

Larval Anuran Stable Isotope Signatures and Stoichiometry Across Multiple Geographically Isolated Wetlands in the Southeastern United States

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Abstract - Animals live in complex environments that vary spatially and temporally. This heterogeneity strongly influences the availability and quality of food resources and has strong impacts on growth and survival of consumers. Geographically isolated wetlands provide an interesting system to study trophic relationships because they vary spatially and temporally in hydrology and vegetation. Larval anurans play an important role in these wetland systems because they are often the most abundant consumers. Yet, little is known about larval anuran diet. Here we assessed the diet of 3 larval anurans (*Acris gryllus* [Southern Cricket Frog], *Hyla gratiosa* [Barking Treefrog], and *Lithobates sphenoccephalus* [Southern Leopard Frog]), across 2 isolated wetland types (marsh and cypress savanna) using a stable isotope mixing model, stable isotope analysis in R (SIAR). Furthermore, we assessed variation in basal resource and anuran tissue stoichiometry (C:N). Our analyses suggested that larvae of these 3 species primarily function as herbivores and detritivores. All fed on a mix of algal resources, detrital particulate organic matter, and litter originating from the wetland canopy. Barking Treefrog had a lower C:N than the other two species, suggesting their dietary N requirements may be greater. Understanding the trophic roles of these animals is essential in determining their ecological significance and contributes to a more complete view of isolated wetlands in the surrounding landscape.

Introduction

Understanding food-web structure and trophic relationships is central to ecology, but can be difficult due to the complexity of natural communities. Organisms live in spatially and temporally heterogeneous systems that can vastly differ in resource abundance and productivity. Thus, food-resource availability and quality interact to influence food and habitat selection by consumers and overall ecosystem function (Marecchelli et al. 2011, Sitters et al. 2015). Furthermore, the availability and quality of food resources can strongly influence the growth and survival of consumers. Given this complexity, consumers often feed on a variety of resources that may span trophic levels. Wetlands present a challenging environment to explore these interactions because they vary in structure, hydrology (depth and permanence of water), vegetation, and diversity and abundance of primary consumers even when they are in close proximity to one another.

Larval anurans often play an important role in wetland food webs because they can reach high densities and biomass, exhibit high per-capita consumption rates, and

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serve as important prey for other species (Gibbons et al. 2006, Pryor 2003). Phenotypic plasticity of foraging behavior in response to resource variability can be one of the principal causes of variation in growth and life-history parameters (Ghalambor et al. 2007, Gotthard and Nylin 1995, Newman 1998, Nylin and Gotthard 1998). Both the quantity and quality of food resources in wetlands can influence phenotypic expression, determining the duration of larval stage and the timing of metamorphosis, which are critical aspects of development (Hall and Wake 1999, Kupferberg 1997, Stoler and Relyea 2013). Food quality influences growth and time of metamorphosis of amphibians (e.g., Alvarez and Niecieza 2002, Pryor 2003, Skelly and Golon 2003), and larvae often forage actively for high-quality resources, yet will change their feeding and behavior in the presence of predators (Bestion et al. 2015, Lawler 1989, Relyea and Werner 1999). Many amphibian larvae have been regarded as microphagous, suspension-feeding herbivores, and detritivores (Altig et al. 2007, Duellman and Trueb 1986, Whiles and Altig 2010) and viewed as feeding unselectively (Heyer 1976, Seale 1980), exhibiting little feeding-niche differentiation (but see Kupferberg 1997). While larval anurans are an abundant and diverse component of freshwater wetlands, we still know relatively little about the trophic status and feeding ecology of many species (Altig et al. 2007), especially of larval anurans in geographically isolated wetlands, where diversity and biomass can be exceptionally high (Gibbons et al. 2006, Leibowitz 2003), there are few grazing invertebrates (Battle and Golladay 2002), and amphibians likely provide significant transport of nutrient subsidies via movements between wetlands and terrestrial habitats (Capps et al. 2015, Earl and Semlitsch 2012). Previous studies (Opsahl et al. 2010, Verburg et al. 2007, Whiles et al. 2006) have suggested a periphyton/larval anuran link, but this has not been examined for individual species. Additionally, little has been done to examine the quality of these resources.

Stable isotopes are commonly used in aquatic ecology to study food webs and trophic structure (Finlay 2001, Post 2002, Vander Zanden and Rasmussen 1999). The stable carbon isotope ratio ($\delta^{13}\text{C}$) of an animal's tissues is a composite of the food items assimilated by that individual (Fry 2006). Because ^{13}C is a diet tracer that is incorporated into body tissues with little fractionation, it has also been used to examine inter- and intraspecific variations in diet (Hentschel 1998, Rossi et al. 2004). The nitrogen isotope ratio, as $\delta^{15}\text{N}$ of tissues, also provides information about an organism's diet; because consumers are enriched in ^{15}N by ~2–6‰ relative to their food sources, $\delta^{15}\text{N}$ provides useful information about trophic position (Post 2002, Vanderklift and Ponsard 2003).

Here we investigated the food resources larval anurans selected and assimilated into their tissues in seasonally ponded, geographically isolated wetlands in the southeastern US. Additionally, we examined anuran tissue stoichiometry (C:N) across species and wetlands. We focused on 3 common species of anurans across 2 wetland vegetation types (cypress savannas and marshes) in a relatively undisturbed *Pinus palustris* Mill. (Longleaf Pine) forest in southwestern Georgia. The goals of our study were to: (1) determine what food resources anurans were primarily selecting and if these resources varied in quality, (2) discern if there was

variation in diet across wetland types, and (3) assess whether there were differences in tissue stoichiometry across species.

