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What can turtles teach us about the theory of ecological stoichiometry?

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SUMMARY

1. Vertebrate skeletons have high phosphorus (P) content relative to other tissues. Variation in skeletal investment within and among species is hypothesised to predict variation in P demand, standing stock and recycling. These relationships have been examined among fish, but not in vertebrates with more robust skeletons, such as turtles.

2. Our objectives were to (i) describe freshwater turtle stoichiometry relative to skeletal mass, (ii) compare turtle body and excreta stoichiometry to patterns among fish and (iii) relate turtle skeletal stoichiometry to turtle nutrient storage and recycling.

3. Skeleton constituted 82% of turtle dry mass. Total body %P increased ontogenetically with turtle mass and 93% of all P resided in the skeleton. Phosphorus storage within turtle assemblages was high (0.2–0.45 kg ha^{-1}). Turtles excreted lower concentrations of P than fish. Excreta N:P was positively correlated with body N:P, suggesting that increased skeletal P investment was inversely related to P demand.

4. Our results demonstrate that P stored in the bone of turtles can represent a large standing stock of P in fresh waters. Further, our work suggests skeletal investment alone is not sufficient to predict an animal's P demand and, by extension, their effects on nutrient recycling. Instead, our results indicate P demand is determined by both skeletal investment and growth rate. Consequently, taxa with high body P, extreme longevity and slow growth rates, such as adult turtles, may serve as stable standing stocks of nutrients while also contributing proportionately to nutrient remineralisation.

Keywords: nutrient recycling, nutrient storage, phosphorus, reptile, skeleton

Introduction

Freshwater biota can affect the spatial and temporal dynamics of nutrient cycling through the import and export of elements across system boundaries (Vanni, 2002; Regester, Lips & Whiles, 2006) and through the capture, retention and recycling of elements over time (Carpenter, Cottingham & Schindler, 1992; Small, Helton & Kazanci, 2009; Schmitz, Hawlena & Trussell, 2010). Trophic specialisation and physiology can also influence spatial and temporal nutrient dynamics (Vanni, 2002). Ecological stoichiometry (ES) proposes that imbalances between an animal's diet and demand for an element can be used to predict ratios and rates of nutrient recycling as animals (especially vertebrates) are thought to be relatively stoichiometrically homeostatic (Sterner & Elser, 2002; Vanni, 2002). Depending on their stoichiometry and biomass, particular species can have large effects on nutrient dynamics (Elser *et al*., 1996; Vanni, 1996; Small *et al*., 2011). For example, Small *et al.* (2011) found that *Astyanax aeneus* represented only 18% of the total fish biomass in phosphorus (P)-limited Costa Rican streams, but contributed up to 90% of the P remineralised by the fish community.

A number of factors determine taxon- or age-specific variation in tissue stoichiometry. Ecological stoichiometry is partially founded upon 'stoichiometric invariance', which refers to the fact that nutrient concentrations of most tissues are relatively constant across a range of taxa (see review by Allen & Gillooly, 2009); thus, differences in tissue mass should determine the capacity for nutrients to limit growth. For example, RNA is P-rich

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and larger, faster growing taxa produce more RNA (Sterner & Elser, 2002). Work with microbes and small invertebrates has suggested that rapidly growing organisms become P-limited due to the high P demand of RNA production (Elser *et al.*, 2003). However, in vertebrates, the P demand of RNA is thought to be much smaller than the demand from P-rich tissues, such as bone, and has the potential to alter the relationships between tissue stoichiometry and body mass (Elser *et al.*, 1996; Allen & Gillooly, 2009).

The presence of bone is a key trait that distinguishes the stoichiometry of vertebrates from other animals, and variation in skeletal investment among vertebrates is probably a trait that determines taxon-specific effects on nutrient dynamics (Reiners, 1986; Sterner & Elser, 2002; Hendrixson, Sterner & Kay, 2007). Bone accounts for >20% of biomass of some vertebrates and is composed of collagen and mineral hydroxyapatite ($(Ca_{10}(PO_4)_6(OH_2)$) that has a distinctive stoichiometry (i.e. nitrogen (N): phosphorus (P) ratio = ~0.8) compared to other tissues (Anderson, Rahn & Prange, 1979; Iverson, 1984; Sterner & Elser, 2002). Additionally, bone can have a very low turnover rate such that vertebrates with robust skeletons, large body sizes or standing biomass, or high longevity may have unique effects on ecosystem processes, such as maintaining large biotic pools of limiting nutrients and slowing downstream spiralling of nutrients (Kitchell *et al.*, 1979; Small *et al.*, 2009; Vanni, Boros & McIntyre, 2013).

Following the assumptions of ES, we propose that increased skeletal investment implies increased P demand and increased P demand should result in disproportionately high P retention and low P excretion (Sterner & George, 2000; Vanni *et al.*, 2002; Hendrixson *et al.*, 2007). For example, some fish families (e.g. Salmonidae and Cyprinidae) have features such as smooth cycloid scales and modest internal skeletons, resulting in relatively low body P content (Sterner & George, 2000; Hendrixson *et al.*, 2007). In contrast, heavily armoured catfish (e.g. Loricariidae and Aspredinidae), which invest in bony plates and robust, dorsolaterally flattened skulls, have the highest overall P content reported for fish (Vanni *et al.*, 2002; Hood, Vanni & Flecker, 2005; Hendrixson *et al.*, 2007).

Ecological stoichiometry predicts that differences in skeletal investment among fish species or age classes within species can explain differences in body N:P ratios. Body N:P is thought to be inversely correlated with the N:P ratio of excreta. Thus, we predict that individuals with more bone have lower body N:P and excrete higher N:P due to increased P sequestration rates for skeletal maintenance and growth (Sterner & Elser, 2002). However, one can also make a paradoxical counter-prediction about the relationship between skeletal composition and P demand. Because bone has a slow turnover rate relative to other tissues (Daelerum & Angerborn, 2005), the maintenance of bone among mature individuals with relatively determinant growth is likely to create little demand for P in adults while creating a high P demand among growing juveniles. Consequently, one could predict that the relationship between body P and P demand depends on an individual's growth rate and the overall contribution of the skeleton to its mass.

The relationships between skeletal investment, body stoichiometry and nutrient recycling have not been adequately examined across vertebrate taxa; nearly all knowledge on vertebrate stoichiometry has come from studies of fish. Thus far, work on fish supports the prediction that increased skeletal investment is positively related to P demand (Pilati & Vanni, 2007; McIntyre & Flecker, 2010). However, we note that fish bones are typically not dense and that fish skeletons generally represent a small proportion of total tissue mass compared to other vertebrates, such as mammals and reptiles (Iverson, 1984). The question remains whether the stoichiometric relationships, particularly the effects of skeletal investment and indeterminate growth on nutrient demand, can be generalised from fish to other vertebrates.

