

STABLE ISOTOPE ANALYSIS REVEALS FOOD WEB STRUCTURE AND WATERSHED IMPACTS ALONG THE FLUVIAL GRADIENT OF A MESOAMERICAN COASTAL RIVER

KIRK O. WINEMILLER,^{a*} DAVID J. HOEINGHAUS,^{a,b} ALLISON A. PEASE,^a PETER C. ESSELMAN,^c RODNEY L. HONEYCUTT,^{a†} DONMALE GBANAADOR,^a ELIZABETH CARRERA^a and JOSIAH PAYNE^a

^a *Section of Ecology, Evolution and Systematic Biology, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258, USA*

^b *Department of Biological Sciences and the Institute of Applied Sciences, University of North Texas, 1155 Union Circle #310559, Denton, TX 76203-5017, USA*

^c *Department of Fisheries and Wildlife, Michigan State University, 13 Natural Resources Building, East Lansing, MI 48824-1222, USA*

ABSTRACT

Ecosystem processes and biological community structure are expected to change in a relatively predictable manner along fluvial gradients within river basins. Such predictions are heavily based on temperate rivers, and food web variation along fluvial gradients in Mesoamerican rivers has received limited attention. In this study, we analyzed carbon and nitrogen stable isotope ratios of basal carbon sources and dominant consumer species to examine aquatic food web structure along the fluvial gradient of the Monkey River Basin, Belize. Similar to previous studies in other regions, consumer species richness and functional diversity increased along the downstream fluvial gradient, due in part to the addition of estuarine species in lower reaches and increasing diversity of piscivorous species along the gradient. Aquatic food webs in upstream reaches were primarily supported by allochthonous production sources, and in-stream sources increased in importance along the downstream gradient. Our study system traversing the Maya Mountain Marine Area Transect also provided a unique opportunity to test the utility of primary consumer $\delta^{15}\text{N}$ as an indicator of watershed impacts within a tropical basin with a diverse biota and a different type of agricultural impact than typically studied (i.e. banana plantations vs. tilled row cropping). As expected, primary consumer $\delta^{15}\text{N}$ at sites draining impacted watersheds was enriched compared to values from forested reference sites. Assessment of primary consumer $\delta^{15}\text{N}$ may be a feasible option for monitoring watershed impacts on aquatic food webs in service of the ridge-to-reef conservation strategy adopted for this watershed as well as in other tropical river basins. Copyright © 2010 John Wiley & Sons, Ltd.

KEY WORDS: banana plantation; Belize; Bladen River; fish; fluvial gradient; Maya Mountain Marine Area Transect; pollution; river continuum; trophic ecology

Received 15 August 2009; Revised 14 December 2009; Accepted 4 March 2010

INTRODUCTION

Ecologists have long recognized that ecosystem processes and biological community structure change along fluvial gradients within river basins (e.g. Hynes, 1970). Availability of production sources, organismal responses to discharge variation and species interactions all vary in relation to abiotic environmental factors that undergo transition from headwaters to downstream reaches and coastal habitats. The structure of aquatic food webs also is expected to change along the course of a river basin from inland tributaries to the sea (Power and Dietrich, 2002). For example, the importance of terrestrial versus in-stream

sources of carbon varies with position along the upstream–downstream fluvial gradient of stream systems (Vannote *et al.*, 1980; Thorp and Delong, 1994, 2002). In watersheds exposed to urbanization or agricultural land use, pollution and anthropogenic nutrient enrichment also can affect resources and alter aquatic food webs (e.g. Clements *et al.*, 2000; DeBruyn and Rasmussen, 2002; Anderson and Cabana, 2005; Simon *et al.*, 2007). A number of studies have shown that alteration of the surrounding landscape for agriculture, urban development, and other uses can have important effects on the ecological integrity of rivers (reviewed in Allan, 2004).

Ratios of stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotopes have been used extensively to examine aquatic food webs, and they can reveal variation in food web structure across longitudinal fluvial gradients (e.g. Hoeinghaus *et al.*, 2007a; Saito *et al.*, 2007) as well as in relation to anthropogenic allochthonous inputs from the watershed (e.g. Cabana and Rasmussen, 1996; Anderson and Cabana,

*Correspondence to: Kirk O. Winemiller, Section of Ecology, Evolution and Systematic Biology, Department of Wildlife and Fisheries Sciences, Texas A&M University, College Station, TX 77843-2258.
E-mail: k-winemiller@tamu.edu

†Present address: Natural Science Division, Seaver College, Pepperdine University, 24255 Pacific Coast Highway, Malibu, CA 90263 USA.

2005; Saito *et al.*, 2008). Here we examine variation in food web structure in the Monkey River, Belize, at several locations spanning a longitudinal fluvial gradient from a headwater stream to the coast. Previous studies have documented changes in fish assemblage composition and species diversity along longitudinal fluvial gradients in Mesoamerican rivers (Winemiller and Leslie, 1992; Rodiles-Hernández *et al.*, 1999; Esselman *et al.*, 2006). However, food web studies of Mesoamerican streams are scarce, and stable isotope analyses have been conducted for very few aquatic communities in the region (Kilham and Pringle, 2000; Verburg *et al.*, 2007). Using stomach contents of fishes, Winemiller (1990) examined temporal variation in food web properties in two Costa Rican streams, but little is known about corresponding changes in tropical fluvial food webs at the watershed scale.

We employed stable isotope methods to examine aquatic food web structure and potential local watershed influences along a longitudinal gradient in the Monkey River Basin. The Monkey River Basin in southeastern Belize is the largest basin in the Maya Mountain Marine Area Transect (MMMAT; Heyman and Kjerfve, 1999), a corridor of reserves and private lands extending from the ridge of the Maya Mountains to the Belize Barrier Reef in the Caribbean Sea. Sixty-two per cent of the Monkey River Basin is protected in ecological reserves. The Upper Bladen branch of the Monkey River drains a nearly pristine forested landscape within the Bladen Nature Reserve. In contrast, the Swasey catchment yields more than 50% of the bananas grown in Belize. In addition to revealing potential influence of land characteristics on isotopic composition of aquatic biota, our description of aquatic food webs along this corridor will provide important basic information for the unique 'ridge-to-reef' conservation strategy that was adopted for this region (Heyman and Kjerfve, 1999).

METHODS

Study area

The study region is the Monkey River watershed, including its two largest tributaries, the Bladen and Swasey Rivers, in southeastern Belize (Figure 1). This watershed delivers freshwater from the Maya Mountains to the coast adjacent to the Belize Barrier Reef and the Sapodillas Cayes Marine Reserve. During December 2005 and January 2006, seven areas were surveyed: (1) Firetail Creek, a small tributary of the Upper Bladen River draining pristine forested hills within the Bladen Nature Preserve; (2) Upper Bladen River, a segment of pristine aquatic habitat located just downstream from the southeast border of the Bladen Nature Preserve; (3) Upper Swasey River 3 km upstream of Red Bank Village; (4) Lower Swasey River, a segment

adjacent to banana plantations; (5) Lower Bladen River 1–2 km upstream from the junction with the Swasey River which delivers waters draining a heavily agricultural catchment; (6) Lower Monkey River, an estuarine segment 0.25–2 km above the mouth; (7) Mangrove and seagrass habitats in an area 0.25–2 km north of the Monkey River mouth.

