ECOLOGY OF
FRESHWATER FISH

Basin geochemistry and isotopic ratios of fishes and basal production sources in four neotropical rivers

Jepsen DB, Winemiller KO. Basin geochemistry and isotopic ratios of fishes and basal production sources in four neotropical rivers.
Ecology of Freshwater Fish 2007: 16: 267–281. © 2007 The Authors.
Journal compilation © 2007 Blackwell Munksgaard

Abstract – We analysed stable carbon and nitrogen isotopic ratios of dissolved inorganic carbon (DIC), plants, detritus and fishes to estimate the relative importance of dominant production sources supporting food webs of four Venezuelan rivers with divergent geochemical and watershed characteristics. Based on samples taken during the dry season at each site, fishes from two nutrient-poor, blackwater rivers had significantly lower $\delta^{13}C$ values (mean $= -31.4\%_o$ and $-32.9\%_o$) than fishes from more productive clearwater and whitewater rivers (mean $= -25.2\%_o$ and $-25.6\%_o$ respectively). Low carbon isotopic ratios of fishes from blackwaters were likely influenced by low $\delta^{13}C$ of DIC assimilated by aquatic primary producers. Although floodplains of three savanna rivers supported high biomass of C_4 grasses, relatively little carbon from this source appeared to be assimilated by fishes. Most fishes in each system assimilated carbon derived mostly from a combination of microalgae and C_3 macrophytes, two sources with broadly overlapping carbon isotopic signatures. Even with this broad overlap, several benthivorous grazers from blackwater and whitewater rivers had isotopic values that aligned more closely with algae. We conclude that comparative stable isotopic studies of river biota need to account for watershed geochemistry that influences the isotopic composition of basal production sources. Moreover, isotopic differences between river basins can provide a basis for discriminating spatial and temporal variation in the trophic ecology of fishes that migrate between watersheds having distinct geochemical characteristics.

David B. Jepsen and Kirk O. Winemiller

Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX, USA

David B. Jepsen, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843, USA;
e-mail: david.jepsen@oregonstate.edu

Present address: David B. Jepsen, Oregon Department of Fish and Wildlife, Corvallis Research Lab, 28655 Hwy 34, Corvallis, OR 97333, USA.

Introduction

Tropical floodplain rivers are dynamic ecosystems which support diverse fish communities and important fisheries (Lowe-McConnell 1987; Welcomme 1989). Rivers also provide critical migration routes for fishes and other aquatic organisms (Barbarino-Duque *et al.* 1998; Dettmers *et al.* 2001). In South America, many of the major rivers drain watersheds with forests or savannas that remain little impacted by humans. Tropical rivers have been classified as whitewater, clearwater and blackwater systems, based on water physicochemical properties and productivities (Sioli 1975). Given increasing levels of habitat alteration, natural resource exploitation and land use changes in many South American river basins (Bayley & Petrere 1989; Agostinho *et al.* 2000), there is an urgent need to understand aquatic food web ecology, including the influence of watershed attributes on food web structure and dynamics.

Tributaries of the Orinoco River in Venezuela and Colombia drain landscapes with widely contrasting geochemistry and geology (Edmond *et al.* 1995, 1996) that yield large inter-basin differences in water chemistry, alluvial sediments and vegetation (Stallard 1985; Lewis *et al.* 2000). Carbon isotope geochemistry in

these basins reflects these differences. The $\delta^{13}C$ of dissolved inorganic carbon (DIC) is higher in rivers draining savanna regions dominated by Andean alluvial deposits compared with rivers draining heavily forested, nutrient-poor watersheds of the Guyana Shield (Tan & Edmond 1993). Aquatic primary producers using this DIC source reflect its isotopic signature, and aquatic autotrophs from productive savanna rivers have higher ratios of $^{13}C/^{12}C$ relative to autotrophs in forested basins (Tan & Edmond 1993). Because stable carbon isotopic ratios are highly conserved as material is passed through food chains (Fry & Sherr 1984), differences in autotroph carbon isotopic ratios should be reflected in tissues of resident consumers. Thus, carbon isotopic data are particularly useful for estimating energetic links between local production sources and consumers, and simultaneously reflect interbasin differences in geochemistry. Isotopic ratios of other elements, such as nitrogen and sulphur, can aid in resolving consumer–resource links, provided that reasonable estimates of trophic fractionation are taken into account (Phillips 2001).

Isotopic data for tropical blackwater food webs are scarce (Benedito-Cecilio & Araujo-Lima 2002), which is unfortunate given that these systems support very high-level biodiversity (Goulding *et al.* 1988). Aquatic primary production is low in most tropical black waters (Wissmar *et al.* 1981), but bacterial abundance and respiration rates are similar to other water types (Benner *et al.* 1995). Terrestrial vegetation seems to have low direct nutritive value for fishes (Forsberg *et al.* 1993); however, diets of many Amazonian fishes, blackwater fishes in particular, have been shown to include terrestrial primary and secondary production sources (Walker 1985; Goulding *et al.* 1988).

Here, we analyse stable isotopes in plants and fishes to estimate carbon/energy sources and trophic linkages in food webs of four Venezuelan rivers with divergent biogeochemistry and watershed geology. These rivers were chosen because they encompassed the extremes of geochemical variability in Neotropical rivers (whitewater Apure and blackwater Pasimoni) as well as intermediate conditions (clearwater Aguaro and dilute blackwater Cinaruco). Three rivers drain savannas in the llanos region, and the Pasimoni drains a densely forested, low-gradient region of the Guyana Shield (Fig. 1). All four regions are sparsely populated, with three of the rivers (Aguaro, Cinaruco and Pasimoni) draining watersheds designated as national parks. A previous analysis of trophic guild structure in these rivers based on stable isotope and C/N ratios revealed that nutrient-poor rivers had lower within-guild variability of isotopic ratios and top piscivores with higher trophic positions (Jepsen & Winemiller 2002). Higher within-guild variability presumably results from greater diversity of basal inputs linked

to upper food web elements, and higher trophic position of top piscivores implies longer food chains. Piscivores also had less variable isotopic ratios than fishes from other trophic guilds. Here we examine within- and between-system variations in isotopic ratios of dominant production sources to infer potential contributions to consumers.

Differences in the ranges of carbon isotopic ratios of DIC for whitewater (−8.1‰ to −12.5‰) and blackwater (−9.5‰ to −23.0‰) rivers may be the foundation for assemblage-wide differences in carbon isotopic ratios (Winemiller & Jepsen 2004). Tan & Edmond (1993) reported higher carbon isotopic ratios of particulate organic carbon (POC) from savanna rivers of the Orinoco Basin relative to blackwater and clearwater rivers draining the Guyana Shield formation. Fishes from these systems would thus be expected to reflect these heavier carbon sources. In contrast, fishes from rivers draining densely forested watersheds of the Guyana Shield would be expected to feed more heavily on aquatic and terrestrial resources with lower carbon isotopic ratios. Our study further examines these isotopic differences for inorganic and biotic components of river ecosystems with contrasting geochemical attributes, and estimates basal production sources supporting the major fish populations in each river.

Study sites

Whitewater Apure River

The Apure River (Apure State) is a whitewater river with large suspended sediment loads, high-dissolved nutrient concentrations and moderate pH (Table 1). The Apure (basin 167,000 km^2) delivers only 6.6% of total discharge to the Orinoco, yet it contributes over 30% of dissolved ions at high water (Edmond *et al.* 1996). This high suspended load is derived from tributaries draining the relatively young eastern slopes of the Andes. Weathering processes affect solute content and impart a relatively high DIC concentration to the river (Edmond *et al.* 1996). Abundant nutrients and slow velocities in floodplain areas promote extensive growth of emergent and floating macrophytes (e.g., *Paspalum* spp. and *Eichhornia* spp.) that are similar in composition and ecology to the 'floating meadows' present in the Amazon and Orinoco floodplains (Junk 1970). Despite the high nutrient availability, phytoplankton production in the main channel of the Apure is moderate (26.2 mg·C·m^{-2}·day^{-1}, Edmond *et al.* 1996), presumably due to low light penetration (Lewis *et al.* 2000).