Turtles are unique in morphology, physiology, life history and ecology, which may add a novel perspective to ES. Turtles have evolutionarily modified their ribs and repositioned the pectoral and pelvic girdles, resulting in an encased axial skeleton [shell] made of dermal and endochondral bone and covered by keratinised scutes (Gilbert, Cebra-Thomas & Burke, 2008). High nutrient demand to produce a shell is hypothesised to place a significant growth constraint on juvenile turtles (Clark & Gibbons, 1969). In contrast, adult turtles exhibit negligible growth and extreme longevity, which is predicted to alleviate nutrient demands for shell production (Clark & Gibbons, 1969; Gibbons, 1987; Congdon *et al.*, 2013). Turtles occur globally in nearly all cool-temperate to tropical freshwater environments, and populations can reach remarkably high biomass (Iverson, 1982; Vitt & Caldwell, 2009). High skeletal investment, high biomass, negligible adult growth and extreme longevity may make the effects of turtles on nutrient storage and recycling within freshwater ecosystems distinctive from other aquatic taxa. The objectives of our study were to (i) provide the first description of the ecological stoichiometry of freshwater turtles relative to skeletal mass and tissue-specific stoichiometry, (ii) examine the relationship between turtle

body and excreta stoichiometry and compare it to freshwater fish and (iii) provide the first estimates of nutrient storage and recycling of common freshwater turtle species in streams and ponds of the south-eastern United States.

Methods

Study site and focal species

Turtle populations were studied in three sites from three habitat settings: the North Oconee River (NOR), a complex of man-made ponds within the Whitehall Experimental Forest (WEF) near the NOR and two stream tributaries (Ichawaynochaway and Spring Creeks) of the Lower Flint River Basin (LFRB; detailed site descriptions available in Sterrett, 2014). Sampled study ponds were <1 ha in wetted area, and sampled stream reaches were 0.5 km in length. The three settings had similar turtle assemblages but differed in species' relative abundance (see Sterrett *et al*., 2011 and Sterrett, 2014). All habitats were generally dominated by omnivorous yellow-bellied sliders (Emydidae, *Trachemys scripta*) and musk turtles (Kinosternidae, *Sternotherus* spp.). All three habitats contained omnivorous common snapping turtles (Chelydridae, *Chelydra serpentina*). WEF ponds were the only sites that contained abundant, omnivorous painted turtles (Emydidae, *Chrysemys picta*), while the NOR and LFRB river habitats contained herbivorous river cooters (Emydidae, *Pseudemys* spp.) and carnivorous softshell turtles (Trionychidae, *Apalone* spp.). The LFRB streams also contained molluscivorous Barbour's map turtles (Emydidae, *Graptemys barbouri*) and omnivorous alligator snapping turtles (Chelydridae, *Macrochelys temminckii*). For this study, we focused on four focal species that were the most commonly encountered and thus provided sufficient sample sizes for estimating body and excretion nutrients: *T. scripta*, *C. picta*, *S. odoratus* and *S. minor*.

Estimating turtle biomass, nutrient standing stocks and nutrient excretion

To estimate turtle standing biomass, nutrient standing stock and excretion rates, we first estimated turtle densities using a capture–mark–recapture robust sampling approach (Pollock, 1982). We sampled turtles for 3–5 consecutive days between May and August from 2010 to 2012 in WEF ponds and from 2011 to 2012 in NOR and LFRB streams. At each site, turtles were captured using hoop traps (0.9 m diameter, three hoops, 3.8 cm mesh) baited with sardines (Legler, 1960).

Twenty traps were set *c.* 25 m apart on alternating banks of each stream, and ten traps were set 20 m apart along the margins of each pond. Our traps were placed closer than recommended (Rodda, 2012), which allowed for more saturated trapping. We recorded each captured turtle's species and sex, measured maximum carapace length, plastron length and wet mass and uniquely marked individuals by filing or drilling the marginal scutes of the carapace (Cagle, 1939). A subset of turtles (n = 92, *C. picta* = 14, *T. scripta* = 27, *S. odoratus* = 32, *S. minor* = 19) was held temporarily for excretion trials, and subsets of those turtles (n = 32, 7–9 per species) were sacrificed for body stoichiometry measurements after the final day of trapping. An additional *S. odoratus* that died in a hoop trap was used for body stoichiometry measurements. All living turtles were released at their point of capture following processing.

We estimated turtle densities in each habitat type for each species and sex. Male and female turtles can be highly sexually dimorphic in size; thus, sex-specific abundances were expected to more accurately reflect total biomass. To do this, we estimated abundance and capture probabilities (p) during consecutive sampling days, while allowing for individuals to leave or die (i.e. apparent survival) and recruit into the site between years (Williams, Nichols & Conroy, 2002; Meador, Peterson & Wisniewski, 2011). Data were not adequate to estimate p for all species–sex combinations; therefore, we pooled capture histories for all species to evaluate support for three alternative time-varying models for estimating p (i.e. capture varies by day, year or constant). In each model, we allowed apparent survival and recruitment to vary among years. We fit candidate models using a Markov chain Monte Carlo (MCMC) Bayesian modelling approach and a dynamic occupancy formulation of the Jolly–Seber *ad hoc* robust design model (Jolly, 1965; Seber, 1965; Kery & Schaub, 2012). We used data augmentation with 500 unobserved individuals as null capture histories (Kery & Schaub, 2012) and evaluated the relative support of each model using Akaike's information criterion with a small-sample bias adjustment (AIC_c) and Akaike model weights (w; Hurvich & Tsai, 1989; Burnham & Anderson, 2002). Models with Akaike weights within 10% of the best-approximating model were considered plausible capture models, which is comparable to the minimum cut-off (i.e. 1/8) suggested by Royall (1997) for evaluating strength of evidence. Using the best-supported capture model (or set of plausible models), we re-ran species- and sex-specific models (n = 14) to estimate abundances within each site during each sampling year. We calculated the average density of individuals

(ind ha^{-1}) across years and sites within each habitat type by dividing average site-specific abundances by site-specific wetted area. Uncertainty in abundance estimates was included in turtle density estimates because average density was calculated within the MCMC framework.

