Larval anurans follow predictions of stoichiometric theory: implications for nutrient storage in wetlands

**Daniel D. Knapp**, Lora L. Smith, and Carla L. Atkinson

1Department of Biological Sciences, The University of Alabama, 1325 Science and Engineering Complex, Tuscaloosa, Alabama 35487 USA
2Jones Center at Ichauway, 3988 Jones Center Drive, Newton, Georgia 39870 USA

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**Abstract.** Ecological stoichiometry provides a framework to predict how animals regulate nutritional balances within their tissue and, as a result, how animal biomass affects ecosystem processes through nutrient cycling. However, most interspecific and developmental stoichiometric studies in animals focus on invertebrates, and the few vertebrate studies are largely fish-centric. Larval anurans are ideal vertebrates to test predictions of developmental and interspecific stoichiometry as they undergo a complex development, exhibit broad arrays of life-history traits, and can constitute high animal biomass in wetlands, implying major roles in wetland nutrient storage and cycling. We examined (1) patterns of body stoichiometry across larval developmental stages within multiple anuran species, (2) whether key predictors of stoichiometric change, specifically body size, developmental period, and breeding season, influence interspecific stoichiometric variation, and (3) natural magnitudes and fluctuations of larval anuran nutrient storage in geographically isolated wetlands (GIWs). We measured carbon (C), nitrogen (N), and phosphorus (P) tissue content in larval anurans across five developmental stages within 11 species collected from four GIWs to examine patterns of developmental and interspecific stoichiometry. Within species, we found broad developmental stoichiometric patterns in which later developmental stages were lower in %N, but higher in %P than earlier stages. Patterns in %C were inconsistent but were generally lower in later stages, while tissue C:N ratios increased, and C:P and N:P decreased in later developmental stages. Interspecific stoichiometric variation was partially explained by body size and developmental period which positively affected %C and C:N ratios. We observed spatial and temporal fluctuations in species-specific biomass which dictated nutrient storage patterns within larval anuran assemblages, though stoichiometric identity played a major role. Our estimated magnitudes of larval anuran areal nutrient storage also greatly exceeded that of other wetland fauna with the maximum estimated areal P storage reaching over 200 times that of a similar-density co-occurring invertebrate group. These results highlight stoichiometric patterns of development and interspecific variation in a diverse group of amphibians while providing critical baseline information for elucidating the role of anurans in wetland nutrient dynamics.

**Key words:** biomass; consumer-driven nutrient dynamics; development; ecological stoichiometry; interspecific variation; larval anuran; life history; nutrient storage; wetlands.

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† E-mail: dknapp109@gmail.com
**INTRODUCTION**

Recent research has highlighted the importance of animals to nutrient storage and cycling across ecosystems through their ability to maintain and transform nutrient balances via ingestion, assimilation, excretion, and egestion (Vanni et al. 2013, Capps et al. 2015a, Atkinson et al. 2017a). Ecological stoichiometry provides a framework for understanding how animals affect nutrient availability (Sterner and Elser 2002). Studies of ecological stoichiometry typically concentrate on three elements—carbon (C), nitrogen (N), and phosphorus (P). As C, N, and P play different roles in an organism’s chemical composition, ecological stoichiometric theory predicts that nutrient requirements correlate with developmental and morphological changes (Elser et al. 1996, El-Sabaawi et al. 2016, Stephens et al. 2017).

Studies examining developmental and interspecific stoichiometry in vertebrates have primarily focused on fish (Vanni 2002) and found that body stoichiometry is influenced by ontogeny, morphological differences, body size, and diet (Sterner and George 2000, Pilati and Vanni 2007, Boros et al. 2015, El-Sabaawi et al. 2016). Anurans provide unique perspectives for studying ontogenetic stoichiometric patterns because they undergo a complex development during which skeletal, muscular, and visceral anatomy drastically change (Gosner 1960, McDiarmid and Altig 1999), consequently leading to variation in stoichiometric demands (Tiegs et al. 2016, Stephens et al. 2017, McLeay et al. 2019). For example, larvae of two common North American anurans increase in %P throughout development, likely related to P-rich osteological growth (Tiegs et al. 2016, McLeay et al. 2019). Adult anurans also exhibit a suite of interspecific life-history characteristics, from small to large body size and terrestrial to scansorial locomotion (Altig and McDiarmid 2015), which are likely reflected in larval tissue stoichiometry across development. However, the few studies of anuran tissue stoichiometry have focused on single species, limiting tests of interspecific stoichiometric predictions. Furthermore, skeletal, muscular, and visceral development influence morphological variation both inter- and intraspecifically in larval anurans (Emerson 1978, Larson 2005), which may lead to differences in tissue stoichiometry.

