A stable isotope study of a neotropical stream food web prior to the extirpation of its large amphibian community

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Abstract: Rapid and massive amphibian population declines have been reported throughout upland areas of the Neotropics. The abundance and species richness of Neotropical amphibian communities suggest that losses of this magnitude are likely to have strong effects at the ecosystem level. To improve understanding of the implications of their loss we used stable isotope analysis to examine trophic relationships in an ecosystem in which amphibians are dominant in a second-order forest stream at 750 m asl in Parque Nacional Omar Torrijos Herrera, Panama. We analysed δ^{13}C, δ^{15}N and C:N ratios of major biotic components (basal resources, invertebrates, amphibians, fish and reptiles) in the stream and of the adjacent riparian food web. Tadpoles (mean δ^{15}N = 4.49‰) and adult amphibians (mean δ^{15}N = 5.45‰) were intermediate links in the aquatic and terrestrial food web respectively. High δ^{15}N signatures identified fish as top predators in the aquatic food web and snakes and the toad *Bufo* as top predators in the terrestrial food web. Isotopic signatures clearly distinguished between trophic groups of tadpoles: microbial feeders (Centrolenidae, δ^{15}N range = 0.91–3.05‰), herbivores (*Rana* and *Hyla*, δ^{15}N range = 4.74–5.15‰) and neuston feeders (*Colostethus*, δ^{15}N range = 5.31–6.40‰). Dependence on autotrophic production was indicated by enriched signatures of carbon isotopes in pool dwellers versus those that reside in faster-flowing sections of the stream. High nitrogen concentrations in detrital matter (average 0.8%, C:N = 10.3) suggested that grazing tadpoles enhanced nitrogen fluxes and improved the quality of organic matter available to detritivores.

Key Words: δ^{13}C, δ^{15}N, amphibian declines, aquatic invertebrates, Panama, stable isotopes, tadpoles

INTRODUCTION

Challenges to understanding ecosystem dynamics arise when major functional groups are removed from communities for which there are few if any long-term data. Such is the case with the extirpation of frogs in the Americas (Lips *et al.* 2003). Amphibian species are threatened with extinction from a variety of factors, including habitat loss, overexploitation, disease and climate change (Stuart *et al.* 2004). Neotropical regions have the highest diversity of amphibians, and have shown the greatest number of losses at the population, species and regional level (Lips *et al.* 2006). In Central America, many declines have been attributed to an exotic

pathogenic fungus that can persist in the environment indefinitely. In these cases, arrival of the fungus typically results in the permanent loss of 80–90% of adult and larval amphibian abundances and a 50% reduction in species richness within 6 mo (Lips *et al.* 2006). These populations are unlikely to recover (Lips 1998, 1999; Lips *et al.* 2003), and the few remaining upland cloud-forest sites with healthy amphibian communities are likely to suffer declines in the near future (Lips *et al.* 2006). This has allowed us to predict the arrival of the fungus to particular areas with a relative degree of certainty, and thus has allowed us to establish baseline levels of trophic resources and relationships among components prior to local extinctions for comparison to post-decline levels. Neotropical tadpoles are abundant, occupy multiple habitats, and are often present year round (McDiarmid & Altig 1999). They also exhibit various behavioural and morphological adaptations thought to represent diverse feeding strategies, diets and trophic levels (Altig *et al.*

2007, Petranka & Kennedy 1999). These studies indicate that both adult and larval amphibians are likely to play important roles in their ecosystems. However, the scarcity of studies which examined the ecological roles of amphibians (Altig *et al.* 2007), particularly in the Neotropics where their diversity is high, has prevented an understanding of the impacts of amphibian population declines on ecosystem structure and function. Our goal was to use analyses of stable isotopes to identify food-web connections and to examine processes in, and functioning of, the ecosystem in a neotropical headwater stream that still harboured healthy, diverse amphibian assemblages.

Carbon isotopes (δ^{13}C) identify major energy sources, and nitrogen isotopes (δ^{15}N) indicate trophic position within a food web. In many cases, nitrogen isotopes have proven to be more effective tools than gut analyses for interpreting diet relationships because they account for what has been assimilated over a period of growth. In addition, temporal changes in δ^{13}C and δ^{15}N in basal resources can reveal various processes that are important for the functioning of the ecosystem (Fry 2006). While stable isotopes have become a standard tool in food-web studies, few stable isotope studies on amphibians and reptiles exist (Dalerum & Angerbjörn 2005, Vanderklift & Ponsard 2003). Kupfer *et al.* (2006) was one of the first studies incorporating these vertebrates. The objective of our study was to investigate trophic relations within the aquatic and riparian food web and in particular we wanted to determine whether we could distinguish different trophic groups among species of tadpoles by their isotope signatures. We wanted to test the hypothesis that distinct trophic groups among tadpoles such as the centrolenids and herbivorous species differ significantly in their stable isotope signatures. In addition, we wanted to compare the quality of organic detritus in the stream in terms of its nutrient concentration with that which is reported from streams without abundant tadpole communities. We would expect that detritus in an amphibian-dominated stream ecosystem is relatively enriched in nitrogen by nutrient recycling by the abundant herbivorous tadpoles, compared with streams where amphibians are not abundant. Another aim of the study was to examine δ^{13}C at similar trophic levels. We wanted to test the hypothesis that consumption of autotrophic production in a neotropical stream dominated by amphibian grazers can be demonstrated by interspecific differences in δ^{13}C at similar trophic levels.