We estimated habitat–sex–species biomass, nutrient standing stock and nutrient excretion rates within the above MCMC model framework to include uncertainty in our estimates. We estimated turtle biomass (wet mass kg ha^{-1}) by multiplying turtle density by species-, sex- and habitat-specific mean wet mass (kg) of captured animals. Nutrient standing crops (C, N, P; kg ha^{-1}) were estimated as the product of dry biomass, AFDM (%) and whole body nutrient content (%C, %N, %P; methods described below). AFDM conversions were estimated using a subset of tissue samples. Each sample was weighed, placed in a ceramic crucible covered with aluminium foil, ashed at 500 °C and reweighed to determine % organic material. Total individual AFDM was calculated as sum of the product of individual tissue % organic material and dry mass divided by total dry mass. Field-collected data provided relationships for estimating dry mass from wet mass for each species (*T. scripta*, $y = 0.3268x - 0.121$, $r^2 = 0.99$; *C. picta*, $y = 0.3563x - 0.121$, $r^2 = 0.99$; *S. odoratus*, $y = 0.4482x - 10.004$, $r^2 = 0.92$; *S. minor*, $y = 0.3609x + 1.1785$, $r^2 = 0.89$). We included uncertainty into biomass and nutrient estimates by randomly selecting a wet mass, dry mass, AFDM and nutrient concentration (%) value during each MCMC iteration using each variable's mean and standard deviation and assuming values are normally distributed. Nutrient excretion of all focal species (μg ha^{-1} h^{-1}) was modelled as the product of density (ind ha^{-1}) and mean habitat-, sex- and species-specific individual nutrient excretion (μg L^{-1} h^{-1}) of total N and P collected from field experiments (described below). Because we had excretion measurements from only one *T. scripta* captured in the NOR, we used the mean and standard deviation of N and P excretion among all *T. scripta* to estimate excretion rates within the NOR. We calculated the total (male and female) density, biomass, nutrient standing crop and nutrient excretion for each habitat type using the sex-specific means and standard deviations for each metric and 10 000 iterations in R (version 3.0.0). We report the mean and 95% credibility intervals of turtle density (ind ha^{-1}), biomass (kg ha^{-1}), nutrient standing crop (total C, N and P (kg ha^{-1})) and nutrient excretion (total N and P μg L^{-1} h^{-1}) of each habitat type. All models were run using JAGS (version 3.2.0; Plummer, 2003) and the R2Jags package in R with three parallel chains each consisting of 20 000 iterations, 5 000 burn-in, and a thinning rate of three. Convergence was assessed using *R-hat* (Brooks & Gelman, 1998), visual inspection of chain mixing and posterior distribution plots.

Estimating individual excretion

Excretion collection occurred in a shaded area at each site using appropriate modifications of methods used to measure fish excretion rates (Schaus *et al.*, 1997). Bait in turtle traps was held in a perforated bag that allowed for dispersal of odour but did not allow turtles to feed. Immediately following capture, turtles were cleaned by removing leeches from all parts of the body, scrubbing off algae and debris from the carapace and plastron and rinsing debris from inguinal and axillary regions with filtered water. Turtles were placed in individual 19-L sterilised (acid-washed or autoclaved) polyethylene bins (45.7 × 30.4 × 22.8 cm; Rubbermaid®, Atlanta, GA, U.S.A.) covered with window screening. Two litres of pond or stream water, which covered most of the carapace of every turtle, was filtered using a portable pump (0.45 m; Masterflex® Peristaltic Pump, Vernon Hills, IL, U.S.A.) to remove suspended particles and added to each bin. A control bin without a turtle was included in each set of excretion trials. In 2011, we collected hourly 60 mL water samples over 7 h and determined that 5–7 h of incubation was sufficient for consistent excretion estimates for three of our four focal species; however, 6–7 h was better for one species (Fig. S1 in Supporting Information). Therefore, we used 6-h incubations for turtles sampled in 2012. All water samples were collected using a new Luer-lock syringe and immediately filtered (0.45 μm) into a Nalgene container and frozen. We minimised handling of animals and used shaded containers to minimise stress and temperature fluctuations (Vanni, 2002; Whiles *et al.*, 2009). Excretion samples were analysed for total dissolved nitrogen (TDN) and total dissolved phosphorus (TDP) following a persulfate digestion at the University of Georgia Analytical Chemistry Laboratory. Excretion was estimated as the difference between the experimental and control samples, and rates were estimated as the changes in TDN and TDP per volume (2 L), per unit time (6 h).

We removed three measurements of TDN and five measurements of TDP that were equal to or less than the values of the control measurements. We used linear regression to quantify allometric relationships between wet body mass (log-transformed) and mass-specific excretion rates (log-transformed). All analyses were completed in Statistica (version 10; StatSoft, Inc.© 2011, Tulsa, OK, U.S.A.).

Estimating tissue and whole body nutrient content

To measure body nutrient content, turtles were euthanised with intravenous injections of zylazine (1 mg kg^{-1}), decapitated once fully anesthetised and immediately frozen following American Veterinary Medicine Association (AVMA) (2007) guidelines. Tissues were later thawed and dissected into categories: shell (carapace and plastron), organs (included all major organs except gastrointestinal tract) and body. Body samples included a mixture of muscle and smaller bones, a subset of which were dissected and separated for analyses. Tissues were dried to a constant mass at 15.5 °C, grounded in a ball mill and re-dried for storage. Carbon and N content was measured by subsampling dry, milled tissues into tins and analysed by micro-Dumas combustion using a Carlo Erba 2NA 1500 CHN analyser (CarloErba, Milan, Italy). Phosphorus content was measured by weighing a subsample of dry, milled tissue into an acid-washed ceramic crucible, ashed at 500 °C, acid-digested and analysed using the ascorbic acid method of spectrophotometry (Jones, Wolff & Mills, 1991). Total body nutrient content was determined as the product of the proportion of nutrient in a tissue, the proportion of the tissue to total mass and total mass. We used a one-way ANOVA to test for differences in nutrient content among species, and Tukey's honestly significant difference was used *post hoc* to compare nutrient concentrations between species. We used linear regression to quantify allometric relationships between body wet mass (log-transformed) and whole body nutrient content (log-transformed). One *S. minor* sample was removed from analyses because nutrient measurements deviated from the mean by >2 standard deviations and were deemed suspect.

