OIKOS 96: 46–55, 2002

Structure of tropical river food webs revealed by stable isotope ratios

David B. Jepsen and Kirk O. Winemiller

Jepsen, D. B. and Winemiller, K. O. 2002. Structure of tropical river food webs revealed by stable isotope ratios. – Oikos 96: 46–55.

Fish assemblages in tropical river food webs are characterized by high taxonomic diversity, diverse foraging modes, omnivory, and an abundance of detritivores. Feeding links are complex and modified by hydrologic seasonality and system productivity. These properties make it difficult to generalize about feeding relationships and to identify dominant linkages of energy flow. We analyzed the stable carbon and nitrogen isotope ratios of 276 fishes and other food web components living in four Venezuelan rivers that differed in basal food resources to determine 1) whether fish trophic guilds integrated food resources in a predictable fashion, thereby providing similar trophic resolution as individual species, 2) whether food chain length differed with system productivity, and 3) how omnivory and detritivory influenced trophic structure within these food webs. Fishes were grouped into four trophic guilds (herbivores, detritivores/algivores, omnivores, piscivores) based on literature reports and external morphological characteristics. Results of discriminant function analyses showed that isotope data were effective at reclassifying individual fish into their pre-identified trophic category. Nutrient-poor, black-water rivers showed greater compartmentalization in isotope values than more productive rivers, leading to greater reclassification success. In three out of four food webs, omnivores were more often misclassified than other trophic groups, reflecting the diverse food sources they assimilated. When fish $\delta^{15}N$ values were used to estimate species position in the trophic hierarchy, top piscivores in nutrient-poor rivers had higher trophic positions than those in more productive rivers. This was in contrast to our expectation that productive systems would promote longer food chains. Although isotope ratios could not resolve species-level feeding pathways, they did reveal how top consumers integrate isotopic variability occurring lower in the food web. Top piscivores, regardless of species, had carbon and nitrogen profiles less variable than other trophic groups.

D. B. Jepsen and K. O. Winemiller, Dept of Wildlife and Fisheries Sciences, Texas A&M Univ., College Station, TX 77843, USA (present address of DBJ: Oregon Cooperative Fish and Wildlife Research Unit. 104 Nash Hall, Dept of Fisheries and Wildlife Sciences, Oregon State Univ., Corvallis, OR 97331-3803, USA [jepsend@ucs.orst.edu]).

Food webs are macrodescriptors of community feeding interactions that can be used to map the flow of materials and nutrients in ecosystems. Extracting and interpreting this information in a meaningful manner has been one of the most active areas in ecological research over the last 30 yr. One approach is to identify the most important feeding links within an assemblage of consumers, then define a trophic structure that can be compared with other systems. Such comparative food web studies have been used to address theoretical questions such as 'does greater trophic connectivity increase system stability?' (Pimm 1982, Cohen et al. 1990), and 'do number of trophic levels increase with productivity?' (Pimm 1982, Briand and Cohen 1987). Answers for such questions have obvious applications for natural resources management, and food web studies are cen-

Accepted 16 July 2001

ISSN 0030-1299

tral to research on ecosystem function, because predator-prey interactions can influence important ecosystem processes such as nutrient cycling (DeAngelis 1992, Huntly 1995, Vanni et al. 1997, Wilhelm and Suttle 1999).

Estimation of the spatial extent or long-term dynamics of trophic pathways in a food web is difficult, because most dietary data are obtained from short-term feeding observations and stomach contents analyses. Without continuous sampling over long periods, these techniques provide only static representations of trophic relationships and energy pathways. Detritivory and omnivory (feeding at more than one trophic level) are two foraging modes that are particularly difficult to quantify with conventional diet analyses, often resulting in an inadequate representation of their importance in food webs and ecosystems. Here we analyze stable isotope signatures among tropical fish feeding guilds to reveal trophic structure and patterns of detritivory and omnivory within river food webs.

Detritivory is an important feeding mode in many, if not most, food webs, and detritus/detritivore interactions can strongly influence food web dynamics in many ecosystems (DeAngelis 1992, Polis and Strong 1996, Vanni and DeRuiter 1996). Yet detritus is often treated as an aggregate pool of organic matter derived from diverse sources, mostly because the separate organic components in a detrital matrix (or within stomach contents) are difficult to identify. As a result, we know little about the nutritional importance of alternative detrital sources, which may differ seasonally depending upon the relative contributions of autochthonous and allochthonous inputs. Omnivory can theoretically have a stabilizing or destabilizing effect on ecosystems, depending to some extent on where it occurs in the food chain (Yodzis 1981, Pimm 1982). For example, omnivory at higher trophic levels may mitigate top-down control in many aquatic environments (Vadas 1990). In theory, trophic specialization should be more common in constant food webs, compared to those with intense or frequent fluctuations, so that omnivory may be less prevalent in stable systems.

Detritivory and omnivory are particularly common foraging modes for tropical freshwater fishes. Detritus dominates the organic matter pool in most tropical rivers, and detritivorous fish comprise a significant portion of the ichthyobiomass (Bayley 1973, Winemiller 1995). Despite the prevalence of omnivory, tropical rivers contain fish assemblages with an impressive diversity of specialized anatomical and behavioral feeding adaptations that result in complex food webs (Lowe-McConnell 1987, Winemiller 1990, 1995). Omnivory is probably an adaptive response to strong seasonal fluctuations in water level that influence the availability of food resources (Knöppel 1970, Lowe-McConnell 1987, Bayley 1988, Goulding et al. 1988, Winemiller 1990). A seasonal increase in dietary niche breadth is particularly common in spatially heterogeneous floodplain river ecosystems where seasonal water fluctuations control the type and amount of resources available to consumers (Junk et al. 1989, Winemiller 1989). Considering the complex spatio-temporal dynamics of tropical lowland rivers, and the diversity of food resources available to consumers at any given time, it is difficult to link fluxes in food resources with consumer population dynamics. Yet from a multi-species fisheries standpoint, there is a great need to understand consumer-resource dynamics within complex trophic networks

Several workers have used trophic classifications (based on stomach contents) to examine the structure of tropical fish assemblages (Angermeier and Karr 1983, Bayley 1988, Goulding et al. 1988, Winemiller 1989, 1990, 1991). Lacking are comparative studies from large rivers that support the greatest production and largest fisheries. Given the high degrees of detritivory, omnivory, taxonomic diversity, and spatiotemporal variation in trophic interactions in large rivers, conventional dietary analysis has practical limitations. For example, in the case of detritivory, the portion of the detrital material found in alimentary tracts that is actually assimilated is usually not known.