Clearwater Aguaro River

Although chemically diverse, clearwater rivers have high transparencies, acidic to neutral pH, and usually drain flat, forested or savanna regions with latosol soils
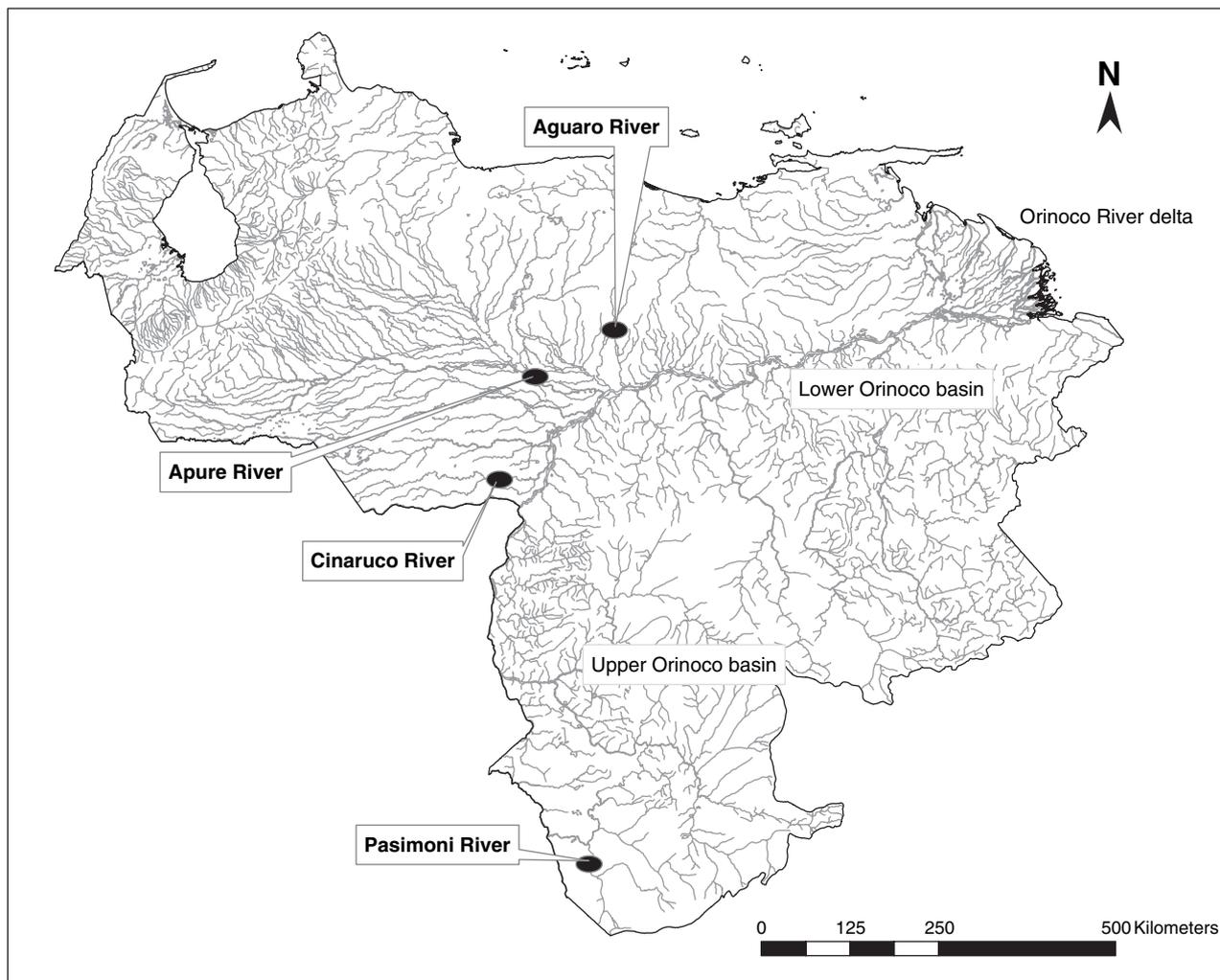
Fig. 1. Map of Venezuela showing locations of sampling sites in the Aguaro (clearwater), Apure (whitewater), Cinaruco (dilute blackwater) and Pasimoni (blackwater) rivers.

Table 1. Geochemical data for four Venezuelan rivers in the present study.

River	Water type	pH	Conductivity (s·cm^{-1})	Secchi depth (m)	Alkalinity (eq·l^{-1})	Reference
Apure	White	7.7–8.2	98–190	0.2	700–1719	1, 3, 4
Aguaro	Clear	6.5	16	1.5	–	5
Cinaruco	Dilute black	5.5–6.3	5.9–10.5	1.0	6–68	1, 3, 4
Pasimoni	Black	4.2–4.5	<20–24	1.2–1.8	–53	2, 4, 5

1, Depetris & Paolini (1991); 2, Edmond *et al.* (1995); 3, Edmond *et al.* (1996); 4, Jepsen (1999); 5, Yanes & Ramírez (1988).

(Sioli 1975). High transparency and low colour are due to low concentrations of suspended sediments and humic compounds. The Aguaro River (Guarico State) was chosen to represent a clearwater river (Table 1). The Aguaro is geochemically similar to the Cinaruco River (discussed below), but differs in having higher transparency, higher pH, denser growth of aquatic macrophytes (mostly *Egeria* sp. and Charophyceae) and a coarser substrate. Riparian vegetation consists of a narrow gallery forest, with upland areas composed of savanna grasses (*Trachypogon* spp. and others).

Dilute blackwater Cinaruco River
The Cinaruco River (Apure State) was chosen to represent an intermediate position on a gradient between clearwater and blackwater conditions (i.e., dilute blackwater). Blackwater rivers are nutrient-poor systems with few suspended solids and low pH

(Table 1). Transparency is reduced in black waters by high levels of humic acids and other dissolved organic compounds that stain the water. Black waters usually originate in deep sandy soils and are associated with the absence of suspended clay particles and other solids. In contrast to whitewater and clearwater ecosystems, blackwater rivers generally support little aquatic macrophyte growth.

The Cinaruco is unusual among left-bank tributaries of the Orinoco River. Unlike the larger Apure, Arauca and Meta Rivers that also traverse savannas of the western llanos, the headwaters of the Cinaruco do not extend to the slopes of the Andes Mountains, but drain entirely from sandy alluvia within the savanna. The Cinaruco is considered a geochemically dilute floodplain river, with a strong deficit of inorganic compounds as in other blackwater rivers (Edmond *et al.* 1996). Detailed descriptions of the Cinaruco River appear in Cotner *et al.* (2006) and Montoya *et al.* (2006).

Blackwater Pasimoni River
The Pasimoni River drains a forested lowland region on the western fringe of the Guayanan shield in southern Amazonas State. The Pasimoni is part of a network of rivers in extreme southern Venezuela that forms the northern headwaters of the Rio Negro via the Casiquiare River. The Pasimoni represents an extreme blackwater ecosystem (Table 1) with low pH and transparency derived from high levels of dissolved organic carbon (DOC), podzolic soils and low concentration of inorganic ions. The watershed is covered by sandy soils and humid tropical rainforest, and aquatic macrophytes are scarce.

Methods

Field collections

From each of the four river ecosystems, we collected samples of sediments, suspended POC, benthic algae, macrophytes (both aquatic and terrestrial) and fishes for stable isotopic analysis. Collections were carried out during the dry season (January to April) in 1997 and 1998. Samples were collected in lentic backwater habitats (connected lagoons and channel shoreline areas) where aquatic production is believed to be greatest (Thorp & Delong 1994; Reynolds & Descy 1996; Thorp & Delong 2002). It was not possible to collect the same vascular plant species for each system due to regional floristic differences. Instead, we collected a subset of the most dominant plants (based on visual estimation) from both terrestrial and aquatic portions of the littoral zone. Tree and shrub diversity was high in riparian areas of all four rivers. Because we could only analyse a few plant samples and had no

obvious basis to select one plant species over another, we also collected composite leaf samples from the forest floor and marginal slack-water areas. Plant and litter samples were washed with water while scrubbing with a toothbrush to ensure that no debris or algae was present.

We attempted to capture fishes of the same species from each site to facilitate intersite comparisons, but this was not possible in many cases due to differences in regional and local assemblage composition. For most species we collected five to 10 individuals of the most common species from four broad trophic categories. Herbivores were species with special dentition modified for tearing leaves or crushing seeds, and for which the literature reports for the genus indicated over 50% vascular plant matter in gut contents. Detritivores/algivores possessed morphological adaptations for sucking or scraping organic material from soft or firm substrates. Invertivores/omnivores included species found with significant amounts of plant and animal matter in their stomachs, plus more specialised species that consume mostly invertebrates and little or no plant material. These two functional groups (true omnivores vs. species that are essentially invertivores) were combined, because the fraction of vegetation in diets varies as a continuum among the species we captured. Piscivores have jaws and dentition designed for grasping/impaling fish (*Acestrorhynchus* and *Hydrolycus*), swallowing whole fish (*Cichla* and *Pseudoplatystoma*) or tearing pieces of flesh from prey fish (*Pygocentrus* and *Serrasalmus*).

Triplicate DIC samples were collected in 250-ml glass bottles (acid washed) and preserved with 0.5 ml of $HgCl_2$ solution. Our DIC samples from the Aguaro and Apure Rivers were destroyed during shipping, and therefore we have no direct estimates of $\delta^{13}C_{DIC}$ for these two rivers. Coarse particulate organic matter (CPOM) was collected from shallow areas and sorted with sieves (>250 μm). Water-column POC samples (with phytoplankton assumed as a main component) were collected by passing water samples through a 50-μm mesh filter (to remove zooplankton) followed by filtering onto pre-combusted (450 °C for 8 h) glass fibre filters (Whatman GF/F). Samples within a site were combined, and then filters were frozen until further processing in the laboratory. Our sample of organic matter filtered from the water column from the Pasimoni River (having extremely low conductivity and nutrient content to support water-column production) was insufficient for POC analysis. From several sites at locations where fish collecting was concentrated, attached algae were carefully scraped or washed from surfaces of firm substrata including submersed plants, isolated from coarse detrital material when possible, then combined and collected onto filters as described for POC. The most conspicuous C_3 (using

the ribulose bisphosphate (RuBP) carboxylation pathway) and C_4 (phosphoenopyruvate (PEP) carboxylation pathway) submerged macrophytes were collected from littoral zones of the same locations, and scrubbed and rinsed to remove epiphyton.

Fishes were collected with a combination of experimental gillnets, seines, castnets and hook-and-line. Muscle tissue (skinned and boneless; ca 2.5 g) was excised from the dorso-lateral region of larger fishes, whereas whole specimens were collected for species <30 mm. Individual samples were either frozen with dry ice or placed in tissue culture cells and packed with NaCl. The salt preservation method induces negligible isotopic change in muscle tissue (Arrington & Winemiller 2002). In the laboratory, plant and fish tissues were rinsed in distilled H_2O and dried at 60 °C for 48 h. Bones and scales were removed with the aid of a microscope to obtain only soft tissue samples. After drying, samples were ground to a fine powder with a pestle and mortar and stored in glass vials.