Field-site Description

Our study wetlands were located at Ichauway, an 11,500-ha privately owned property of the Joseph W. Jones Ecological Research Center, in the Dougherty Plain district of the Southeastern Coastal Plain physiographic province in southwestern Georgia (Fig. 1). Ichauway has been managed primarily for forest and wildlife since the 1920s and consists of multi-aged second-growth Longleaf Pine forest with large areas of undisturbed native groundcover (Kirkman et al. 1999). Active limestone dissolution and subsequent land subsidence in the region have resulted in the development of numerous irregular-shaped geographically isolated wetlands (Hicks et al. 1987). The wetlands are described as geographically isolated because they are surrounded entirely by uplands and lack persistent and direct connections to regional surface waters (Leibowitz 2003). The hydroperiod of wetlands in the region is primarily controlled by precipitation, evapotranspiration, and shallow groundwater (Kirkman et al. 1999); thus, the wetlands are intermittently inundated, typically filling in late fall or winter and drying down in spring or summer. All wetlands were inundated following heavy rains in March 2005 and remained flooded throughout the study (May–August; Fig. 2). Wetlands began to dry down beginning in August, and most were dry by September 2005. The climate in this region is humid subtropical with an average annual precipitation of 132 cm and mean annual low and high temperatures of 12.9 and 26.3 °C, respectively (University of Georgia Weather Network 2016).

Prior classification of isolated wetlands based on characterization of vegetation, soils, and hydrology, identified 3 vegetation types (i.e., marshes, cypress savannas, and cypress gum swamps; Kirkman et al. 2000). Our study wetlands were limited to marshes and cypress savannas due to greater anuran abundance in those wetland types (Liner et al. 2008). Marshes are characterized by sandy soil, a treeless open canopy, and diverse emergent vegetation dominated by a mixture of C₃ and C₄ grasses and sedges (Opsahl et al. 2010). Cypress savannas are characterized by sandy-clay to clay soil, an open canopy of *Taxodium ascendens* Brongn. (Pond Cypress) and other C₃ woody species, and an understory of mixed emergent vegetation similar to marshes. The wetlands are surrounded by Longleaf Pine and *Aristida stricta* Michx. (Wiregrass) forests that have been maintained with prescribed fire (frequency of every 1–2 years) for the past 80 years.

Our focal anuran species were *Acris gryllus* (Leconte) (Southern Cricket Frog), *Hyla gratiosa* Leconte (Barking Treefrog), and *Lithobates sphenoccephalus* (Cope) (Southern Leopard Frog). Larvae of the 3 species differ in body size and feeding behavior. Larval Southern Cricket Frogs attain a total length of 42 mm and have been described as benthic-feeding periphyton consumers (Altig and McDiarmid 2015, Altig et al. 1975). Southern Leopard Frog larvae range from 65 to 83 mm in total length and are described as benthic omnivore/detritivores (Altig et al. 1975), whereas larval Barking Treefrogs can attain 70 mm in total length and feed in the

water column (Altig and McDiarmid 2015, Altig et al. 1975). All 3 species breed in marshes and cypress savannas from winter through spring in the study area (Liner et al. 2008, Smith et al. 2006) and were the most common species encountered during the study.