Finally, most larval anurans within southeastern United States graze on periphyton and other organic matter (Atkinson et al. 2017b) and, in high abundance, directly and indirectly affect important ecosystem processes such as primary production and litter decomposition (Seale 1980, Whiles et al. 2006, Rugenski et al. 2012). Therefore, changes and differences in larval anuran tissue stoichiometry may influence ecosystem nutrient cycling as shown in other taxa (Hawlena et al. 2012, Atkinson et al. 2017a).

While animal-driven nutrient studies largely highlight nutrient remineralization through excretion or egestion (Atkinson et al. 2017a), there is increasing recognition of the role of animal-mediated nutrient storage in ecosystem nutrient cycling (Vanni et al. 2013, Capps et al. 2015a). For example, larval anuran nutrient storage may represent not only an important sink of nutrients from aquatic ecosystems but an important import of nutrients into terrestrial ecosystems because of their biphasic life cycle (Gibbons et al. 2006, Capps et al. 2015a, b, Fritz and Whiles 2018). Furthermore, animal-mediated nutrient storage may influence nutrient availability within ecosystems limited in external nutrient sources such as isolated wetlands (DeAngelis et al. 1989, Battle and Golladay 2001, Capps and Flecker 2013).

Geographically isolated wetlands (GIWs) are typically small and highly productive systems with most nutrient cycling occurring internally by macrophytes and phytoplankton (Battle and Golladay 2001, Smith et al. 2017). These wetlands typically do not support fish (Tiner 2003, Smith et al. 2017), but they often support high densities of larval anurans, with the potential for high magnitudes of nutrient storage in these organisms (Smith et al. 2017, McLeay et al. 2019), which could effectively drive temporal fluxes of nutrients and regulate GIW nutrient cycles (Seale 1980). Amphibian diversity is notably high in GIWs (Semlitsch and Bodie 1998, Smith et al. 2017), but species-specific contributions of anurans to nutrient storage are relatively unknown. Thus, determining whether stoichiometry varies within and across taxa, linking species identity to nutrient storage, and identifying patterns and fluxes of nutrient storage are critical to understanding their role in ecosystem nutrient cycles.
Our goal for this study was to test ecological stoichiometry in a diverse order of vertebrates by examining patterns of ontogenetic change and determining predictors of interspecific variation in the tissue stoichiometry (%C, %N, %P, C:N, C:P, and N:P) of larval anurans. Specifically, we tested whether developmental stages, body size, length of developmental periods, and breeding season affect tissue stoichiometry of larval anurans from 11 species collected from four GIWs. We predicted that (1) progressing developmental stages in larval anurans would significantly affect larval stoichiometry similarly among species, (2) interspecific larval stoichiometry would significantly differ as a result of variable life-history traits, and (3) species-specific stoichiometry influences magnitudes of larval anuran nutrient storage within GIWs with storage fluctuating temporally and spatially.

**Materials and Methods**

**Study site**

We conducted this study at the Jones Center at Ichauway (hereafter Ichauway) located in Baker County, Georgia, USA. Ichauway is an 11,736-ha research site dominated by longleaf pine (*Pinus palustris*) forest with native ground cover and more than 100 GIWs. Marsh GIWs are dominated by grasses and sedges (Kirkman et al. 2000), and have highly variable sediment and nutrient accumulations (Craft and Casey 2000), short hydroperiods (Battle and Golladay 2001), and high amphibian species richness (Liner et al. 2008). For example, Liner et al. (2008) detected larvae from a total of 16 anuran species across multiple seasons and marsh GIWs.

**Larval anuran collection**

We used a dip net (3 mm mesh size; Memphis Net and Twine, Memphis, Tennessee, USA) to collect larval anurans intermittently across the hydroperiod from February 2017 to September 2018 at the four study GIWs. We timed our sampling to collect samples of all available larvae across five developmental stages. The five stages were simplified as follows based on Gosner (1960): stage 1, no limbs (Gosner stages 23–25); stage 1.5, developing hind limbs (Gosner stages 26–37); stage 2, fully developed hind limbs (Gosner stages 38–40); stage 2.5, developing forelimbs (Gosner stage 41); and stage 3, developed forelimbs, but retaining a tail (Gosner stages 42–46; Fig. 1). We euthanized larval anurans using 5 g/L tricaine methanesulfonate (MS-222) solution (Torreilles et al. 2009) and froze them at −20°C for later stoichiometric analysis.