Mass spectrometry

Organic samples were analysed for isotopic ratios ($\delta^{13}C$ and $\delta^{15}N$) and total carbon and nitrogen content at the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia. Subsamples for each sample were combusted in a Carlo Erba CHN elemental analyser, and the resultant gases (CO_2 and N_2) were analysed with a Finnigan Delta C mass spectrometer (Finnigan MAT Bremer, Germany). Standard deviations of replicate samples were $\pm 0.2\%_o$ for both $\delta^{13}C$ and $\delta^{15}N$. The DIC isotope analysis was performed at the Stable Isotope Laboratory, Department of Oceanography at Texas A&M University following the methods in Hassan (1980).

Isotopic ratios are reported in per mil difference (‰) relative to standards (PeeDee Belemnite for carbon and atmospheric N for nitrogen; see Craig 1957):

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

where $R = {}^{15}N/{}^{14}N$ or ${}^{13}C/{}^{12}C$. Carbon and nitrogen elemental ratios (C:N) were <4.0 for most fishes, indicating a low lipid content in samples. Therefore, isotopic data were not corrected for lipid content.

Classification to species level was not possible for a few fish species due to ongoing revisions within the genus. For statistical procedures, we used isotopic values of individual fishes to calculate mean values for a species at a site. No error estimate is available for nine species for which either a single individual was captured or several small individuals were combined into a bulk sample. In some statistical analyses, we grouped fishes within a genus to increase sample size

and facilitate comparisons among river systems. We used random-effects ANOVA's, Tukey's multiple range test, and where appropriate, the Student's t-test to compare differences in mean isotopic signatures for fish species at different sites. Statistical analyses were conducted with SAS statistical software (SAS Inst., Cary, NC, USA). We used a two end-member mixing model adapted from Phillips & Gregg (2001) to estimate the median per cent (and 95% CI) of C_4 grass carbon assimilated into muscle tissue of fishes at each site, relative to the percentage of carbon from C_3 autotrophs. The C_4 end-member was set at $-13.1\%_o$, which was the mean $\delta^{13}C$ of 30 samples from this study and reported in the literature for South American rivers (Table 2a). For the C_3 end-member we used $-31.2\%_o$, the mean of 114 C_3 plant samples collected during this study and literature reports (Table 2a). Combination of $\delta^{13}C$ values from these diverse sources into a mean is valid for contrast with C_4 plants because the former constitute a functional group (C_3 autotrophs) with broadly overlapping values that do not overlap with C_4 plants (Phillips $et\ al.$ 2004).

Results

Significant differences in carbon and nitrogen isotopic signatures were observed among some of the main production source categories within locations and also between equivalent fish trophic groups from different rivers and river types. The $\delta^{13}C$ of C_3 plants measured in this study (algae and submerged and emergent macrophytes) ranged from -32.8 to $-24.1\%_o$, providing a wide contrast with Neotropical C_4 grasses that ranged from $-12.3\%_o$ to $-11.1\%_o$ at our sites. These values are consistent with those reported in the literature from other Neotropical rivers (Table 2 Fig. 2). At the Apure River site, C_4 grasses (mostly $Paspalum\ fasciculatum$) and C_3 water hyacinths ($Eichhornia\ azurea$) dominated aquatic macrophyte-standing biomass in near-shore areas, and large woody vegetation was rare in the adjacent riparian zone. C_4 grasses (mostly $Panicum$ and $Trachypogon$ spp.) dominated the extensive floodplain of the Cinaruco River and uplands of the Aguaro River, but shrubs and trees formed a thick riparian corridor along both of these rivers. $Digitaria$ sp. was the only notable grass species from the heavily forested Pasimoni site and had $\delta^{13}C$ values (ca $-31.5\%_o$) indicating a C_3 photosynthetic pathway. The dominant tree species on the banks of the Pasimoni River channel ($Malouetia\ glandulifera$) had $\delta^{13}C$ values ca $2\%_o$ greater than a composite leaf sample from the same location ($-29.4\%_o$ vs. $-31.8\%_o$).

The mean carbon isotopic signature of CPOM from the Pasimoni River ($-30.5\%_o$) was lower than CPOM values from the three savanna rivers (Apure

Table 2. Summary of stable carbon and nitrogen isotopic values (‰) of basal sources from Neotropical freshwaters.

	Whitewater			Blackwater			
	N	$\delta^{13}C$	SD	N	$\delta^{13}C$	SD	Source
(a)							
POC, lentic zones	21	−33.9	2.0				1, 2, 3
POC, lotic zones	11	−25.4	1.2	33	−28.9	1.9	6
Attached algae	11	−27.7	2.7	15	−32.2	2.7	1, 2
C_3 macrophytes	18	−28.7	1.7	46	−31.4	3.1	1, 4, 5
C_4 grasses	28	−13.3	1.3	2	−11.5	–	1, 3, 4
	N	$\delta^{15}N$	SD	N	$\delta^{15}N$	SD	
POC	7	3.8	1.0				1, 2
Attached algae	11	3.1	1.8	15	2.1	1.9	1, 2
C_3 macrophytes	7	2.0	1.7	23	2.4	1.7	1
C_4 grasses	2	6.7	0.8	2	4.7	–	1
(b)	N	$\delta^{13}C$	Minimum	Maximum			
POC	40	−33.3	−26	−40			7
Attached algae	22	−27.4	−21.5	−34.8			8
Attached algae	15	−26.2	−22	−30			7
C_3 macrophytes	82	−29.9	−24.7	−34.7			8
C_4 grasses	68	−13.1	−11.7	−15.7			8

Section (a) are mean values calculated from individual samples reported from the literature sources. Section (b) summarises reported mean values of $\delta^{13}C$ from the literature. Data are organised into whitewater and blackwater categories, based on descriptions of study sites in the respective reports.
1, This study; 2, Hamilton & Lewis (1992); 3, Victoria *et al.* (1992); 4, Hedges *et al.* (1986); 5, Medina & Minchin (1980); 6, Tan & Edmond (1993); 7, Forsberg *et al.* (1993); 8, Waichman (1996); POC, particulate organic carbon.
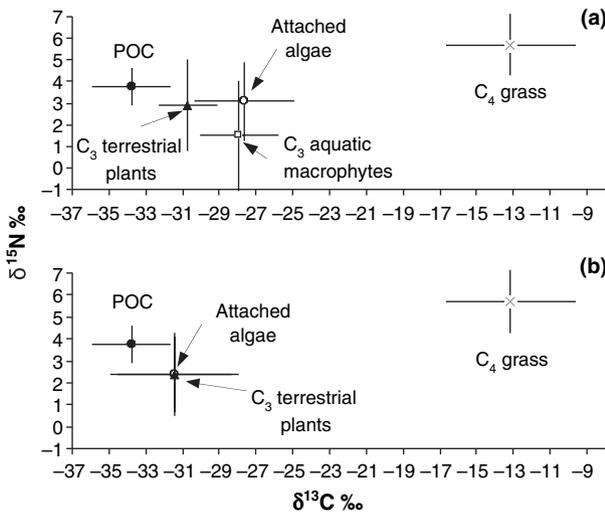

Fig. 2. Scope of potential basal $\delta^{13}C$ and $\delta^{15}N$ isotopic ratios (mean and SD) from two types of tropical river systems. Panel (a) represents sources in nutrient-rich white water rivers, and panel (b) represents sources in nutrient-poor blackwater rivers. Data for mean values were derived from multiple published sources, summarised in Table 2.

mean = −23.0, SD = 0.8; Aguaro mean = −27.3, SD = 1.5; Cinaruco mean = −27.5, SD = 1.6). Pasimoni River sediments consisted mostly of sand, and sediment CPOM was mostly decomposing leaf litter. We augmented our basal resource data set by summarising reported stable isotopic values (Table 2) for source pools from other Neotropical freshwater sites where we could distinguish between sites having

nutrient-rich white or mixed waters and sediments rich in clays and fine particulate organic matter (FPOM), versus those associated with nutrient-poor black waters and sandy sediments with relatively little FPOM.

In total, tissue samples from 281 fish specimens from 52 species were analysed, with 21 of the genera collected from more than one river (Table 3). Assemblage-wide values of fish $\delta^{13}C$ and $\delta^{15}N$ were significantly different among the four rivers (Fig. 3; ANOVA: $\delta^{13}C$: $F = 117.0$, $P < 0.0001$; $\delta^{15}N$: $F = 65.6$, $P < 0.0001$). Mean $\delta^{13}C$ and $\delta^{15}N$ values were not significantly different between the Aguaro and Apure rivers, but fishes from these two sites had higher $\delta^{13}C$ (Apure mean = −25.2‰, Aguaro mean = −25.6‰) compared with fishes from the two blackwater rivers (Cinaruco mean = −31.4‰, Pasimoni mean = −32.9‰; Tukey's HSD test with Bonferroni correction, $P < 0.05$). Pasimoni fishes had significantly lower $\delta^{13}C$ and higher $\delta^{15}N$ than fishes from the other three rivers. Cinaruco fishes had significantly lower $\delta^{15}N$ (Cinaruco mean = 7.6‰) compared with fishes from the other three rivers (Aguaro mean = 8.2‰, Apure mean = 8.5‰, Pasimoni mean = 10.0‰).

Mean $\delta^{13}C$ values among species ranged from −13.7‰ for *Schizodon isognathus* (herbivore – Apure) to −38.1‰ for *Curimata vittatus* (detritivore – Pasimoni). The invertivores/omnivore guild had the largest range of $\delta^{13}C$ values (Table 3, Fig. 4), reflecting the diverse food resources exploited by this group. However, Aguaro piscivores also had a large range of

Table 3. Mean δ^{13}C and δ^{15}N (‰ and SD) and mass C:N elemental ratios of fishes from four rivers in Venezuela.