METHODS

Research was conducted along a 200-m reach of a second-order tributary of the Río Guabal in the headwater valley of the Río San Juan in Parque Nacional Omar Torrijos Herrera, Panama (8°40′04″N, 80°35′36″W). The site is at 750 m asl on the eastern extent of the Cordillera Central and 8 km north of the town of El Copé, in Coclé Province. The habitat is cloud forest and selective logging in the late 1970s created a matrix of primary and secondary forest in the region. The climate is transitional, between the wetter rain forests of the Atlantic slope and the drier forests of the Pacific slope. Mean monthly temperature is between 19 °C and 26 °C and mean annual precipitation is 3500 mm; daily mean air temperature was 21 °C (range 16–31 °C) during out study period. The study stream is a high-gradient, second-order stream with a heterogeneous mix of organic and inorganic substrates. Canopy cover is uniformly dense (∼75%) with occasional treefall gaps. The stream has a neutral pH and mean annual water temperature is 21–25 °C; temperatures ranged from 23–25 °C during our study.

At the time of our study, the park harboured a fauna of 65 amphibian species, 40 of which occurred in riparian habitats, and 23 of which had stream-dwelling tadpoles (Lips *et al.* 2003). Amphibians were abundant throughout the year, with average capture rates of 0.36 adult anurans per m of stream and 0.13 adults per m of terrestrial trail (Lips unpubl. data). Tadpoles are abundant in the stream. Ranvestel *et al.* (2004) observed densities of *R. warszewitschii* and *Hyla* spp. tadpoles of $23.6 \pm 3.1 \, \mathrm{m}^{-2}$ (mean ± 1 SE) in runs, $28.3 \pm 0.3 \, \mathrm{m}^{-2}$ in riffles, and $36.7 \pm 7.9 \, \mathrm{m}^{-2}$ in pools in May–July 2001.

Samples were collected from May to August 2000, which represents the transition from the dry to rainy season. The rainy season of 2000 started at the end of May (daily average rainfall = 5.14 mm, range = 0–40 mm). During the sampling period, we recorded minimum and maximum air temperature and rainfall twice daily and estimated discharge daily using a staff gauge installed at the upper end of the study reach. Composite samples of basal resources were collected each month from five sites along the stream. We analysed three samples per month of filamentous green algae (FGA), and of periphyton (PERI). Samples of seston (SES) and fine benthic organic matter (FBOM) were collected once per month. The FBOM was rinsed from a rock taken from the stream bed and PERI was scrubbed from a rock from the stream bed which had been rinsed with filtered water. Seston was filtered from 2–3 L of fast-flowing water from the stream and FGA was plucked from stream rocks. Filamentous green algae was dried in scintillation vials while other samples were filtered and dried onto Whatman glass microfibre filters. We collected three monthly grab samples of leaf packs from the stream bed (CPOM, composite from 10 sites). We removed all aquatic invertebrates from CPOM samples and rinsed leaves before drying.

Samples of common amphibians, reptiles and stream invertebrates were collected by either combining individuals of small taxa or by taking tissue samples from the larger organisms. Tadpole collections included several

species of *Colostethus* and centrolenids which could not be identified to species level, *Hyla colymba*, and *Rana warszewitschii*. *Atelopus zeteki* Dunn was abundant in our study stream, but we did not collect this species because it is CITES protected. Aquatic insects were assigned to functional feeding groups (Merritt & Cummins 1996, Perez 1996). We used unbaited minnow traps to collect three individuals of each of three size classes (carapace length < 20, 21–30, > 30 cm) of the shrimp *Macrobrachium* sp. and a kick seine to collect three specimens of each of five classes (<10, 11–20, 21–30, 31–40, and > 41 mm) of the poeciliid fish *Brachyrhaphis roswithae* and three samples of the fossorial catfish *Trichomycterus striatus*.

We collected three individuals each of three riparian snake species (*Imantodes cenchoa*, *Oxybelis brevirostris*, *Rhadinea vermiculaticeps*) that are known to feed on frogs (Savage 2002), a riparian snake (*Sibon argus*) that feeds on arboreal frog eggs (Ryan & Lips 2004) and probably on slugs and snails like several of its congeners (Savage 2002), a riparian lizard (*Norops lionotus*), and six species of adult riparian anurans (*Bufo haematiticus*, *Centrolene prosoblepon*, *Eleutherodactylus talamancae*, *Hyla palmeri*, *H. colymba*, *Rana warszewitschii*). We anaesthetized tadpoles, frogs and fish by immersion in MS222, and anaesthetized the snakes and lizards with Oragel applied to the head. For small vertebrates (tadpoles, fishes, small frogs), viscera were removed and the samples were rinsed with freshwater and then dried whole. For larger organisms (large frogs, snakes, lizards and shrimps), we dried samples of muscle tissue. The gut contents of all vertebrates, including tadpoles, were removed and preserved in formalin for identification. Vouchers were collected for all vertebrate species and deposited in the Southern Illinois University Wet Vertebrate Collection.

All samples were dried in a drying oven within 24 h of collection at ∼50 °C for 3 d and processed for analysis of carbon and nitrogen contents and stable isotopes. Analyses were performed with a Carlo Erba NA 1500 CHN analyzer coupled to a Finnigan Delta C mass spectrometer at the Institute of Ecology Analytical Chemistry Laboratory, University of Georgia. Isotope ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ (with units of ‰) according to the equation $\delta^{13}C$ or $\delta^{15}N = ((R_{sample}/R_{standard}) - 1) \times 1000$, where R is $^{13}C{:}^{12}C$ or $^{15}N{:}^{14}N$. The standard is PeeDee belemnite carbonate for $\delta^{13}C$ and atmospheric N for $\delta^{15}N$.

Statistical tests were performed with SPSS.