The most upstream site, Firetail Creek, has a relatively narrow channel, steep bed gradient, large coarse substrate and closed canopy. The Upper Bladen River also drains the pristine forested terrain of the Bladen Nature Reserve, but has a wider channel, more heterogeneous substrate and receives more sunlight. The Lower Bladen River has a lower gradient, finer substrates and a more impacted watershed than the Upper Bladen. The Upper Swasey is slightly larger than the Bladen Branch, and flows through a constrained channel dominated by bedrock and boulder substrates covered with aquatic macrophytes (*Marathrum oxycarpum*). In the coastal plain the river widens, the macrophytes disappear and substrates gradually change to fine gravel and sand. Banana agriculture occurs in patches on the floodplain adjacent to the Bladen and Swasey Branches in the upper portion of the coastal plain, and is more extensive on the Swasey branch (Figure 1). The Lower Monkey River is under tidal influence. Its broad channel is lined with sedges and has little canopy cover. Sediments are fine, and diverse freshwater and estuarine fishes and macroinvertebrates inhabit open waters of the channel, dense beds of floating plants and lentic secondary channels. Adjacent coastal mangrove and seagrass habitats at the mouth of the Monkey River are broad and shallow and contain estuarine and marine species.

Sample collection and stable isotope analyses

The most conspicuous and abundant fish species, invertebrates, and in-stream and riparian producers were collected at each survey site. Benthic algae, detritus, aquatic and terrestrial plants (including seeds) were crushed and preserved in salt. For fish and invertebrate specimens, muscle tissue (taken from large specimens following euthanasia via whole body immersion in MS 222) or whole body minus viscera or shell (for small specimens) was obtained and preserved in salt following Arrington and Winemiller (2002). In the lab, tissue samples were soaked and rinsed in distilled water to remove all salt, then dried in an oven at 60°C for 48 h (Arrington and Winemiller, 2002). Dried samples were ground to a fine powder with mortar and pestle then stored in clean glass vials. Sub-samples of each ground sample were weighed to the nearest 0.01 mg and pressed into Ultra-Pure tin capsules (Costech, Valencia, CA), and sent to the Analytical Chemistry Laboratory of the Institute of Ecology, University of Georgia, for analysis of

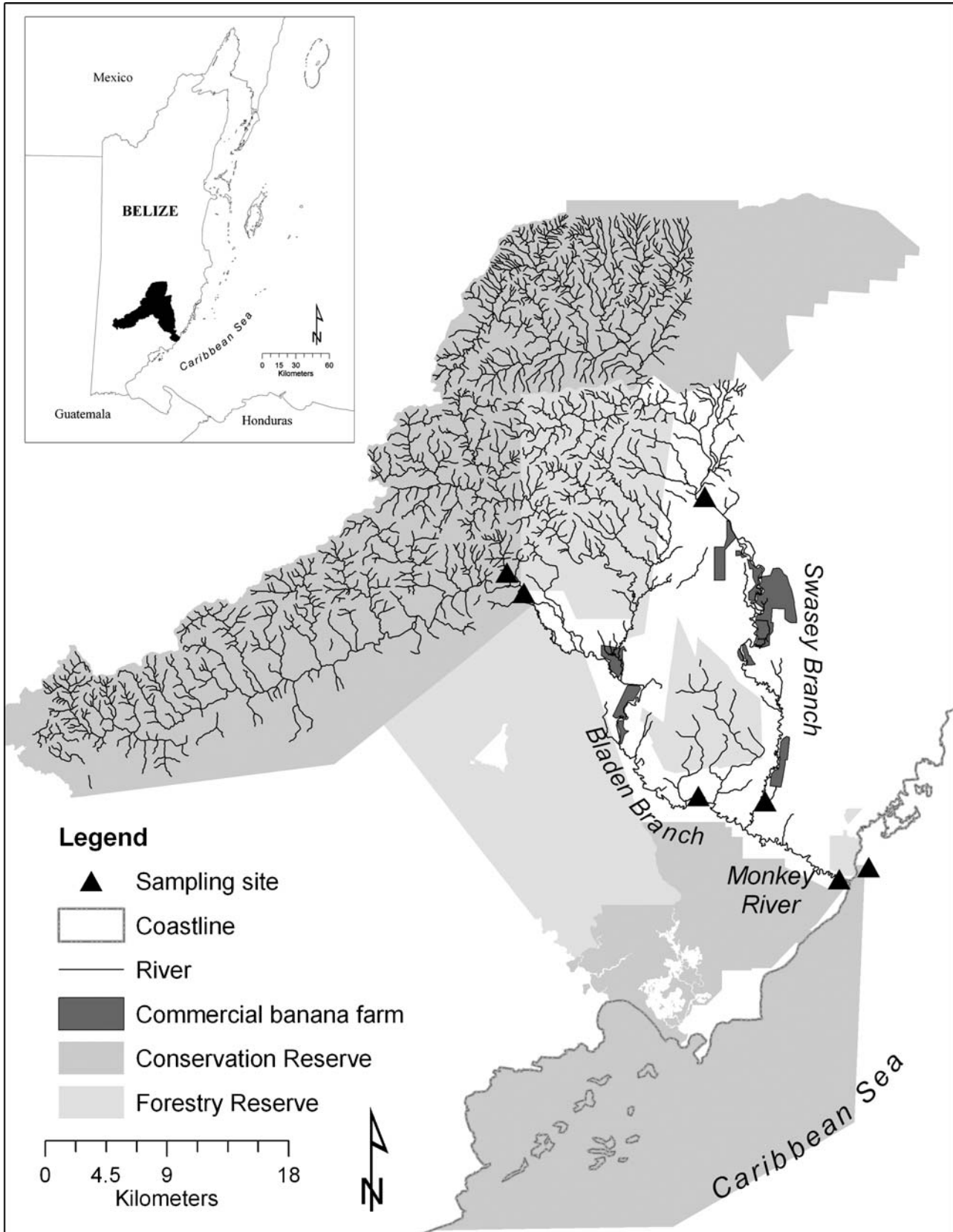


Figure 1. Map of the Monkey River drainage basin in Belize, depicting sampling locations, conservation and forestry reserve boundaries and banana plantations

carbon and nitrogen stable isotope ratios. Results are expressed in delta notation (parts per thousand deviation from a standard material): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000$; where $R = {}^{13}\text{C}/{}^{12}\text{C}$ or ${}^{15}\text{N}/{}^{14}\text{N}$. The standard material for carbon is Pee Dee Belemnite (PDB) limestone, and the nitrogen standard is atmospheric nitrogen. Standard deviations of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analyses were 0.09 and 0.12‰, respectively for animal replicates, and 0.44 and 0.48‰, respectively for primary producer replicates.

Statistical analyses

Carbon isotopic signatures of dietary items are conserved within 1‰ in consumer tissues (McCutchan *et al.*, 2003), and allow evaluation of the relative importance of carbon sources ultimately supporting secondary production when source isotopic values are distinct (Peterson and Fry, 1987). Nitrogen isotope ratios of consumer tissues can be used to determine consumer trophic position because they are typically enriched 2.5–3.4‰ relative to their food (Post 2002; Vanderklift and Ponsard, 2003). Bi-plots of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of basal resources and consumers were used to depict food web structure and patterns of isotopic variation along the longitudinal fluvial gradient of the Bladen–Monkey River. Relative positions of taxa on the $\delta^{13}\text{C}$ axis allow for comparison of the basal carbon sources supporting secondary production in the species assemblages, and the positions of taxa within communities for $\delta^{15}\text{N}$ indicate trophic position (Peterson and Fry, 1987). Carbon and nitrogen isotope ratios of consumer assemblages (fishes and invertebrates) were compared among sites using MANOVA. Bonferroni-corrected pairwise comparisons were calculated for significant main effects. Observed differences in assemblage-level C and N isotope ratios of consumers were interpreted relative to source isotope ratios at each site along the fluvial gradient.