River/species	N	Average SL (mm)	δ^{13}C	δ^{15}N	C:N	Trophic group	Source
Apure River							
Brycon whitei	1	160	−24.1	10.5	3.38	H, **O**	6, 12
Aequidens pulcher	4	86	−22.7	8.8	3.13	I, **O**	5, 12, 13
Caquetaia kraussi	4, 3	82	−26.8 (3.9)	9.8 (1.7)	3.14	I, **P**	12
Hoplias malabaricus	2	259	−23.7 (0.7)	9.1 (0.4)	3.13	P	2, 5, 12
Hydrolycus armatus	7	328	−24.5 (1.8)	9.4 (0.3)	3.22	P	4, 7, 10
Moenkausia dichroura	2	66	−27.9 (1.5)	9.8 (1.0)	3.14	O	5, 10
Mylossoma duriventre	3, 3	151	−20.6 (3.0)	6.0 (1.2)	3.36	H	1, 7, 10
Piaractus brachypomus	4, 2	192	−23.0 (2.4)	5.2 (0.8)	3.23	**H**, O	7, 10, 12
Plagioscion squamosissimus	3	333	−24.6 (0.7)	10.2 (0.3)	3.94	P	2, 4, 11
Prochilodus mariae	5	237	−29.3 (3.5)	7.3 (0.6)	3.31	H, **D**	5, 10, 12
Psuedoplatystoma fasciatum	5	554	−26.0 (1.6)	9.0 (0.5)	3.11	P	2
Pygocentrus cariba	2, 3	206	−22.6 (0.5)	9.8 (0.4)	3.33	P	8, 12
Rineloricaria caracasensis	5	124	−27.8	7.9	3.15	H, **D**	12
Schizodon isognathus	10	323	−13.7 (4.3)	8.9 (0.7)	3.24	H	7, 10
Triportheus angulatus	3	102	−22.9	8.2	3.19	I, **O**	1, 7
Aguaro River							
Brycon sp.	8	188	−22.9 (1.1)	9.3 (0.8)	3.17	H, **O**	1, 4, 10
Bryconops sp.	3, 3	<50	−21.7 (0.2)	8.6 (0.1)	3.26	H, **I**	2, 5, 12
Cichla orinocensis	1	198	−23.1	8.5	3.07	P	3, 11
Crenicichla sp. af. *lugubris*	4	283	−20.5 (1.9)	8.9 (0.4)	3.18	I, **P**	2, 4, 5
Heros sp.	6, 4	137	−20.8 (2.0)	7.7 (0.5)	3.14	I	2, 5, 12
Hoplias malabaricus	3	357	−22.0 (3.5)	8.6 (0.1)	3.16	P	2, 5, 12
Hoplerythrinus unitaeniatus	9	227	−17.9 (1.6)	9.5 (0.5)	3.14	I, **P**	5, 10, 12
Hydrolycus armatus	10	388	−25.7 (1.8)	9.1 (0.2)	3.33	P	4, 7, 10
Prochilodus mariae	1	190	−28.9	5.2	3.24	H, **D**	5, 10, 12
Rineloricaria caracasensis	4	105	−26.8	7.1	3.36	H, **D**	12
Satanoperca daemon	5	138	−22.2 (2.0)	6.3 (0.4)	3.13	I	2, 5
Semaprochilodus kneri	9, 2	137	−28.5 (2.6)	6.9 (0.6)	3.17	H, **D**	2, 12
Tatia sp.	2	<45	−24.2 (1.0)	9.5 (1.4)	3.61	I	2
Cinaruco River							
Acestrorhynchus sp.	1	110	−29.6	8.4	3.14	P	2
Ancistrus sp.	1	61	−29.2	7.2	3.29	H, **D**	2, 5, 12
Boulengerella lucius	3	310	−29.9 (1.7)	8.9 (0.7)	3.27	P	2, 4
Brycon sp.	4	141	−25.2 (0.6)	8.0 (0.9)	3.26	O	4, 5
Caenotropus labyrinthicus	3	94	−30.2 (0.5)	8.3 (0.5)	3.35	H, **D**	2, 12
Cichla intermedia	4	364	−29.8 (0.3)	9.3 (0.6)	3.37	P	3
Cichla orinocensis	6	286	−29.9 (0.8)	8.8 (0.5)	3.20	P	3, 11
Cichla temensis	7	457	−28.3 (1.3)	9.2 (0.4)	3.28	P	3, 11
Crenicichla sp. af. *lugubris*	2	280	−28.7 (2.0)	8.6 (0.4)	3.16	I, **P**	4, 5
Cyphocharax spirulus	1		−33.0	6.5	3.30	D	9, 10, 12
Dekeyseria sp.	2, 3		−30.5 (0.6)	6.0 (0.3)	3.28	H, **D**	4, 12
Geophagus sp.	5	200	−25.9 (2.3)	6.2 (0.2)	3.13	I	4, 5
Heros sp.	2	153	−29.4 (4.4)	7.0 (0.7)	3.17	I	2, 4, 5
Hydrolycus armatus	5	375	−28.0 (1.8)	9.6 (1.0)	3.32	P	4, 7, 10
Laemolyta taeniatus	3	240	−31.1 (0.6)	7.8 (0.5)	3.22	H, D, **O**	2
Leporinus fasciatus	1		−31.2	7.9	3.21	O	4, 5
Myleus rubripinnis	5	144	−22.8 (1.1)	5.4 (0.8)	3.19	H	2
Orinocodoras eigenmanni	1		−30.4	8.5	3.20	I, **O**	12
Piaractus brachypomus	2	225	−24.2 (1.0)	5.0 (0.3)	3.42	**H**, O	7, 10
Plagioscion squamosissimus	2		−28.7 (0.8)	9.8 (0.2)	3.21	P	2, 4, 11
Semaprochilodus kneri	18	236	−32.3 (2.0)	6.1 (0.6)	3.26	H, **D**	4, 12
Semaprochilodus laticeps	2	390	−31.6 (0.1)	7.0 (0.1)	3.13	H, **D**	4, 12
Serrasalmus manueli	4	230	−27.5 (0.6)	9.7 (0.4)	3.11	P	4, 8
Tetragonopterus chalceus	5	90	−28.8 (1.4)	8.1 (0.3)	3.20	H, I, **O**	2
Triportheus angulatus	5	<100	−25.8 (1.2)	7.8 (1.0)	3.23	O	1
Pasimoni River							
Acestrorhynchus falcirostris	5		−29.0 (0.9)	12.1 (0.3)	3.24	P	2
Bryconops sp.	5		−29.4 (0.9)	9.3 (0.7)	3.25	O	2, 12
Chalceus macroleptidotus	5		−25.7 (0.4)	8.5 (0.2)	3.17	I	4, 12
Cichla temensis	9	566	−29.1 (1.7)	12.3 (0.5)	3.32	P	
Crenicichla sp. af. *lugubris*	5		−30.9 (1.5)	10.8 (0.4)	3.14	I, **P**	4, 5
Curimata vittatus	2		−38.1 (0.8)	8.6 (0.6)	3.32	H, **D**	2, 12
Cyphocharax spirulus	5		−33.8 (1.1)	8.4 (0.6)	3.11	H, **D**	9, 10, 12

Table 3. (Continued)

River/species	N	Average SL (mm)	$\delta^{13}C$	$\delta^{15}N$	C:N	Trophic group	Source
Hoplias malabaricus	5		−30.3 (1.0)	11.0 (0.3)	3.18	P	3, 10, 13
Hydrolycus pectoralis	3		−29.1 (0.1)	11.9 (0.2)	3.58	P	2, 5, 12
Hydrolycus armatus	2		−30.3 (0.2)	12.7 (0.4)	3.61	P	2, 4
Laemolyta sp.	1		−34.0	9.0	4.06	H, D, **O**	2
Lasiancistrus sp.	5		−34.4 (1.1)	8.9 (0.4)	3.37	H, **D**	4, 12
Leporinus sp.	4		−31.3 (0.5)	8.9 (0.2)	3.23	D, **O**	2
Myleus rubripinnis	5		−29.6 (0.7)	5.7 (0.2)	3.24	H	2
Pseudoplatystoma fasciatum	2		−30.4 (0.6)	11.3 (0.1)	3.19	P	2
Satanoperca daemon	8		−30.8 (0.5)	8.8 (0.3)	3.19	I	5
Serrasalmus gouldingi	5		−29.0 (0.9)	11.5 (0.4)	3.70	P	8

Trophic group was assigned by reports from the literature sources or unpublished data for that species or genus: H, herbivore; D, algivore/detritivore; I, insectivore; O, omnivore; P, piscivore (bold letters indicate how species were grouped in the present study for graphical and statistical comparisons). Family names for each species appear in Jepsen & Winemiller (2002).
1, Goulding (1980); 2, Goulding *et al.* (1988); 3, Jepsen *et al.* (1997); 4, D.B. Jepsen, unpublished data; 5, Knöppel (1970); 6, Lilyestrom & Taphorn (1983); 7, Machado-Allison (1987); 8, Nico & Taphorn (1988); 9, Peterson (1997); 10, Taphorn (1992); 11, Williams *et al.* (1998); 12, Winemiller (1989) and personal communication; 13, Winemiller (1990).
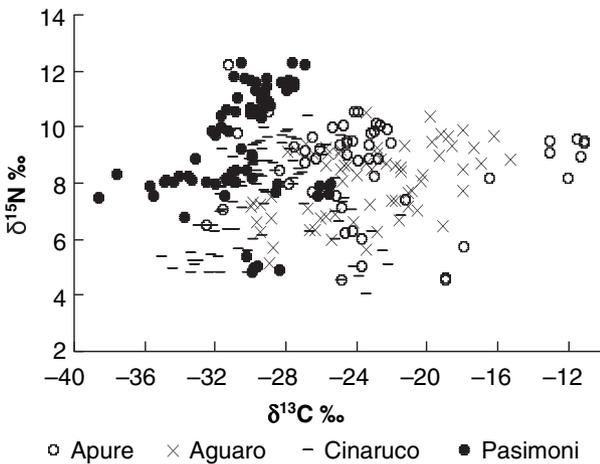

Fig. 3. Biplot of $\delta^{13}C$ and $\delta^{15}N$ values from muscle tissue samples of fishes from four Venezuelan rivers that drain landscapes with different geochemical profiles and vegetation characteristics.

