anuran intestinal performance, the advantage of gut regulation for estivating anurans, and four questions to be further explored.

Metabolic response to feeding

Meal digestion is accompanied by an increase in metabolic rate, which represents the specific dynamic action of meal, which in its entirety represents the combined energy expended on meal ingestion, digestion and assimilation (Kleiber, 1975). The six anuran species of this study all experience significant postprandial increases in metabolic rate by as much as 3.5- to 11.6-fold. Metabolic increases associated with feeding have previously been documented for the anurans *B. marinus* (Secor and Faulkner, 2002; Andersen and Wang, 2003), *Bufo boreas*, *Bufo terrestris* and *Bufo woodhousei* (Secor and Faulkner, 2002), *Ceratophrys cranwelli* (Powell et al., 1999) and *R. catesbeiana* (Busk et al., 2000). Anderson and Wang (2003) fed adult *B. marinus* rat pups equal in mass to 8.5% of toad body mass and observed a 2.8-fold increase in $\dot{V}_{O_2}$. The larger postprandial increase in $\dot{V}_{O_2}$ observed in this study for *B. marinus* (3.9-fold) simply reflects their larger meals (15% of body mass), and that the postprandial metabolic response increases with meal size (Secor and Faulkner, 2002). For *B. boreas*, *B.*

Table 2. *Body mass and wet and dry masses of organs removed from fasted and fed estivating anuran species*

	Bufo alvarius			*Ceratophrys ornata*			*Pyxicephalus adspersus*		
Mass (g)	Fasted	Fed	*P*	Fasted	Fed	*P*	Fasted	Fed	*P*
Body	150±23	139±24	0.751	178±48	200±65	0.795	235±36	246±18	0.806
Lung wet	1.684±0.211	1.825±0.403	0.499	1.197±0.511	1.496±0.239	0.650	1.734±0.434	1.931±0.313	0.591
Lung dry	0.250±0.053	0.246±0.049	0.667	0.200±0.090	0.207±0.040	0.780	0.255±0.064	0.282±0.041	0.699
Heart wet	0.676±0.064	0.736±0.131	0.443	0.868±0.236	1.083±0.177	0.304	1.215±0.300	1.140±0.097	0.132
Heart dry	0.136±0.010	0.140±0.042	0.844	0.115±0.033	0.136±0.022	0.571	0.174±0.039	0.148±0.014	0.090
Liver wet	3.846±0.550	5.168±0.922	0.042	9.591±3.404	10.59±4.11	0.594	4.637±0.574	7.003±0.645	0.231
Liver dry	1.144±0.169	1.352±0.253	0.152	2.559±0.806	2.900±1.334	0.787	1.604±0.203	2.288±0.398	0.278
Stomach wet	2.673±0.284	3.475±0.484	0.099	2.861±0.852	6.774±1.880	0.009	2.424±0.400	6.182±0.741	0.012
Stomach dry	0.410±0.050	0.486±0.076	0.313	0.572±0.187	0.920±0.290	0.005	0.416±0.060	0.784±0.117	0.048
Gallbladder wet	0.112±0.010	0.056±0.018	0.010	0.390±0.015	0.114±0.007	0.0006	0.352±0.064	0.197±0.024	0.010
Gallbladder dry	0.022±0.005	0.039±0.003	0.070	0.059±0.008	0.018±0.001	0.014	0.044±0.008	0.026±0.006	0.114
Spleen wet	0.044±0.018	0.057±0.022	0.611	0.028±0.003	0.039±0.002	0.061	0.046±0.005	0.050±0.004	0.487
Spleen dry	0.011±0.005	0.011±0.004	0.859	0.006±0.001	0.009±0.001	0.037	0.010±0.001	0.010±0.001	0.604
Pancreas wet	0.152±0.016	0.182±0.045	0.186	0.281±0.064	0.424±0.049	0.023	0.232±0.040	0.326±0.030	0.037
Pancreas dry	0.031±0.004	0.037±0.008	0.227	0.062±0.020	0.090±0.008	0.023	0.047±0.007	0.058±0.006	0.037
Large intestine wet	0.543±0.067	0.899±0.200	0.047	0.890±0.180	1.331±0.317	0.048	0.758±0.120	1.356±0.137	0.049
Large intestine dry	0.079±0.010	0.128±0.028	0.035	0.149±0.031	0.185±0.049	0.278	0.115±0.020	0.176±0.009	0.094
Kidneys wet	0.676±0.049	0.752±0.098	0.007	0.644±0.183	1.083±0.208	0.005	0.636±0.161	0.908±0.033	0.022
Kidneys dry	0.113±0.009	0.149±0.017	0.010	0.099±0.031	0.166±0.028	0.013	0.101±0.024	0.147±0.017	0.083