**Stoichiometric analysis**

We dissected euthanized larvae to remove the gastrointestinal tract below the manicotto glandular to the rectum (McDiarmid and Altig 1999), measured total length (body plus tail) to the nearest 1 mm, and weighed larvae to the nearest 0.001 g. Whole-body (body and tail) larval anurans were freeze-dried for ≥72 h using a Labconco FreeZone 1 (Labconco, Kansas City, Missouri, USA) and then re-weighed to determine larval dry mass. We used either a mortar and pestle or a Retsch Mixer Mill (Retsch GmbH, Haan, Germany) to homogenize dried larvae. To measure total %C and total %N tissue content, we analyzed a ~1–3 mg subset of each homogenized larva with a FLASH 2000 NC Analyzer (Thermo Fisher Scientific, Waltham, Massachusetts, USA). We combusted a further ~1–3 mg subset of each homogenized larva in a Thermolyne muffle furnace (Thermo Fisher Scientific) at 500°C for 1 h, digested each in 1 N HCl at 105°C for 2 h, and used the ascorbic acid–molybdate method to determine total %P (Murphy and Riley 1962, Solórzano and Shar 1980). Larvae <4 mg dry mass were homogenized together with like species and stages collected from the same wetland to increase particulate material for our analysis.

**Statistical analyses**

To test for differences in tissue stoichiometry across larval developmental stages within a species, we used mixed-effect ANOVA models with stage as a fixed effect and wetland identity as a random effect to control for wetland-specific variability. For species collected from a single wetland, we tested for differences in tissue stoichiometry across developmental stages within a species using one-way ANOVAs with stage as a factor. To test for interspecific variation in stoichiometry for larvae at stage 3, we used one-way
ANOVAs with species as a factor. Means used in the ANOVA were calculated by pooling data for larvae across all four wetlands for each species and stage. Significant ANOVA results ($P < 0.05$) were followed by Tukey’s HSD post hoc tests to determine significant differences in tissue stoichiometry for pairwise comparisons between stages within a species and among species. Analyses were conducted using R 3.6.0 (R Core Team 2019) with mixed-effect ANOVA models performed using package lme4 and Tukey HSD post hoc tests performed using package multcomp.

To further test predictors of interspecific stoichiometric variation, we examined whether life-history traits affected tissue stoichiometry. As we expected pronounced morphological variation in larvae immediately prior to metamorphosis (Emerson 1978, Larson 2005), we tested interspecific stoichiometric variation at developmental stages closest to metamorphosis. We considered four life-history traits, commonly considered influential to tissue stoichiometry, from published sources along with our data—average developmental period (days), average length (mm), average dry mass (mg), and first collected (number of days from January 1st; Wright and Wright 1995, Wright 2002, Jensen et al. 2008, Altig and McDiarmid 2015). Life-history trait data for each species measured can be found in Appendix S1: Table S1. We chose traits based upon availability of consistent published records for all tested species, as well as potential influences of traits on larval stoichiometry. We were not able to quantify precise larval developmental rates from this study as amphibian developmental rates vary widely within cohorts (Wilbur and Collins 1973). Therefore, for the
developmental trait average developmental period (days), we took the average between reported minimum and maximum ranges of time to metamorphose from each source and used the mean across sources. For two body size traits, average length (mm) and average dry mass (mg), we averaged the length and dry mass of collected stage 3 larvae for each species. The breeding season trait, first collected (number of days from January 1st), was calculated as the number of days since January 1st of each year that we first observed each species during our two-year collection period. Then, we used principal component analyses (PCA) on log-transformed trait data to determine which combination of life-history traits explained the most variation among species. We used linear regression to test whether life-history traits loaded on principal component 1 (PC1; see Results) were drivers of %C, %N, and %P body content at stage 3 across anuran species. We also tested the relationships between PC1 and C:N, C:P, and N:P ratios at stage 3 using linear regression. A significant relationship indicated by linear regression between PC1 species scores and stoichiometry suggests that stoichiometry may be predicted by life-history traits loaded on PC1. All analyses were performed using R 3.6.0 (R Core Team 2019), with PCA performed using package prcomp.