$\delta^{13}C$, suggesting consumption of isotopically divergent prey. The large range of $\delta^{13}C$ among herbivorous fishes in the Apure River was due to the relatively high $\delta^{13}C$ of *Schizodon isognathus*, which clearly assimilated C_4 grass carbon (Table 4). Other herbivores in this system had lower $\delta^{13}C$ values (Table 3) that would be consistent with assimilated carbon from C_3 plants or algae (Table 4). Although herbivores, detritivores/algivores, and invertivores/omnivores from the Cinaruco River tended to have higher $\delta^{13}C$ than their counterparts in the Pasimoni River [Kolmogorov–Smirnov (K-S) test: $P < 0.05$ in all three comparisons], piscivores within these two systems had similar isotopic values (K-S test: $P > 0.05$).

Carbon isotopic ratios indicated that carbon originating from C_4 grasses was not a major component of fish tissues in the two blackwater rivers (Table 4, Fig. 4). For the Apure, Aguaro, Cinaruco and Pasimoni rivers, respectively, 54%, 41%, 94% and 97% of all fishes sampled had $\delta^{13}C$ within the range of $\delta^{13}C$ for C_3 plants measured in this study. Several fishes from the Apure and Aguaro rivers had $\delta^{13}C$ values intermediate between C_3 plants and C_4 grasses, indicating a contribution of both sources to these species.

Several fish species were collected at more than one site, allowing a direct evaluation of the influence of local conditions on consumer isotope composition. For example, *Hydrolycus armatus* was found at all four sites, but specimens from the Pasimoni had significantly lower $\delta^{13}C$ relative to conspecifics from the Aguaro and Apure sites (Table 4). The significant 6.8‰ difference in $\delta^{13}C$ for herbivorous *Myleus rubripinnis* from the Cinaruco (−22.8‰) and Pasimoni (−29.6‰) rivers indicates some dependence of Cinaruco *Myleus rubripinnis* on a basal source with higher $\delta^{13}C$ (e.g., C_4 grasses, which are rare or possibly absent in the dense riparian forest of the Pasimoni). The two piscivorous species shared by the Aguaro and Apure rivers (*Hoplias malabaricus* and *Hydrolycus armatus*) revealed no significant between-site differences in $\delta^{13}C$ or $\delta^{15}N$ isotopic ratios.

Discussion

Our results emphasise the need to consider both watershed/geochemical setting and the potential for between-system isotopic differences in comparative research with stable isotopes. This is particularly relevant for investigations of trophic dynamics across ecosystem boundaries (i.e., food web subsidies). Stable isotopic ratios of fishes revealed significant
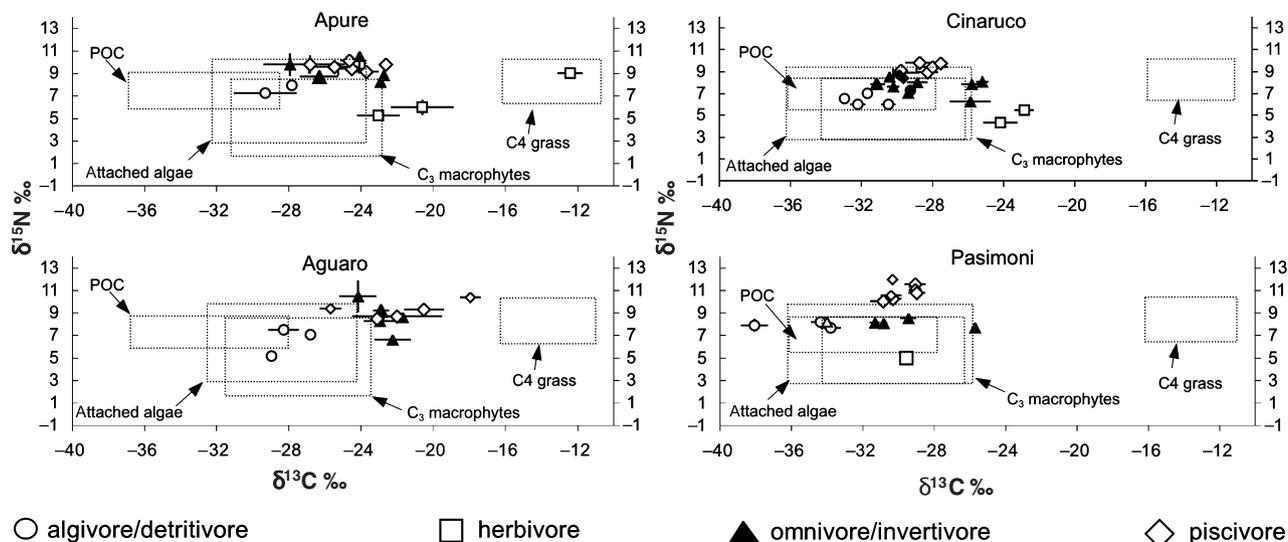
Fig. 4. Distribution of mean $\delta^{13}C$ and $\delta^{15}N$ values (and SE bars) for fishes species within trophic groups from four Venezuelan rivers. Reference boxes indicate isotopic range of basal sources collected in these rivers and reported in the literature, based on Fig. 2 and Table 2 (e.g., a range for microalgae could not be established for the Pasimoni due to small sample size). The boxed areas for C_4 grasses represent a composite range based on grass samples from the Apure, Aguaro and Cinaruco Rivers.

between-river differences that were consistent with our expectations based on between-river differences in geochemistry (Tan & Edmond 1993) and watershed vegetation. For example, the higher $\delta^{13}C$ value for CPOM from the Apure River presumably reflects a greater contribution of detrital C_4 grass carbon relative to the contribution received by the other rivers. The relative influence of biogenic (respired) CO_2 on the overall $\delta^{13}C_{DIC}$ pool may be greater in forested blackwaters than in whitewater savanna rivers, which could have contributed to between-river differences in plant and fish isotope ratios. Respired CO_2 is isotopically light, and in forested oligotrophic systems it is probably the major source for carbon fixation in aquatic photosynthesis (Rau 1978). Because of the extremely low alkalinities of blackwater rivers (Table 1) and the rarity of marine deposits in the Guayana Shield, isotopically heavy carbonates probably do not constitute a large source of inorganic carbon for photosynthesis. Pasimoni $\delta^{13}C_{DIC}$ was −16.6‰, which lies within the range of values reported for other blackwater rivers (Tan & Edmond 1993).

In contrast to black waters, savanna white waters, like the Apure River, probably have DIC pools with smaller proportions of respired CO_2, and larger CO_2 inputs from dissolution of carbonate-rich soils and atmospheric exchange that would contribute to an isotopically heavier DIC pool (Tan & Edmond 1993). Although we did not acquire $\delta^{13}C_{DIC}$ samples for the Apure, reported values in the dry season are −8.7‰ (Tan & Edmond 1993). Assuming these $\delta^{13}C_{DIC}$ estimates are accurate, DIC is about 8‰ higher in the Apure than the Pasimoni. Due to the conservative

nature of carbon isotopes in metabolic exchange, these differences are at least partially responsible for the isotopic distinction between blackwater and whitewater fishes. As expected, the $\delta^{13}C_{DIC}$ of the dilute blackwater Cinaruco River (−11.8‰) was intermediate between the above extremes, probably reflecting the influence of low alkalinity moderated by low biogenic input or high atmospheric exchange. Nevertheless, Cinaruco fishes were more similar isotopically to fishes from the blackwater Pasimoni River in the Rio Negro Basin than fishes from the Apure River, an Orinoco tributary located <200 km to the north of the Cinaruco.

Sources of whitewater fish production

Most fishes in the Apure system could not be assigned to a particular basal source, because benthic algae, POC and C_3 plants had broadly overlapping ranges for $\delta^{13}C$. One clear exception was the herbivorous anostomid *Schizodon isognathus*, which was estimated to have assimilated almost all of its carbon from C_4 grasses. Attached algae $\delta^{13}C$ (mean −27.5‰) was only slightly higher than the dominant C_3 macrophyte (*Eichhornia azurea*, −29.3‰), and many fishes could have assimilated a combination of these sources, thereby obscuring a direct trophic connection to either source. In other tropical freshwaters, attached algae show a large range of $\delta^{13}C$ (ca −20.0‰ to −34‰) that varies with season and habitat (Hamilton & Lewis 1992; Forsberg *et al.* 1993; Hecky & Hesslein 1995). Our most extensive algal sampling came from the Cinaruco River where $\delta^{13}C$ of benthic microalgae

Table 4. Estimates of C_4 grass carbon (with 95% CI for values based on multiple samples) assimilated by fish collected from four rivers in Venezuela.