Differences in baseline $\delta^{15}\text{N}$ among local species assemblages may indicate anthropogenic nitrogen inputs, such as from sewage or agricultural practices (Cabana and Rasmussen, 1996; Anderson and Cabana, 2005). Human population density and agricultural intensity in watersheds exhibit a positive correlation with baseline $\delta^{15}\text{N}$ in aquatic food webs. We used herbivorous and detritivorous fishes as indicators of the N baseline at each site because they are generally larger and more mobile primary consumers than invertebrates, and therefore provide greater temporal and spatial integration of source N variability (Cabana and Rasmussen, 1996; Hoeninghaus *et al.*, 2008). Nitrogen isotope signatures of herbivorous and detritivorous fishes were compared among sites using ANOVA. Two samples of white mullet, *Mugil curema*, collected at the Lower Monkey River were excluded from this analysis because their carbon

and nitrogen isotope signatures indicated that they recently moved into this site from the seagrass/mangrove zone (Tables I and II). Differences in surface geology affect baseline N conditions between the Bladen and Swasey watersheds (Esselman *et al.*, 2006). Therefore, in our comparisons of baseline $\delta^{15}\text{N}$ in aquatic food webs in relation to watershed impacts, comparatively pristine upper reaches of each branch (i.e. Upper Swasey, Firetail Creek and Upper Bladen) are considered reference conditions for lower portions of the respective watersheds that are impacted by banana plantations and human populations.

RESULTS

A total of 269 samples were collected for isotope analysis, comprised by 53 fish species, 6 general invertebrate groups, and 9 basal carbon source groups (Tables I and II). Fewer consumer species were present, and thus collected, at upland sites (Firetail Creek, Upper Bladen and Upper Swasey) than at middle and lower reaches of the watershed (Tables I and II, Figure 2). Species and feeding guild composition of consumer assemblages changed along the fluvial gradient. Sampling sites located lower in the fluvial gradient (Lower Monkey River, seagrass/mangrove) had greater numbers of estuarine and marine species (e.g. Great barracuda, Mayan sea catfish, several species of grunt), and piscivores became more conspicuous components of the assemblages when moving from upland sites towards the coast (Tables I and II, Figure 2).

The majority of basal sources collected along the fluvial gradient had carbon isotope ratios between –30 and –20‰ (Table I). $\delta^{13}\text{C}$ of riparian vegetation was approximately –30‰ across all sites, and coarse detritus had similar carbon isotope ratios indicating that it was derived from leaf fall from the riparian zone. Aquatic macrophytes had slightly enriched carbon isotope ratios (between –22 and –29‰) relative to riparian vegetation. Contrary to the consistency observed for $\delta^{13}\text{C}$ of riparian vegetation along the fluvial gradient, filamentous algae became more enriched along the downstream fluvial gradient, ranging from approximately –38‰ at Firetail Creek to approximately –14‰ at the seagrass/mangrove site. Along with ${}^{13}\text{C}$ -enriched filamentous algae, other dominant basal carbon sources in the seagrass/mangrove zone (macroalgae and submerged aquatic vegetation) were also comparatively ${}^{13}\text{C}$ -enriched relative to dominant sources in the freshwater sites. $\delta^{15}\text{N}$ of basal sources was comparable across sites, with the notable exception that in-stream production sources, namely filamentous algae and aquatic macrophytes, became considerably ${}^{15}\text{N}$ -enriched at middle reaches of the fluvial gradient relative to upper reaches and riparian sources (Table II).

Table I. Carbon stable isotope ratios for fishes, invertebrates and basal resources collected at each site. Standard deviations are reported for replicated samples within sites. Similar taxa are combined at each site for both invertebrate and basal resource samples due to limited taxonomic resolution

Species	Common name	Firetail Creek	Upper Bladen	Upper Swasey	Lower Bladen	Lower Swasey	Lower Monkey	Seagr./ Mangr.
Fish								
<i>Achirus lineatus</i>	Lined sole						-24.60	
<i>Agonostomus monticola</i>	Mountain mullet	-27.25			-26.35			
<i>Amphilophus robertsoni</i>	False firemouth cichlid						-20.28 ± 5.21	
<i>Anchoa belizensis</i>	Belize anchovy		-24.17 ± 0.81					
<i>Asyanax aeneus</i>	Banded tetra	-27.43			-28.11 ± 2.21			
<i>Atherinella</i> sp.	Silverside	-25.07			-28.50 ± 0.43			
<i>Awaous banana</i>	River goby				-29.74	-22.57 ± 2.21		
<i>Bairdiella ronchus</i>	Ground croaker					-24.90 ± 0.60	-29.78	
<i>Belonesox belizanus</i>	Pike killifish	-25.63	-20.25		-27.30 ± 1.17	-25.69 ± 0.82	-22.93	
<i>Brycon guatemalensis</i>	Machaca	-24.98 ± 0.46	-22.21		-24.66 ± 0.19	-23.63 ± 0.51	-23.13	
<i>Centropomus ensiferus</i>	Swordspine snook					-25.65 ± 0.46		
<i>Centropomus parallelus</i>	Fat snook					-26.97		
<i>Centropomus pectinatus</i>	Tarpon snook						-16.67	
<i>Centropomus undecimalis</i>	Common snook				-26.27		-20.39	
<i>Centropomus</i> sp.	Unidentified juvenile snook							
<i>Cichlasoma salvini</i>	Yellowbelly cichlid	-24.75 ± 2.18	-21.16 ± 0.99		-27.59	-26.88 ± 1.56	-26.74	
<i>Cryptoheros spilurus</i>	Blue-eye cichlid	-22.37	-21.44 ± 0.25			-25.85 ± 0.81	-21.56	
<i>Ctenogobius pseudofasciatus</i>	Slashcheek goby						-25.75	
<i>Diapterus</i> sp.	Mojarra							
<i>Eleotris pisonis</i>	Spinycheek sleeper					-31.85 ± 0.91		-20.18
<i>Epinephelus itajara</i>	Itajara							-14.42
<i>Eucinostomus melanopterus</i>	Flagfin mojarra					-31.90		-13.29 ± 0.32
<i>Eucinostomus jonesii</i>	Slender mojarra							
<i>Eugerres brasilianus</i>	Brazilian mojarra					-26.70 ± 0.61		
<i>Eugerres plumieri</i>	Striped mojarra					-29.89		-16.18 ± 1.03
<i>Gambusia luna</i>	Sleek mosquitofish							
<i>Gobiomorus dormitor</i>	Bigmouth sleeper		-20.90 ± 1.22		-31.65	-25.00 ± 1.43	-20.44	
<i>Haemulon aurolineatum</i>	Tomtate grunt				-26.65			-15.37
<i>Haemulon flavolineatum</i>	French grunt							-13.63
<i>Haemulon plumieri</i>	Grunt							-14.73
<i>Harengula jaguana</i>	Scaled herring							-15.69
<i>Heterandria bimaculata</i>	Twospot livebearer			-22.84 ± 0.46				
<i>Hyphessobrycon compressus</i>	Mayan tetra				-30.26			
<i>Luijanus griseus</i>	Gray snapper							
<i>Luijanus synagris</i>	Lane snapper							-14.59 ± 0.53
<i>Micropphis brachyurus brachyurus</i>	Short-tailed pipefish							-10.24
<i>Mugil curema</i>	White mullet							
<i>Oligoplites saurus</i>	Leatherjack							
<i>Ophisternon aenigmaticum</i>	Obscure swamp eel				-25.05			
<i>Petenia splendida</i>	Bay snook	-25.91 ± 1.11	-18.80		-28.49 ± 0.68		-22.23	
<i>Poecilia mexicana</i>	Shortfin molly	-24.41			-27.09 ± 2.86	-28.03 ± 1.11	-25.64	-15.86
		-23.40						