Larval anuran density, biomass, and nutrient storage sampling

We measured biomass of larval anurans monthly during the hydroperiods (March–June 2018) of the four study wetlands using an aluminum 0.37 m² throw trap (Kushlan 1981). We sampled a minimum of 0.025% of the wetland surface area (calculated from wetland contour data and water-level data; Jones Center at Ichauway, unpublished data); throws were concentrated in water <0.5 m in depth. For each throw, larval anurans were removed from the trap using a dip net (3 mm mesh size) and we continued dip netting until we had 10 consecutive sweeps without captures. Each larva was identified to species, measured for total length to the nearest 1 mm, weighed to the nearest 0.01 g, assigned to one of five developmental stage categories as described above, and released.

We estimated average monthly density (number of individuals per m²) and areal biomass (mg of larval dry mass per m²) of larvae by species and stage in each wetland. To estimate dry mass of field-sampled anurans, we created log-transformed length–dry mass regressions for larvae of each species at each stage (Liess et al. 2015, Tiegs et al. 2016). Coefficients for each species- and stage-specific regression equation can be found in Appendix S1: Table S2. To estimate species/ stage-weighted nutrient storage in larval anurans, we calculated the product of each species- and stage-specific %C, %N, and %P value by corresponding dry masses for each larva caught in the throw trap. Using the estimated nutrient masses, we calculated monthly average species-specific C, N, and P areal storage (mg/m²) for each wetland.

RESULTS

We collected the larvae of Acris gryllus, Gastrophryne carolinensis, Hyla cinerea, Hyla gratiosa, Hyla squirella, Lithobates capito, Lithobates catesbeianus, Lithobates sphenoecephalus, Pseudacris nigrita, Pseudacris ornata, and Scaphiopus holbrookii. When available, larvae of five different metamorphic stages were collected for each species, for a total of 594 samples. Of the 594 samples, 123 fell below the 4 mg dry mass threshold and were homogenized with like species and stages. Three species had incomplete data for at least one stage and nutrient: A. gryllus (stages 1 and 1.5; %C and %N), H. cinerea (stage 2.5; %C, %N, and %P), and S. holbrookii (stages 1 and 2; %C, %N, and %P). For full descriptive statistics of developmental tissue stoichiometry for each species, see Appendix S1: Table S3.

Developmental stoichiometry

Tissue stoichiometry significantly changed throughout development for all species (Fig. 2; Appendix S1: Tables S4, S5). Average %C showed inconsistent patterns throughout development but was generally lower in later stages than earlier stages in nine of 11 species, with five of those species experiencing significant decreases in average %C (Fig. 2A). Ten of 11 species significantly decreased in %N throughout development (Fig. 2B), and 10 of 11 species significantly increased in %P throughout development (Fig. 2C). C:N ratios generally
increased throughout development in 10 of 11 species with a significant increase in eight species (Fig. 2D), while C:P ratios significantly decreased throughout development in 10 of 11 species (Fig. 2E) and N:P significantly decreased in all species (Fig. 2F).
Interspecific stoichiometric variation and the influence of life-history traits

Species varied significantly in tissue nutrient content at stage 3 (Fig. 3; Appendix S1: Tables S6, S7). Percent carbon ranged from 40.8% ± 1.1% (standard deviation) in G. carolinensis (N = 11) to 50.3% ± 1% in L. catesbeianus (N = 8; Fig. 3A).

For %N, species ranged from 9.4% ± 0.02% in S. holbrooki (N = 3) to 11% ± 0.3% in H. squirella (N = 5; Fig. 3B). Percent phosphorus ranged from 0.9% ± 0.2% in P. ornata (N = 16) to 1.9% ± 0.1% in G. carolinensis (N = 11; Fig. 3C). C:N ratios ranged from 4.7 ± 0.1 in G. carolinensis (N = 11) to 6.3 ± 1.0 in L. catesbeianus (N = 8; Fig. 3D). C:P ratios varied from 200 to 150 in H. squirella (N = 5; Fig. 3E) to 150 to 100 in S. holbrookii (N = 3; Fig. 3F).

Fig. 3. Interspecific variation in tissue stoichiometry of larvae at stage 3 (Gosner stages 42–46) for 11 species of anurans naturally occurring within southwestern Georgia, USA. (A) %C, (B) %N, and (C) %P tissue contents are arranged from lowest to highest %P, and (D) C:N, (E) C:P, and (F) N:P tissue ratios are arranged from lowest to highest N:P tissue ratio. Lowercase letters that do not share a mean are statistically different as determined by Tukey’s HSD test.