	Apure	Aguaro	Cinaruco	Pasimoni
Herbivores				
Schizodon isognathus	100 (95.2–100)			
Myleus rubripinnis			46.4 (38.8–54.0)	8.8 (3.7–13.9)
Mylossoma duriventre	58.6 (32.1–85.0)			
Piaractus brachypomus	45.3 (28.9–61.7)		38.7	
Detritivores				
Curimata vittatus				0
Cyphocharax spilurus			0	0
Ancistrus sp.			11.0	
Dekeyseria sp.			3.9 (0–9.8)	
Lasiancistrus sp.				0
Rineloricaria caracasensis	18.8 (14.6–23.0)	24.3 (20.1–28.5)		
Prochilodus mariae	10.5 (0–34.9)	12.7		
Semaprochilodus kneri		16.0 (5.6–26.4)	0	
Semaprochilodus laticeps			0	
Omnivores				
Laemolyta sp.			0.6 (0–6.7)	0
Leporinus sp.			0	0
Tatia sp.		38.8		
Brycon sp.	39.2	45.9 (40.4–51.3)	33.1 (28.0–38.3)	
Byconops sp.		52.5		9.9 (3.7–16.2)
Chalceus macrolepidotus				30.4 (27.3–33.5)
Moenkausia dichroura	18.2			
Tetragonopterus chalceus			13.3 (5.5–21.0)	
Triportheus angulatus	45.9		29.8 (21.5–38.2)	
Caenotropis labrynthicus			5.5 (2.0–10.9)	
Aequidens pulcher	47.0			
Caquetia kraussi	24.3 (0–51.0)			
Geophagus sp.			29.3 (12.9–45.6)	
Heros sp.		45.9 (35.1–56.6)	9.9	
Satanoperca daemon		49.7 (35.7–63.7)		2.2 (0–6.1)
Orinocodoras eigenmanni			4.4	
Piscivores				
Acestrorhynchus sp.			8.8	12.2 (6.1–18.2)
Pygocentrus cariba	47.5 (42.0–53.1)			
Serrasalmus gouldingi				12.2 (5.8–18.5)
Serrasalmus manueli			20.4 (15.5–25.4)	
Cichla intermedia			7.7 (4.2–11.3)	
Cichla orinocensis		44.8	7.2 (2.3–12.1)	
Cichla temensis			16.0 (8.5–23.6)	11.6 (4.0–19.1)
Crenicichla sp. af. *lugubris*		59.1 (42.1–76.1)	13.8	1.7 (0–11.9)
Boulengerella lucius			7.2 (0–30.9)	
Hydrolycus pectoralis				11.6 (8.6–14.6)
Hydrolycus armatus	37.0 (27.6–46.4)	30.4 (22.9–37.9)	17.7 (4.9–30.5)	5.0
Hoplerythrinus unitaeniatus		73.5 (66.4–80.6)		
Hoplias malabaricus	41.4	50.8 (2.6–99.1)		5.0 (0–12.0)
Psuedoplatystoma fasciatum	31.5			4.4
Plagioscion squamosissimus	36.5 (28.1–44.8)			

Estimates are percentages from a two-source mixing model using $\delta^{13}C$ of fish muscle tissue and values for C_4 grasses and the mean of particulate organic carbon, attached algae and C_3 macrophytes from Neotropical whitewater and blackwater rivers from values given in Table 2(a).

ranged from −29.0‰ to −36.0‰ ($n = 12$). Algal samples from the Orinoco floodplain reveal an even wider range, from −24.2‰ (epiphyton) to −37.2‰ (seston) (Hamilton & Lewis 1992). Several studies have shown significant seasonal variation in benthic algal isotopic ratios in rivers of North America (Fisher *et al.* 2001; Herwig *et al.* 2004). Both carbon and nitrogen isotopic ratios of benthic cyanobacteria (*Cladophora*) varied between spring and late summer in floodplain lakes of temperate Australia (Bunn & Boon 1993). In the Apure and Aguaro rivers, the specialised periphyton grazer *Rineloricaria caracasensis* had $\delta^{13}C$ values (−27.8‰ and −26.8‰ respectively) that closely matched those of local benthic algae samples. Most Apure fishes had $\delta^{13}C$ closer to attached algae than C_3 macrophytes.

The role of phytoplankton as a carbon source in the Apure food web is unresolved. Our $\delta^{13}C_{POC}$ was 3.5‰ lower than zooplankton (Table 2), and the detritivorous characiform *Prochilodus mariae* had the next lowest value (Table 3). Our single POC estimate (−32.8‰) did not permit examination of

variation in $\delta^{13}C_{POC}$ in this system. Tan & Edmond (1993) recorded $\delta^{13}C_{POC}$ between $-24.4‰$ and $-27.9‰$ from the Apure, and $-24.1‰$ to $-28.2‰$ from the whitewater Meta River, another major river draining the piedmont and llanos of Columbia and Venezuela. Applying this higher range would account for most $\delta^{13}C$ values for Apure fishes. However, this range overlaps that of $\delta^{13}C$ of attached algae and some C_3 macrophytes, thereby offering little resolution of carbon source for fishes in whitewaters. Given the above patterns, and the fact that algae provide a higher quality protein source than C_3 and C_4 macrophytes (Table 2 in Forsberg *et al.* 1993), algae appear to be a principal production source of fish biomass in tropical whitewater rivers during the dry season.

Phytoplankton carbon has been shown to be an important carbon source for fishes in other Neotropical rivers in which $\delta^{13}C$ of phytoplankton was lower than values recorded for the Apure River. Among Amazonian floodplain lakes, mean $\delta^{13}C$ of phytoplankton for 40 samples was $-33.3‰$, with only a few values near $-28.0‰$ (Fig. 2 in Forsberg *et al.* 1993). However, those samples were collected from floodplain lakes in drainages with variable geochemical attributes (e.g., blackwater Rio Negro and whitewater Rio Solimoes), and it is therefore unclear if these $\delta^{13}C$ values would be representative of a savanna river like the Apure. In a study of the Orinoco River floodplain, phytoplankton ranged from -34.0 to $-37.2‰$, and many fishes had $\delta^{13}C$ within this range (Hamilton *et al.* 1992; Hamilton & Lewis 1992). However, this site differed from the Apure in having less alkalinity (190–380 $\mu eq·l^{-1}$), lower pH, and greater water clarity (Hamilton & Lewis 1992). These factors likely induce less ^{13}C enrichment in the inorganic carbon pool, relative to conditions in the Apure. This appears to be the case, as fishes from the Orinoco floodplain (Hamilton *et al.* 1992) had lower $\delta^{13}C$ than fishes from the Apure.

Sources of blackwater fish production

Fishes in blackwater rivers seem to assimilate little carbon from C_4 grasses. This would be expected in the heavily forested Pasimoni watershed where grasses are scarce, but not over the vast floodplains of the Cinaruco River where C_4 grasses are dominant. The maximum estimate of C_4 carbon contribution for Cinaruco fishes was for the characiforms *Myleus rubripinnis* and *Piaractus brachypomus*, two genera that feed on seeds, fruits and leaves of terrestrial vegetation (Goulding 1980). Therefore, our carbon isotopic data add to the growing evidence that C_4 grasses play a relatively minor role in nutritional support of tropical freshwater fishes (Araujo-Lima

et al. 1986; Hamilton *et al.* 1992; Forsberg *et al.* 1993).

Our carbon and nitrogen isotopic data indicate that blackwater food webs are mostly dependent upon autochthonous primary production. Although most fishes from the Pasimoni and Cinaruco rivers had mean $\delta^{13}C$ values within the range of C_3 vascular plants measured in this study or reported in the literature (Hedges *et al.* 1986; Hamilton & Lewis 1992; Forsberg *et al.* 1993), most detritivores/algivores had $\delta^{13}C$ values more negative than the C_3 vascular plants we sampled. However, other (unsampled) C_3 terrestrial sources with low $\delta^{13}C$ cannot be discounted as possible energy sources. Forest floor vegetation near San Carlos del Rio Negro (same forest zone as Pasimoni, within 100 km) had low $\delta^{13}C$ (down to $-37.5‰$), presumably resulting from carbon fixation of depleted CO_2 derived from soil respiration in this heavily forested region (Medina *et al.* 1986). Therefore, ^{13}C-depleted terrestrial plants could partially account for low $\delta^{13}C$ values of Pasimoni fishes. Terrestrial vegetation dominates carbon inputs to blackwater rivers (580–790 $g·m^{-2}·yr^{-1}$ for Río Negro *igapó* forests, Adis *et al.* 1979), and secondary productivity in lowland blackwater streams has been linked to large inputs of allochthonous detritus and DOC from bordering floodplains (Walker 1985; Wallace *et al.* 1987). Yet in most blackwater systems, aquatic macrophytes have low biomass, and most terrestrial vegetation has poor nutritional quality (Forsberg *et al.* 1993). In tropical waters, particulate and DOC from macrophytes may enter the metazoan food web after processing by the microbial loop, in which microbes consume detritus and in turn serve as a food resource for elements of the metazoan food web (Benner *et al.* 1995; Waichman 1996), a pathway that may be particularly dominant in blackwater systems (Meyer 1990). In Orinoco River floodplain lakes, most organic carbon (ca 98%) is thought to enter a microbial dead end, with little contribution to consumers (Lewis *et al.* 2001), yet it is unclear if this is the case for black waters. Our $\delta^{15}N$ data of C_3 vascular plants and fishes suggest that microbial processing would have to be an important step in this food web linkage. Vascular plants from the Pasimoni had very low $\delta^{15}N$ ($-1.0‰$ to $0.5‰$, Table 2) and direct assimilation of this source alone would result in primary consumers with $\delta^{15}N$ values in the range 1.5–3.0‰ (assuming an average trophic enrichment of 2.5‰, Lewis *et al.* 2001). Yet all Pasimoni detritivores had $\delta^{15}N$ values $>8‰$, suggesting either that C_3 macrophytes were not directly assimilated to any great extent, or that plant detritus is colonised by a microbial community comprising two to three trophic levels (essentially conserving the substrate carbon signature

but enriching ^{15}N), which in turn is assimilated by detritivores.