RESULTS

Fine benthic organic matter had a significantly higher (t-test, t = 2.92, df = 42, P < 0.01) $\delta^{15}N$ value (average 4.23‰) than other basal resources (average $\delta^{15}N$ = 2.15‰), while the $\delta^{15}N$ values were on average

Table 1. Stable isotopes of carbon and nitrogen (mean ± 1 SD) and C to N ratios in the basal resources of the food web of the stream at El Cope, Panama. Values are averages for each month and averages for each category over the study period. N is the number of samples collected each month. Total average is the mean for all basal resources.

	N	$\delta^{15}N$‰	$\delta^{13}C$‰	molar C:N
Periphyton (PERI)				
May	3	4.22 ± 2.47	−29.4 ± 2.10	7.0
June	3	2.27 ± 0.75	−30.1 ± 2.41	5.2
July	3	1.80 ± 0.15	−30.2 ± 1.25	4.6
August	3	1.55 ± 0.64	−31.0 ± 0.88	3.1
Average		2.46 ± 1.58	−30.2 ± 1.63	5.0
Filamentous algae (FGA)				
May	3	4.04 ± 1.57	−29.7 ± 2.46	12.8
June	3	2.58 ± 2.22	−33.2 ± 2.45	10.2
July	3	0.92 ± 0.09	−34.9 ± 3.23	8.0
August	3	0.56 ± 1.13	−35.2 ± 0.99	7.0
Average		2.03 ± 1.92	−33.2 ± 3.11	9.5
Seston (SES)				
May	1	1.29	−29.4	4.2
June	1	2.60	−30.4	1.7
July	1	1.71	−30.4	3.8
August	1	1.52	−31.3	2.1
Average		1.78 ± 0.57	−30.4 ± 0.78	2.9
Coarse particulate organic matter (CPOM)				
May	3	2.36 ± 0.61	−30.0 ± 1.02	29.6
June	3	2.40 ± 0.71	−29.4 ± 0.25	32.4
July	3	1.61 ± 0.59	−29.9 ± 0.23	29.1
August	3	2.02 ± 0.73	−30.0 ± 0.28	28.5
Average		2.10 ± 0.66	−29.8 ± 0.53	29.7
Fine particulate organic matter (FBOM)				
May	1	3.62	−28.9	14.1
June	1	5.35	−29.1	5.7
July	1	3.86	−29.0	13.0
August	1	4.10	−29.1	8.4
Average		4.23 ± 0.77	−29.0 ± 0.11	10.3
Total average		2.52 ± 1.28	−30.5 ± 1.84	11.5

very similar for both types of alga (FGA and PERI) and for two types of detrital material (CPOM and SES, Table 1). The FBOM differed significantly from CPOM (t-test, t = 2.93, df = 14, P < 0.02) in $\delta^{13}C$ but not from PERI (t-test, t = 1.42, df = 14, P > 0.17).

During the 4-mo sampling period, the $\delta^{15}N$ values in the algal compartments significantly decreased by about 3‰ (Regression, $F_{1,10}$ = 11.9, P < 0.01 for FGA and $F_{1,10}$ = 6.5, P < 0.03 for PERI). C:N ratios decreased significantly between May and August in PERI (Regression, $F_{1,10}$ = 35.2, P < 0.0002) and FGA ($F_{1,10}$ = 11.1, P < 0.01). C:N ratios and $\delta^{15}N$ were positively correlated in FGA (n = 12, r = 0.74, P < 0.01) and in PERI (n = 12, r = 0.73, P < 0.01). Other basal resources (FBOM, CPOM and SES) showed no such temporal pattern and remained relatively constant in nitrogen isotopes.

In the FGA, monthly mean $\delta^{13}C$ decreased 5.5 (Regression, $F_{1,10}$ = 9.4, P < 0.02). $\delta^{13}C$ in PERI, CPOM and FBOM did not change significantly (Regression, P > 0.25). While $\delta^{13}C$ in FGA and PERI were similar in May (−29.7 and −29.4‰ respectively), FGA was on average 3‰ more depleted than PERI (t-test, t = 3.00, df = 22, P < 0.01).
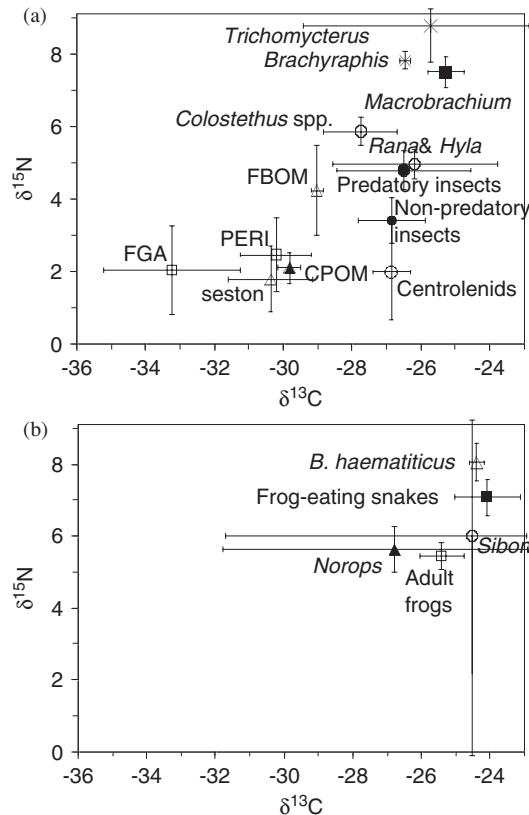
Figure 1. Average stable isotopes of carbon and nitrogen with 95% confidence limits for the major taxa and food resources in a tributary of Río Guabal, El Copé, Panama. The stream food web. *Rana, Hyla, Colostethus* and centrolenids are tadpoles (a). The riparian food web (b).