Interspecific stoichiometric variation and the influence of life-history traits

Species varied significantly in tissue nutrient content at stage 3 (Fig. 3; Appendix S1: Tables S6, S7). Percent carbon ranged from 40.8% ± 1.1% (standard deviation) in G. carolinensis (N = 11) to 50.3% ± 1% in L. catesbeianus (N = 8; Fig. 3A).
ratios ranged from 57.1 ± 3.8 in *G. carolinensis* (*N* = 11) to 136 ± 27.9 in *P. ornata* (*N* = 16; Fig. 3E). N:P ratios ranged from 11.9 ± 0.2 in *S. holbrookii* (*N* = 3) to 26.1 ± 5.2 in *P. ornata* (*N* = 16; Fig. 3F).

Our PCA showed separation in life-history traits across species. Principal component 1 loadings were primarily composed of body size and developmental traits and explained 69% of variation in life-history traits, while PC2 loadings were primarily composed of breeding season traits and explained 23.6% of variation (Fig. 4; Appendix S1: Table S8). Percent C was predicted by PC1 (*F*<sub>1,9</sub> = 5.258, *P* = 0.048, Adj-*R*<sup>2</sup> = 0.30) with larger and slower-developing species having higher %C (Fig. 5A). Principal component 1 did not predict %N and %P at stage 3 (Fig. 5B, C). However, *Lithobates* spp., which are morphologically distinct, appeared as influential points potentially affecting the fitted curve within the % P and PC1 regression. If removed, %P at stage 3 was predicted by PC1 (*F*<sub>1,6</sub> = 7.784, *P* = 0.024, Adj-*R*<sup>2</sup> = 0.53; Fig. 5D). Principal component 1 predicted C:N ratios (*F*<sub>1,9</sub> = 13.97, *P* = 0.0046, Adj-*R*<sup>2</sup> = 0.56) with larger and slower-developing species having higher C:N ratios (Fig. 5E). However, neither C:P or N:P were predicted by PC1 (Fig. 5F, G). As *Lithobates* spp. also appeared...
Fig. 5. Relationship between principal component 1 (PC1) scores and tissue stoichiometry of larvae from 11 species of anurans within southwestern Georgia, USA. Principal component 1 (69%) was comprised of the traits: average length (mm), average dry mass (mg), and average developmental period (days). (A) %C, (B) %N, and (C) %P, (D) %P (with Lithobates spp. excluded), (E) C:N ratio, (F) C:P ratio, (G) N:P ratio, and (H) N:P (with Lithobates spp. excluded) ratio at stage 3 regressed over PC1 scores.
as influential points within the N:P ratios and PC1 regression, there appeared to be a positive trend in the relationship between N:P ratios and PC1 once removed (Fig. 5H), but the relationship was not significant.

Larval anuran density, biomass, and nutrient storage

Hydroperiods across all wetlands ranged from two months (March–April 2018) to four months (March–June 2018) with wetland surface area ranging from 507.7 m² in April at Wetland 21 to 4304.8 m² in March at Wetland 46 (Appendix S1: Table S9). Species composition, density, areal biomass, and areal nutrient storage varied monthly and among wetlands (Figs. 6, 7; Appendix S1: Table S9). Areal biomass primarily influenced areal nutrient storage, with species composition also influencing magnitudes of nutrient storage (Figs. 6, 7). Density ranged from 5.4 ± 7.6 individuals/m² in April at Wetland 46 to 442.1 ± 423.8 individuals/m² in March at Wetland 21 (Fig. 6A). Areal biomass ranged from 28.1 ± 62.4 mg/m² in March at Wetland 46 to 14481.4 ± 2245.4 mg/m² in May at Wetland 37 (Fig. 6B). Wetland 46 had the lowest areal C (13.7 ± 30.3 mg/m²), N (3.4 ± 7.6 mg/m²), and P (0.2 ± 0.5 mg/m²) storage in March, while Wetland 37 had the highest areal C (6884.6 ± 1065.3 mg/m²), N (1639.2 ± 250.5 mg/m²), and P (151.9 ± 25.3 mg/m²) storage in May (Fig. 7A–C).

Fig. 6. (A) density and (B) biomass of larval anurans across hydroperiods of four geographically isolated wetlands within southwestern Georgia, USA. Red letters display large spikes in density and biomass and indicate a split in y-axis scales. 3M = March, 4A = April, 5M = May, and 6J = June.