In contrast, stable isotope analysis is particularly well suited to identify dominant pathways of carbon and nutrient transfer in food webs, because the method can estimate assimilation of food resources over time (Peterson and Fry 1987). Although coarse in taxonomic resolution, isotope data can quickly reveal important feeding links among consumers, and can overcome some of the methodological limitations associated with stomach contents analysis. Provided there is sufficient divergence in isotope ratios of primary producers, isotope data can identify previously unrecognized nutritional sources.

We examined carbon and nitrogen stable isotope data from four tropical river systems to illustrate the pathways of energy and nutrients between consumers at both the species and trophic guild levels, to explore the following hypotheses:

H_1: Food chain lengths are longer in more productive white-water rivers than nutrient- poor black-waters, based on the general supposition that productive systems support more feeding links (Briand and Cohen 1987). ^{15}N is fractionated at each trophic transfer, and has been used in many studies as an indicator of trophic level (Vander Zanden et al. 1997), and top predators in most food chains have higher $\delta^{15}N$ than consumers lower on the chain. Therefore, productive rivers should have a greater difference in $\delta^{15}N$ values between autotrophs at the base of the food web and the top predator, relative to comparable autotrophs and predators in nutrient-poor waters.

H_2: Isotope ratios will be more similar within trophic guilds than between trophic guilds. It is assumed that members within guilds are consuming similar food resources, relative to consumers outside that guild, which will be reflected in isotope signatures.

H_3: Trophic levels will be isotopically more distinct in black-water systems, due to lower potential omnivory than in more productive rivers. Productive rivers support more invertebrate biomass, with several levels of consumers having a potentially broader spectrum of isotope values. Fishes consuming these assemblages will reflect this variability, which will be transmitted to upper-level piscivores. Therefore, fishes in white-waters will have more variable isotope values, reflecting their greater food resource spectrum.

Methods

Study design and site selection

To examine the relationship between system productivity and food chain length, we collected stable isotope data from several fish species from each of four trophic guilds (see below) in four river systems in Venezuela. These species were among the most dominant biomass components of the fish assemblage at each site. The four lowland rivers were chosen to represent a broad spectrum of geochemical attributes and productivities, yet they shared fish families and some fish species. Limnological data and site descriptions for these rivers can be found in Jepsen and Winemiller (unpubl.). The Apure River is a turbid, nutrient-rich river (white-water) in the western llanos (savanna) region of Venezuela, and supports dense growth of emergent and floating macrophytes in marginal and backwater habitats. The Aguaro River is a clear-water system that also drains savanna vegetation in the central llanos, but differs from the Apure in having fewer dissolved nutrients and greater water clarity. Submerged macrophytes and filamentous green algae dominate primary production in the Aguaro. The Cinaruco River is an oligotrophic river in the southern llanos with sandy substrates and almost no aquatic macrophytes. This river has high amounts of dissolved organic carbon (mostly humic and fulvic acids) that stain the water; and low pH (5.5–6.3) hence it conforms to Sioli's (1975) description of a black-water river. The Pasimoni River is an extreme black-water river with low pH (4.2–4.5) that drains an area of highly weathered bedrock (Guyana Shield) and dense rainforest in southern Venezuela. Ionic content and aquatic primary production is very low in the Pasimoni. The Pasimoni and Apure are on opposite ends of a nutrient and primary production gradient, with the Aguaro (moderate productivity) and Cinaruco (low productivity) lying between these two extremes.

Fish guild membership was assigned from Neotropical literature reports, unpublished stomach contents data (D. Jepsen, K. Winemiller, C. Peterson unpubl.), and the ecomorphology of the species or genus (a list of scientific names by guild for each system with dietary information and references appears in Jepsen and Winemiller unpubl.). Due to inter-site differences in assemblage composition, it was not possible to collect the same species from each system for each guild. For isotope analysis, we attempted to collect 5–10 individuals per dominant species per site, but this was not always possible. The species examined were the most important in terms of biomass within the respective systems based on collections using several different collecting methods (see below).

As noted above, tropical freshwater fishes exploit food resources in a variety of ways, and many species can switch diets opportunistically in response to the relative availability of food (Peterson and Winemiller 1997). Stomach contents data can demonstrate this diet diversity, but from an ecosystem energetics perspective, there may be a low number of dominant pathways involving fishes. For example, although consumers of fine detritus (mud containing fine particulate organic matter) and consumers of course particulate organic matter (CPOM) ingest particles of different sizes, it is not clear if the organic matter within these respective foods are of the same or different origin. Given that we examined adult fishes, and analysis of stable isotope ratios in muscle tissue records relatively long-term assimilation, ontogenetic diet shifts were not a significant factor in our study. We grouped fishes into four broad trophic classes. Herbivores were fishes that masticate coarse vegetal material (leaves, seeds) and where literature reports indicated over 50% vascular plant matter in gut contents, during some portion of the year (Jepsen and Winemiller unpubl.). Fishes with morphological adaptations for sucking or scraping fine and coarse particulate organic material from substrates were classed as detritivores/algivores. Omnivores were fishes that feed on invertebrates and varying portions of plant material. Piscivores were an obvious group and included fishes with dentition for grasping/impaling, engulfing, or tearing flesh from prey-fish.