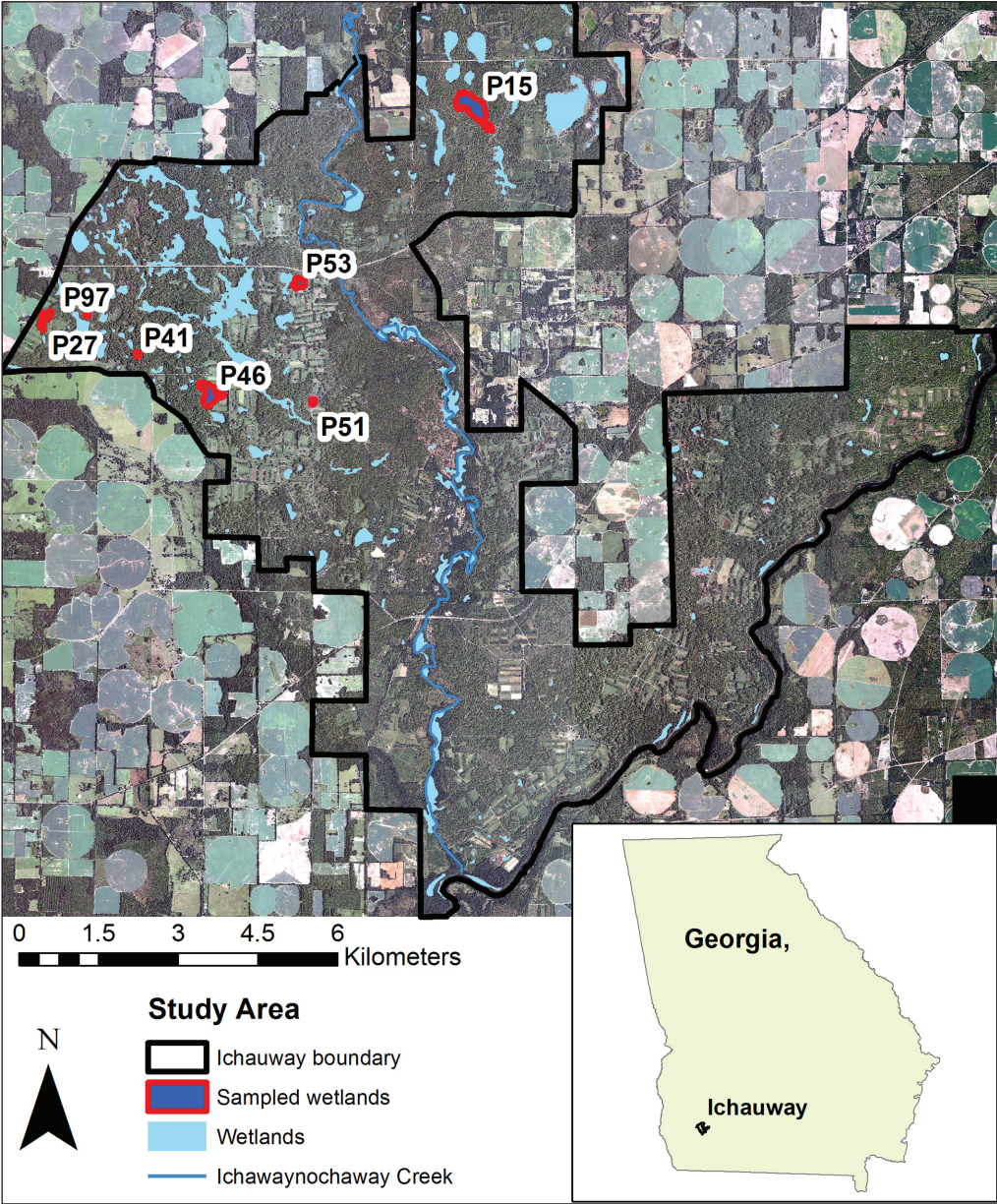


Figure 1. The distribution of wetlands and the study wetlands in the landscape. The wetlands were contained within Ichauway, a ~11,700-ha (~29,000-acre) area composed primarily of Longleaf Pine and Wiregrass uplands. Land use surrounding Ichauway is primarily center-pivot agriculture. Here we show the aerial view of the study area with the study wetlands highlighted.

Methods

Field sampling and sample processing

We selected 4 marshes and 3 cypress savannas ($n = 7$) for food-web analyses of larval anurans, basal resources, and available invertebrates. The wetlands were all in close proximity to one another (>10 km apart). While the cypress wetlands were all on the western portion of the study area, we do not think this biased our study. We sampled each wetland 3 times across the hydroperiod (Fig. 2) at ~6-week intervals during 2005 (May 9–17, June 29–July 7, and August 16–23) for basal resources and presumed primary and secondary consumers. Further, we sampled these wetlands for water chemistry (dissolved organic carbon (DOC), dissolved inorganic carbon (DIC), total dissolved carbon (TDC), ammonium ion ($\text{NH}_4\text{-N}$), nitrate ($\text{NO}_3\text{-N}$), and soluble reactive phosphorus (SRP)) during each collection date (see Supplemental Table S1, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s16-1-S2311-Atkinson-s1>, and, for BioOne subscribers, at <http://dx.doi.org/10.1656/S2311.s1>). Basal resources included periphyton (attached benthic algae and associated microbial communities), large detrital material (CPOM: leaves and other plant detritus >1000 μm), and fine particulate organic matter (FPOM; material of undetermined source <1000 μm and >45 μm) that was suspended in the water column (see Supplemental Table S2, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s16-1-S2311-Atkinson-s1>, and, for BioOne subscribers, at <http://dx.doi.org/10.1656/S2311.s1>). We sampled periphyton with periphytometers, consisting of a plastic slide holder

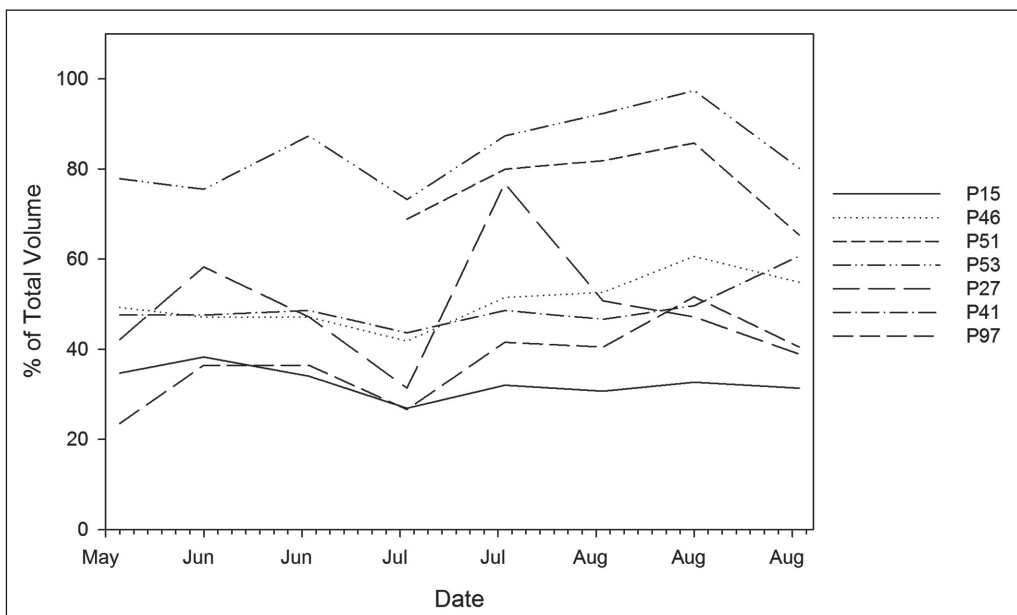


Figure 2. The hydroperiod of the wetlands sampled during the study. The gauge height of each wetland was recorded 8 times (except P51, which was recorded 5 times), biweekly from 25 May 2005 to 30 August 2005 and converted to the percent total volume of inundation of the wetland.

fitted with 5 pre-ashed glass microscope slides, placed in each wetland ~3 weeks prior to sampling. We collected POM by sieving water from open areas at least 10 m away from the shoreline. CPOM was primarily composed of senescent grasses (Poaceae) and sedges (Cyperaceae) in the marshes and grasses, sedges, and Pond Cypress leaves in the savannas. Three replicate samples were taken of each basal resource at each time period. The stable isotope signatures and C:N of basal resources did not significantly vary among the sampling periods (ANOVA with time period as the factor and food resource as a blocking factor; $\delta^{13}\text{C}$: $P = 0.08$, $\delta^{15}\text{N}$: $P = 0.97$, C:N: $P > 0.06$); thus, we averaged basal resources within resource category across the 3 sampling periods. Our rationale for selection of potential basal resources was based on the dominant sources of nutrition available in the wetlands and feeding modes known for larval anurans (Altig and McDiarmid 1999). A broader characterization of wetland primary producer isotopic composition has been previously published (Opsahl et al. 2010).