Estimating skeletal biomass

In addition to measurements from field-collected turtles, museum specimens were used to determine skeletal investment to overall body mass and nutrient composition. All parts of 151 complete turtle skeletons at the Florida Museum of Natural History (Gainesville, Florida) and the Chelonian Research Institute (Oviedo, Florida) were measured and weighed. The maximum carapace length of skeletons was used to estimate 'live' wet mass of a specimen based on length-to-mass relationships from field-collected animals. We used field-collected length and mass data of the focal species fitted with an allometric equation (power function; $Y = aX^b$) to estimate wet mass (Y) from carapace length (X) and a constant percentage body mass X (a) and percentage body mass X (b). Field-collected data provided relationships for estimating body mass of osteological specimens (*T. scripta*: $n = 125$, $y = 0.0003x^{2.86}$, $r^2 = 0.99$; *C. picta*: $n = 23$, $y = 0.0004x^{2.74}$, $r^2 = 0.97$; *S. minor*: $n = 75$, $y = 0.0002x^{2.96}$, $r^2 = 0.97$; *S. odoratus*: $n = 93$, $y = 0.0048x^{2.40}$, $r^2 = 0.87$).

Estimating relationships between body stoichiometry and growth

We used our measurements and published estimates to examine relationships between species-specific body nutrient content and adult growth among fish and turtles. Data on body nutrient content of fish were retrieved from Tanner, Brazner & Brady (2000), Vanni *et al.* (2002), Dantras & Attayde (2007) and Hendrixson *et al.* (2007). Growth rates for fish published in Tanner *et al.* (2000) were used to compare to adult growth rates of adult turtles that grew over a 10-year period (Congdon *et al.*, 2013). We used estimates of *Kinosternon subrubrum* and *K. sonoriensis* from Congdon *et al.* (2013) as proxies for growth of Kinosternidae. We acknowledge that the growth rates of Tanner *et al.* (2000) are absolute growth rates and do not take into account proportional effects (McIntyre & Flecker, 2010). Although it might be better to compare measures of growth in mass, we used measures of growth in length (mm/day) because these rates were available for both taxa. We used linear regressions to quantify relationships between body %P and estimates of growth rate across fish and turtles.

Results

We made 586 captures of 369 individuals across 10 species and three habitat types. As expected, *T. scripta*, *C. picta*, *S. odoratus* and *S. minor* made up most (86%) of captured individuals. In WEF, *S. odoratus*, *T. scripta*, *C. picta*, *C. serpentina* and *K. subrubrum* composed 57, 19, 18, 3 and 3% of total captures, respectively. In NOR, *T. scripta*, *C. serpentina*, *S. odoratus*, *S. minor*, *C. picta* and *A. spinifera* made up 51, 23, 16, 5, 1 and 1% of individuals captured, respectively. In LFRB, *S. minor*, *T. scripta*, *P. concinna*, *M. temminckii*, *A. spinifera* and *A. ferox* made up 51, 38, 7, 2, 2 and 2% of individuals captured, respectively.

Skeletal biomass and contributions to stoichiometry

Among turtle species, the skeleton constituted 28%, on average, of total wet body mass and 82% of total dry mass. The shell constituted 25 to 36% of the total wet mass among families (Table 1; 82 to 93% by dry mass).

Table 1 Mean skeletal investment (% of wet mass) for four freshwater turtle species, all turtles combined and teleost fish. Standard deviations are in parentheses. Some museum specimens were used for only one measurement because of limitations in skeletal parts

	% Shell*	% Skull†	% Appendicular Skeleton†	% Total Skeleton†
Trachemys scripta	35.7 (8.2) n = 39	0.36 (0.09) n = 12	1.4 (0.4) n = 11	34.9 (3.8) n = 9
Chrysemys picta	26.7 (6.6) n = 41	0.37 (0.05) n = 7	1.9 (0.4) n = 12	28.8 (3.8) n = 10
Sternotherus minor‡	26.4 (6.5) n = 43	3.20 (0.70) n = 13	2.3 (0.4) n = 11	27.2 (3.6) n = 10
Sternotherus odoratus‡	24.8 (6.7) n = 50	1.45 (0.38) n = 17	2.5 (0.9) n = 20	23.7 (3.9) n = 15
Turtles	28.1 (8.1) n = 173	1.50 (1.20) n = 49	2.1 (0.7) n = 54	27.5 (5.5) n = 44
Fish§	–	–	–	3.1 (1.3) n = 37

*Based on field-collected data (n = 32) and museum specimens (n = 141).
†Based on museum specimens.
‡Tend towards carnivory (and molluscivory) as adults.
§Estimated from Reynolds & Karlotski (1977) and Casadevall *et al.* (1990) across 18 species.

Table 2 Mean body stoichiometry (%C, %N and %P) and nutrient ratios (C:N, C:P and N:P, by mass) of body tissues (shell, bone, muscle, organs and whole body) of individuals across turtle species. Standard deviation is in parentheses

	n	%C	%N	%P	C:N	C:P	N:P
Shell	33	21.85 (3.36)	5.06 (0.74)	8.57 (1.01)	4.39 (0.87)	2.60 (0.58)	0.60 (0.15)
Bone	33	23.28 (5.77)	5.13 (1.59)	8.48 (1.61)	4.65 (0.92)	2.88 (1.01)	0.64 (0.25)
Muscle	32	47.04 (5.02)	12.77 (1.45)	0.66 (0.13)	3.69 (0.26)	74.66 (19.38)	20.21 (5.04)
Organs	32	51.57 (6.06)	8.25 (1.30)	0.67 (1.55)	6.28 (1.02)	83.33 (31.49)	13.30 (5.05)
Body*	33	42.91 (4.72)	9.93 (0.99)	2.89 (0.98)	4.40 (0.71)	17.23 (8.06)	3.89 (1.53)

*Whole body without shell.

Mean skeleton percentage by wet mass varied by as much as 45% among species (range 24–35%). The skulls of both kinosternid species were 4 to 9 times the proportion of body mass of the two omnivorous emydid species (Table 1). Turtle bone had 9 to 13 times the concentration of P (8.48%) as internal organs (0.94%) or muscle (0.66; Table 2). The shell contained, on average, 86% of P within a turtle. In contrast, bone was 38 to 60% lower in N (5.1%) than internal organs (8.3%) and muscle (12.8%; Table 2). Turtle shells had the lowest N:P of any tissue (0.60%; hereafter presented only as mass ratios), and muscle had the highest (20.21; Table 2). As a result, a turtle with its shell had a N:P of 1.04 (Fig. 1). Excluding the shell, turtles had a N:P of 3.89 (Table 2).