Field collections and tissue isotopic analysis

Fishes were collected with a combination of experimental gillnets, seines, castnets, and hook-and-line. Muscle tissue (skinned and boneless, approx. 5 g) was removed from the dorsum of larger fish, whereas whole specimens were collected for species < 30 mm. Individual samples either were sealed in aluminum foil, then frozen with dry ice, or placed in tissue culture cells and packed with NaCl. In the laboratory, fish tissues were soaked and rinsed in distilled water, then dried in an

oven at 60°C for 48 h, after which they were ground to a fine powder with a pestle and mortar then stored in clean glass vials.

Fish tissue samples were analyzed for isotope ratios ($^{13}C/^{12}C$ and $^{15}N/^{14}N$) and total carbon and nitrogen content at the Analytical Chemistry Laboratory, Inst. of Ecology, Univ. of Georgia. Sub-samples for each sample were weighed to 10^{-6} g, pressed into Ultra-Pure tin capsules (Costech, Valencia, CA), then dry combusted (micro Dumas technique) with a Carlo Erba CHN elemental analyzer. Purified gases (CO_2 and N_2) were then analyzed for isotopic composition with a Finnigan Delta C mass spectrometer. Precision for this machine was ± 0.05‰, and replicates for standards were usually within 0.1‰.

Isotope ratios are reported in parts per thousand (‰) relative to standards (PeeDee Belemnite for carbon, and atmospheric N for nitrogen (see Craig 1957), and defined in delta notation as

$$\delta^{15}N \text{ or } \delta^{13}C = \left(\frac{R_{sample}}{R_{standard}} - 1\right) \times 10^3$$

where $R = {}^{15}N/{}^{14}N$ or $^{13}C/^{12}C$. Samples enriched in the heavier isotope are more positive than other samples, and materials depleted in the heavier isotope are lighter than other samples. Biota tend to have less ^{13}C than PeeDee Belemnite, and have negative $\delta^{13}C$ values (~ 0 to ~ -50‰). Conversely, most biota have more ^{15}N relative to air and have positive $\delta^{15}N$ signatures (~ 0 to $\sim +20$‰).

Data analysis

Carbon and nitrogen isotope data, and total C:N values for each fish sample were used as variables in a discriminant function analysis (DFA) to examine whether these data could accurately reclassify individuals into the predefined trophic group. DFA conducted an a posteriori test to determine the trophic group in which each fish sample had the highest probability of membership. This analysis also produced canonical variates for each sample based on the eigenstructure of the multivariate matrix. The first two variates for each sample were then plotted as conventional *x*-*y* data to examine patterns in the data set.

Mean $\delta^{15}N$ values for each species were used to compare the trophic length of each food web. The heavier ^{15}N accumulates in consumers as nitrogen moves up the food web, and as a result, top consumers tend to have greater $\delta^{15}N$ than consumers near the base of the food web. Rather than inferring a discrete trophic level based on a species $\delta^{15}N$, we calculated a realized trophic position as a continuous variable for each fish. The formula for a species trophic position (TP) was

$$TP = \left(\frac{\delta^{15}N_{fish} - \delta^{15}N_{reference}}{2.8}\right) + 1$$

where $\delta^{15}N_{reference}$ was the mean of sediment and periphyton $\delta^{15}N$ for each system, and the denominator value (2.8) was an estimated ‰ mean trophic enrichment (fractionation) of $\delta^{15}N$ between fish and their food source. Previous workers have used a fractionation value of 3.4‰ (Vander Zanden and Rasmussen 1996), which is a mean value derived from laboratory studies on several animal taxa (DeNiro and Epstein 1981, Minagawa and Wada 1984). The 2.8‰ estimate here is a refinement for tropical fishes, based on the mean $\delta^{15}N$ difference between *Rineloricaria* and bulk periphyton samples in the Apure River and several other comparisons between herbivores and known basal sources (Jepsen and Winemiller unpubl.). The $\delta^{15}N_{reference}$ values in the above expression were based on means of sediment and periphyton $\delta^{15}N$ for each river system, and served as site-specific adjustments for basal $\delta^{15}N$ signatures. This allowed inter-site comparisons of fish trophic position values, used to test the relationship of system productivity on food chain length. Reference basal $\delta^{15}N$ values were 3.1, 4.8, 4.7, and 4.5‰ for the Aguaro, Apure, Cinaruco, and Pasimoni rivers, respectively.

Classification success from the DFA was used to estimate feeding variability within trophic groups and sites. Sites with higher total reclassification error had, by definition, more fishes with overlapping isotope signatures, and by extension, more feeding variability between guilds (i.e., isotope data revealed less trophic guild structure). For purposes here, we defined omnivory occurring within a feeding guild when the range of $\delta^{15}N$ within the guild exceeded 2.8‰. To compare differences in mean isotope signatures among trophic groups at different sites, we used ANOVAs, and Tukey's multiple range tests for means comparisons. Data analyses were conducted with SAS statistical software (Cary, North Carolina).

Results

We analyzed $\delta^{13}C$ and $\delta^{15}N$ values from 276 individual fishes comprising 53 species. Isotope means for each species at each site are reported elsewhere (Jepsen and Winemiller unpubl.). None of the common fish species collected at the Aguaro site fit our criteria for assignment to the herbivore guild. With this exception, all four trophic guilds were well represented in all four rivers.