Table 2. Stable isotopes of nitrogen and carbon (mean $\pm$ 1 SD) in the invertebrates from the stream at El Cope, Panama. N is the number of samples collected.

	N	δ^{15}N‰	δ^{13}C‰
Shredders and Scrapers (SH-SC)			
Coleoptera			
Ptilodactylidae	3	4.78 ± 0.34	-27.0 ± 0.85
Elmidae	6	2.03 ± 1.00	-27.2 ± 0.33
Trichoptera			
Calamoceratidae	1	2.40	-21.7
Ephemeroptera			
Leptophlebiidae	2	3.80 ± 0.36	-27.8 ± 1.07
Average		2.75 ± 1.39	-26.3 ± 2.29
Filterers and Collectors (FL-CO)			
Trichoptera			
Hydropsychidae	3	3.90 ± 0.30	-27.8 ± 0.71
Philopotamidae	1	5.15	-21.8
Diptera			
Simuliidae	1	3.83	-28.3
Chironomidae	1	4.12	-28.0
Average		4.25 ± 0.61	-26.5 ± 3.12
Insect predators			
Odonata			
Calopterygidae	3	4.72 ± 0.41	-25.9 ± 3.51
Polythoridae	3	5.38 ± 0.28	-25.0 ± 2.83
Libellulidae	2	3.58 ± 0.44	-28.9 ± 2.36
Plecoptera			
Perlidae	2	5.20 ± 0.17	-27.3 ± 0.78
Average		4.72 ± 0.81	-26.8 ± 1.70
Macrobrachium			
11–15 cm	3	6.92 ± 0.08	-25.0 ± 0.92
26–28 cm	3	7.67 ± 0.72	-25.8 ± 0.64
30–33 cm	3	7.91 ± 0.57	-25.1 ± 0.82
Average		7.49 ± 0.64	-25.3 ± 0.80

Aquatic vertebrate species (n = 9) were significantly lower in %N (10.9%) and had a higher C:N ratio (4.8) compared with terrestrial vertebrate species (n = 14, 12.1% and 4.2 respectively; t = 2.27, df = 21, P < 0.05 for % N and t = 5.03, df = 21, P < 0.0001 for C:N). Stable isotope data of components of the aquatic and riparian food webs are shown in Figure 1. Aquatic insects (Table 2) were similar in δ^{13}C (about -27‰), with two exceptions, Calamoceratidae and Philopotamidae (-21.7‰). Shredders (SH) and scrapers (SC) showed the greatest variation in δ^{15}N, from 2.03‰ to 3.80‰ for SC (average = 2.33‰), and 2.40‰ to 4.78‰ for SH (average = 3.59‰). The Ptilodactylidae beetle larvae and Leptophlebiidae mayflies had the highest δ^{15}N ratios and elmid beetle larvae had the lowest. The filterers (FL) and collectors (CO) averaged 4.25‰ δ^{15}N. The difference between the average for all basal food types (2.52‰ δ^{15}N) and FL-CO (4.25‰ δ^{15}N) was 1.73‰ δ^{15}N and 0.23‰ for SH-SC (2.75‰ δ^{15}N). It does not seem likely that FBOM (4.23‰ δ^{15}N) made up a large fraction of the food base of insects.

The insect predators had the highest δ^{15}N values among the insects, averaging 4.72‰, which was higher than other insect larvae by 1.37‰. *Macrobrachium* shrimps had the highest δ^{15}N among invertebrates,

and this value increased with increasing size (Table 2). Shrimp δ^{15}N was 7.49‰ on average and shrimps were the most enriched in δ^{13}C of the aquatic food web, averaging -25.3‰. *Macrobrachium* was 1.0‰ higher in δ^{13}C (t = 3.89, df = 22, P < 0.001) than the fish *Brachyrhaphis roswithae* (while their δ^{15}N signatures were similar: 7.50‰ and 7.70‰ respectively, P > 0.2).

Tadpoles averaged 4.49‰ δ^{15}N and -27.0‰ δ^{13}C (Table 3). Herbivorous *Rana warszewitschii* and *Hyla colymba* tadpoles had lower δ^{15}N values (mean 4.95‰) than the neuston-feeding tadpoles of *Colostethus* spp. (Dendrobatidae, mean δ^{15}N = 5.86‰, t-test, t = 3.31, df = 13, P < 0.006). Glass frog tadpoles (Centrolenidae) were lowest in δ^{15}N (1.98‰). The two species of fish in this system averaged 7.97‰ δ^{15}N and -26.2‰ δ^{13}C. The catfish *Trichomycterus striatus* had a higher δ^{15}N (t-test, t = 3.30, df = 16, P < 0.005) and a similar δ^{13}C (t-test, t = 1.25, df = 16, P > 0.23) compared with *B. roswithae*. *Brachyrhaphis roswithae* increased in δ^{15}N with increasing size (Table 3), but δ^{15}N in the yolk stage was similar to that in large adults.

Terrestrial species except *Norops lionotus* (see below) were more enriched in δ^{13}C (average = -25.0‰, n = 12)

Table 3. Stables isotopes of nitrogen and carbon (mean ± 1 SD) in the vertebrates from the stream and riparian zone, El Cope, Panama. N is the number of samples collected.