(Continues)

Table I. (Continued)

Species	Common name	Firetail Creek	Upper Bladen	Upper Swasey	Lower Bladen	Lower Swasey	Lower Monkey	Seagr./ Mangr.
<i>Pomadasys crocro</i>	Burro grunt			-21.42 ± 0.94		-26.06 ± 0.01		
<i>Rhamdia laticauda</i>	Filespine chulin							
<i>Rhamdia quelen</i>	South American catfish	-23.82	-25.84		-25.28			
<i>Roctio octofasciata</i>	Jack Dempsey					-29.12		
<i>Sciades assimilis</i>	Mayan sea catfish					-24.14	-23.05	
<i>Sphaeroides testudineus</i>	Cheekered puffer							-16.70
<i>Sphyraena barracuda</i>	Great barracuda							-14.51
<i>Strongylura timucu</i>	Timucu				-24.23 ± 3.20	-25.91		
<i>Thorichthys meeki</i>	Firemouth cichlid		-25.18 ± 0.75	-22.78	-28.00	-25.43 ± 0.73		
<i>Vieja maculicauda</i>	Blackbelt cichlid		-24.86	-21.41	-26.22 ± 0.35	-26.14 ± 0.61	-26.12 ± 0.83	
<i>Xiphophorus hellerii</i>	Green swordtail	-23.25		-21.73				
Bleenniidae	Unidentified blenny							-13.50 ± 2.00
Invertebrates								
	Bivalves							-21.73
	Crabs		-24.60 ± 1.16				-24.28	-11.91 ± 3.40
	Aquatic insect larvae			-24.25 ± 0.66	-27.73 ± 3.05			
	Shrimp	-24.95 ± 3.70		-22.05 ± 0.99	-27.68 ± 1.47	-25.33 ± 0.87	-25.51	-14.66 ± 2.38
	Snails			-20.49 ± 2.19	-20.13 ± 1.03		-20.37	-15.05 ± 4.12
	Starfish							-17.36
Plants/algae/detritus								
	Aquatic macrophytes			-22.16 ± 0.04	-26.68	-28.91 ± 0.52	-28.92	
	Coarse detritus			-29.93 ± 0.48		-31.33 ± 1.22		
	Filamentous algae	-37.94		-25.84 ± 1.07	-22.02			-14.24 ± 0.29
	FPOM			-27.85				-20.31 ± 5.33
	Macroalgae							
	Organic sediment				-23.40			
	Riparian vegetation		-30.36 ± 1.43		-30.50 ± 1.50		-29.81	
	Seeds from riparian veg	-30.37 ± 0.95						
	Submerged aquatic veg	-28.37 ± 1.79						-8.97 ± 1.36

Table II. Nitrogen stable isotope ratios for fishes, invertebrates and basal resources collected at each site. Standard deviations are reported for replicated samples within sites. Similar taxa are combined at each site for both invertebrate and basal resource samples due to limited taxonomic resolution

Species	Common name	Firetail Creek	Upper Bladen	Upper Swasey	Lower Bladen	Lower Swasey	Lower Monkey	Seagr./ Mangr.
Fish								
<i>Achirus lineatus</i>	Lined sole						10.64	
<i>Agonostomus monticola</i>	Mountain mullet		5.26					
<i>Amphilophus robertsoni</i>	False firemouth cichlid				10.63			
<i>Anchoa belizensis</i>	Belize anchovy			9.22 ± 1.16	10.57 ± 0.22		9.34 ± 0.57	
<i>Asyanax aeneus</i>	Banded tetra		3.64		10.51 ± 0.26			
<i>Atherinella</i> sp.	Silverside		4.83		11.86			
<i>Awaous banana</i>	River goby					11.49 ± 3.21		
<i>Bairdiella ronchus</i>	Ground croaker					13.01 ± 0.89	10.47	
<i>Belonesox belizanus</i>	Pike killifish		8.47	11.75	12.21 ± 0.11	13.83 ± 1.06	10.55	
<i>Brycon guatemalensis</i>	Machaca		5.32 ± 0.88	8.51	8.15 ± 0.30	8.58 ± 0.82	10.09	
<i>Centropomus ensiferus</i>	Swordspine snook					12.56 ± 0.06		
<i>Centropomus parallelus</i>	Fat snook					11.70		
<i>Centropomus pectinatus</i>	Tarpon snook						10.96	
<i>Centropomus undecimalis</i>	Common snook				12.88			
<i>Centropomus</i> sp.	Unidentified juvenile snook						9.03	
<i>Cichlasoma salvini</i>	Yellowbelly cichlid		8.03 ± 3.65	10.36 ± 0.09	9.58	12.87 ± 1.41	10.72	
<i>Cryptoheros spilurus</i>	Blue-eye cichlid		6.35	9.92 ± 0.84		13.14 ± 0.60	10.32	
<i>Ctenogobius pseudofasciatus</i>	Slashcheek goby						7.76	
<i>Diapterus</i> sp.	Mojarra							
<i>Eleotris pisonis</i>	Spinycheek sleeper					9.99 ± 0.63		8.88
<i>Epinephelus itajara</i>	Itajara							8.57
<i>Eucinostomus melanopterus</i>	Flagfin mojarra					11.96		10.21 ± 0.35
<i>Eucinostomus jonesii</i>	Slender mojarra							
<i>Eugerres brasilianus</i>	Brazilian mojarra					13.05 ± 0.55		
<i>Eugerres plumieri</i>	Striped mojarra					10.69		8.37 ± 0.85
<i>Gambusia luma</i>	Sleek mosquitofish						11.00	
<i>Gobiomorus dormitor</i>	Bigmouth sleeper			10.25 ± 0.06	9.22	11.96 ± 0.47		
<i>Haemulon aurolineatum</i>	Tomtate grunt				11.15			9.95
<i>Haemulon flavolineatum</i>	French grunt							10.19
<i>Haemulon plumieri</i>	Grunt							10.90
<i>Harengula jaguana</i>	Scaled herring							8.77
<i>Heterandria bimaculata</i>	Twospot livebearer			9.70 ± 0.42				
<i>Hyphessobrycon compressus</i>	Mayan tetra				8.72			
<i>Lutjanus griseus</i>	Gray snapper					13.13		10.69 ± 0.63
<i>Lutjanus synagris</i>	Lane snapper						9.59	7.67
<i>Micropphis brachyurus brachyurus</i>	Short-tailed pipefish						4.00 ± 0.67	
<i>Mugil curema</i>	White mullet						8.84	
<i>Oligoplites saurus</i>	Leatherjack							
<i>Ophisternon aenigmaticum</i>	Obscure swamp eel				9.71		11.04	
<i>Petenia splendida</i>	Bay snook		7.48 ± 1.48	9.43	12.70 ± 0.26		8.56	
<i>Poecilia mexicana</i>	Shortfin molly		3.39	9.43	9.88 ± 0.43	12.22 ± 0.81		3.82
			3.24					

(Continues)

Table II. (Continued)