Fig. 7. (A) Carbon, (B) nitrogen, and (C) phosphorus nutrient storage of larval anurans across hydroperiods of four geographically isolated wetlands within southwestern Georgia, USA. Red letters display large spikes in nutrient storage and indicate a split in y-axis scales. 3M = March, 4A = April, 5M = May, and 6J = June.
DISCUSSION

Our findings further develop stoichiometric theory and provide a framework for examining other taxonomic groups by identifying mechanisms influencing intraspecific and interspecific variation. Furthermore, our study is novel in that we used patterns of stoichiometric change across development and interspecific variation to demonstrate potential nutrient storage of whole larval anuran assemblages at the wetland scale throughout the hydroperiod. Broadly, we found significant patterns of stoichiometric change within larvae of multiple anuran species across developmental stages transitioning from an aquatic to terrestrial lifestyle, but stoichiometry also varied inter-specifically due to differences in life-history strategy. We found evidence supporting our first hypothesis that developmental stoichiometric patterns of larvae were generally consistent across species. There was weak support for our second hypothesis that life-history traits typically related to stoichiometry in other taxa influence larval anuran stoichiometry as we found that two important life-history traits, body size and developmental period length, influenced interspecific variation in %C content, C:N ratios, and potentially %P content. Finally, our third hypothesis was supported by our observations that species-specific stoichiometry affected magnitudes of larval anuran nutrient storage, which varied spatially and temporally.

Developmental stoichiometry

Larval anuran tissue stoichiometry changed significantly throughout development across most species studied here. Though allometric relationships in developing fish and, to some degree, invertebrate stoichiometry have been found (Hendrixson et al. 2007, Pilati and Vanni 2007, González et al. 2018), larval anurans do not follow a linear body growth pattern (Tiegs et al. 2016). Therefore, we expected changes in stoichiometry to result from physiological and morphological transformations. Our results may signify a selective switch in stoichiometric demands throughout larval anuran development. Similar decreasing C:N patterns have been observed in fish, suggesting that changes in energy allocation throughout development result in shifts from N-rich protein growth to C-rich lipid growth (Deegan 1986, Pilati and Vanni 2007, Boros et al. 2015). While our observations are consistent with this pattern, we cannot confirm whether this mechanism is controlling C:N ratios in larval anurans without directly examining lipid:protein ratios.

Our results suggest that larval anurans increase allocation of %P over %C and %N throughout development. This is supported by ontogenetic stoichiometry studies in fish which revealed decreasing C:P and N:P ratios throughout development was caused by increasing P allocation for bone growth (Hendrixson et al. 2007, Pilati and Vanni 2007). Studies have found that %P and % calcium, another nutrient heavily found in bone (Sterner and Elser 2002), are correlated in both fish (Hendrixson et al. 2007) and larval anurans (McLeay et al. 2019). However, skeletal structure may be highly variable within and among anuran genera (Trueb et al. 2011), and further osteological information is needed to conclude on species-specific patterns and magnitudes of %P tissue content.

We found that anurans did not share the same stoichiometric developmental patterns as invertebrates (González et al. 2018). For example, across 71 species of aquatic invertebrates, González et al. (2018) found increasing body %C and %N and generally decreasing %P with body size, possibly related to ontogenetic growth of physiological traits such as C-rich chitin and N-rich flight muscle development (González et al. 2018). Since we observed developmental patterns of generally decreasing %C, strongly decreasing %N, and strongly increasing %P in larval anuran ontogeny, and as we provide, to our knowledge, the most extensive developmental stoichiometric study within a single vertebrate order, we conclude that larval anuran stoichiometric development strongly diverges with developmental stoichiometric patterns in invertebrates (González et al. 2018). Furthermore, as larval anuran developmental stoichiometry shares many similarities as patterns found in fish (Pilati and Vanni 2007, Boros et al. 2015), regardless of morphological and physiological ontogenetic differences, we would tentatively expect similar developmental stoichiometric patterns in other vertebrate taxa. Future studies focused on both vertebrate and invertebrate ontogenetic stoichiometry will better inform the role of animals in whole ecosystem nutrient cycling.
Our results contrast somewhat with recent studies examining larval anuran developmental stoichiometry. Neither Tieg et al. (2016) nor McLeay et al. (2019) observed decreases %N tissue content across developmental stages in *Lithobates sylvaticus* and *L. sphenoecephalus*, respectively, which was observed for most species here. Furthermore, both studies observed increases in %C across developmental stages in their study species (Tieg et al. 2016, McLeay et al. 2019), while we observed inconsistent developmental patterns of %C across species, with the majority experiencing a slight or significant decrease from the first stage to the last stage. However, as with our study, both Tieg et al. (2016) and McLeay et al. (2019) reported significant increases in %P, general increases in C:N ratios, and decreases in C:P and N:P ratios throughout development. As the ranges for our stoichiometric values fall within previously reported values, our contrasting stoichiometric developmental patterns likely reflect differences in study design, particularly since we measured more discrete changes in stoichiometry throughout larval anuran development.