Table 3. *Body mass and wet and dry masses of organs removed from fasted and fed non-estivating anuran species*

	Bufo marinus			*Leptodactylus pentadactylus*			*Rana catesbeiana*		
Mass (g)	Fasted	Fed	*P*	Fasted	Fed	*P*	Fasted	Fed	*P*
Body	142±20	142±28	0.998	139±31	147±29	0.866	330±32	299±3	0.392
Lung wet	1.679±0.153	1.556±0.449	0.776	1.264±0.256	1.221±0.212	0.063	1.754±0.100	1.549±0.133	0.576
Lung dry	0.241±0.027	0.234±0.076	0.916	0.186±0.049	0.186±0.041	0.194	0.242±0.018	0.254±0.024	0.409
Heart wet	0.753±0.117	0.759±0.201	0.964	0.920±0.208	0.929±0.265	0.766	1.141±0.082	1.277±0.078	0.171
Heart dry	0.124±0.012	0.143±0.047	0.604	0.126±0.031	0.134±0.041	0.970	0.140±0.012	0.168±0.019	0.242
Liver wet	3.936±0.045	4.856±2.072	0.600	1.620±0.429	2.700±0.827	0.080	5.743±0.377	5.876±1.227	0.826
Liver dry	1.067±0.030	1.061±0.434	0.989	0.405±0.119	0.644±0.207	0.141	1.327±0.209	1.581±0.414	0.588
Stomach wet	2.497±0.465	3.089±0.590	0.175	1.991±0.531	2.323±0.421	0.332	7.664±0.462	8.076±0.595	0.577
Stomach dry	0.463±0.089	0.529±0.097	0.438	0.414±0.113	0.462±0.085	0.556	1.324±0.148	1.344±0.023	0.620
Gallbladder wet	0.314±0.069	0.082±0.025	0.017	0.145±0.067	0.035±0.003	0.043	1.371±0.294	0.300±0.109	0.032
Gallbladder dry	0.052±0.010	0.014±0.003	0.011	0.021±0.009	0.006±0.001	0.139	0.179±0.054	0.053±0.012	0.047
Spleen wet	0.082±0.014	0.087±0.019	0.776	0.083±0.027	0.068±0.010	0.444	0.283±0.075	0.117±0.005	0.080
Spleen dry	0.018±0.003	0.018±0.004	0.994	0.019±0.006	0.015±0.001	0.418	0.062±0.015	0.025±0.001	0.058
Pancreas wet	0.284±0.306	0.306±0.050	0.348	0.086±0.010	0.087±0.019	0.765	0.190±0.059	0.318±0.046	0.116
Pancreas dry	0.050±0.005	0.052±0.009	0.147	0.019±0.002	0.019±0.004	0.732	0.039±0.012	0.064±0.006	0.103
Large intestine wet	0.975±0.219	1.232±0.245	0.114	0.631±0.245	0.783±0.235	0.233	0.957±0.096	1.291±0.093	0.104
Large intestine dry	0.152±0.036	0.179±0.036	0.094	0.116±0.042	0.125±0.040	0.955	0.147±0.023	0.192±0.018	0.208
Kidneys wet	0.748±0.148	0.813±0.111	0.600	0.466±0.086	0.583±0.103	0.059	1.123±0.217	1.201±0.128	0.290
Kidneys dry	0.129±0.026	0.148±0.034	0.510	0.083±0.015	0.106±0.022	0.074	0.171±0.034	0.214±0.016	0.100