Species	Common name	Firetail Creek	Upper Bladen	Upper Swasey	Lower Bladen	Lower Swasey	Lower Monkey	Seagr./ Mangr.
<i>Pomadasy crocro</i>	Burro grunt			11.47 ± 0.08		14.17 ± 0.28		
<i>Rhamdia laticauda</i>	Filespine chulin							
<i>Rhamdia quelen</i>	South American catfish	7.22	8.78		10.89			
<i>Rocio octofasciata</i>	Jack Dempsey					9.13		
<i>Sciades assimilis</i>	Mayan sea catfish					12.43	11.32	
<i>Sphaeroides testudineus</i>	Checked red puffer							6.29
<i>Sphyraena barracuda</i>	Great barracuda							9.73
<i>Strongylura timucu</i>	Timucu				12.31 ± 1.67	12.72		
<i>Thorichthys meeki</i>	Firemouth cichlid		7.49 ± 1.27	9.19	10.44	11.71 ± 0.01		
<i>Vieja maculicauda</i>	Blackbelt cichlid		5.75	10.25	10.86 ± 0.56	10.93 ± 1.65	8.64 ± 0.77	
<i>Xiphophorus hellerii</i>	Green swordtail	4.70		8.72				
Blenniidae	Unidentified blenny							9.80 ± 0.58
Invertebrates								
	Bivalves							6.04
	Crabs		6.20 ± 1.14				6.27	8.24 ± 0.51
	Aquatic insect larvae			5.63 ± 0.77	7.27 ± 0.56			
	Shrimp	6.29 ± 1.14		7.66 ± 0.01	9.37 ± 1.32	10.69 ± 1.62	6.71	7.02 ± 0.55
	Snails			6.15 ± 0.77	7.53 ± 0.26		8.06	6.49 ± 2.14
	Starfish							10.43
Plants/algae/detritus								
	Aquatic macrophytes			4.95 ± 0.44	5.41	5.97 ± 1.04	7.09	
	Coarse detritus			1.00 ± 0.80		2.59 ± 3.16		
	Filamentous algae	0.92		4.32 ± 1.40	5.01			6.87 ± 1.09
	FPOM			3.24				7.83 ± 0.54
	Macroalgae							
	Organic sediment				6.54			
	Riparian vegetation	1.88 ± 1.53	1.81 ± 0.40		1.62 ± 1.79		0.16	
	Seeds from riparian veg	2.62 ± 1.41						
	Submerged aquatic veg							6.68 ± 1.15

FLUVIAL GRADIENTS IN TROPICAL AQUATIC FOOD WEBS

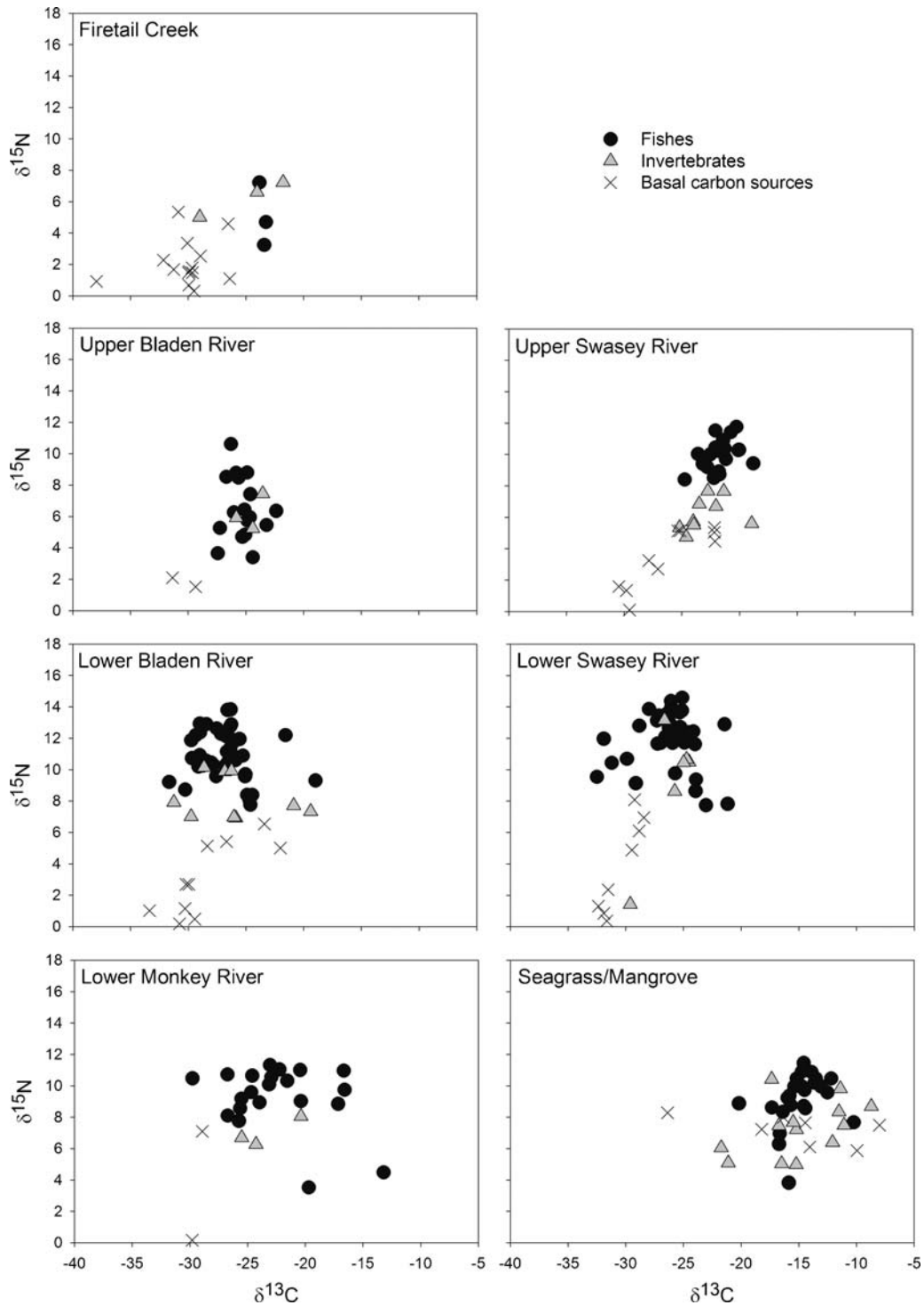


Figure 2. Bi-plots of carbon and nitrogen stable isotope signatures of fishes, invertebrates and basal carbon sources at each location along the fluvial gradient (Figure 1)

Carbon isotope ratios for most consumers were between -30 and -20‰ , with samples from the Lower Monkey River and the coastal zone beyond the river mouth (seagrass/mangrove) more enriched in ^{13}C (Figures 2 and 3). Higher

consumer $\delta^{15}\text{N}$ values were generally observed at sites at middle elevations in the watershed (Figures 2 and 3). Significant differences in carbon and nitrogen isotope signatures were observed at the assemblage level

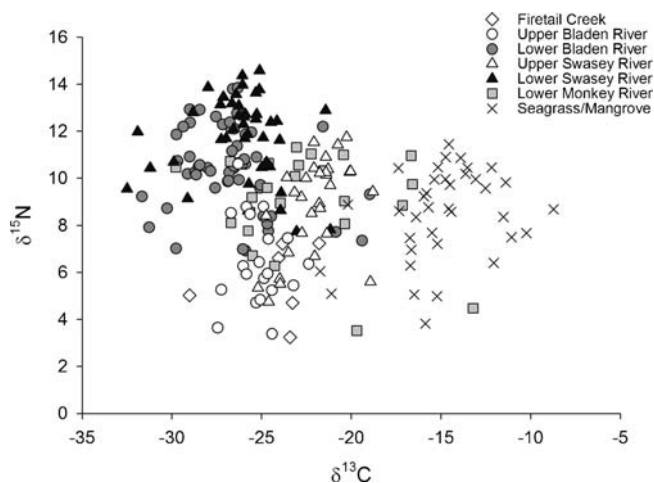


Figure 3. Carbon and nitrogen stable isotope ratios of aquatic consumers across survey sites. MANOVA results comparing assemblage-level isotope ratios among sites are summarized in Table III. See Figure 1 for locations along the fluvial gradient and in relation to watershed impacts

($F_{12,412} = 53.04$, $p < 0.001$). The seagrass/mangrove site was significantly more enriched in $\delta^{13}\text{C}$ than all other sites (Figure 3, Table III). *Post-hoc* tests for $\delta^{15}\text{N}$ distinguished the enriched Lower Swasey River and Lower Bladen River from all other sites (Table III). The remaining sites separated into two groups comprised by the Lower Monkey River, Upper Swasey and seagrass/mangrove with intermediate $\delta^{15}\text{N}$ values, and the Upper Bladen River and Firetail Creek with the lowest $\delta^{15}\text{N}$ values (Figure 3, Table III).