	N	$\delta^{15}N$‰	$\delta^{13}C$‰
Aquatic			
Fish			
Brachyrhaphis roswithae Meyer & Etzel			
0–10 mm (yolk stage)	3	8.25 ± 0.14	−26.2 ± 0.09
11–20 mm	3	7.39 ± 0.36	−26.6 ± 0.36
21–30 mm	3	7.49 ± 0.28	−26.3 ± 0.28
31–40 mm	3	7.88 ± 0.63	−26.5 ± 0.37
41+ mm	3	8.04 ± 0.30	−26.6 ± 0.30
Trichomycterus striatus Meek & Hildebrand	3	8.77 ± 0.40	−25.7 ± 1.49
Average fish		7.97 ± 0.58	−26.2 ± 0.68
Tadpoles			
Rana warszewitschii Schmidt	3	5.15 ± 0.37	−25.5 ± 3.15
Hyla colymba Dunn	3	4.74 ± 0.34	−26.9 ± 1.26
Colostethus sp. 1	3	5.86 ± 0.04	−26.1 ± 0.25
Colostethus sp. 2	3	6.40 ± 0.41	−29.2 ± 0.67
Colostethus sp. 3	3	5.31 ± 0.57	−27.9 ± 1.65
Average non-centrolenids		5.49 ± 0.68	−27.1 ± 2.00
Centrolenidae sp. 1	3	3.05 ± 0.58	−26.7 ± 0.73
Centrolenidae sp. 2	3	0.91 ± 0.24	−27.0 ± 0.23
Average centrolenids		1.98 ± 1.24	−26.9 ± 0.52
Terrestrial			
Adult anurans			
Colostethus flotator Dunn	3	5.11 ± 0.92	−24.2 ± 2.17
Colostethus panamensis Dunn	3	5.99 ± 0.77	−26.0 ± 1.07
Colostethus nubicola Dunn	3	5.77 ± 0.33	−26.8 ± 2.09
Centrolene prosoblepon Boettger	3	5.27 ± 0.15	−25.1 ± 1.79
Hyla colymba Dunn	3	4.58 ± 0.05	−25.1 ± 0.60
Rana warszewitschii Schmidt	3	6.29 ± 0.64	−25.4 ± 0.26
Hyla palmeri Boulenger	2	4.39 ± 0.02	−25.0 ± 0.39
Eleutherodactylus talamancae Dunn	1	6.67	−25.8
Average frogs (excl. *Bufo*)		5.45 ± 0.83	−25.4 ± 1.42
Bufo haematiticus Cope	3	8.05 ± 0.21	−24.4 ± 0.08
Snakes and lizards			
Imantodes cenchoa Linné	3	6.90 ± 0.19	−24.4 ± 0.38
Oxybelis brevirostris Cope	2	7.15 ± 0.81	−24.3 ± 1.69
Rhadinea vermiculaticeps Boulenger	3	7.21 ± 0.89	−23.6 ± 1.61
Average frog-eating snakes		7.08 ± 0.59	−24.1 ± 1.16
Sibon argus Cope	3	6.00 ± 2.85	−24.5 ± 2.88
Norops lionotus Dunn	3	5.63 ± 0.25	−26.8 ± 2.02

than species in the aquatic food web (−26.4‰, n = 30; t-test, df = 40, t = 2.98, P < 0.005). Adult frog species (excluding *Bufo*) had a $\delta^{15}N$ averaging 5.45‰ which was similar to that of tadpole species (5.49‰, not including centrolenids) and an average $\delta^{13}C$ of −25.4‰, which was 1.7‰ more enriched than tadpole species (t-test, t = 2.98, df = 12, P < 0.05). The two adult *Hyla* species had the lowest $\delta^{15}N$ values. The toad *Bufo haematiticus* had a much higher $\delta^{15}N$ than the other anurans, and a more enriched $\delta^{13}C$. *Bufo haematiticus* was highest in $\delta^{15}N$ (8.05‰), of all collected terrestrial organisms (0.97‰ higher than the three frog-eating snake species, t = 2.69, df = 9, P < 0.05).

The riparian lizard, *Norops lionotus*, had a nitrogen isotopic signature similar to that of adult frogs, all of which are assumed to be insectivores. However, the $\delta^{13}C$ of *N. lionotus* was 1.4‰ more depleted than adult frogs. *Norops lionotus* had lower $\delta^{15}N$ (5.63‰) than the three frog-eating snake species (t = 3.99, df = 9, P < 0.004). Riparian snakes (excluding *Sibon argus*) had isotopic values averaging about 7.1‰ $\delta^{15}N$ and −24.1‰ $\delta^{13}C$. The $\delta^{15}N$ in riparian snake species (excluding *Sibon argus*) was significantly higher by 1.69‰ (t = 4.07, df = 8, P < 0.004) compared with adult frog species (excluding *Bufo*). Isotopic values of *Sibon argus* were more variable than for other snakes (Figure 1) and its $\delta^{15}N$ did not differ significantly from $\delta^{15}N$ of frogs (t = 0.84, df = 21, P > 0.4).

DISCUSSION

Basal resources

Patterns observed in stable isotope values of basal resources reflect the supply and processing of nitrogen and carbon in the system. The higher $\delta^{15}N$ values in FBOM compared with other basal resources (Table 1) are likely a result of decomposition and high denitrification rates in stream sediments, processes that favour the removal of the lighter isotope (Fry 2006). In contrast to autotrophs, the other basal resources in our study stream (SES, CPOM and FBOM) did not change significantly in $\delta^{15}N$ over time. The decrease in $\delta^{15}N$ in algae during our study may be linked to the transition from dry-season (May) to wet-season conditions (June–August) and concomitant increases in stream flow. Trudeau & Rasmussen (2003) showed that $\delta^{15}N$ and $\delta^{13}C$ values in periphyton and FGA decreased with increasing flow conditions in laboratory experiments. In addition, in the wet season, inputs of nitrogen to the stream are likely higher and inorganic dissolved nitrogen is more abundant in the stream as indicated by the large decreases in C:N ratio in PERI and FGA towards August while C:N remained similar in SES, CPOM and FBOM. Increased supply of nitrogen would further increase fractionation and decrease algal $\delta^{15}N$ (Fry 2006) which would explain the decrease in $\delta^{15}N$ in periphyton and FGA. In addition, higher rates of denitrification leading to higher $\delta^{15}N$ in inorganic dissolved nitrogen may explain higher algal $\delta^{15}N$ in the dry season. At low flow oxygen near and in the sediment is depleted at faster rates allowing obligate anaerobic denitrifiers to reduce the NO_3 pool while increasing $\delta^{15}N$ of the remaining NO_3. Higher stream velocities and more oxygenated water would reduce denitrification in the wet season. As a result $\delta^{15}N$ in NO_3 and in autotrophs would be expected to decrease in the wet season. The reduced availability of nitrogen by denitrification in the