Trophic group comparisons

Isotope data reclassified 86 and 99% of the fish species into their pre-defined trophic categories in the Cinaruco and Pasimoni systems, respectively (Table 1). Most errors for Cinaruco samples were due to reclassification of omnivorous fishes into the piscivore (19.2% of time) and detritivore guilds (10.5% of time). Isotope data from the Pasimoni River revealed well-defined trophic structure, with only one individual fish, an omnivore, being reclassified as a detritivore. Classification errors in the two productive ecosystems (Apure, Aguaro) were higher than in the black-water food webs. Although detritivores and herbivores were successfully classified $>83\%$ of time for species in both the Apure and Aguaro systems, omnivores and piscivores often had overlapping isotope signatures that resulted in poorer reclassification success for these groups. For each river, both mean $\delta^{13}C$ and $\delta^{15}N$ were significantly different between trophic groups (ANOVA, $p < 0.0001$ in all cases).

In the Pasimoni River food web, there were large between-guild differences in mean $\delta^{15}N$, mostly resulting from ^{15}N enrichment of upper-level consumers ($r^2 = 0.91$, $F = 238$, $p < 0.0001$, df = 73,3). In tests among trophic groups, mean $\delta^{13}C$ values between omnivores and piscivores were never significantly different at any river, indicating these guilds used a similar carbon source, and demonstrating the integrative effect of carbon as a conservative isotope tracer (Tukey tests, Table 2). However, at all sites except the Apure, detritivores had significantly lower $\delta^{13}C$ than other groups, indicating these fishes assimilated a carbon source that was isotopically light. Benthic algae or sedimented phytoplankton (reported in Jepsen and Winemiller unpubl.) were the only food sources that had $\delta^{13}C$ low enough to explain the low values in detritivores.

With the exception of fishes from the Apure, piscivores always had higher mean $\delta^{15}N$ than any other group. At the Apure, mean omnivore $\delta^{15}N$ values were actually slightly higher than piscivores, though not statistically significant (Table 2). If differences in mean $\delta^{15}N$ are used as the only criteria to indicate trophic levels, then only two trophic levels are identified among Apure River fishes (herbivore/detritivores and omnivorous/piscivores), and three levels are observed in the other three fish assemblages (Table 2).

Bivariate plots of canonical loadings of isotope data from the discriminant function analysis revealed that feeding guilds have distinct isotope properties (Fig. 1). With the exception of the Aguaro River food web, canonical axis 1 described a gradient of $\delta^{15}N$, with piscivores having positive canonical axis loadings, while lower trophic levels had lower loadings. In all cases, herbivorous fishes had the most negative axis-1 scores, illustrating their lower position in the food web. In the Apure, Cinaruco, and Pasimoni rivers, axis 2 mostly described differences in $\delta^{13}C$, with positive axis values corresponding to fishes with lower $\delta^{13}C$ signatures. For these three assemblages, piscivores were positioned near the 0 value on canonical axis 2, an indication that top consumers integrate isotopic variability even from diverse basal carbon sources. As expected, omnivores in all systems had widely varying carbon isotope signatures, which was reflected in the dispersed canonical loadings for this guild in Fig. 1. For the Aguaro food web, DFA loadings yielded no clear patterns in bivariate space. In this river, piscivorous species appeared as

Table 1. Classification summary of trophic groups from discriminant function analysis from four Venezuelan river systems. H = herbivore, D = detritivore/algivore, O = omnivore/insectivore, P = piscivore.

Site	*n*	Predicted trophic groupings reclassification success (%)				Error	Total error
		H	D	O	P		
Aguaro							0.30
D	12		83.3	16.7		0.17	
O	24		16.7	53.3	30.0	0.47	
P	27			25.9	74.1	0.26	
Apure							0.26
H	18	94.4	5.6			0.06	
D	6		100			0.0	
O	10		20.9	58.3	20.9	0.42	
P	21			55.0	45.0	0.55	
Cinaruco							0.14
H	8	100				0.0	
D	25		88.5	11.5		0.12	
O	29	8.3	10.5	62.0	19.2	0.38	
P	33			6.1	93.9	0.06	
Pasimoni							0.01
H	5	100				0.0	
D	12		100			0.0	
O	23		5.0	95.0		0.05	
P	36				100	0.0	

Table 2. Results of Tukey's Standarized Range tests for means of isotope ratios for fish in four river systems. Reading across rows, trophic group mean values with common underlines are not significantly different; reading down columns, mean values for fishes of same trophic group but different sites with common letters are not significantly different. $\alpha = 0.05$.

Site	Herbivores	Detritivores	Omnivores	Piscivores
		$\delta^{15}N$ (‰)		
Aguaro		6.7 a	7.8 c	9.1 b
Apure	7.2 a	7.4 b	9.6 a	9.4 b
Cinaruco	5.5 b	6.2 a	7.7 c	9.2 b
Pasimoni	5.7 ab	8.7 c	8.9 b	11.7 a
		$\delta^{13}C$ (‰)		
Aguaro		−28.0 a	−22.2 a	−21.8 a
Apure	−17.8 a	−29.1 a	−26.0 b	−24.5 b
Cinaruco	−23.0 b	−31.9 b	−27.8 c	−28.8 c
Pasimoni	−29.6 c	−34.8 c	−29.6 d	−29.6 c

two isotopic clusters on the DFA plot, suggesting fish in this guild assimilated matter from two different dominant trophic pathways. An alternate explanation is that the fish represented by these disparate clusters were feeding at different trophic levels. The greater trophic discreteness displayed in the Pasimoni and to a lesser extent the Cinaruco, appears to be related to greater isotopic homogeneity within species of these rivers. Intraspecific standard deviations for $\delta^{13}C$ and $\delta^{15}N$ support this contention, where isotopic variability within species was less in the nutrient-poor systems, compared to the more productive rivers (Fig. 2). In addition, Fig. 2 illustrates that in general, variability in $\delta^{13}C$ was greater than $\delta^{15}N$ for species in all four systems.