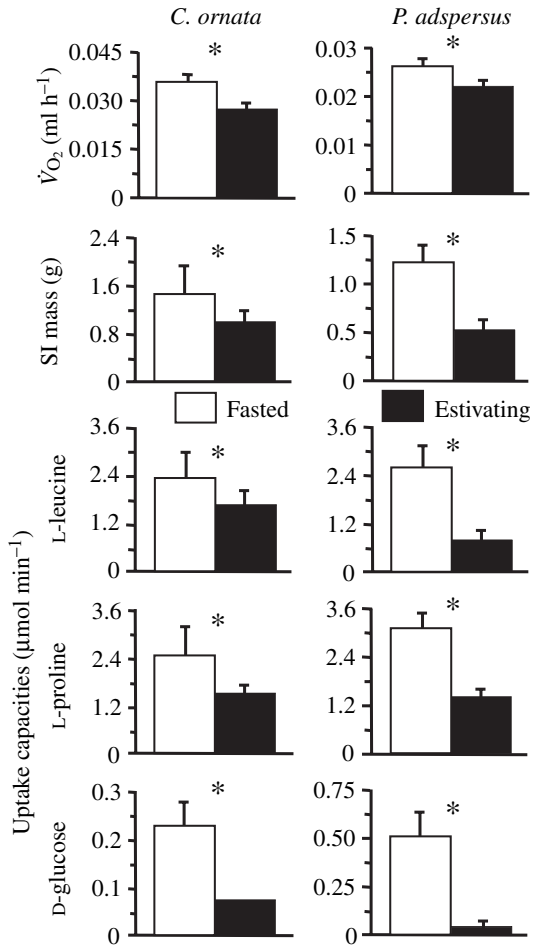

Fig. 8. Mean $\dot{V}_{O_2}$ at 30°C, small intestinal (SI) mass, and total small intestinal uptake capacity of L-leucine, L-proline and D-glucose, of *Ceratophrys ornata* and *Pyxicephalus adspersus* either following a 2 week fast (fasted, open bars) or following 1 month of laboratory-induced estivation (estivated, closed bars). Note that with estivation, both species experienced significant decreases in metabolic rate, intestinal mass and performance.

terrestris and *B. woodhousei* the digestion of cricket meals equaling 10% of body mass generated an average threefold increase in $\dot{V}_{O_2}$, and mass-specific SDA values (kJ kg^{-1}) similar to those experienced by the non-estivating species of this study (Secor and Faulkner, 2002).

The three- and fourfold increases in $\dot{V}_{O_2}$ observed for adult *R. catesbeiana* are well matched to meal sizes of 10% (Busk et al., 2000) and 15% (this study) of body mass, respectively. Interestingly, under similar experimental treatment (rodent meals 17% of body mass at 30°C), *C. cranwelli* experienced only a fourfold increase in metabolic rate, less than half of the magnitude of response observed for *C. ornata* in this study. One explanation for this difference in the metabolic response is the much smaller body size of *C. cranwelli* (mean=23.2 g) used in the Powell et al. (1999) study compared to *C. ornata* of this study, and the findings that the scope of postprandial peak $\dot{V}_{O_2}$ increases with body mass (Secor and Diamond, 1997; Secor and Faulkner, 2002).

The significantly greater SDA of the estivating species compared to the non-estivators is due to the estivators' lower SMR, higher postprandial peak in $\dot{V}_{O_2}$, and longer duration of significant metabolic response. In combination, these differences account for a 55% greater SDA for the estivating species compared to the non-estivators. In a similar fashion, sit-and-wait foraging snakes that feed infrequently possess SMR that are 50% less, postprandial peak $\dot{V}_{O_2}$ that are 25% greater, and twice the duration of the SDA response compared to frequently feeding, active-foraging species (Secor and Diamond, 2000). Thereby, independent of meal size and body temperature, sit-and-wait foraging snakes experience an SDA that is 80% greater than that of active foraging species. Connected by natural long episodes of aphagia, estivating anurans and sit-and-wait foraging snakes appear to exhibit parallel metabolic responses to fasting and feeding.

Proximate mechanisms of intestinal regulation

As quantified, intestinal nutrient uptake capacity is the product of small intestinal mass and mass-specific rates of nutrient uptake. For the three estivating species, the significant postprandial increase in their intestinal uptake capacity is a shared function of an averaged 120% increase in nutrient uptake rates and a 160% increase in small intestinal mass. Although matched by family, the non-estivating species exhibit much more modest postprandial increases in both nutrient uptake (26±4% increase) and intestinal mass (49±11% increase). Whereas intestinal function and morphology contribute relatively equally to the upregulation of intestinal performance for estivating anurans, the relative contribution of nutrient uptake is much greater for infrequently feeding snakes. The five- to 30-fold increase in nutrient uptake capacity that infrequently feeding snakes experience with feeding is predominately due to an averaged sevenfold increase in uptake rates, and to a lesser extent, to the doubling of small intestinal mass (Secor and Diamond, 2000). Hypertrophy of the intestinal mucosa is largely responsible for the postprandial increases in small intestinal mass for both snakes and anurans. After feeding, intestinal enterocytes of the infrequently feeding sidewinder rattlesnake *Crotalus cerastes* increase in width and volume by 75% and 400%, respectively (Secor et al., 1994), increases that are similar to those experienced by estivating anurans (Fig. 6).