Larval anurans were captured using 30-cm D-frame dip nets with 3-mm mesh from all wetlands (sample sizes shown in Table 1). We attempted to collect larval anurans each time, but all species were not present at each wetland on each date. Macroinvertebrates were collected using dip nets with mesh sizes ranging from 500 μm to 5 mm and sorted in the field to compare trophic level with larval anurans. All samples were immediately frozen at -20 $^{\circ}\text{C}$, then dried at 50 $^{\circ}\text{C}$ and weighed for isotopic analysis. We used whole specimens for macroinvertebrates and larval anurans, and only grouped multiple individuals per wetland in cases where individuals provided insufficient mass for analyses. Further details on field collection and sample processing of detrital plant material, particulate organic matter, periphyton, larval anurans, and macroinvertebrates have been previously described by Opsahl et al. (2010).

Table 1. Results showing the mean percent contribution (and 95% confidence intervals) to larval anuran diet for each food resource and wetland from the SIAR mixing model for 3 species: *Hyla gratiosa* (Barking Treefrog), *Acris gryllus* (Southern Cricket Frog), and *Lithobates sphenoccephalus* (Southern Leopard Frog).

Wetland type/ Wetland			Mean % contribution to diet (95% CIs)		
Species	<i>n</i>		Periphyton	FPOM	CPOM
Marsh					
P15	<i>H. gratiosa</i>	5	43.9 (33.9–53.8)	42.6 (32.4–52.8)	13.5 (5.0–19.6)
P46	<i>A. gryllus</i>	4	38.2 (27.3–48.8)	27.0 (17.1–36.2)	34.8 (22.4–45.9)
	<i>H. gratiosa</i>	9	30.7 (21.1–40.5)	43.1 (37.5–48.8)	26.2 (14.3–37.0)
P51	<i>H. gratiosa</i>	5	32.7 (18.4–44.6)	33.4 (19.2–45.4)	33.9 (19.8–46.1)
P53	<i>H. gratiosa</i>	10	40.3 (34.8–46.1)	33.8 (27.1–40.8)	25.9 (16.4–34.8)
	<i>L. sphenocephalus</i>	3	46.5 (37.1–56.5)	24.1 (13.1–33.6)	29.4 (17.1–40.4)
Cypress Savanna					
P27	<i>L. sphenocephalus</i>	3	38.9 (27.7–49.4)	29.7 (16.1–41.1)	31.4 (19.6–41.9)
P41	<i>A. gryllus</i>	3	60.3 (44.5–78.0)	20.9 (7.5–31.9)	18.7 (6.3–28.5)
	<i>H. gratiosa</i>	3	75.0 (66.6–85.8)	13.7 (4.3–20.1)	11.3 (4.2–16.1)
	<i>L. sphenocephalus</i>	4	63.7 (51.9–77.1)	20.8 (8.0–31.2)	15.4 (5.5–22.2)
P97	<i>H. gratiosa</i>	5	64.3 (48.6–79.3)	19.9 (6.9–29.1)	15.8 (5.6–23.0)
	<i>L. sphenocephalus</i>	3	44.3 (37.2–50.8)	33.0 (23.8–42.9)	22.7 (10.2–33.5)

Samples were analyzed for total C and N and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ at the University of Georgia's Ecology Analytical Laboratory. The analytical precision for the stable isotope measurements was ± 0.1 for $\delta^{13}\text{C}$ and ± 0.2 for $\delta^{15}\text{N}$. To quantify the quality of the basal resources, we calculated the molar C:N ratios of periphyton, FPOM, CPOM, and larval anurans. Furthermore, we corrected for lipid content of the larval anuran tissue when C:N exceeded 3.5, as recommended by Post et al. (2007). Following this approach, we normalized $\delta^{13}\text{C}$ values to account for lipid variation in $\delta^{13}\text{C}$ following the equation in Caut et al. (2013) derived for amphibian tadpoles: $\delta^{13}\text{C}_{\text{normalized}} = -1.11 (\delta^{13}\text{C}_{\text{untreated}}) + 0.37(\text{C:N})$. Our statistical analysis was not limited to composite samples of all species by wetland type as in Opsahl et al. (2010). Our analysis was expanded to include 3 individual species per wetland: Barking Treefrog, Southern Cricket Frog, and Southern Leopard Frog. Based on the $\delta^{15}\text{N}$ signatures of larval anurans in comparison to both basal resources and macroinvertebrates in the wetlands (Fig. 3), we estimated the potential contribution of basal food resources to larval anurans.

Statistical analysis and isotope mixing model

We used a MANOVA to test for differences in the isotope signatures ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$; dependent variable matrix) across the wetland types (marsh vs. cypress savanna) and resource types (periphyton, FPOM, CPOM). To determine if there was a statistically significant difference in the quality (C:N) of basal resources available to larval anurans, we used a 2-way ANOVA with resource type and wetland type as main effects. We also used a 2-way ANOVA (without an interaction) to determine if C:N of larval anurans varied across species and wetland types (means per species across wetland types). Significant ANOVAs were followed by Tukey's HSD multiple comparisons to determine if significant differences existed among resource types and/or wetland types ($\alpha = 0.05$).