Trophic position and mean food chain length

Mean trophic positions (TP) for each fish species are listed in Table 3. Because the trophic position estimates are corrected for differences in $\delta^{15}N$ among basal sources in each river food web, the TP values are directly comparable across systems. In general, fish species of the lower food web from the Cinaruco had lower TPs than comparable species at other sites. Averaged TPs for detritivores and omnivores from the Apure and Aguaro rivers were essentially the same, but Aguaro top piscivores were 0.4 TP units greater than those from the Apure. Thus, even though Apure piscivores had a greater mean absolute $\delta^{15}N$ than Aguaro piscivores (9.4‰ and 9.1‰, respectively, for Apure and Aguaro fish; Table 2), Apure piscivores apparently feed lower in the web than Aguaro piscivores (i.e., there is less isotopic enrichment in the Apure system). For Pasimoni fishes, ^{15}N enrichment is even more pronounced. Although trophic positions were similar near the base of the web for the Apure, Cinaruco and Pasimoni herbivores, Pasimoni detritivores were apparently assimilating a greater proportion of material with higher TPs within the detrital pool relative to fishes in the Apure and Cinaruco. A more direct comparison involves the case of ecomorphologically similar detritivorous/algivorous *Dekeyseria* sp. and *Lasiancistrus* sp. from the Cinaruco and Pasimoni rivers, respectively. Pasimoni *Lasiancistrus* had a mean TP 1.1 units greater than *Dekeyseria* from the Cinaruco. In isotopes units, this is a difference of about 2.6‰, a value close to the 2.8‰ enrichment per trophic transfer. This suggests that Pasimoni *Lasiancitrus* foraged one trophic level higher than the very similar *Dekeyseria* from the Cinaruco. From a systems energetic perspective, Pasimoni detritivores were functioning as omnivores, consuming both basal and primary consumer organic matter.

Comparisons among mean TP for *Hydrolycus armatus* at each site indicated that Pasimoni fish were feeding about one trophic level higher than conspecifics from the Cinaruco and Apure, with *Hydrolycus* from the Aguaro intermediate between these estimates. Contrary to the hypothesis of greater system productivity supporting longer food chains, the least productive river in this study (Pasimoni) contains food chains of longer average length (leading to top predator *Hydrolycus*) than the three more productive rivers. For the Pasimoni food web, there was a 2.2 TP-unit increase from herbivores to piscivores, representing about three trophic levels.

A comparison of the trophic position data from the Pasimoni and Apure suggests that omnivory can influence the length of the food chain. As noted above, Pasimoni detritivores/algivores have an elevated trophic position, likely due to the partial assimilation of microheterotrophs (primary consumers, which would elevate $\delta^{15}N$) associated with phytobenthos. Hence, Pasimoni detritivores achieve the same trophic position as omnivores in this system (TP = 1.5 and 1.6, respectively). In

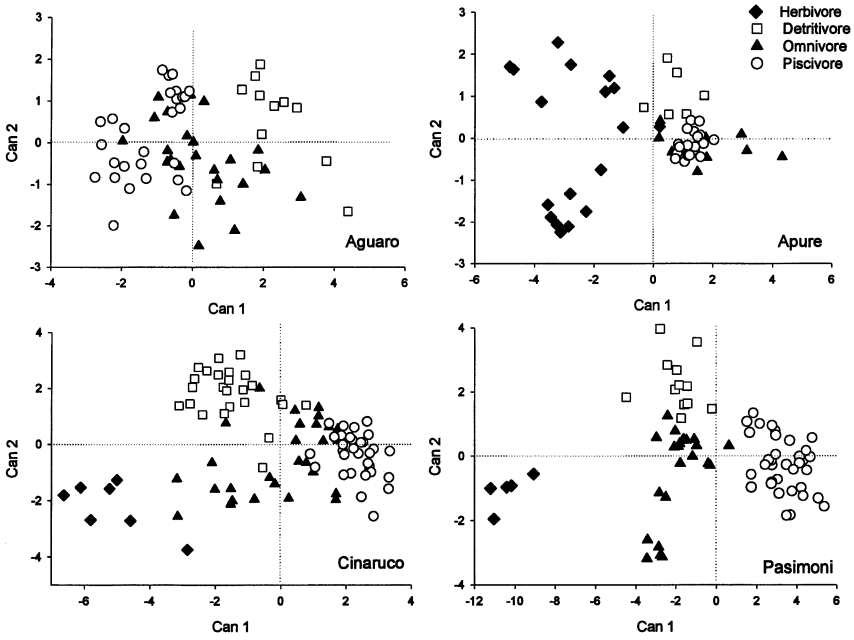

Fig. 1. Canonical variates derived from a discriminant function analysis using stable isotope data for four tropic groups of fishes from a clear-water river (Aguaro), white-water river (Apure), and two black-water rivers (Cinaruco and Pasimoni) in Venezuela. Can 1 and Can 2 refer to the first two canonical functions of a multivariate data set.

contrast, only one trophic transfer may be involved with Apure detritivores, and species in this group are essentially primary consumers (presumably algivorous, TP = 1). It is not clear why detritus, as a food source in the Apure has a lower trophic status. In effect, there is probably a greater relative abundance of prey fish with isotopically higher $\delta^{15}N$ available to piscivores in the Pasimoni, than in the Apure, resulting in a more direct mechanism of ^{15}N enrichment in Pasimoni piscivores. In this case, omnivory occurring lower in the food chain probably promotes greater ^{15}N accumulation in top consumers.