Benthic algae are probably an important carbon source supporting blackwater food webs. The δ^{13}C of algae on firm substrates were similar to those of several fish species in the Pasimoni and Cinaruco rivers. Detritivorous/algivorous fishes scrape/suck organic material from substrates, and should have δ^{13}C values reflecting a bulk sample of periphyton. However, mean δ^{13}C values for Pasimoni detritivores were 3.1‰ lower than periphyton samples from this site, suggesting either the importance of another carbon source with lower δ^{13}C, or that Pasimoni periphyton samples have a lower δ^{13}C range than reported here. Phytoplankton is the most probable alternate carbon source. Although direct grazing on plankton is unlikely for these benthivorous fishes, phytoplankton may become available when algal cells settle onto substrates (Winemiller *et al.* 2006). Although we were not able to collect sufficient phytoplankton samples from the Pasimoni, it can be estimated from $\delta^{13}C_{DIC}$ samples (−16–6‰). Assuming river phytoplankton have an isotopic fractionation range of 21.2–24.3‰ from a DIC source (Tan & Strain 1983), Pasimoni phytoplankton would have δ^{13}C values between −37.8‰ and −40.9‰, thereby accounting for low δ^{13}C in Pasimoni detritivores/algivores.

Sources of clearwater fish production

Counter to our expectation that the clearwater Aguaro River would have a fish assemblage isotopically intermediate between whitewater and blackwater rivers, most fishes in the Aguaro had higher δ^{13}C than ecologically similar fishes from the other rivers. Some of the Aguaro C_3 sources had higher δ^{13}C than sources in the other three systems. However, even if most Aguaro fishes assimilated the C_3 source with the highest δ^{13}C (littoral sedge, −24.1‰), they still would have to assimilate some portion of carbon derived from C_4 grasses. Phytoplankton density was extremely low as evidenced by extremely high water transparency; however, benthic microalgae and filamentous green algae were present on submerged macrophytes and rocks.

Aguaro detritivores/algivores had δ^{13}C values similar to benthic algae, but in order to account for their isotopic signatures, most invertivorous/omnivorous and piscivorous fishes must have assimilated some C_3 macrophyte carbon and, in some cases, C_4-grass carbon. A possible route of this material may be from consumption of herbivorous insects, such as grasshoppers, that feed on savanna grasses. Savannas of the llanos generally have high orthopteran densities, and the reliance of some species on C_4 grasses is confirmed by direct feeding observations and the similarity of

their δ^{13}C (e.g., −12.9‰ for a composite sample of several orthopterans from the Cinaruco site) with that of local C_4 grasses (−11.6 ± 1.9‰). However, terrestrial insects as a major dietary component probably cannot explain the high δ^{13}C of the cichlids *Heros* sp. and *Satanoperca daemon*, species that consume mostly aquatic invertebrates. Beyond these general patterns, it is difficult to resolve trophic linkages in the Aguaro.

Isotopic discrimination of trophic groups and food web subsidies

Our analysis emphasizes the need to evaluate biogeochemical differences among fluvial ecosystems when making ecological comparisons and interpretations based on isotopic data. A multivariate analysis of fishes from these four rivers revealed that species could be classified into the appropriate trophic guild based on their stable isotope and C/N ratios, but only when ecosystem type was included as a factor or local assemblages were analysed separately (Jepsen & Winemiller 2002). Fishes from the blackwater Pasimoni River had the most isotopically distinctive guilds that yielded greatest classification success. Analysis of samples collected from rivers with divergent biogeochemical attributes likely would produce erroneous estimates of carbon sources supporting consumer biomass.

All our samples were collected during the annual dry season when water levels were lowest. The effect of seasonal variation in isotopic signatures of production sources and consumers in tropical rivers needs to be investigated. Several producer and consumer taxa in backwater habitats of two temperate North American rivers revealed significant seasonal variation in both carbon and nitrogen isotopic ratios (Herwig *et al.* 2004). Ontogenetic niche shifts are another potential source of variation for consumer isotopic signatures. It is important to emphasize that our findings are based on the examination of adult size classes.

Isotopic variation among consumers that is associated with regional or ecosystem characteristics affords opportunities to examine material transfer between regions (Anderson & Polis 1998). Some Neotropical fishes (e.g., Prochilodontidae) undergo annual long-distance migrations, and a few species forage in different water types during different life-cycle stages and hydrologic periods (Winemiller & Jepsen 1998, 2004; Benedito-Cecilio & Araujo-Lima 2002). In the Cinaruco River, the largest size classes of the resident piscivore *Cichla temensis* consume juvenile *Semaprochilodus kneri* that immigrate seasonally into the blackwater river from nursery areas in productive floodplain habitats of the whitewater Orinoco River. During several months each year, *Semaprochilodus kneri* are the dominant dietary component of large

Cichla temensis (Jepsen *et al.* 1997). Tissues of smaller *Semaprochilodus kneri* have higher δ^{13}C than the larger conspecifics (Winemiller & Jepsen 2004). Thus carbon from both blackwater (*in situ*) and whitewater (spatial subsidies) production sources are assimilated by large *Cichla* and influence δ^{13}C values of its tissues. Based on a simple mixing model, it was estimated that about 45% of the carbon assimilated by large *Cichla* during the annual falling-water period is derived from whitewater floodplains of the Orinoco, an estimate that closely matched the volumetric consumption of *Semaprochilodus kneri* based on stomach contents analysis (Winemiller & Jepsen 2004). Future studies of tropical river food webs should be able to exploit isotopic differences of basal sources, watersheds and species life histories to address a variety of ecological questions.

Acknowledgements

References

Adis, J., Furch, K. & Irmler, U. 1979. Litter production of a Central Amazonian black water inundation forest. Tropical Ecology 20: 236–245.

Agostinho, A.A., Thomaz, S.M., Minte-Vera, C.V. & Winemiller, K.O. 2000. Biodiversity in the high Paraná River floodplain. In: Gopal, B., Junk, W.J. & Davis, J.A., eds. Wetlands biodiversity. Leiden, the Netherlands: Backhuys Publishers, pp. 89–118.

Anderson, W.B. & Polis, G.A. 1998. Marine subsidies of island communities in the Gulf of California: evidence from stable carbon and nitrogen isotopes. Oikos 81: 75–80.

Araujo-Lima, C., Forsberg, B.R., Victoria, R. & Martinelli, L.A. 1986. Energy sources for detritivorous fishes in the Amazon. Science 234: 1256–1258.

Arrington, D.A. & Winemiller, K.O. 2002. Preservation effects on stable isotope ratios of fish muscle. Transactions of the American Fisheries Society 131: 337–342.

Barbarino-Duque, A., Taphorn, D.C. & Winemiller, K.O. 1998. Ecology of the coporo, *Prochilodus mariae* (Characiformes, Prochilodontidae), and status of annual migrations in western Venezuela. Environmental Biology of Fishes 53: 33–46.

Bayley, P.B. & Petrere, M. Jr 1989. Amazon fisheries: assessment methods, current status, and management options. In: Dodge, D.P., ed. Proceedings of the international large river symposium. Ottawa, ON: Canadian Special Publication in Fisheries and Aquatic Sciences, vol. 106, pp. 385–398.

Benedito-Cecilio, E. & Araujo-Lima, C.A.R.M. 2002. Variation in the carbon isotope composition of *Semaprochilodus insignis*, a detritivorous fish associated with oligotrophic and eutrophic Amazonian rivers. Journal of Fish Biology 60: 1603–1607.

Benner, R., Opsahl, S., Chin-Leo, G., Richey, J.E. & Forsberg, B.R. 1995. Bacterial carbon metabolism in the Amazon River system. Limnology and Oceanography 40: 1262–1270.

Bunn, S.E. & Boon, P.I. 1993. What sources of organic carbon drive food webs in billabongs? A study based on stable isotope analysis. Oecologia 96: 85–94.

Cotner, J.B., Montoya, J.V., Roelke, D.L. & Winemiller, K.O. 2006. Seasonal variability in benthic and water column productivity in a Venezuelan llanos river. Journal of the North American Benthological Society 25: 171–184.

Craig, H. 1957. Isotopic standards for carbon and oxygen and correction factors for mass-spectrometric analysis of carbon dioxide. Geochimica et Cosmochimica Acta 12: 133–149.

Depetris, P.J & Paolini, J.E. 1991. Biogeochemical aspects of South American rivers: the Paraná and Orinoco. In: Degens, E.T., Kempe, S. & Richey, J.E., eds. Biogeochemistry of major world rivers. SCOPE Report 42. Chichester/New York: Wiley and Sons, Ltd, pp. 105–125.

Dettmers, J.M., Wahl, D.H., Soluk, D.A. & Gutreuter, S.G. 2001. Life in the fast lane: fish and foodweb structure in the main channel of large rivers. Journal of the North American Benthological Society 20: 255–265.

Edmond, J.M., Palmer, M.R., Measures, C.I., Grant, B. & Stallard, R.F. 1995. The fluvial geochemistry and denudation rate of the Guyana Shield in Venezuela, Colombia, and Brazil. Geochimica et Cosmochimica Acta 59: 3301–3325.

Edmond, J.M., Palmer, M.R., Measures, C.I., Brown, E.T. & Yuh, Y. 1996. Fluvial geochemistry of the eastern slope of the northeastern Andes and its foredeep in the drainage of the Orinoco in Colombia and Venezuela. Geochimica et Cosmochimica Acta 60: 2949–2976.

Fisher, S.J., Brown, M.L. & Willis, D.W. 2001. Temporal food web variability in an upper Missouri River backwater: energy origination points and transfer mechanisms. Ecology of Freshwater Fish 10: 154–167.