Total body element concentrations and ratios varied little among turtle species we studied and did not differ across habitats. Body composition (as % of dry mass) among all individuals was 29.72 ± 3.69% C (mean ± SD; range 24.46–37.65), 6.63 ± 0.50% N (range 5.81–7.93) and 6.48 ± 0.84% P (range 4.62–8.12). There was no difference in %N and %P content between species (d.f. = 3, MS = 0.370, F = 1.516, P = 0.233 and d.f. = 3, MS = 0.985, F = 1.706, P = 0.189, respectively). In contrast, %C varied significantly among species (d.f. = 3, MS = 52.31, F = 5.82, P = 0.003) and was driven largely by a higher C content of *C. picta* (Table 3). Specifically, pairwise comparisons (Tukey's HSD) indicated that *C. picta* was significantly higher in %C body content than *T. scripta*, *S. minor* and *S. odoratus* (P = 0.03, 0.002 and 0.05, respectively). Across all four focal species, C:P was 4.69 ± 1.07 (3.01–8.08), C:N was 4.49 ± 0.69 (3.55–5.74), and N:P was 1.04 ± 0.20 (0.76–1.70).

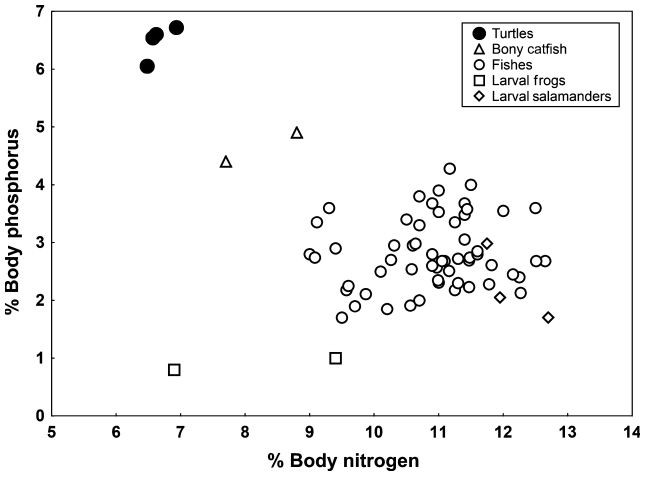

Fig. 1 Mean nitrogen (N) and phosphorus (P) content as a percentage of dry mass for five aquatic vertebrate taxonomic groups. Each point represents the mean of a species or family groups from a study locality. Bony catfish represents Aspredinidae and Loricariidae separately from other fish. Data are compiled from this study, Penczak (1985), Tanner *et al.*, 2000; Vanni *et al.*, 2002; Hendrixson *et al.*, 2007; Dantras & Attayde (2007) and Milanovich (2010).

Table 3 Diet, longevity (years), mean and range (parentheses) size of animals (plastron length (mm), wet mass (g)) and mean and standard deviation (parentheses) body stoichiometry (%C, %N, %P, by dry mass, and N:P ratio, by mass) of four species within two families in this study

Family and species	Diet*	Longevity† (yrs)	Plastron length (mm)	Wet mass (g)	Body stoichiometry			
					%C	%N	%P	N:P
Emydidae								
Trachemys scripta	O (H)	31+	149 (84–223)	767 (132–2050)	29.10 (3.78)	6.93 (0.64)	6.72 (1.04)	1.07 (0.30)
Chrysemys picta	O (H)	40+	111 (81–143)	220 (93–340)	33.51 (1.71)	6.48 (0.54)	6.05 (0.19)	1.07 (0.12)
Kinosternidae								
Sternotherus minor	O (C)	21+	75 (63–86)	143 (79–200)	27.22 (1.82)	6.62 (0.26)	6.60 (1.02)	1.03 (0.21)
Sternotherus odoratus	O (O)	28+	63 (56–74)	95 (69–163)	29.33 (4.10)	6.47 (0.47)	6.61 (0.79)	1.01 (0.12)

*General diet (primary adult diet in parentheses); O = omnivore; C = carnivore; H = herbivore.
†Longevity based on survival estimates in wild populations (see Ernst & Lovich, 2009).

Variation in body nutrient content and mass-specific excretion stoichiometry among turtles was related to body mass, which explained any apparent differences among species or habitats. Because we had measurements only for adult-sized *Sternotherus* spp., we lacked sufficient variation in body size to examine ontogenetic relationships with stoichiometry for species in this genus. Therefore, we conducted analyses on all turtles combined and just on the two emydid species to draw inferences about the generality of relationships specifically within and among species. Among all turtles, there was no significant relationship between body mass and body N:P ($P = 0.41$), although we found this relationship significant among individual emydids ($y = 0.51 - 0.21x$, $r^2 = 0.29$, $P = 0.03$; Fig. 2). Among all turtles and within emydids, body mass was negatively (but weakly) correlated with excretion N:P (all turtles: $y = 0.1798 - 0.3925x$, $r^2 = 0.03$, $P = 0.03$; Emydidae only: $y = 1.4077 - 0.8217x$, $r^2 = 0.15$, $P = 0.01$; Fig. 2). Among all turtles, body mass was negatively correlated with mass-specific P excretion rate ($y = -0.1372 - 0.5905x$, $r^2 = 0.10$, $P = 0.0031$), but not mass-specific TDN excretion rate ($P = 0.44$). Among emydids, there was a significant negative relationship between body mass and mass-specific N excretion ($y = 1.1452 - 0.3976x$, $r^2 = 0.10$, $P = 0.05$), but not TDP excretion ($P = 0.21$). There was a positive correlation between body N:P and mass-specific N:P excretion among all turtles ($y = -0.7422 + 4.3865x$, $r^2 = 0.20$, $P = 0.02$) and within emydids ($y = -0.6781 + 5.7136x$, $r^2 = 0.52$, $P = 0.0025$; Fig. 2).

Biomass, nutrient standing stock and nutrient excretion

Turtle densities varied across sampling sites (mean ± SD; 97 ± 77 ind ha^{-1}) and did not correlate with variations in turtle biomass (34 ± 14 kg wet mass ha^{-1}; Fig. 3) because of differences in relative abundance and intraspecific variation in body size among habitats (S. Sterrett, unpubl. data). Higher densities of turtles within WEF ponds were caused by the high density of *S. odoratus* (Fig. 3), a relatively small turtle. Mean standing crop nutrients for the turtle assemblage across all habitat types were 1.4 ± 0.58 kg C ha^{-1}, 0.33 ± 0.14 kg N ha^{-1} and 0.33 ± 0.14 kg P ha^{-1}. Standing crop C, N and P estimates of turtle biomass varied among sites generally in proportion to differences in turtle biomass and, to a lesser degree, species composition (Table 4; Fig. 2). Because of its high density and biomass among habitat types, *T. scripta* contributed the most to assemblage-level standing crop of C, N and P (Table 4).