Hypertrophy of enterocytes generates a lengthening of the intestinal villi and a resulting increase in functional surface area. The role of increasing luminal surface area to upregulate intestinal performance is evident for the infrequently feeding Burmese python *Python molurus*. The python's eightfold postprandial increase in nutrient uptake rates is contributed to by a 40% increase in villus length and a 4.8-fold increase in microvillus length (Lignot et al., 2005). From electron micrographs of intestinal microvilli of a fasted and a fed individual, I did not observe any significant postprandial

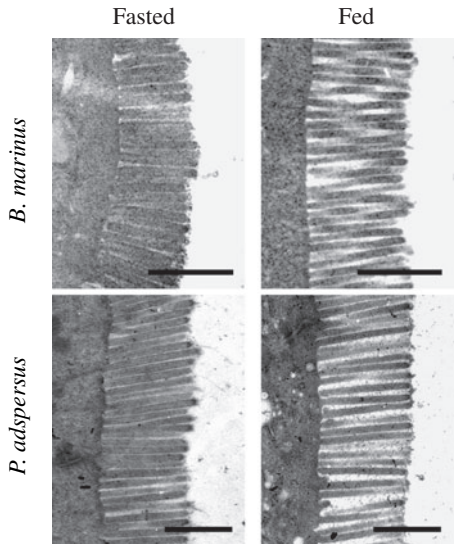

Fig. 9. Electron micrographs of intestinal microvilli of fasted and fed *Bufo marinus* and *Pyxicephalus adspersus*. Note the lack of a significant postprandial change in microvillus length for either species. Bars, 1 μm.

change in microvillus length for any of the species (Fig. 9). Among the six species, microvilli of fasted and fed individuals averaged 1.38±0.39 μm and 1.48±0.24 μm in length, respectively. Hence, anurans apparently elevate intestinal performance by increase luminal surface area at the villus level and potentially by increasing nutrient transport activity and/or density at the cellular level.

The adaptive advantage of gut regulation

For infrequently feeding snakes, the fasting-related downregulation of gut performance is proposed to be an adaptive mechanism to reduce metabolic rate and thus energy expenditure between their infrequent meals. An objective of this study was to determine whether anurans that estivate and are aphagic for long intervals exhibit similar integrated responses. For three anuran species that estivate, fasting was characterized by the significant downregulation of intestinal performance and relatively low rates of metabolism. Compared to similar-sized members of the same family that do not estivate, the estivating species possess intestinal uptake capacities and SMR's that are, respectively, 40% and 62% lower. In considering that whole-animal rates of metabolism represent the summed metabolic output of all tissues, a downregulated gut would contribute to their lower SMR. The ecological significance of a lower SMR is apparent when calculating the longevity without feeding of *P. adspersus* with *R. catesbeiana*. Assuming equal body size (250 g), 3% body fat, and an average body temperature of 25°C, an inactive, non-estivating, *P. adspersus* would theoretically be able to survive 145 days on its fat stores alone, more than twice the duration (60 days) predicted for an inactive *R. catesbeiana*. Possessing lower SMR would similarly allow *B. alvarius* and *C. ornata* to survive longer without food than *B. marinus* and *L. pentadactylus*.

For estivating anurans, the lowered metabolic rate and downregulation of intestinal performance with fasting can be viewed as a precursor to the further depression of their physiology with estivation. The depression in metabolic rate with estivation observed in this study and others have been attributed to reductions in cellular activity, including decreases in RNA synthesis, protein synthesis, enzyme activity, ion pumping and protein phosphorylation (Guppy and Withers, 1999; Storey, 2002). The respective 45% and 74% decline in enterocyte performance for *C. ornata* and *P. adspersus* following laboratory-induced estivation likely contributes to the concurrent decrease in metabolic rate. In addition to the intestine, other tissues (i.e. stomach, liver, kidneys) are also expected to be downregulated during estivation because of their lack of use. For the anuran, *Neobatrachus centralis*, the 67% decline in liver protein synthesis during estivation contributes to the 55% decrease in liver metabolism, which is responsible for 5% of the reduction in whole-animal metabolism (Fuery et al., 1998). Organs such as the heart and lungs may remain unregulated given that cardiac and pulmonary performance are still maintained during estivation for *P. adspersus* (Loveridge and Withers, 1981).