We used a Bayesian isotope mixing model in the stable isotope analysis in R (SIAR, v3.2.0) to determine the source contributions to larval anuran diet using lipid-corrected data. SIAR projects probability distributions of sources to consumer diets while accounting for uncertainty in source isotope signatures and in trophic fractionation (Moore and Semmens 2008). Bayesian mixing models allow the variation and uncertainties associated with isotopic estimates and trophic enrichment to be propagated through the model, with outputs being more reflective of the natural variability within a system. The SIAR model is fit via Markov chain Monte Carlo methods producing simulations of source dietary proportions using a Dirichlet prior distribution (Parnell et al. 2010). The probability distributions are estimated using a sampling–resampling algorithm. We used average fractionation for primary consumers \pm the standard deviation values ($\delta^{13}\text{C}$: 0.4 ± 1.2 ; $\delta^{15}\text{N}$: 3.4 ± 1.61) calculated from multiple stable isotope studies (Post 2002, Vanderklift and Ponsard 2003). We used periphyton, CPOM, and FPOM as potential basal resources in the mixing models because our $\delta^{15}\text{N}$ values, which are indicative of trophic level, have a similar trophic position as primary consumer aquatic macroinvertebrates (Figs. 3, 4). Additionally, previous

studies indicate that larval anurans are primarily herbivorous (Altig et al. 2007, Whiles and Altig 2010). We averaged the basal resources through time because $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N did not vary with sampling date. We ran the SIAR mixing model for 500,000 iterations, discarding the first 50,000 samples. The resulting distributions of probability–density functions of feasible foraging solutions produced by SIAR allowed direct identification of the most probable solution for the sources supporting each species within a wetland (Parnell et al. 2010). We used upper and lower 95% confidence intervals to describe the contribution for each diet item (Phillips and Gregg 2003).

Results

Isotope signatures

Stable isotope signatures varied across basal resource type ($F_{\text{approx}} = 17.8$, $\text{df} = 4$, $P < 0.0001$) and between wetland types ($F_{\text{approx}} = 30.4$, $\text{df} = 2$, $P < 0.0001$), and there was an interaction between wetland type and resource type ($F_{\text{approx}} = 4.1$, $\text{df} = 4$, $P = 0.003$) (Figs. 3, 4). Larval anurans were enriched in $\delta^{15}\text{N}$ by 0.52–5.6‰ relative

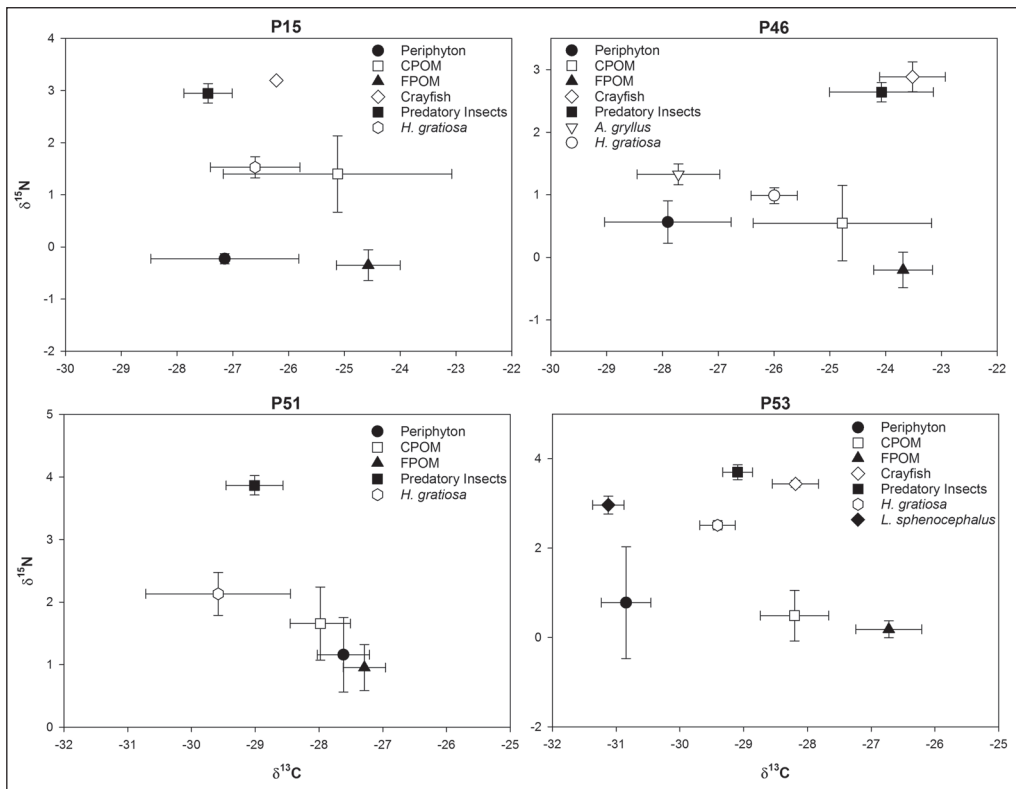
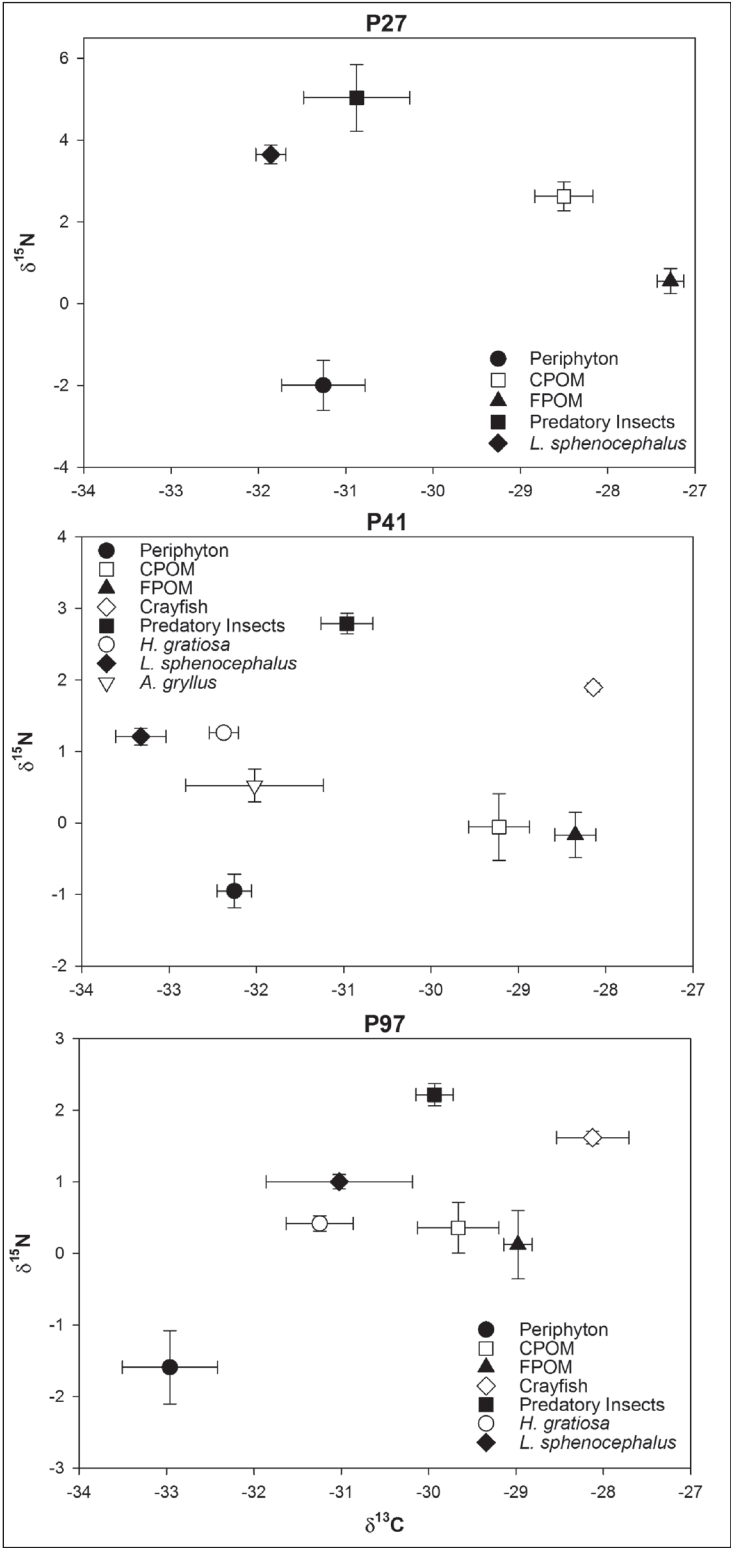