aquatic compared with the terrestrial environment may be indicated by the significantly lower %N and higher C:N ratio in aquatic vertebrates compared with terrestrial vertebrates. Furthermore, the decrease of $\delta^{15}N$ in FGA and PERI in the wet season may be explained in part by relatively depleted $\delta^{15}N$ of inorganic fixed nitrogen in precipitation. Values of $\delta^{15}N$ in precipitation can range from -18 to $+8‰$ (Fry 2006).

The most depleted $\delta^{13}C$ values observed in our study were for FGA, which was collected primarily from fast-flowing habitats, and these values decreased during our study. Finlay *et al.* (2002) reported that carbon isotope ratios of algae and their consumers decreased with increasing water velocity in streams, especially in productive streams where CO_2 may limit primary production. Discrimination against ^{13}C-CO_2 during photosynthesis increases with CO_2 availability (Hecky & Hesslein 1995). Finlay *et al.* (2002) found algae (and herbivores) in pool habitats to be enriched in $\delta^{13}C$ by 5–8‰ compared to riffle habitats. They suggested that water stagnation could enrich algal $\delta^{13}C$ through boundary-layer effects and by reducing CO_2 concentrations as the water warms.

Increasing stream flows during the course of our study likely increased the supply of CO_2 to algae. However, periphyton growth is limited to very short distances from the substrate surface where flows are limited in the boundary layer. Therefore, as we observed, changes in $\delta^{13}C$ of PERI may be small or may not occur with increasing CO_2 supply rates in the stream. In contrast, longer strands of FGA should respond to increasing CO_2 supplies by a decrease in $\delta^{13}C$ as was the case in our study (by 5.5‰). These processes explain both the much lower average $\delta^{13}C$ of FGA compared with PERI and the different temporal patterns in $\delta^{13}C$ between PERI and FGA (Table 1).

A small amount of fractionation of $\delta^{13}C$ naturally occurs in food webs, and herbivores show high variability in this regard (Hecky & Hesslein 1995, Vander Zanden & Rasmussen 2001), but it seems unlikely that FGA was heavily utilized as a food resource by any of the vertebrate or invertebrate taxa that we examined because the difference in $\delta^{13}C$ between FGA (average $= -33.2‰$) and primary consumers (average $= -27.0‰$) was too large. In addition, the C:N ratio of FGA (9.5) agreed less with that of aquatic invertebrates (C:N $= 4.8$) and aquatic vertebrates (C:N $= 4.8$) than the C:N ratio of PERI (C:N $= 5.0$, Table 1), indicating that PERI is a higher-quality food source than FGA (Pringle *et al.* 1999).

While PERI and CPOM are both likely important food sources in this stream (March & Pringle 2003, Wallace *et al.* 1997), they were quite similar in $\delta^{13}C$ and $\delta^{15}N$ (Figure 1) and thus we were unable to use isotopic signatures as tracers to distinguish the relative importance of autochthonous and allochthonous food sources. However, in view of the fact that PERI and FBOM were similar in $\delta^{13}C$ while CPOM was significantly different from FBOM and because the difference between

the C:N ratios of PERI (5.0 ± 1.7) and FBOM (10.3 ± 3.9) was smaller than the difference between the C:N ratios of FBOM and CPOM (29.7 ± 2.5), it seems possible that FBOM was for a large part derived from PERI. A high proportion of autochthonous material in FBOM in neotropical streams may indicate a high turnover rate of periphyton enhanced by grazing tadpoles. Fine benthic organic matter may in large part consist of tadpole faeces. The proportion of nitrogen in FBOM in our study stream (C:N $= 10.3$) was much higher than in temperate streams (C:N > 30, Cross *et al.* 2003). Detritivores in streams can increase the quality of FBOM by decreasing the C:N ratio (Pringle *et al.* 1999), while decreasing the quantity of organic material in the stream by decreasing its retention (Pringle *et al.* 1999, Taylor *et al.* 2006).

Invertebrates

Stream invertebrates had fairly similar $\delta^{13}C$ values with two exceptions, the Calamoceratidae and Philopotamidae, which were much more enriched. Both of these caddisflies are typically found in slow-flowing habitats; philopotamids filter fine particles in slow-flowing water (Malas & Wallace 1977) and *Phylloicus*, the calamoceratid in our study stream, feeds on detritus accumulations in depositional habitats (Rincon & Martinez 2006). Finlay *et al.* (1999) showed that current velocity in pools and riffles influences $\delta^{13}C$ signatures of invertebrate herbivores and that this signal was transferred to invertebrate predators in these habitats as well. We did not measure current velocities at points of collection, but it is likely that the enriched $\delta^{13}C$ values we observed in philopotamids and *Phylloicus* were linked to habitat preferences.