The trend in $\delta^{15}\text{N}$ observed at the assemblage level was also observed for the primary consumer taxa used as indicators of watershed impacts. Primary consumer $\delta^{15}\text{N}$ differed among sites ($F_{6,10} = 23.22$, $p < 0.001$), with the Lower Swasey River exhibiting the highest $\delta^{15}\text{N}$ value (Figure 4). Nitrogen isotope values of primary consumers at the Lower Bladen River, Upper Swasey River and Lower Monkey River were also considerably higher (5‰) than those of primary consumers at the remaining sites (Figure 4). The pattern observed for all primary consumer species combined was also observed for the Shortfin molly, *Poecilia mexicana*, the only primary consumer species to occur at all

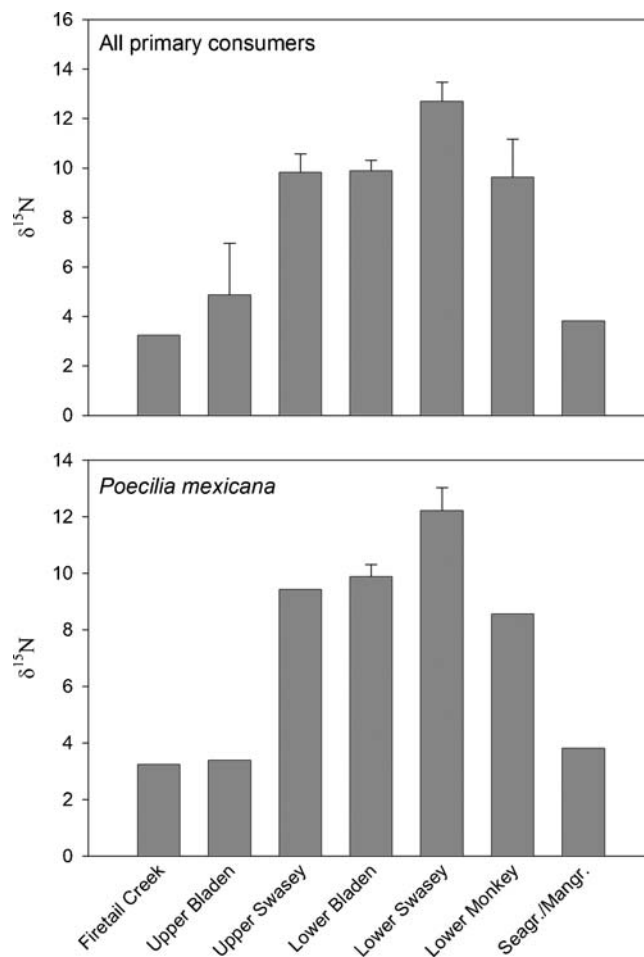


Figure 4. Mean (± 1 S.D.) nitrogen stable isotope signatures of all primary consumer taxa together and *Poecilia mexicana* alone (the only primary consumer species to occur at all sites). Comparatively higher $\delta^{15}\text{N}$ values of primary consumers at different sites in the same watershed may indicate anthropogenic impacts associated with agricultural land use or human population density

sites (Figure 4). For both the Bladen and Swasey branches, primary consumer $\delta^{15}\text{N}$ values at sites with watersheds impacted by banana plantations (i.e. Lower Bladen and Lower Swasey) were enriched compared with reference

Table III. Summary of MANOVA results comparing carbon and nitrogen isotope ratios of aquatic consumers among sites. Bonferroni-corrected pairwise comparisons are provided for significant main effects

Effect	df	F	p	Pairwise						
Among sites	12, 412	53.04	< 0.001							
$\delta^{13}\text{C}$	6	96.91	< 0.001	LB	LS	UB	FC	LM	US	SM
$\delta^{15}\text{N}$	6	28.34	< 0.001	FC	UB	SM	US	LM	LB	LS

Shared underline signifies no significant difference at $p < 0.05$. Sites are arranged left to right in order of increasing mean value of the consumer assemblage for $\delta^{13}\text{C}$ or $\delta^{15}\text{N}$ (Firetail Creek (FC), Upper Bladen (UB), Upper Swasey (US), Lower Bladen (LB), Lower Swasey (LS), Lower Monkey (LM), Seagrass/Mangrove (SM)).

conditions for each branch (i.e. Firetail Creek and Upper Bladen, and Upper Swasey, respectively; Figure 4).

DISCUSSION

Fluvial gradients in tropical aquatic food webs

Longitudinal changes in fish species richness and assemblage composition are common in most rivers (Matthews, 1998). Although our sampling methodology was designed to survey only the dominant members of the assemblages for food web analyses, we nonetheless observed a general trend of greater species richness at lower positions in the fluvial gradient. The addition of estuarine and marine species in lower reaches contributes to greater fish species richness. Previous studies examining longitudinal gradients in fish assemblages of both temperate (e.g. Sheldon, 1968; Horwitz, 1978; Rahel and Hubert, 1991) and tropical regions (e.g. Ibarra and Stewart, 1989; Toham and Teugels, 1998; Abes and Agostinho, 2001; Hoeinghaus *et al.*, 2004; Petry and Schulz, 2006; Araújo *et al.*, 2009), including work by Esselman *et al.* (2006) for the Monkey River Basin in Belize, found similar patterns of species richness in relation to the fluvial gradient.

Variation in fish species richness along fluvial gradients is often concomitant with changes in functional composition, including greater richness of detritivores and large predators in lower reaches (e.g. Oberdorff *et al.*, 1993; Poff and Allan, 1995; Hoeinghaus *et al.*, 2007b; Ibanez *et al.*, 2007). For example, two recent studies conducted across a large number of sites and river longitudinal positions in Texas, U.S.A. (Hoeinghaus *et al.*, 2007b), and Gabon in western Africa (Ibanez *et al.*, 2007) both found that the relative abundance or species richness of piscivores increased along the downstream fluvial gradient and that headwaters were dominated by insectivorous species. Similarly, we observed greater species richness of piscivores at lower elevation sites in the Monkey River watershed. Longitudinal gradients in functional composition of the consumer assemblage interact with changes in the relative abundance of basal carbon sources to influence food web structure (e.g. Romanuk *et al.*, 2006).