Discussion

The comparative analysis of fish trophic groups using a dual isotope approach identified strong patterns within fish trophic networks that would not have been possible using conventional stomach contents data or general knowledge of fish ecomorphology and feeding behavior. Although coarse in resolution, isotope data demonstrated that the stable carbon and nitrogen ratios of two broad classes of basal production (algae and terrestrial vegetation) can be traced into piscivores through two distinct classes of primary consumers. In nutrient-poor systems, $\delta^{13}C$ and $\delta^{15}N$ data easily identified herbivore and detritivore guilds near the bottom of the food web, an omnivore/insectivore guild in the middle, and piscivores at the top. Considering the great taxonomic, morphological, and behavioral diversity within each of these groups, it is remarkable that carbon and nitrogen signatures within trophic groups were so well defined. For example, although the piscivorous trophic groups in the Pasimoni and Cinaruco contained fish orders with very different feeding behaviors (*Cichla*-engulfers; *Serrasalmus*-shearers), the isotopic carbon and nitrogen compositions of these fishes were similar.

As predicted, fishes in the nutrient-rich Apure River exhibited an isotopically broader resource base than fish in the Cinaruco and Pasimoni systems. This complicated interpretations of the Apure isotope data. Upper trophic guilds in the Apure were poorly defined isotopically, due to large variation in $\delta^{13}C$ and $\delta^{15}N$ within pre-assigned guilds, and to similarities in $\delta^{15}N$ among omnivores and piscivores. This variability may have been due to inclusion within the omnivore guild of species feeding from two different subwebs in the aquatic environment. The Apure collection site encom-

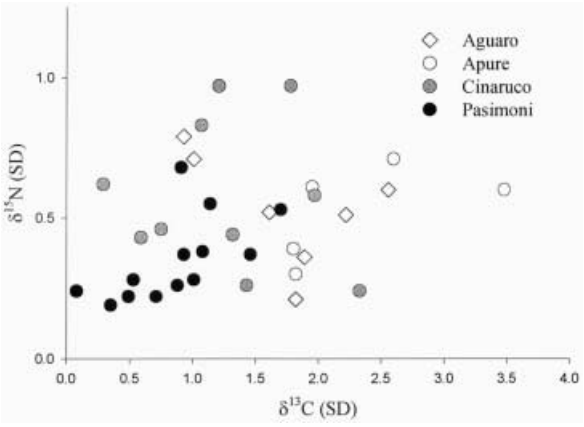

Fig. 2. Standard deviations of mean $\delta^{13}C$ and $\delta^{15}N$ data for fish species in four Venezuelan river systems.

Table 3. Mean trophic positions for fish species of four river ecosystems in Venezuela, estimated from $\delta^{15}N$ data. Species are organized by trophic groups. Value in parentheses is mean excluding *Schizodon isognathus* for Apure system.

Species	Family	River System			
		Aguaro	Apure	Cinaruco	Pasimoni
Herbivores					
Schizodon isognathus	Anostomidae		1.5		
Myleus rubripinnis	Characidae			0.3	0.4
Mylossoma duriventre	Characidae		0.4		
Piaractus brachypomus	Characidae		0.2	0.1	
	average		0.7 (0.3)	0.2	0.4
Detritivores					
Curimata vittatus	Curimatidae				1.5
Cyphocharax spilurus	Curimatidae			0.6	1.4
Ancistrus sp.	Loracariidae			0.9	
Dekeyseria sp.	Loracariidae			0.5	
Lasiancistrus sp.	Loracariidae				1.6
Rineloricaria caracasensis	Loracariidae	1.4	1.1		
Prochilodus mariae	Prochilodontidae	0.7	0.9		
Semaprochilodus kneri	Prochilodontidae	1.3		0.5	
Semaprochilodus laticeps	Prochilodontidae			0.8	
	average	1.1	1.0	0.7	1.5
Omnivores					
Laemolyta sp.	Anostomidae			1.1	1.6
Leporinus sp.	Anostomidae			1.2	1.6
Tatia sp.	Auchenipteridae	2.3			
Brycon sp.	Characidae	1.8	2.1	1.2	
Byconops sp.	Characidae	2.0			1.7
Chalceus macrolepidotus	Characidae				1.4
Moenkausia dichroura	Characidae		1.8		
Tetragonopterus chalceus	Characidae			1.2	
Triportheus angulatus	Characidae		1.2	1.1	
Caenotropis labrynthicus	Chilodontidae			1.3	
Bujurquina pulcher	Cichlidae		1.4		
Caquetia kraussi	Cichlidae		1.8		
Geophagus sp.	Cichlidae			0.6	
Heros sp.	Cichlidae	1.7		0.8	
Satanoperca daemon	Cichlidae	1.2			1.5
Orinocodoras eigenmanni	Doradidae			1.4	
	average	1.8	1.7	1.1	1.6
Piscivores					
Acestrorynchus sp.	Characidae			1.3	2.7
Pygocentrus cariba	Characidae		1.8		
Serrasalmus gouldingi	Characidae				2.5
Serrasalmus manueli	Characidae			1.8	
Cichla intermedia	Cichlidae			1.7	
Cichla orinocensis	Cichlidae	1.9		1.5	
Cichla temensis	Cichlidae			1.6	2.8
Crenicichla cf. *o-lugubris*	Cichlidae	2.1		1.4	2.3
Boulengerella lucius	Ctenuluciidae			1.5	
Hydrolycus pectoralis	Cynodontidae				2.7
Hydrolycus armatus	Cynodontidae	2.2	1.6	1.7	2.9
Hoplerythrinus unitaeniatus	Erythrinidae	2.3			
Hoplias malabaricus	Erythrinidae	2.0	1.6		2.3
Psuedoplatystoma fasciatum	Pimelodidae		1.5		2.4
Plagioscion squamosissimus	Sciaenidae		1.9	1.8	
	average	2.1	1.7	1.6	2.6

passed two biotopes. Most fishes were collected from open water habitats using large-mesh seines, but several species were captured from 'floating meadows' (see Junk 1970, Araujo-Lima et al. 1986), primarily composed of *Eichhornia azurea* and *Paspalum fasciculatum*. Aquatic macroinvertebrates were abundant in the floating meadows, but uncommon in open waters. Trophic networks in these biotopes could have been different, with predaceous insects inserting an extra trophic level in vegetated areas, thereby elevating $\delta^{15}N$ in carnivo-

rous fish in this habitat. Dual isotope data support this hypothesis. Two insectivorous fish species captured within floating vegetation, *Moenkausia dichroura* and *Caquetaia krausii*, had mean $\delta^{15}N$ values higher than the piscivores *Hydrolycus armatus* and *Pseudoplatystoma fasciatum* captured from open water areas. The two insectivorous species also had lower $\delta^{13}C$ relative to all piscivores in this system, and more aligned with *Eichhornia azurea* and associated periphyton, carnivorous belostomatid hemipterans, and shrimp (Jepsen and Winemiller unpubl.). Exclusion of *Moenkausia dichroura* and *Caquetaia krausii* from the DFA data probably would improve the reclassification success between Apure piscivores and omnivores.