The large shrimp *Macrobrachium* was the invertebrate top predator in our study stream and the increase in $\delta^{15}N$ values with body size of individuals was likely a result of increasing carnivory with size. *Macrobrachium* was probably enriched in $\delta^{13}C$ ($-25.3‰$) because it lives primarily in deeper sections of the stream where flow is slow and the supply of CO_2 is lower and therefore $\delta^{13}C$ in primary producers is expected to be high. The $\delta^{13}C$ of *Macrobrachium* was 1.4‰ higher than of the predatory fish *B. roswithae* (while their $\delta^{15}N$ signatures were similar), which lives and feeds in faster-flowing water. The fact that $\delta^{13}C$ values of *Macrobrachium* were determined by spatial distribution of flow speeds, and their effect on algal $\delta^{13}C$, also indicates that *Macrobrachium* depends on organisms that feed on autochthonously produced matter for a significant part of its diet. This agrees with the findings in a study of streams in Puerto Rico where consumers and in particular shrimps relied more on algal-based than on terrestrially derived resources (March & Pringle 2003).

Although we did not distinguish between fast- and slow-flowing habitats when we sampled PERI, it appears

that there is a strong spatial compartmentalization of species subcommunities with little exchange between sections of our study stream. While shrimps dwell in deeper and more stagnant pools, its prey and the diet of its prey also reside in these deeper sections of the stream. On the other hand, *B. roswithae* and their prey live in riffles. Although the similar average isotopic values of PERI and CPOM do not allow quantification of the relative importance of these food sources for organisms in the stream, dependence on autotrophic production is indicated by distinct signatures of carbon isotopes in pool dwellers versus those that reside in faster-flowing sections of the stream. Differences in $\delta^{13}C$ between taxa at similar trophic levels are best explained by consumption of autotrophic producers either by the taxa themselves or by prey items further down the food chain leading to the respective taxa. Because the $\delta^{13}C$ of these autotrophic producers is determined by flow rates, the isotopic signature of their consumers will be representative for the habitat in which they feed.

Vertebrates

Tadpole stable isotope signatures were distinct and the highest of the primary consumers in the system. Tadpoles of *R. warszewitschii* and *H. colymba* which were the most abundant amphibians in the stream had $\delta^{15}N$ values consistent with a primarily herbivorous diet, supporting gut analyses on individuals from this same stream. Ranvestel *et al.* (2004) found that *R. warszewitschii* and *H. colymba* fed on periphyton ($\sim$1/3 of gut contents), amorphous detritus ($\sim$50%) and leaf material ($\sim$15%). The variety of mouthpart morphologies in this tadpole community indicated the presence of various feeding guilds (McDiarmid & Altig 1999), and the isotopic range of tadpoles reflected variation in the diet.

The $\delta^{15}N$ values for all non-centrolenid tadpoles averaged 5.49‰ $\delta^{15}N$. Our results suggest that PERI and CPOM (which were very close in $\delta^{15}N$ and $\delta^{13}C$ and thus difficult to separate in food web analyses), are the most important food sources for most tadpoles in this system, with some interesting exceptions. Tadpoles of *Colostethus* species had a higher $\delta^{15}N$, consistent with a diet consisting in part of neuston (Savage 2002). Tadpoles of the diverse and abundant glass frogs (Centrolenidae), which burrow deep into detritus deposits and submerged leafpacks, had much lower $\delta^{15}N$ values than the other tadpoles, indicating they may derive much of their nutrition from microbial sources, as has been suggested for some other tropical tadpoles (Heyer 1973, Inger 1986).

Of the two carnivorous fishes, *Trichomycterus striatus* was enriched in $\delta^{15}N$ relative to *B. roswithae*. *Trichomycterus striatus* has been reported to feed primarily on benthic insects in riffles (Power *et al.* 1988). Gut contents of *B. roswithae* from this same stream suggested it feeds primarily on terrestrial insects (M.

Whiles, pers. comm., unpubl. data). This, however, was not reflected in our analyses, as the low $\delta^{13}C$ signature of -26.4‰ of *B. roswithae* suggested it fed on aquatic prey as opposed to, for instance, insectivorous, terrestrial frogs that had a relatively high signature, -25.4‰ $\delta^{13}C$. This difference could be related to variability in insect prey resources, as availability of terrestrial insects to stream systems can vary greatly in time and space (Edwards & Huryn 1995). *Brachyrhaphis roswithae* also showed a pattern of increasing $\delta^{15}N$ with size, likely because larger individuals consume larger invertebrates from higher trophic levels and perhaps vertebrates. High $\delta^{15}N$ values for the yolk-sac stage (0–10-mm size class) of *B. roswithae* reflect maternal contributions in this live-bearing species.

While carbon isotopes of non-centrolenid amphibians were more enriched after metamorphosis and the transit from the aquatic to the terrestrial habitat, reflecting a change to a diet of mostly terrestrial prey, their $\delta^{15}N$ signatures remained similar. The similar $\delta^{15}N$ signatures of non-centrolenid tadpoles and adult anurans (excluding the toad *Bufo*) were probably not an effect of slow turnover of isotopic body content after transit from the aquatic to the terrestrial habitat and also did not reflect similarity in trophic levels. Instead, the similar $\delta^{15}N$ signatures were likely caused by differences in $\delta^{15}N$ at the base of the aquatic and terrestrial food webs. Higher $\delta^{15}N$ at the base of the aquatic food webs may be a result of higher rates of denitrification in the aquatic sediments which increases $\delta^{15}N$ in NO_3, or of lower availability of nitrogen which decreases fractionation during uptake by algae, or both. Centrolenid frogs, on the other hand, were much higher in $\delta^{15}N$ values than their tadpoles, indicating a shift from an aquatic microbial diet to a terrestrial insect diet.