Mean turtle assemblage (four focal species) excretion was 47.01 ± 16.28 mg N ha^{-1} h^{-1} and 2.52 ± 117 mg P ha^{-1} h^{-1} across sites (Fig. 4). In stream sites, N and P excretion was dominated by *T. scripta* (Fig. 4; see Appendix Table S1 in Supporting Information). *T. scripta* contributed 54–87% of total N excretion across habitat types. However, *S. odoratus* and *S. minor* rivalled P excretion by *T. scripta* with 43% of total P excretion at WEF and LFRB, respectively (Fig. 4).

A comparison of our measurements and published values of species-specific body nutrient content and adult growth rates of turtles and fish shows a significant negative relationship for turtles and fish combined ($y = 0.1721 - 0.0263x$, $r^2 = 0.92$, $P = 0.0003$; Fig. 5), but an insignificant negative relationship for fish only ($y = 0.294 - 0.078x$, $r^2 = 0.13$, $P = 0.1850$).

Discussion

Variation in skeletal investment is hypothesised to be a key trait determining taxon-specific effects on nutrient dynamics (Reiners, 1986; Sterner & Elser, 2002; Hendrixson *et al.*, 2007). Compared with other tissues, bone can

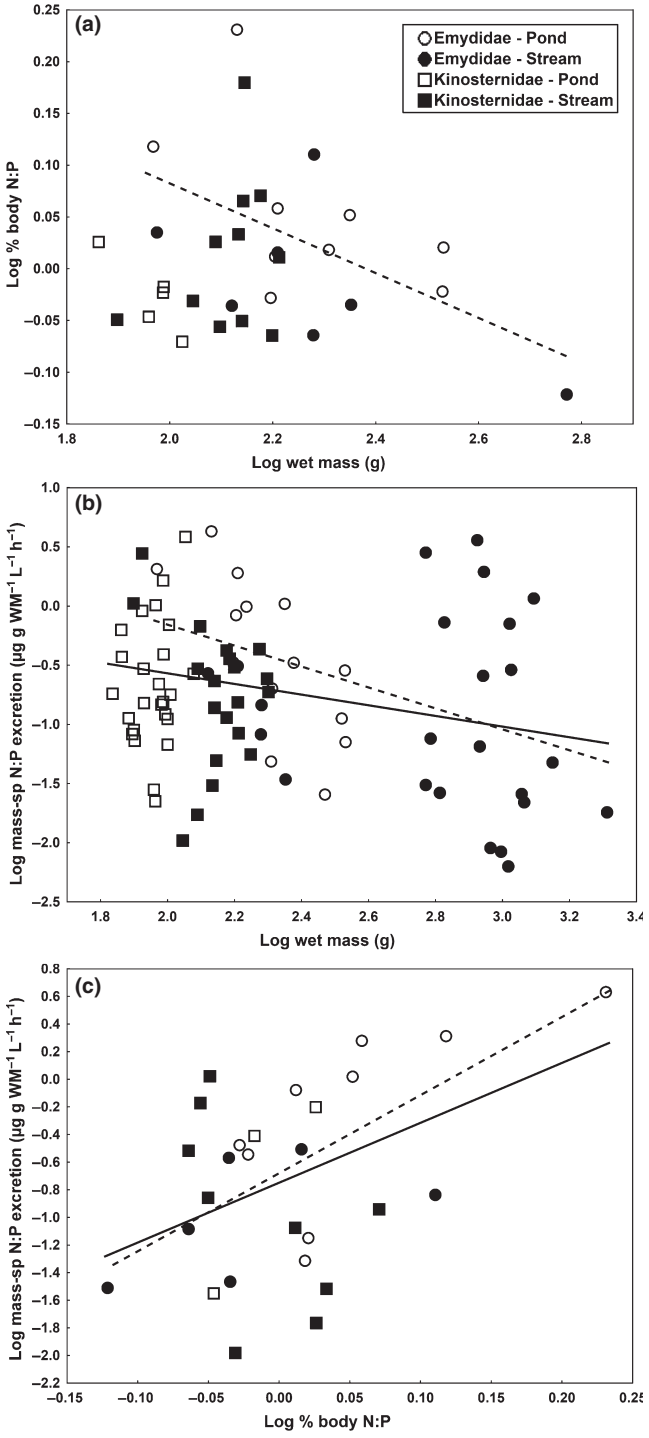

Fig. 2 Body mass and body nitrogen (N):phosphorus (P) versus mass-specific total N:P excretion and body N:P for Emydidae (circles) and Kinosternidae (squares) in pond (open symbols) and stream (shaded symbols) habitats. Significant relationships are shown for all turtles (solid line) and Emydidae (dotted line).

have a very low turnover rate, so vertebrates with large population biomass, robust skeletons, large body sizes or greater longevity may store substantial amounts of nutrients in tissues and subsequently affect nutrient limitation in fresh waters (Small *et al.*, 2009; Vanni *et al.*, 2013; cf. Atkinson & Vaughn, 2014 for a comparable example with invertebrates). Our study suggests that P demand by consumers is strongly influenced by four factors: tissue composition, diet, tissue turnover and individual growth potential. The latter two are not generally discussed in ES literature.

We found that turtle body and excretion N:P stoichiometry was largely related to ontogenetic shifts in body size, specifically to changes in P demand related to shell production in smaller turtles versus maintenance of shell in older, larger individuals. Among the emydids, for which we studied a sufficient range of body sizes, body mass was negatively correlated with body N:P, with larger turtles having a higher mass-specific P content, which is consistent with smaller turtles having less P relative to body mass because their skeleton is not fully developed. Under the theory of stoichiometric invariance and assuming turtles are homeostatic, higher body P content would imply that larger turtles have a higher P demand. Clark & Gibbons (1969) found a positive relationship between body size and calcium in the shell of juvenile *C. picta*, implying greater Ca demand among juvenile turtles. Because Ca and P are linked in the construction of bone mineral (i.e. $Ca_{10}(PO_4)_6(OH_2)$), it is also implied that smaller turtles have a higher P demand to support skeletal growth. Once produced, bone has a turnover rate an order of magnitude slower than other tissues (Chisholm, Nelson & Schwarcz, 1982; Daelerum & Angerborn, 2005) and adult turtles show negligible growth. This suggests, in turtles, body P content is ontogenetically inversely related to P demand. This idea contradicts published patterns in fish (Pilati & Vanni, 2007), yet is supported by the allometric relationships of nutrient excretion among turtles we documented in this study. Here, N:P of excretion was negatively correlated with body mass and body N:P was positively correlated with excretion N:P, implying lower P demand among adult turtles with higher body P content. These patterns may also reflect ontogenetic diet shifts, as juvenile turtles are more carnivorous and potentially consume more P than omnivorous or herbivorous adults (Clark & Gibbons, 1969; Bouchard & Bjorndal, 2006).