Carbon isotope signatures of the consumer assemblage as a whole changed very little along the fluvial gradient (generally between -30 and -20‰), with the exception of the seagrass/mangrove site and to a lesser degree the Lower Monkey River. Even though the range of carbon isotope signatures of consumer assemblages was fairly consistent in the middle to upper reaches of the watershed, basal source signatures differed along the gradient. Most importantly, filamentous algae at Firetail Creek was much more ^{13}C depleted (approximately -38‰) than at the other freshwater sites (between -26 and -22‰), which suggests that it contributes

only a minor fraction of the carbon assimilated by consumers in Firetail Creek. As might be expected from the river continuum concept (Vannote *et al.*, 1980), consumers at this uppermost site with a closed canopy appear to rely primarily on allochthonous carbon sources. As the channel broadens heading downstream and more light reaches the stream, algae seem to contribute to the food webs to a greater degree. For all of the freshwater sites excluding Firetail Creek, a mixture of filamentous algae and detritus from leaf fall could have produced the observed isotope signatures of aquatic consumers. The estuarine seagrass/mangrove site had the most distinct (^{13}C -enriched) consumer carbon isotope signatures. Enrichment of $\delta^{13}\text{C}$ signatures of aquatic consumers is often observed when transitioning from freshwater to estuarine habitats (e.g. Garcia *et al.*, 2007). Carbon isotopic signatures of algae typically become more enriched along the freshwater to estuarine gradient, and many other aquatic primary producers, such as submerged aquatic vegetation and saltmarsh grasses, are greatly enriched compared with their freshwater analogs (C_4 saltmarsh grasses vs. C_3 aquatic macrophytes; e.g. Deegan and Garritt, 1997; Garcia *et al.*, 2007). Comparatively enriched carbon isotope signatures suggest that individuals of a few species collected at the Lower Monkey River, such as the white mullet, tarpon snook and leatherjacket, likely recently colonized the site from the coastal zone.

At the assemblage level, nitrogen isotope signatures were significantly higher at the Lower Swasey and Lower Bladen Rivers than at all other sites. However, this higher assemblage-level $\delta^{15}\text{N}$ does not necessarily indicate longer food chains because the bases of these food webs were also enriched compared to other sites (see discussion of watershed impacts and $\delta^{15}\text{N}$ below). Coarse evaluation of the difference between maximum observed $\delta^{15}\text{N}$ and approximate baseline $\delta^{15}\text{N}$ at each site suggests that food-chain length changed very little along the fluvial gradient, with the exception of Firetail Creek which has a comparatively simple food web (fewer species and links) with short chains. Assuming a trophic fractionation of approximately $+2.5\text{‰}$ (Vanderklift and Ponsard, 2003), which may be more appropriate for tropical food web studies (e.g. Jepsen and Winemiller, 2002; Hoeinghaus *et al.*, 2007a, 2008) than the higher estimate of $+3.4\text{‰}$ often used in temperate systems (e.g. Post, 2002), food-chain lengths appear to range between four and five trophic levels (except Firetail Creek which has approximately three trophic levels). The vast majority of river food-web studies have observed between three and four trophic levels (Winemiller, 2004; Vander Zanden and Fetzer, 2007). The shorter food-chain length of Firetail Creek was due to low abundance and diversity of piscivores; *Rhamdia quelen* was the only piscivorous species captured in this tributary, and this generalist predator also consumes aquatic invertebrates.

Watershed impacts and $\delta^{15}\text{N}$

Previous research in temperate river systems has found positive correlations between baseline $\delta^{15}\text{N}$ in aquatic food webs and human population density and agricultural intensity in the watershed (e.g. Cabana and Rasmussen, 1996; Lake *et al.*, 2001; Anderson and Cabana, 2005). Nitrogen isotope signatures of primary consumer species may therefore be useful as indicators of watershed impacts when compared with a non-impacted reference or historic condition. Our study system in the Maya Mountain Marine Area Transect provided a unique opportunity to test this generalization within a tropical watershed with a diverse biota and different agricultural impacts relative to temperate settings (i.e. banana plantations vs. tilled row cropping). Large tracts of the Monkey River Basin remain in nearly pristine condition and are protected within reserves (e.g. the region drained by Firetail Creek and the Upper Bladen River), providing suitable reference conditions for comparison with other sites (e.g. Upper and Lower Swasey River sites) with watersheds impacted by banana plantations and human settlement.

As predicted, based on findings from temperate regions, $\delta^{15}\text{N}$ of primary consumers in impacted sites was significantly greater than in pristine reference sites. The Lower Swasey River adjacent to banana plantations had the highest observed nitrogen isotope values for primary consumers (approximately 13‰), and watershed impacts appear to continue downstream from the Swasey River into the Monkey River mainstem. Primary consumers in upper reaches of the Bladen watershed had $\delta^{15}\text{N}$ values of approximately 4‰, whereas primary consumers in the Lower Bladen River above the confluence with the Swasey River had $\delta^{15}\text{N}$ values of approximately 10‰. Similarly for the Swasey branch, $\delta^{15}\text{N}$ values of primary consumers at the impacted site were enriched compared to the upstream reference site. Effects of geology on N- versus P-limitation of aquatic primary production between the Bladen and Swasey branches (Esselman *et al.*, 2006) may affect the magnitude of N-enrichment in aquatic food webs at sites within impacted watersheds. Our findings support previous research from temperate systems, and highlight the utility of $\delta^{15}\text{N}$ as an indicator of watershed impacts in diverse tropical river networks. Continued surveys of target taxa may allow natural resource managers to identify increases in watershed impacts or expansion of impacted zones into formerly unimpacted watersheds.

Conclusions and conservation implications

Fluvial gradients in river networks affect aquatic food webs through changes in the relative availability of basal carbon sources and the richness and functional composition of the consumer assemblage. Our findings from the Monkey

River drainage in Belize are consistent with food web studies conducted in temperate regions that found similar patterns of carbon source availability and functional composition of the consumer assemblage along fluvial gradients. Stable nitrogen isotope ratios of primary consumers were strongly associated with the level of agricultural and human settlement in their watersheds. Assessment of primary consumer $\delta^{15}\text{N}$ may be a feasible option for monitoring watershed impacts on aquatic food webs in service of the ridge-to-reef conservation strategy for the MMMAT and in other tropical regions.

ACKNOWLEDGEMENTS

Institutional Support in Belize was provided by Jacob and Kelly Marlin of the Belize Foundation for Research and Environmental Education (BFREE), the Belize Forestry Department and the Belize Fisheries Department. Funding for this project was provided by NSF Undergraduate Mentoring in Environmental Biology grant #0203992, NSF DEB grant #0089834 and the International Sportfish Fund.

REFERENCES

- Abes SS, Agostinho AA. 2001. Spatial patterns in fish distributions and structure of the ichthyocenosis in the Água Nanci stream, Upper Paraná River Basin, Brazil. *Hydrobiologia* **445**: 217–227.
- Allan JD. 2004. Landscapes and riverscapes: the influence of land use on stream ecosystems. *Annual Review of Ecology, Evolution & Systematics* **35**: 257–284.
- Anderson C, Cabana G. 2005. $\delta^{15}\text{N}$ in riverine food webs: effects of N inputs from agricultural watersheds. *Canadian Journal of Fisheries and Aquatic Sciences* **62**: 333–340.
- Araújo FG, Pinto BCT, Teixeira TP. 2009. Longitudinal patterns of fish assemblages in a large tropical river of southeastern Brazil: evaluating environmental influences and some concepts in river ecology. *Hydrobiologia* **618**: 89–107.
- Arrington DA, Winemiller KO. 2002. Preservation effects on stable isotope analysis of fish muscle. *Transactions of the American Fisheries Society* **131**: 337–342.
- Cabana G, Rasmussen JB. 1996. Comparison of aquatic food chains using nitrogen isotopes. *Proceedings of the National Academy of Sciences of the United States of America* **93**: 10844–10847.
- Clements DH, Carlisle DM, Lazorchak JM, Johnson PC. 2000. Heavy metals structure benthic communities in Colorado mountain streams. *Ecological Applications* **10**: 626–638.
- Deegan LA, Garritt RH. 1997. Evidence for spatial variability in estuarine food webs. *Marine Ecology Progress Series* **147**: 31–47.
- DeBruyn AM, Rasmussen JB. 2002. Quantifying assimilation of sewage-derived organic matter by riverine benthos. *Ecological Applications* **12**: 511–520.
- Esselman PC, Freeman MC, Pringle CM. 2006. Fish-assemblage variation between geologically defined regions and across a longitudinal gradient in the Monkey River Basin, Belize. *Journal of the North American Benthological Society* **25**: 142–156.