The $\delta^{13}C$ in snakes was 1.2 higher than in frogs confirming both their higher trophic level and terrestrial diet. That the toad *Bufo haematiticus* was higher in $\delta^{15}N$ (8.05‰) than the three frog-eating snake species (7.08‰) suggested that it feeds on average at a higher trophic level than the sampled snakes. It appears that the prey species of *B. haematiticus* are relatively high in $\delta^{15}N$. *Bufo haematiticus* is known to feed primarily on ants and on other arthropods (Savage 2002). The toad may feed largely on carnivorous soldier ants which can be higher in $\delta^{15}N$ than frogs (6.8‰; Verburg unpubl. data). Ants were however not sampled at El Cope.

The isotopic signatures of the lizard *Norops lionotus* agreed with a diet of emerging aquatic insects, confirming observations of its feeding on stream margin arthropods (Savage 2002). The $\delta^{15}N$ signatures of the three snakes that have been reported (Savage 2002) to feed on frogs (Table 3), were consistent with feeding on frogs. The diet of the snake *Sibon argus* consists of slugs, snails and frog eggs (Ryan & Lips 2004, Savage 2002), which would explain its lower and more variable $\delta^{15}N$.

Whole food-web fractionation

The fractionation of $\delta^{13}C$ from basal food sources (excluding FGA) to the herbivore level in our study was larger than expected ($+3‰$). A small general enrichment of $\delta^{13}C$ is expected in aquatic food webs because of trophic fractionation resulting in consumers being more enriched than their diet by an average $+0.5$ to $+0.6‰$ $\delta^{13}C$ per trophic step (McCutchan *et al.* 2003, Vander Zanden & Rasmussen 2001). Vander Zanden & Rasmussen (2001) reported a negative fractionation for herbivores ($-0.4‰$), significantly lower than for non-herbivores, which does not agree with our data.

Nitrogen isotope signatures are often used to determine trophic levels and food chain lengths (Kupfer *et al.* 2006) because of the large fractionation that occurs per trophic step, typically $+3.4‰$ $\delta^{15}N$ (Vander Zanden & Rasmussen 2001). With an average expected $3.4‰$ enrichment in $\delta^{15}N$ ($\Delta\delta^{15}N$) and $0.5‰$ enrichment in $\delta^{13}C$ ($\Delta\delta^{13}C$) between trophic levels in the food web (Vander Zanden & Rasmussen 2001), the ratio $\Delta\delta^{15}N/\Delta\delta^{13}C = 6.8$. This is much higher than that which we observed between periphyton and CPOM at the bottom of the food web and the top consumer in our study stream *T. striatum* ($\Delta\delta^{15}N/\Delta\delta^{13}C = 1.4$ and 1.6 respectively). This low ratio $\Delta\delta^{15}N/\Delta\delta^{13}C$ is not just restricted to the aquatic biota; the $\Delta\delta^{15}N/\Delta\delta^{13}C$ ratio was 1.22 for terrestrial frogs and frog-eating snakes. It is not clear why $\Delta\delta^{15}N$ in the food web is relatively small and $\Delta\delta^{13}C$ relatively large compared to temperate systems (Vander Zanden & Rasmussen 2001), but this pattern appears to be common in tropical systems (Kilham & Pringle 2000). Kilham & Pringle (2000) reported only about $+1.8‰$ $\Delta\delta^{15}N$ fractionation per trophic level in neotropical streams. In a recent review of fractionation in food webs, McCutchan *et al.* (2003) found that the trophic enrichment in $\delta^{15}N$ depended on the items being consumed, with nitrogen fractionation being lower for invertebrate diets ($+1.5‰$ $\Delta\delta^{15}N$), intermediate for algal diets ($+2.3‰$ $\Delta\delta^{15}N$) and higher for high protein diets ($+3.4‰$ $\Delta\delta^{15}N$).

Consequences of amphibian extirpations on frog-dominated stream food webs

As predicted (Lips *et al.* 2003), the pathogen arrived at our study site in 2004 infecting $> 70\%$ of the amphibian species and caused a massive die-off of all species in all habitats, after which approximately 80% of amphibian abundance had disappeared across the region (Lips *et al.* 2006, Whiles *et al.* 2006). The loss of biodiversity can severely impair ecosystem services (Worm *et al.* 2006). Nevertheless, and although there is great concern over the losses of amphibian populations and much effort has been put into studying causes, little is known of the

ecological consequences of these extirpations. Here we provide important baseline stable isotope data on the food web of a tropical highland stream ecosystem prior to the loss of its amphibian community. Our stable isotope data reflect the trophic structure of the animal community in a stream habitat prior to the extinction of the amphibians and indicate that tadpoles and adult amphibians are an important prey base for invertebrates and snakes, in both terrestrial and aquatic habitats. In addition, higher trophic levels such as carnivorous birds and mammals (for instance otters), not included in our study, will be affected by the loss of this source of prey. Likewise, our $\delta^{15}N$ data provide evidence for insectivory by adult amphibians and for herbivory by tadpoles, linking terrestrial and aquatic food webs. The high nutritional quality of the detrital organic matter in our study stream may result from grazing by tadpoles which enhances nitrogen fluxes. Losses of 90% of amphibian biomass throughout highland tropical areas will affect nutrient cycling and energy flow, within and between habitats. Hence, catastrophic losses, including the loss in 2004 (Lips *et al.* 2006) of the diverse and abundant amphibian assemblage from the highland tropical stream that we examined here, while reducing ecosystem complexity should result in changes in processes and functioning such as N-cycling and energy exports to riparian food webs.

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