Our work demonstrates that skeletal investment is a key trait determining the potential effects of vertebrate species on freshwater nutrient dynamics. The relationships between body and excretion stoichiometry discussed above affect our understanding of taxon-specific effects on nutrient recycling within freshwater systems. Because of their bony skeleton and generally large body

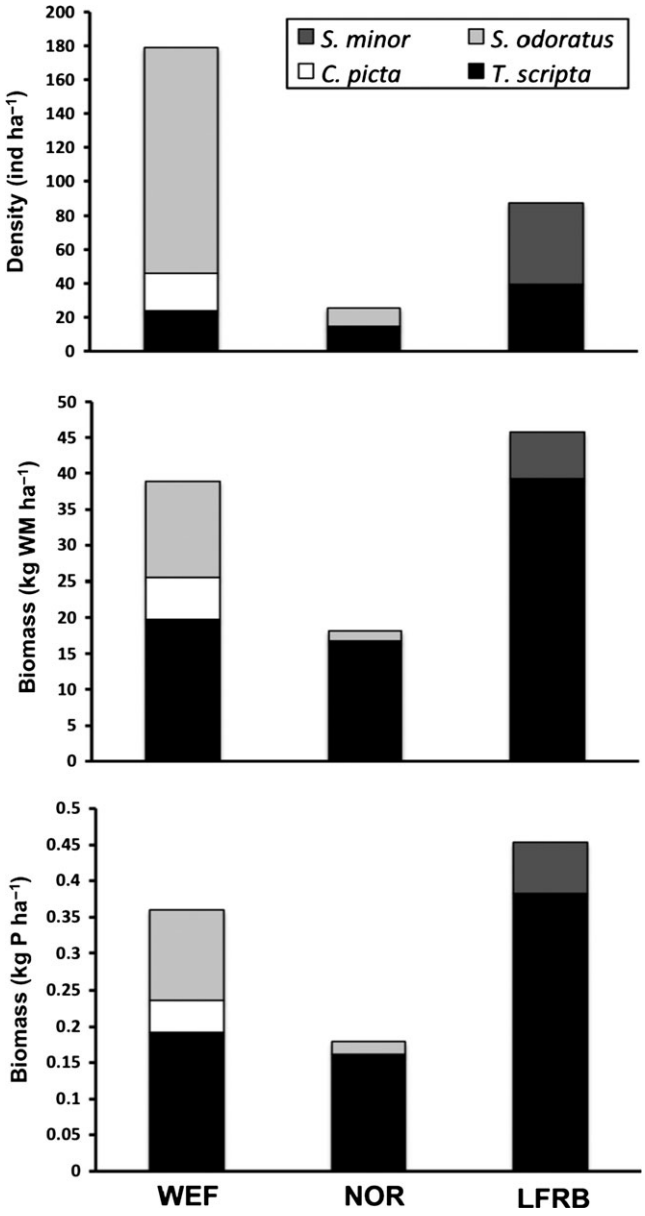

Fig. 3 Mean density, biomass and standing crop of phosphorus of four focal turtle species in each habitat type ((Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB)) in GA, U.S.A.

sizes and/or standing biomass, vertebrates can represent a large pool of nutrients in aquatic systems (Vanni, 2002; Small *et al.*, 2009; Vanni *et al.*, 2013). Our estimates of turtle standing stock biomass (0.6–1.5 g dry mass m^{-2}) were within the range of values published for other communities of turtles (up to 31 g dry mass m^{-2}; converted from wet mass in Congdon, Greene & Gibbons, 1986) and fish (0.2–27.6 g dry mass m^{-2}; reviewed in Turner *et al.*, 1999). Within the LFRB, turtle biomass was 47% greater than the highest biomass estimate for the introduced flathead catfish (*Pylodictis olivaris*) in 1985 and 700% greater than current catfish biomass estimates (Kaeser *et al.*, 2011). Because turtles have, on average, twice the P content of freshwater fish (Fig. 1), turtles can represent comparable or greater standing stocks of P and probably contribute to large and stable standing stocks of P within freshwater systems.

Our measures of the stoichiometry of turtles do not fully support current ES predictions about the effect of skeletal investment on nutrient excretion. Our estimates of total N and P excretion rates by turtle communities were similar to the lowest estimates for freshwater fish [despite comparable biomass], benthic macroinvertebrates and zooplankton (Vanni, 2002). Among all turtles, mean N:P of excretion (by mass) was c.26, higher than values reported for armoured catfish (c.16; Hood *et al.*, 2005; Table 4). This pattern is consistent with ES and data for fish and amphibians (Vanni *et al.*, 2002), which predict that taxa with a lower body N:P should excrete a greater proportion of N to P. However, individual measurements of excretion rates among turtles were variable, ranging by more than an order of magnitude among more omnivorous species. Mean mass-specific excretion of P and N was lower for turtles sampled in this study (0.41 μg dry g^{-1} h^{-1} and 5.70 μg dry g^{-1} h^{-1}, respectively) than armoured catfish (1.35 μg dry g^{-1} h^{-1} and 19.45 μg dry g^{-1} h^{-1}, respectively; Hood *et al.*, 2005). Nevertheless, excretion N:P of turtles and armoured catfish excretion may be quite similar. Our data indicate less P demand by larger, P-rich turtles. Yet, we did not collect the appropriate evidence to determine whether lower P excretion rates of turtles than fish were due to greater P demand to support a large skeleton.

We believe that the high variation in N:P of excretion among turtle taxa may be explained by differences in diet. Specifically, ES would predict that a diet with greater N:P could cause greater excretion N:P. Our species-specific turtle excretion data support this hypothesis. *Sternotherus* spp., which are carnivorous and probably have a diet rich in P, had the lowest N:P excretion ratios of our four focal turtle species. The N:P excretion of *Sternotherus* spp. was similar to the reported N:P of aquatic macroinvertebrates (Cross *et al.*, 2003), which are their primary prey, and was comparable to the reported N:P of fish excretion. In contrast, *T. scripta* and *C. picta*, which are omnivorous, consuming large amounts of aquatic macrophytes and algae (Parmenter & Avery, 1990), had the highest excretion N:P.