Although isotope studies are few for tropical freshwater systems, and until now entirely lacking for tropical black-water systems, the isotope patterns here suggest that nutrient-poor, black-water food webs have more discrete trophic levels than those of productive white-water rivers. This type of insight would have been very difficult to achieve using conventional diet data, and emphasizes a unique benefit of stable isotope studies. For complex trophic networks that are poorly studied, isotope analysis can rapidly indicate the most important energy pathways, and lead to more refined studies that incorporate seasonal and spatial heterogeneity. Black-water rivers may be particularly good systems to model energy flow using stable isotope data.

Contrary to the hypothesis that food chain length increases with productivity, isotope data collected here suggest that mean chain length was lower for the most productive tropical rivers. Top piscivores in the nutrient-poor Pasimoni had $\delta^{15}N$ $\sim 8‰$ higher than basal sources, whereas piscivores in the nutrient-rich Apure were only $\sim 5‰$ greater than local basal resources. Assuming our $\delta^{15}N$ estimates for basal sources are accurate, the 3‰ difference between top piscivores in these systems constitutes about one tropic level. A likely explanation is that Apure piscivores were consuming from a more omnivorous-based food web.

In the absence of additional data sources, the use of carbon and nitrogen isotope analyses to infer species-level trophic relationships within tropical river food webs is limited. Although basal sources are sufficiently distinct in $\delta^{13}C$ to partition many primary consumers, the broad diets of many fishes at intermediate trophic levels obscure many piscivore/herbivore links. Analysis of isotope data using less aggregated trophic guilds (i.e., defining >4 trophic guilds) could improve resolution of food web structure. This might be particularly the case for our omnivore guild, which included insectivores, scavengers, and granivores to varying degrees. Piscivores also could be subdivided into finer level trophic guilds (Winemiller 1989). For example, isotope data might be able to discriminate between large, open water species (*Hydrolycus*, *Pellona*, and *Pseudoplatystoma*), and ambush predators associated with littoral zones or aquatic vegetation (*Hoplias*, *Cichla*, and *Crenicichla*).

Isotope approaches have been used successfully in relatively simple food webs with distinct basal sources and few interacting consumers (Estep and Vigg 1985, Kling et al. 1992, Yoshioka et al. 1994). In diverse assemblages with long-lived, upper-level consumers, linkages to lower trophic levels and basal sources are more difficult to identify. Tropical fishes respond to environmental variability with behavioral and physiological attributes that introduce variation in their isotope signatures. Given the high species diversity, feeding plasticity, and foraging opportunism that are inherent in many tropical aquatic food webs, detailed estimation of community feeding links remains a challenge. We feel that isotope analysis of trophic aggregations (trophospecies) provides an appropriate resolution of food use and foraging history that can lead to greater understanding of energy compartmentalization and other details of food web structure. In addition, we suggest that stable isotope studies will have greater utility if more attention were given to the proper scaling of isotope data associated with ecological phenomena.

For example, isotope data may contribute to a greater understanding of patch dynamics and the spatial extent of fish foraging activities. A dominant ecological feature in tropical floodplain rivers are seasonal fish movements across mesohabitats (macrophyte beds, lagoons, channels) (Winemiller and Jepsen 1998), and isotope data of tissues with different turnover times may detect these localized fish feeding patterns. Benthic fishes and those with limited mobility may feed in isotopically distinct patches, and sampling the isotope composition of these species along environmental gradients (depth, current velocity, temperature, substrate coarseness) may detect long-term intraspecific and interspecific habitat partitioning (Bootsma et al. 1996).

Acknowledgements – Valuable assistance during field work in Venezuela was provided by D. Taphorn, A. Barbarino Duque, B. Stergios, D. Rodríguez Olarte, G. Webb, C. Lofgren, L. Nico, A. Arrington, H. López Fernández, and S. Walsh. DBJ offers special thanks to L. Roelke, R. Best, G. Salata, and R. Downer of the Stable Isotope Lab at TAMU for training and assistance in sample preparation and mass spectrometry. T. Maddox of the Inst. of Ecology, Univ. of Georgia performed additional isotope analysis. We thank J. Cotner and L. Cifuentes for helpful comments on the manuscript. Funding was provided by National Geographic Society grants 5609-96 and 6074-97 and grants from the Texas Agriculture Experiment Station and Texas A&M Office of Research (KOW); and a Tom Slick Doctoral Research Fellowship, and grants from Sigma Xi and the TAMU Office Graduate Studies (DBJ).

References

Angermeier, P. L. and Karr, J. R. 1983. Fish communities along environmental gradients in a system of tropical streams. – Environ. Biol. Fishes 9: 117–135.