The reduction in metabolic rate during estivation will serve to further extend an anuran's survivorship. The decrease in metabolism observed in this study for *C. ornata* and *P. adspersus* would theoretically add another month to their dormancy. Loveridge and Withers (1981), studying the metabolic rate of dormant and cocooned *P. adspersus*, estimated that a 500 g individual should be able to survive dormant for almost 9 months on 15 g of body fat at 20°C. Although I did not measure the metabolic rate and intestinal performance of estivating *B. alvarius*, which unlike *C. ornata* and *P. adspersus* do not form a cocoon, I would expect this species to exhibit similar adaptive responses to estivation.

In light of the advantages of a downregulated gut during fasting, there are two potential setbacks. First is the added cost of upregulation, which would contribute to the larger postprandial metabolic responses of estivating species compared to non-estivating species. For the infrequently feeding *P. molurus* it is estimated that the upregulation of the gastrointestinal tract is equivalent in cost to approximately 5% of the ingested energy (Secor, 2003). For an estivating anuran, the first meal would require the upregulation in structure and function of the GI tract; the intestine alone increases sixfold in mass and triples in nutrient uptake rates. Second, the potential reduction in digestive performance and efficiency with the first post-estivation meal. Following 3 months of laboratory estivation, passage time for the first meal doubles for *Cyclorana alboguttata*, although without any loss of energy extraction efficiency (Cramp and Franklin, 2003). For a migratory bird (*Sylvia atricapilla*) that has not fed for several days, refeeding is characterized by an initial low rate of nutrient assimilation (Karasov and Pinshow, 2000). Following 10 months of estivation, an anuran's first meal will probably

require more energy and time to process, with or without a reduction in extraction efficiency.

Further insights in amphibian digestive physiology

Interest in amphibian physiology has had a recent reinsurgence with the global realization that amphibian populations and species are rapidly disappearing (Stuart et al., 2003). While there are several proposed causes for the demise of amphibian populations (parasites, UV radiation, pesticides, herbicides), they are all based on a common mechanism, the inability of the amphibian's physiology to cope with the new and in many cases unnatural environmental perturbation. Exploring the mechanisms by which amphibians can endure natural perturbation and regulate tissue performance would provide insights into their ability to survive environmental challenges. The findings of this and other studies spark many questions regarding amphibian physiology, but the following four are relevant to their digestive physiology.

First, given the energetic advantage of gut downregulation, why don't all anurans widely regulate intestinal performance with feeding and fasting, as observed for the three estivating species? To borrow from the explanation on the narrow regulation of intestinal performance by frequently feeding snakes, it may in fact be energetically inefficient for a more frequently feeding anuran to be constantly up- and downregulating the gut with each frequent meal (Secor, 2001). By maintaining the gut in a state of readiness, the frequent meals can be digested rapidly and efficiently. Field data on feeding frequency and energetics would be valuable in constructing energy models that demonstrate the benefits in linking feeding ecology with gut regulation.

Second, how general is the combination of depressed metabolism and downregulated gut performance among estivating amphibians? Among practically all estivating amphibians studied, estivation is characterized by a depression in metabolic rate (Pinder et al., 1992; Guppy and Withers, 1999). One study that has documented a change in gut structure or function with estivation, found a 85% decline in small intestinal mass for estivating *Cyclorana alboguttata* (Cramp and Franklin, 2003). Further studies on other estivating amphibians (i.e. *Scaphiopus*, *Neobatrachus*, *Amphiuma*, *Siren*) will determine the extent that metabolic depression is aided by gut downregulation.

Third, hibernation is another life-history feature of amphibians that entails long episodes of aphagia. To what extent does the amphibian intestine respond to hibernation? Metabolic depression during hibernation is largely achieved by the substantial decrease in body temperature (Storey and Storey, 1990). In addition, the downregulation of the gut could supplement the depressed metabolism, allowing the animal to survive longer on endogenous energy stores. This question could be addressed by comparing metabolic rates and intestinal morphology and function at various times during hibernation.

Fourth, what are the mechanisms that underlie the regulation of intestinal performance for anurans? Tissue, cellular and molecular studies could ascertain the cascade of events that result in the enterocyte hypertrophy and increase in nutrient uptake with feeding, and the subsequent atrophy and downregulation with estivation. Combined with studies on the cellular mechanisms of metabolic depression (Storey, 2002), an integrated picture could emerge on the estivating-induced reduction in tissue function and metabolism.

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