The variability in excretion N:P may have been influenced by fasting that was associated with the trapping

Table 4 Mean standing crop (kg ha^{-1}) of carbon (C), nitrogen (N) and phosphorus (P) of each focal species and across all species in each habitat type (Whitehall Experimental Forest (WEF), N. Oconee River (NOR) and Lower Flint River Basin (LFRB)) in GA, U.S.A. Standard deviations are in parentheses

	WEF			NOR			LFRB		
	C	N	P	C	N	P	C	N	P
Trachemys scripta	0.80 (0.34)	0.19 (0.08)	0.19 (0.08)	0.67 (0.32)	0.16 (0.08)	0.16 (0.08)	1.58 (0.74)	0.38 (0.18)	0.38 (0.18)
Chrysemys picta	0.25 (0.10)	0.04 (0.01)	0.04 (0.01)	–	–	–	–	–	–
Sternotherus odoratus	0.48 (0.14)	0.12 (0.04)	0.12 (0.04)	0.07 (0.04)	0.02 (0.001)	0.02 (0.001)	–	–	–
Sternotherus minor	–	–	–	–	–	–	0.29 (0.10)	0.07 (0.02)	0.07 (0.02)
Total	1.53	0.35	0.35	0.74	0.18	0.18	1.87	0.45	0.45

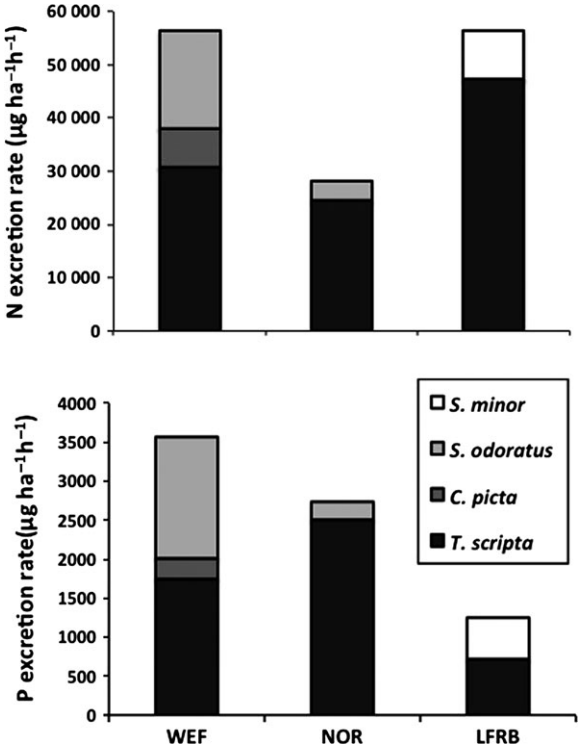

Fig. 4 Mean total dissolved nitrogen (N) and phosphorus (P) excretion rates of four focal turtle species in each habitat type ((Whitehall Experimental Forest (WEF), N. Oconee River (NOR), Lower Flint River Basin (LFRB)) in GA, U.S.A.

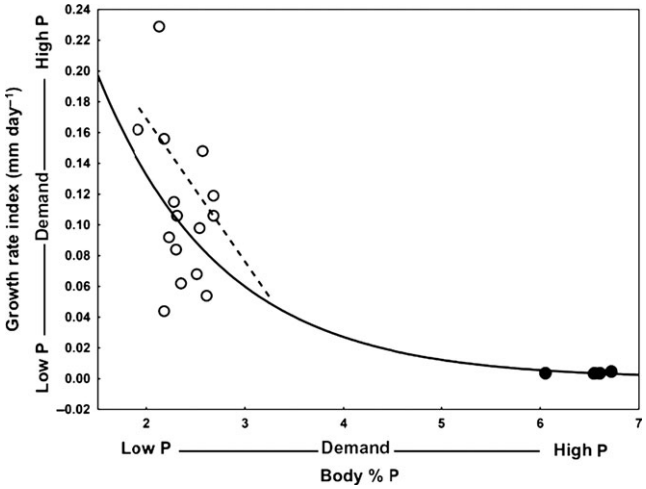

Fig. 5 Relationship between body % phosphorus (P) and growth for fish (linear; dotted line; open symbols) and fish and turtles (exponential; solid line; turtles in closed symbols).

of the turtles. A number of logistical factors, such as fasting and handling stress, can make quantifying excretion rates challenging (Vanni, 2002; Whiles *et al*., 2009). Among amphibians and fish, fasting and handling stress can decrease and increase excretion estimates, respectively (Vanni, 2002; Whiles *et al*., 2009). We are fairly confident that turtles were not able to feed after they entered a trap, so they may not have had access to food for 12–18 h. However, this potential fasting time is low compared to published estimates of digestive turnover for our focal species (49–61 h; Parmenter, 1981) and relatively short compared to digestion times for our study species. If fasting or handling stress did influence our excretion estimates, we expect them to underestimate true excretion rates and think we would not have found such strong relationships between body and excretion nutrients. Further research should compare our results with excretion rates of turtles captured using direct capture methods (i.e. dipnetting or snorkelling; Sterrett *et al*., 2010).

Turtles are unique among vertebrates in their skeletal investment, and our estimates suggest that freshwater turtles have the potential to represent large standing stocks of P. Because turtles have exceptional longevity (relative to many freshwater taxa) and are primarily composed of P-rich bone, the turnover of P from turtle standing stocks to other parts of the ecosystem is likely to be significantly slower than turnover from fish, amphibian or invertebrate stocks. Phosphorus is often naturally limiting in freshwater ecosystems (Elser *et al*., 2007), and uptake and retention of P by turtles may be very important as a store for P. Conversely, our excretion estimates

suggest that turtles do not remineralise nutrients at rates comparable to other freshwater taxa, and thus may be not as important for supplying nutrients to support primary or heterotrophic production. It is unclear whether turtles have an effect on remineralised nutrient ratios disproportionate to their mass, and our results do not support the idea that high skeletal investment of an element implies high demand for that element through time. Moreover, our findings suggest that taxon-specific traits and ontogenetic shifts in diet may be especially important for predicting nutrient ratios of species' excretions and availability of remineralised nutrients.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Cumulative hourly N excretion rates (mg L^{-1}) from four focal turtle species and a control tank. Value at time 0 represents N concentration of habitat-specific water.

Table S1. Mean N and P excretion rate (μg L^{-1} h^{-1}), mass-specific N and P excretion rate (μg L^{-1} g dry mass h^{-1}) and mean N:P excretion (mass ratio) for each focal species in each habitat type (Whitehall Experimental Forest (WEF), N. Oconee River (NOR) and the Lower Flint River Basin (LFRB)) in GA, U.S.A.

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