Araujo-Lima, C. A. R. M., Portugal, L. P. S. and Ferreira, E. G. 1986. Fish-macrophyte relationship in the Anavilhanas Archipelago, a blackwater system in the Central Amazon. – J. Fish Biol. 29: 1–11.
Bayley, P. B. 1973. Studies on the migratory characin, *Prochilodus platensis* Holmberg 1889, (Pisces, Characoidei) in the River Pilcomayo, South America. – J. Fish Biol. 5: 25–40.
Bayley, P. B. 1988. Factors affecting growth rates of young tropical floodplain fishes: seasonality and density-dependence. – Environ. Biol. Fishes 21: 127–142.
Bootsma, H. A., Hecky, R. A., Hesslein, R. H. and Turner, D. F. 1996. Food partitioning among Lake Malawi nearshore fishes as revealed by stable isotope analyses. – Ecology 77: 1286–1290.
Briand, F. and Cohen, J. E. 1987. Environmental correlates of food chain length. – Science 238: 956–960.
Cohen, J. E., Briand, F. and Newman, C. M. 1990. Community food webs: data and theory. – Springer-Verlag.
Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. – Geochim. Cosmochim. Acta 12: 133–149.
DeAngelis, D. L. 1992. Dynamics of nutrient cycling and food webs. – Chapman and Hall.
DeNiro, M. J. and Epstein, S. 1981. Influence of diet on the distribution of nitrogen isotopes in animals. – Geochim. Cosmochim. Acta 45: 341–351.
Estep, M. L. F. and Vigg, S. 1985. Stable carbon and nitrogen isotope tracers of trophic dynamics in natural populations and fisheries of the Lahontan lake system, Nevada. – Can. J. Fish. Aquat. Sci. 42: 1712–1719.
Goulding, M. M., Carvalho, L. and Ferreira, E. G. 1988. Río Negro: rich life in poor water. – SPB Acad. Publ.
Huntly, N. 1995. How important are consumer species to ecosystem functioning. – In: Jones, C. G. and Lawton, J. H. (eds), Linking species and ecosystems. Chapman and Hall, pp. 72–83.
Junk, W. 1970. Investigations of the ecology and production-biology of the "floating Meadows" (Paspalo-Echinochloetum) of the Middle Amazon. – Amazoniana 2: 449–495.
Junk, W. J., Bayley, P. B. and Sparks, S. E. 1989. The flood pulse concept in river-floodplain systems. – In: Dodge, D. P. (ed.), Proceedings of the International Large River Symposium. Canadian Special Publication Fisheries and Aquatic Sciences 106, pp. 110–127.
Kling, G. W., Fry, B. and O'Brien, W. D. 1992. Stable isotopes and planktonic trophic structure in Arctic lakes. – Ecology 73: 561–566.
Knöppel, H.-A. 1970. Food of Central Amazon fishes: contribution to the nutrient-ecology of Amazonian rain-forest streams. – Amazoniana 2: 257–352.
Lowe-McConnell, R. H. 1987. Ecological studies in tropical fish communities. – Cambridge Univ. Press.
Minagawa, M. and Wada, E. 1984. Stepwise enrichment of ^{15}N along food chains: further evidence and the relation between ^{15}N and animal age. – Geochim. Cosmochim. Acta 48: 1135–1140.
Peterson, B. J. and Fry, B. 1987. Stable isotopes in ecosystem studies. – Annu. Rev. Ecol. Syst. 18: 293–320.
Peterson, C. C. and Winemiller, K. O. 1997. Ontogenetic diet shifts and scale-eating in *Roeboides dayi*, a neotropical characid. – Environ. Biol. Fishes 49: 111–118.
Pimm, S. L. 1982. Food webs. – Chapman and Hall.
Polis, G. A. and Strong, D. R. 1996. Food web complexity and community dynamics. – Am. Nat. 147: 813–846.
Sioli, H. 1975. Tropical rivers as an expression of their terrestrial environment. – In: Golly, F. B. and Medina, E. (eds), Tropical ecological systems. Springer-Verlag, pp. 275–288.
Vadas, R. L., Jr. 1990. The importance of omnivory and predator regulation of prey in freshwater fish assemblages in North America. – Environ. Biol. Fishes 27: 285–302.
Vander Zanden, M. J., Cabana, G. and Rasmussen, J. B. 1997. Comparing trophic position of freshwater fish calculated using stable isotope ratios ($\delta^{15}N$) and literature dietary data. – Can. J. Fish. Aquat. Sci. 54: 1142–1158.
Vander Zanden, M. J. and Rasmussen, J. B. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. – Ecol. Monogr. 66: 451–477.
Vanni, M. J. and DeRuiter, P. C. 1996. Detritus and nutrients in food webs. – In: Polis, G. A. and Winemiller, W. O. (eds), Food webs: integration of patterns and dynamics. Chapman and Hall, pp. 25–29.
Vanni, M. J., Layne, C. D. and Arnott, S. E. 1997. "Top-down" trophic interactions in lakes: effects of fish on nutrient dynamics. – Ecology 78: 1–20.
Wilhelm, S. W. and Suttle, C. A. 1999. Viruses and nutrient cycles in the sea. – BioScience 49: 781–788.
Winemiller, K. O. 1989. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. – Environ. Biol. Fishes 26: 177–199.
Winemiller, K. O. 1990. Spatial and temporal variation in tropical fish trophic networks. – Ecol. Monogr. 60: 331–367.
Winemiller, K. O. 1991. Comparative ecology of *Serranochromis* species (Teleostei: Cichlidae) in the Upper Zambezi River floodplain. – J. Fish Biol. 39: 617–639.
Winemiller, K. O. 1995. The structural and functional aspects of fish diversity. – Bull. Fr. Peche Piscic. 337/338/339: 23–45.
Winemiller, K. W. and Jepsen, D. B. 1998. Effects of seasonality and fish movement on tropical river food webs. – J. Fish Biol. 53 (Suppl. A): 267–296.
Yodzis, P. 1981. The stability of real ecosystems. – Nature 289: 674–676.
Yoshioka, T., Wada, E. and Hayashi, H. 1994. A stable isotope study on seasonal food web dynamics in a eutrophic lake. – Ecology 75: 835–846.