- Garcia AM, Hoesinghaus DJ, Vieira JP, Winemiller KO. 2007. Isotopic variation of fishes in freshwater and estuarine zones of a large subtropical coastal lagoon. *Estuarine Coastal and Shelf Science* **73**: 399–408.
- Heyman WD, Kjerfve B. 1999. Hydrological and oceanographic considerations for integrated coastal zone management in Southern Belize. *Environmental Management* **24**: 229–245.
- Hoesinghaus DJ, Winemiller KO, Agostinho AA. 2007a. Landscape-scale hydrologic characteristics differentiate patterns of carbon flow in large-river food webs. *Ecosystems* **10**: 1019–1033.
- Hoesinghaus DJ, Winemiller KO, Agostinho AA. 2008. Hydrogeomorphology and river impoundment affect food-chain length in diverse neotropical food webs. *Oikos* **117**: 984–995.
- Hoesinghaus DJ, Winemiller KO, Birnbaum JS. 2007b. Local and regional determinants of stream fish assemblage structure: inferences based on taxonomic vs. functional groups. *Journal of Biogeography* **34**: 324–338.
- Hoesinghaus DJ, Winemiller KO, Taphorn DC. 2004. Compositional change in fish assemblages along the Andean piedmont—Llanos floodplain gradient of the río Portuguesa, Venezuela. *Neotropical Ichthyology* **2**: 85–92.
- Horwitz RJ. 1978. Temporal variability patterns and the distributional patterns of stream fishes. *Ecological Monographs* **48**: 307–321.
- Hynes HBN. 1970. *Ecology of Running Waters*. University of Toronto Press: Toronto.
- Ibanez C, Oberdorff T, Teugels G, Mamononekene V, Lavoué S, Fermon Y, Paugy D, Toham AK. 2007. Fish assemblages structure and function along environmental gradients in rivers of Gabon (Africa). *Ecology of Freshwater Fish* **16**: 315–334.
- Ibarra M, Stewart DJ. 1989. Longitudinal zonation of sandy beach fishes in the Napo River Basin, Eastern Ecuador. *Copeia* **1989**: 364–381.
- Jepsen DB, Winemiller KO. 2002. Structure of tropical river food webs revealed by stable isotope ratios. *Oikos* **96**: 46–55.
- Kilham SS, Pringle CM. 2000. Food webs in two neotropical stream systems as revealed by stable isotope ratios. *Verhandlungen Internationale Vereinigung für theoretische und angewandte Limnologie* **27**: 1768–1775.
- Lake JL, McKinney RA, Osterman FA, Pruell RJ, Kiddon J, Ryba SA, Libby AD. 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. *Canadian Journal of Fisheries and Aquatic Sciences* **58**: 870–878.
- Matthews WJ. 1998. *Patterns in Freshwater Fish Ecology*. Chapman Hall: New York.
- McCutchan JH Jr, Lewis WM, Kendall C, McGrath CC. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. *Oikos* **102**: 378–390.
- Oberdorff T, Guilbert E, Lucchetta JC. 1993. Patterns of fish species richness in the Seine River Basin, France. *Hydrobiologia* **259**: 157–167.
- Peterson BJ, Fry B. 1987. Stable isotopes in ecosystem studies. *Annual Review of Ecology, Evolution and Systematics* **18**: 293–320.
- Petry AC, Schulz UH. 2006. Longitudinal changes and indicator species of the fish fauna in the subtropical Sinos River, Brazil. *Journal of Fish Biology* **69**: 272–290.
- Poff NL, Allan JD. 1995. Functional organization of stream fish assemblages in relation to hydrologic variability. *Ecology* **76**: 606–627.
- Post DM. 2002. Using stable isotopes to estimate trophic position: models, methods, and assumptions. *Ecology* **83**: 703–718.
- Power ME, Dietrich WE. 2002. Food webs in river networks. *Ecological Research* **17**: 451–471.
- Rahel FJ, Hubert WA. 1991. Fish assemblages and habitat gradients in a Rocky Mountain-Great Plains stream: biotic zonation and additive patterns of community change. *Transactions of the American Fisheries Society* **120**: 319–332.
- Rodiles-Hernández R, Díaz-Pardo E, Lyons J. 1999. Patterns in the species diversity and composition of the fish community of the Lacanja River, Chiapas, Mexico. *Journal of Freshwater Ecology* **14**: 455–467.
- Romanuk TN, Jackson LJ, Post JR, McCauley E, Martinez ND. 2006. The structure of food webs along river networks. *Ecography* **29**: 3–10.
- Saito L, Redd D, Chandra S, Atwell L, Fritsen CH, Rosen MR. 2007. Quantifying foodweb interactions with simultaneous linear equations: stable isotope models of the Truckee River, USA. *Journal of the North American Benthological Society* **26**: 642–662.
- Saito L, Rosen MR, Chandra S, Fritsen CH, Arufe JA, Redd C. 2008. Using semi-permeable membrane devices and stable nitrogen isotopes to detect anthropogenic influences on the Truckee River, USA. *Environmental Engineering Science* **25**: 585–600.
- Sheldon AL. 1968. Species diversity and longitudinal succession in stream fishes. *Ecology* **49**: 193–198.
- Simon KS, Niyogi DK, Frew RD, Townsend CR. 2007. Nitrogen dynamics in grassland streams along a gradient of agricultural development. *Limnology and Oceanography* **52**: 1246–1257.
- Thorp JH, Delong MD. 1994. The riverine productivity model: an heuristic view of carbon sources and organic processing in large river ecosystems. *Oikos* **70**: 305–308.
- Thorp JH, Delong MD. 2002. Dominance of autochthonous autotrophic carbon in food webs of heterotrophic rivers. *Oikos* **96**: 543–550.
- Toham AK, Teugels GG. 1998. Diversity patterns of fish assemblages in the Lower Ntem River Basin (Cameroon), with notes on potential effects of deforestation. *Archiv für Hydrobiologie* **141**: 421–446.
- Vander Zanden MJ, Fetzner WW. 2007. Global patterns of aquatic food chain length. *Oikos* **116**: 1378–1388.
- Vanderklift MA, Ponsard S. 2003. Sources of variation in consumer-diet $\delta^{15}\text{N}$ enrichment: a meta-analysis. *Oecologia* **136**: 169–182.
- Vannote RL, Minshall GW, Cummins KW, Sedell JR, Cushing CE. 1980. The river continuum concept. *Canadian Journal of Fisheries and Aquatic Sciences* **37**: 130–137.
- Verburg P, Kilham SS, Pringle CM, Lips KR, Drake DL. 2007. A stable isotope study of a neotropical stream food web prior to the extirpation of its large amphibian community. *Journal of Tropical Ecology* **23**: 643–651.
- Winemiller KO. 1990. Spatial and temporal variation in tropical fish trophic networks. *Ecological Monographs* **60**: 331–367.
- Winemiller KO. 2004. Floodplain river food webs: generalizations and implications for fisheries management. In *Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries Volume II*. Welcomme R, Petr T (eds). Regional Office for Asia and the Pacific: Bangkok, Thailand; 285–309. RAP Publication 2004/16.
- Winemiller KO, Leslie MA. 1992. Fish assemblages across a complex, tropical freshwater/marine ecotone. *Environmental Biology of Fishes* **34**: 29–50.