Forsberg, B.R., Araujo-Lima, C., Martinelli, M.A., Victoria, R.L. & Bonassi, J.A. 1993. Autotrophic carbon sources for fish of the central Amazon. Ecology 74: 643–652.

Fry, B. & Sherr, E.B. 1984. δ^{13}C measurements as indicators of carbon flow in marine and freshwater ecosystems. Contributions in Marine Science 27: 13–47.

Goulding, M. 1980. The fishes and the forest: explorations in Amazonian natural history. Berkeley, CA: University of California Press.

Goulding, M., Carvalho, M.L. & Ferreira, E.G. 1988. Río Negro: rich life in poor water. The Hague: SPB Academic Publishing.

Hamilton, S.K. & Lewis, W.M. Jr 1992. Stable carbon and nitrogen isotopes in algae and detritus from the Orinoco River floodplain, Venezuela. Geochimica et Cosmochimica Acta 56: 4237–4246.

Hamilton, S.K., Lewis, W.M. Jr & Sippel, S.J. 1992. Energy sources for aquatic animals in the Orinoco river floodplain: evidence from stable isotopes. Oecologia 89: 324–330.

Hassan, A.A. 1980. Methodologies for extraction of dissolved inorganic carbon isotope studies: evaluation and alternatives. Reston, VA: U.S. Geological Survey Water Resources Investigations, pp. 82–86.

Hecky, R.E. & Hesslein, R.H. 1995. Contributions of benthic algae to lake food webs as revealed by stable isotope analysis. Journal of the North American Benthological Society 14: 631–653.

Hedges, J.I., Clark, W.A., Quay, P.D., Richey, J.E., Devol, A.H. & Santos, U.M. 1986. Composition and fluxes of particulate organic material in the Amazon River. Limnology and Oceanography 31: 717–738.

Herwig, B.R., Soluk, D.A., Dettmers, J.M. & Wahl, D.H. 2004. Trophic structure and energy flow in backwater lakes of two large floodplain rivers assessed using stable isotopes. Canadian Journal of Fisheries and Aquatic Sciences 61: 12–22.

Jepsen, D.B. 1999. Analysis of trophic pathways in freshwater ecosystems using stable isotope signatures. PhD dissertation. College Station, TX: Texas A&M University.

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

Jepsen, D.B., Winemiller, K.O. & Taphorn, D.T. 1997. Temporal patterns of resource partitioning among *Cichla* species in a Venezuelan blackwater river. Journal of Fish Biology 51: 1085–1108.

Junk, W. 1970. Investigations of the ecology and production-biology of the 'floating Meadows' (Paspalo-Echinochloetum) of the Middle Amazon. Amazoniana 2: 449–495.

Knöppel, J.-A. 1970. Food of central Amazonian fishes: contribution to the nutrient ecology of Amazonian rain-forest-streams. Amazoniana 2: 257–352.

Lewis, W.R. Jr, Hamilton, S.K., Lasi, M.A., Rodríguez, M. & Saunders, J.F. III 2000. Ecological determinism on the Orinoco floodplain. BioScience 50: 681–692.

Lewis, W.R. Jr, Hamilton, S.K., Rodriquez, M.A., Saunders, J.F. III & Lasi, M.A. 2001. Food web analysis of the Orinoco floodplain based on production estimates and stable isotope data. Journal of the North American Benthological Society 20: 241–254.

Lilyestrom, C. & Taphorn, D.C. 1983. Aspectos sobre la biología de la palambra *Brycon whitei* Myers & Weitzman. Revista UNELLEZ Ciencia y Tecnología 1: 53–60.

Lowe-McConnell, R.H. 1987. Ecological studies in tropical fish communities. Cambridge: Cambridge University Press.

Machado-Allison, A. 1987. Los peces de los llanos de Venezuela: Un ensayo sobre su historia natural. Caracas: Universidad Central de Venezela.

Medina, E. & Minchin, P. 1980. Stratification of $\delta^{13}C$ levels in Amazonian rain forests. Oecologia 45: 377–378.

Medina, E., Montes, G., Cuevas, E. & Rokzandic, Z. 1986. Profiles of CO_2 concentration and $\delta^{13}C$ values in tropical rain forests of the upper Rio Negro Basin, Venezuela. Journal of Tropical Ecology 2: 207–217.

Meyer, J.L. 1990. A blackwater perspective on riverine ecosystems. Bioscience 40: 643–651.

Montoya, J.V., Roelke, D.L., Winemiller, K.O., Cotner, J.B. & Snider, J. 2006. Effects of hydrological seasonality and lateral connectivity on environmental variables and algal biomass of a tropical floodplain river. Journal of the North American Benthological Society 25: 157–170.

Nico, L.G. & Taphorn, D.C. 1988. Food habits of piranhas in the low llanos of Venezuela. Biotropica 20: 311–321.

Peterson, C.C. 1997. Food webs of two Venezuelan clear-water streams with seasonal fluctuations in hydrology. Master's thesis. College Station, TX: Texas A&M University.

Phillips, D.L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia 127: 166–170.

Phillips, D.L. & Gregg, G.W. 2001. Uncertainty in source partitioning using stable isotopes. Oecologia 127: 171–179.

Phillips, D.L., Newsome, S.D. & Gregg, J.W. 2004. Combining sources in stable isotope mixing models: alternative methods. Oecologia 144: 520–527.

Rau, G. 1978. Carbon-13 depletion in a subalpine lake: carbon flow implications. Science 201: 901–902.

Reynolds, C.S. & Descy, J.-P. 1996. The production, biomass and structure of phytoplankton in large rivers. Archives fur Hydrobiologie (Supplement) 113: 161–187.

Sioli, H. 1975. Tropical rivers as an expression of their terrestrial environment. In: Golly, F.B., ed. Tropical Ecological Systems. Berlin: Springer-Verlag, pp. 275–288.

Stallard, R.F. 1985. River chemistry, geology, geomorphology, and soils in the Amazon and Orinoco basins. In: Drever, J.I., ed. The chemistry of weathering. Dordrecht: Reidel, pp. 293–316.

Tan, F.C. & Edmond, J.M. 1993. Carbon isotope geochemistry of the Orinoco basin. Estuarine Coastal and Shelf Science 36: 541–547.

Tan, F.C. & Strain, P.M. 1983. Sources, sinks and distribution of organic carbon in the St. Lawrence Estuary, Canada. Geochimica et Cosmochimica Acta 47: 125–132.

Taphorn, D.C. 1992. The characiform fishes of the Apure River drainage, Venezuela. Guanare, Venezuela: Biollania Special Publication 4, pp. 1–537.

Thorp, J.H. & Delong, M.D. 1994. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. Oikos 70: 305–308.

Thorp, J.H. & Delong, M.D. 2002. Dominance of autotrophic autochthonous carbon in food webs of heterotrophic rivers. Oikos 96: 543–550.

Victoria, R.L., Martinelli, L.A., Trivelin, P.C.O., Matsui, E., Forsberg, E.R., Richey, J.E. & Devol, A.H. 1992. The use of stable isotopes in studies of nutrient cycling: carbon isotope composition of Amazonia Varzea sediments. Biotropica 24: 240–249.

Waichman, A.V. 1996. Autotrophic carbon sources for hetero-trophic bacteria bacterioplankton in a floodplain lake of central Amazon. Hydrobiologia 341: 27–36.

Walker, I. 1985. On the structure and ecology of the microfauna in the Central Amazonian forest stream 'Igarapé da Cachoeira'. Hydrobiologia 122: 137–152.

Wallace, J.B., Benke, A.B., Lingle, A.H. & Parsons, K. 1987. Trophic pathways of macroinvertebrate primary consumers in subtropical blackwater streams. Archives fur Hydrobiologie (Supplement) 74: 423–451.

Welcomme, R.L. 1989. Floodplain fisheries management. In: Gore, J.A. & Petts, G.E., eds. Alternatives to regulated river management. Boca Raton, FL: CRC Press, pp. 209–233.

Williams, J.D., Winemiller, K.O., Taphorn, D.C. & Balbas, L. 1998. Ecology and status of piscivores in Guri, an oligotrophic tropical reservoir. North American Journal of Fisheries Management 18: 274–285.

Winemiller, K.O. 1989. Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. Environmental Biology of Fishes 26: 177–199.

Winemiller, K.O. 1990. Spatial and temporal variation in tropical fish trophic networks. Ecological Monographs 60: 331–367.

Winemiller, K.O. & Jepsen, D.B. 1998. Effects of seasonality and fish movement on tropical river food webs. Journal of Fish Biology 53(Suppl. A): 267–296.

Winemiller, K.O. & Jepsen, D.B. 2004. Migratory neotropical fish subsidize food webs of oligotrophic blackwater rivers. In: Polis, G.A., Power, M.E. & Huxel, G.R., eds. Food webs at the landscape level. Chicago, IL: University of Chicago Press, pp. 115–132.

Winemiller, K.O., Montoya, J.V., Layman, C.A., Roelke, D.L. & Cotner, J.B. 2006. Experimental demonstrations of seasonal fish effects on benthic ecology of a Neotropical floodplain river. Journal of the North American Benthological Society 25: 250–262.

Wissmar, R.C., Richey, J.E., Stallard, R.F. & Edmond, J.M. 1981. Plankton metabolism and carbon processes in the Amazon River, its tributaries, and floodplain waters, Peru-Brazil, May-June 1977. Ecology 62: 1622–1633.

Yanes, C.E. & Ramírez, A.J. 1988. Estudio geoquimico de grandes rios Venezolanos. Memoria (Sociedad de Ciencias Naturales La Salle) 48: 41–58.