**Interspecific stoichiometric variation and the influence of life-history traits**

We identified key life-history traits, specifically body size and developmental period, that significantly influenced interspecific %C and C:N ratios in larval anurans. Body size and developmental period impacts are key ecological traits of animals that influence the way in which organisms uptake, store, and transfer nutrients (Woodward et al. 2010, Amundrud and Srivastava 2016). Previous work has shown that faster larval anuran developmental rates and larger body sizes resulted in lipid reduction (specifically fat), suggesting subsequent effects on tissue %C (Scott et al. 2007, Kulkarni et al. 2011). The positive relationship between C:N ratios and body size and developmental period suggests that species with larger-bodied and slower-developing larvae may shift more from N-rich protein growth to C-rich lipid growth.

There were general patterns between life-history traits and interspecific stoichiometry, though one genus did not follow these patterns. Specifically, there was a weak, negative relationship between %P content and life-history traits when *Lithobates* spp. were removed. *H. gratiosa* also appears as a potentially influential point within the relationship between body size, developmental period, and N:P ratios as it was the only species that did not increase in %P from stage 1 to stage 3. While the lower %P and higher %C in species with larger-bodied and longer-developing larvae may be indicative of an increased demand in %C, there was no relationship between body size and C:P ratios. Elser et al. (1996) predicted that N:P ratios increase with longer developmental rates and larger body sizes. When *Lithobates* spp. are excluded, this idea is supported by a significant negative relationship between body size, developmental period, and %P, but not supported by the weak positive relationship between body size, developmental period, and N:P ratios. Unfortunately, because growth rates varied within larval cohorts (Wilbur and Collins 1973) and we sampled intermittently, we were unable to calculate individual- or species-specific growth rates to fully test this hypothesis. Furthermore, though notable that potentially influential points were all species from a single genus, excluding multiple species from our analyses likely implies that %P and N:P ratios are not influenced by our selected traits.

Unknown mechanisms appear to influence patterns in %P tissue content and N:P ratios. For example, larval *Lithobates* spp. contained more %P than species of similar size, which may indicate functional differences from other genera. As adults, *Lithobates* spp. characteristically have high hind limb to snout–vent length ratios compared to other genera which may be expressed during hind limb development in late larval stages (Gomes et al. 2009). Therefore, differences in hind limb development among other species may yield differences in the underlying bone structure and subsequent %P and N:P ratios (Wilson et al. 2009).

Comparative studies of osteological characteristics, such as skeletal structure, among developmental stages of larval anurans of different species may provide insight on species-specific %P tissue content and N:P ratios and consequential effects on nutrient storage and cycling.

**Larval anuran density, biomass, and nutrient storage**

Larval biomass appeared to primarily dictate patterns of nutrient storage. As larval size is a species-specific trait, species composition of
larval anuran assemblages may drive nutrient storage from biomass alone. For example, while the highest density of larval anurans (primarily small-bodied *S. holbrookii*) was found in March at Wetland 21, the highest areal biomass (primarily large-bodied *L. sphenocephalus*) was found in May at Wetland 37, resulting in the highest areal C, N, and P storage. However, species- and stage-specific stoichiometry also played an important role in areal nutrient storage. We estimated that *G. carolinensis* in June at Wetland 21 stored similar magnitudes of P as *L. sphenocephalus* in April at Wetland 37, though *L. sphenocephalus* occurred at more than twice the biomass. As a result, species composition of assemblages may greatly influence nutrient storage magnitude through both species-specific biomass and stoichiometric identities. Species composition, density, areal biomass, and areal nutrient storage in larval anurans also varied by month and among wetlands. This finding was not unexpected given that larval anuran diversity and abundance can directly be affected by wetland hydroperiod (Pechmann et al. 1989) and that GIW hydroperiods can vary both temporally and spatially as a result of landscape position, wetland bathymetry, catchment area, and underlying soils (Park et al. 2014).