Figure 3. Graphs of the raw isotope signatures of the food resources and organisms sampled in marsh wetlands. Shown are the average (mean \pm standard error) isotope signatures of the basal resources (periphyton, fine particulate organic matter, and coarse particulate matter), larval anurans (not lipid corrected), crayfish, and predatory insects (e.g., Libellulidae, Dytiscidae, Anisoptera).

Figure 4. Graphs of the raw isotope signatures of the food resources and organisms sampled in cypress savanna wetlands. Shown are the average (mean \pm standard error) isotope signatures of the basal resources (periphyton, fine particulate organic matter, and coarse particulate matter), larval anurans (not lipid corrected), crayfish, and predatory insects (e.g., Libellulidae, Dytiscidae, Anisoptera).



to periphyton, by 0.46–3.10‰ relative to FPOM, and by 0.22–2.29‰ relative to CPOM across the wetlands (Figs. 3, 4). Furthermore, among macroinvertebrates, crayfish (omnivores) and predatory insects, respectively, had a higher $\delta^{15}\text{N}$ than larval anurans.

C:N

We found a significant difference in C:N among basal resource types ($F_{2,176} = 18.6$, $P < 0.0001$; Fig. 5), but resource C:N did not vary across wetland types ($F_{1,176} = 0.034$, $P = 0.85$). Our Tukey's HSD test showed that periphyton ($P < 0.0001$) and FPOM ($P < 0.0001$) were different (lower C:N) than CPOM, but these 2 resources did not differ in C:N from one another ($P = 0.936$). The C:N of larval anurans varied across species ($F_{2,57} = 12.14$, $P < 0.001$), but not between wetland types ($F_{1,57} = 0.207$, $P < 0.70$). Barking Treefrog had a significantly lower C:N than both Southern Cricket Frog and Southern Leopard Frog ($P < 0.004$; Fig. 6).

Mixing models

Across wetland types, periphyton was generally the dominant resource assimilated by larval anurans (Table 1). In the marshes, periphyton comprised 38.2% of the diet in Southern Cricket Frog (site P46), 30.7–43.9% of the diet in Barking Treefrog (all 4 sites), and 46.5% of the diet in Southern Leopard Frog (site P53). In the cypress savannas, periphyton comprised 60.3% of the diet in Southern Cricket Frog (site P41), 64.3–75.0% of the diet in Barking Treefrog (sites P41 and P97), and 38.9–63.7% of the diet in Southern Leopard Frog (all 3 sites). CPOM and FPOM assimilation varied across wetlands and species, but generally represented an important component in the diet of larval anurans (Table 1).

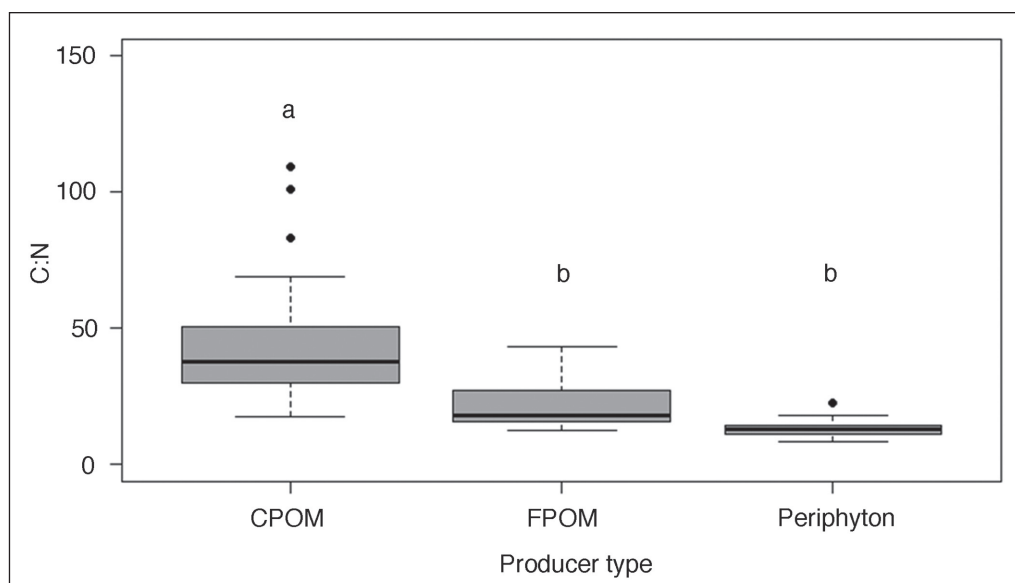


Figure 5. Stoichiometry of the larval anuran food resources. The C:N of each of the basal food resources sampled. CPOM ($n = 63$) had a significantly higher C:N than both periphyton ($n = 59$) and FPOM ($n = 55$), while periphyton and POM did not significantly differ.

Discussion

Larval anurans likely are an important link in wetland food webs (Gibbons et al. 2006, Opsahl et al. 2010). Our results suggest that larvae of each of the 3 anuran species obtained significant nutrition from algal-derived food (i.e., periphyton), but FPOM and, to a lesser degree, vascular plant detritus (CPOM) also are potential food resources. In comparison to other consumer taxa sampled during this study (e.g., crayfish and predatory insects), larval anurans appear to be primary consumers in these isolated wetland systems based on their relatively low $\delta^{15}\text{N}$. We were not able to detect any other potential food resources. Our results suggest that larval anurans likely feed selectively on periphyton and FPOM because they are higher-quality resources (lower C:N) and tend to have more microbes associated with them than CPOM (Atkinson et al. 2009, Bonin et al. 2000, Kamauchi 2005). Previous work has indicated that Barking Treefrog is primarily a pelagic feeder, whereas Southern Cricket Frog and Southern Leopard Frog are both benthic feeders (Altig and McDiarmid 2015, Altig et al. 1975). Our data suggest there is some difference in diet among these 3 species, but all seem to feed on a mixture of the available food resources.

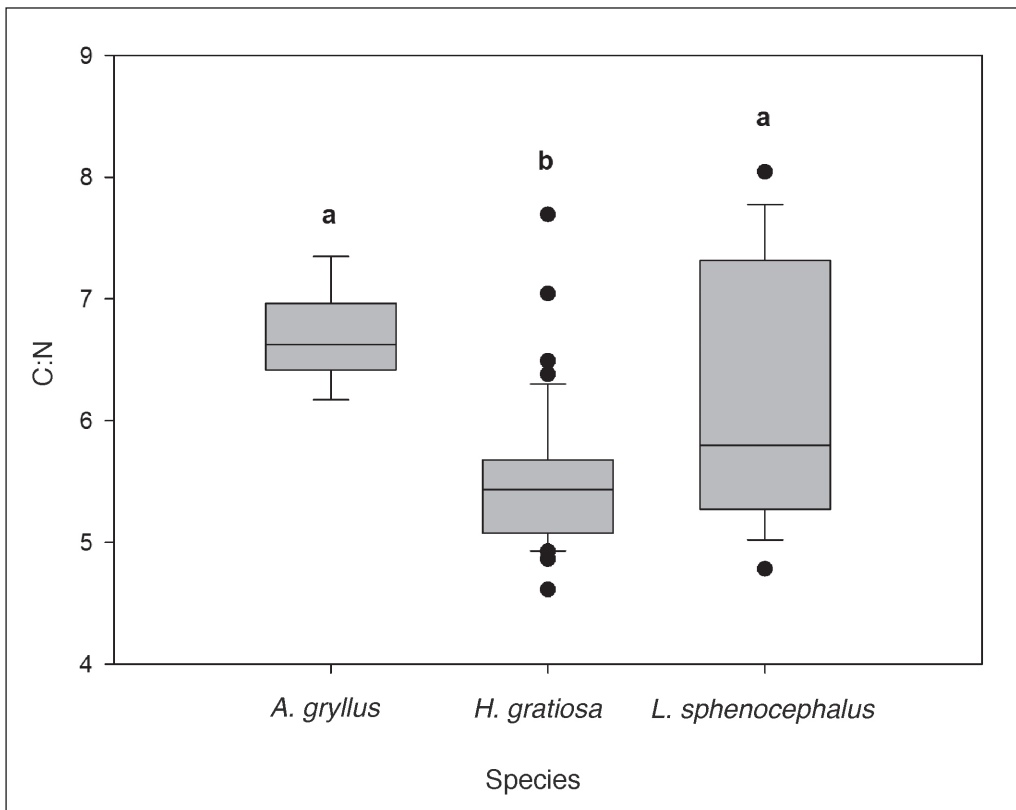


Figure 6. The C:N of the 3 larval anuran species included in the study varied. *Hyla gratiosa* (Barking Treefrog; $n = 37$) had a significantly lower C:N than *A. gryllus* (Southern Cricket Frog; $n = 7$) and *L. sphenoccephalus* (Southern Leopard Frog; $n = 13$).