**Implications of stoichiometric patterns**

Since GIWs typically do not contain fish (Tiner 2003, Smith et al. 2017), amphibians and aquatic invertebrates dominate aquatic faunal biomass (Leeper and Taylor 1998, Gibbons et al. 2006). As a result, understanding the different potential contributions between these two groups is crucial to realizing the role of animals in GIW nutrient cycling. Potentially as a result of divergent ontogenetic %P patterns driven by differences in morphological and physiological developments, late-stage larval anuran tissue is generally P-rich compared to average %P body content of many aquatic invertebrates (Evans-White et al. 2005). For example, Evans-White et al. (2005) found that Chironomidae contain an average %P body content of 0.70, nearly 2.5 times less than late-stage *L. sphenocephalus*. Furthermore, based on prior studies of %P and dry mass, we crudely calculated that average-sized Chironomidae contain approximately 0.003 mg P (Towers et al. 1994, Evans-White et al. 2005). When scaled to densities observed on Ichauway (232.9 individuals/m²; Battle and Golladay 2001), Chironomidae would store roughly 0.70 mg P/m² which is over 200 times less than comparable densities of *L. sphenocephalus* in May at Wetland 37 (191.7 individuals/m²). In fact, the average density of invertebrates in marsh GIWs on Ichauway is more than double that of our highest density of larval anurans (Battle and Golladay 2001), but larval anuran P storage would greatly exceed that of invertebrates as a result of both high biomass and divergent developmental patterns. Though scaled estimations of nutrient storage provide a unique insight into the relative contributions of amphibians and invertebrates in wetland nutrient storage, it is important to note that we sampled only 0.025% of each wetland’s inundated area and our larval anuran assemblage estimates are likely liberal. These admittedly rough calculations highlight the need for further studies to provide a holistic view of nutrient storage across entire wetland communities.

Our results also have implications in ecosystem nutrient cycling, particularly nutrient limitation in isolated wetland ecosystems. As larval anurans are considered nutrient sinks in aquatic ecosystems (Capps et al. 2015a), varying magnitudes of nutrient storage may influence nutrient limitations in GIWs throughout time and space. For example, our observed high areal P storage in larval anurans may intensify P limitation as plant growth in GIWs is typically P-limited or N- and P-co-limited (Craft and Chiang 2002). We may expect assemblages primarily composed of larvae in later developmental stages or from species with low N:P tissue ratios, such as *S. holbrookii* or *G. carolinensis*, to have a greater influence on P availability and P limitation than other stages or species. Furthermore, given the divergent developmental stoichiometric patterns between the dominant GIW fauna, invertebrates, and larval anurans, the relative abundances of each group could influence ecosystem nutrient cycling with potential implications for whole wetland nutrient limitation shifts as indicated in other ecosystems (Allgeier et al. 2013, Atkinson et al. 2013). For example, high biomass of P-rich larval anurans may intensify P limitation in GIWs, but low biomass of larval anurans may intensify N limitation as the dominant aquatic faunal biomass switches to N-rich invertebrates. Stoichiometric theory also suggests that animal
excretion can shift nutrient limitation (Sterner et al. 1992, Capps and Flecker 2013). As larval anurans decrease in tissue N:P throughout development, we expect larvae to excrete at increasing N:P ratios (Elser and Urabe 1999, McLeay et al. 2019), which may alter N:P wetland ratios and affect ecosystem functions such as primary production or litter decomposition (Seale 1980, Whiles et al. 2006, Rugenski et al. 2012 but see McLeay et al. 2019).

**Conclusion**

Our approach revealed important ecological patterns across a diverse assemblage of vertebrates while providing unique estimations of animal nutrient storage in nutrient-limited ecosystems. Specifically, our findings showed a strong ontogenetic pattern in intraspecific stoichiometry of larval anurans, and key life-history traits—body size and developmental period length—predicted interspecific variation that generally followed patterns predicted by theory. We observed several consistent developmental trends across species which most likely result from an increased demand for P for bone growth. Furthermore, stoichiometric developmental patterns directly diverged with patterns found in aquatic macroinvertebrates (González et al. 2018), the other major faunal group in GIWs. We also determined that interspecific differences in body size and developmental period influenced %C and C:N ratios, and potentially %N, %P, and N:P ratios. Finally, we found that the magnitude of nutrient storage varied among months and wetlands suggesting that larval anurans may play a relevant role in driving temporally and spatially variable biogeochemical fluxes. However, it is currently unknown how impactful these magnitudes are and should be measured alongside factors such as leaf litter inputs to determine the true role of larval anuran nutrient storage in wetland nutrient cycling. Further examinations of developmental and interspecific stoichiometric patterns within other taxa will also help ecologists understand direct and accurate contributions of animals within ecosystem nutrient cycling.

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DATA AVAILABILITY

Raw stoichiometry and biomass data are available from Figshare (Knapp et al. 2021): https://doi.org/10.6084/m9.figshare.13683910.v1

SUPPORTING INFORMATION

Additional Supporting Information may be found online at: http://onlinelibrary.wiley.com/doi/10.1002/ecs2.3466/full