Through their foraging activities, larval anurans are contributing to organic-matter processing by ingesting and recycling materials in the wetlands (Seale 1980). Studies on larval anurans in other systems have shown that there is little functional redundancy between larval anurans and invertebrates in aquatic ecosystems (Colon-Gaud et al. 2009, 2010; Whiles et al. 2013); thus, larval anurans are the main primary consumers across multiple wetland systems. Within these geographically isolated wetlands, there also seems to be little functional redundancy; our results show that larval anurans are acting as grazing primary consumers (as indicated by their low $\delta^{15}\text{N}$ in comparison to crayfish and other invertebrates), whereas most of the aquatic invertebrates are filter-feeders and predators (Battle and Golladay 2001a, 2002; Opsahl et al. 2010). While other studies have shown evidence of omnivory or ontogenetic diet shifts in anuran larvae (Schiesari et al. 2009, Schriever and Williams 2013), our results suggest that larval anurans in geographically isolated wetlands of the southeastern Coastal Plain are mainly functioning as primary consumers. Seasonal wetlands are low-nutrient systems (see Supplemental Table S1, available online at <http://www.eaglehill.us/SENAonline/suppl-files/s16-1-S2311-Atkinson-s1>, and, for BioOne subscribers, at <http://dx.doi.org/10.1656/S2311.s1>; Atkinson et al. 2011; Battle and Golladay 2001b, 2003), and the recycling of nutrients bound in plant materials by larval anurans may be a critical process in maintaining wetland productivity (Vanni 2002).

Reproductive output of amphibians in geographically isolated wetlands is considerable, and following metamorphosis, amphibians often move considerable distances (hundreds of meters) between breeding wetlands and upland habitats (Gibbons 2003, Scott et al. 2013, Semlitsch and Bodie 2003, Smith and Green 2005); thus, pulsed migration of anurans may generate a substantial flow of nutrients and energy (Earl and Semlitsch 2012, Seale 1980). For example, Gibbons et al. (2006) showed that a single 10-ha isolated wetland produced >360,000 metamorphic amphibians (>1400 kg of biomass) in 1 year, suggesting that energy and nutrient subsidies by amphibians may be greater than previously realized. The anuran species we studied emerge from the wetlands as terrestrial juveniles and have the ability to move long distances (Semlitsch and Bodie 2003). Our results indicate that the majority of their diet as aquatic larvae originates from wetland-derived periphyton and FPOM. Following metamorphosis, anurans transport a substantial amount of energy and nutrients (as biomass) out of the wetland into the adjacent terrestrial habitat. The surrounding terrestrial Longleaf Pine landscape is nutrient-poor in comparison to the abundant and often aggregated wetlands (Craft and Chiang 2002, Martin et al. 2012), suggesting that migrations of anurans could contribute significant subsidies to support landscape heterogeneity. Furthermore, southeastern species tend to breed throughout the year and are less predictable than northern populations (Pechmann et al. 1991), thus further enhancing the heterogeneity of the movement of energy and nutrients.

Our study also showed that larval amphibians vary in their tissue C:N, which is indicative of nutrient retention and not necessarily diet (Sterner and Elser 2002). Barking Treefrog had a lower C:N than both Southern Cricket Frog and Southern

Leopard Frog, indicating it may have a higher dietary requirement for N. This finding suggests that different species may have different nutrient requirements as has been demonstrated in other studies (Burton and Likens 1975, Seale 1980). Surprisingly, the 2 Hylidae species (Southern Cricket Frog and Barking Treefrog) in the study were different in their C:N. Whether the differences we noted in C:N was species-specific variation or due to differences in developmental stages of the larvae we sampled (Capps et al. 2015) is unclear. Future research is needed to explore the variability in tissue nutrient content among species to document the potential role of the diverse suite of amphibians in wetlands and the surrounding landscape.

While we did not investigate ontogenetic changes in resource use and assimilation, previous studies have shown that larval anurans can vary their diet across developmental stages (Trakimas et al. 2011), but not in all cases (Schriever and Williams 2013). Capps et al. (2015) showed that larval *Lithobates sylvaticus* (LeConte) (Wood Frog) undergo ontogenetic changes in tissue stoichiometry with developmental stage such that they increase in phosphorus content as they grow. Thus, the availability and quality of food and nutrients are likely important constraints on development and metamorphosis of amphibians. This insight has important implications regarding amphibian nutrient requirements and how those demands are met. Further studies incorporating tissue stoichiometry and food selection are needed to better understand production and population dynamics of amphibians.

Diet in larval anurans can also fluctuate in response to intraspecific density, interspecific competition, food availability, predation, and water levels (Arribas et al. 2015, Bestion et al. 2015, Relyea and Werner 1999, Whiles et al. 2010). The ability of anuran larvae to have a large trophic niche breadth demonstrates their capacity for exploiting widely divergent or fluctuating conditions in isolated wetlands. Shifts in the trophic niche of larval amphibians may strongly affect the structure and function of the food web of isolated wetlands. Yet, our results should be taken with some caution. Previous work has shown that gut clearance for stable isotopes is not necessary with primary consumers (Jardine et al. 2005), but we did not allow the gut contents to fully evacuate before freezing, thus potentially leading to some bias. While this study revealed variability in diets of 3 larval anuran species co-occurring in wetlands, it also highlights the need for additional research on the trophic status of other larval anurans in these species-rich sites.

Given the declines in both amphibians (Stuart et al. 2004) and the wetland habitats they depend upon (Batzler and Baldwin 2012), it is essential to characterize their habitat and feeding requirements in developing conservation strategies. Approximately 50% of the remaining geographically isolated wetlands in the southeastern US have been dramatically altered (Lane et al. 2012), and these wetlands are not protected under the US Clean Water Act, which has resulted in consequential reduction in habitat (Leibowitz 2003). Declines in amphibian abundance and diversity are occurring worldwide, yet little is known about the ecological consequences of these losses (Stuart et al. 2004). Studies have shown that primary production, nutrient cycling, and leaf-litter decomposition are affected by larval (Ranvestel et al. 2004, Rugenski et al. 2012) as well as juvenile and adult frogs (Beard et al. 2002).

Understanding the overall trophic status of larval anurans in isolated wetlands is central to determining their ecological role and significance in the larger landscape.

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