

# Food-web structure of coastal streams in Costa Rica revealed by dietary and stable isotope analyses

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**Abstract:** Food webs of streams draining tropical rain forests on Costa Rica's Pacific and Caribbean coasts were examined in the 1980s via dietary analyses, and the same streams were surveyed again in 2004 to compare trophic structure based on analysis of stable isotope ratios of fish, macro-invertebrate and plant tissues. Estimates of species' trophic positions (TP) were calculated from stomach-contents data (51 species; 5420 specimens) and compared with TP estimates derived from analysis of nitrogen isotope ratios (82 taxa; 240 samples). Coefficients of determination for TP based on dietary versus isotopic analysis ranged from 0.18 (Quebrada Camaronal, Corcovado) to 0.73 (Quebrada Estacion, Tortuguero). Consumer taxa within all four streams spanned a broad range of carbon isotope values, indicating assimilation of variable proportions of carbon from periphyton and terrestrial vegetation that in all but one of the streams had similar  $\delta^{13}\text{C}$  values. Approximately half the consumers in all four streams had carbon ratios heavier than any of the *in situ* production sources examined. This pattern could be explained by consumption of other production sources that were not sampled, including periphyton taxa with variable carbon isotope signatures, or migration of prey and/or consumers between these freshwater and coastal marine habitats.

**Key Words:** Central America, detritus, fishes, macro-invertebrate, migration, omnivory, periphyton, production source, shrimp, trophic position

## INTRODUCTION

Because saline coastal waters are a barrier to dispersal by freshwater-adapted organisms, streams of coastal watersheds tend to have fish assemblages that are insular with low richness compared with streams within large continental basins (Hugueny 1989). Certain species of marine crustacean and fish invade tropical coastal streams where they fill vacant freshwater niches and are integral components of food webs (Covich & McDowell 1996, Winemiller 1990). Migratory shrimp have been shown to play important roles in food webs of streams in Central America and the Caribbean (Covich & McDowell

1996, March & Pringle 2003, Pringle & Hamazaki 1998), and amphidromous gobies (e.g. species of the genera *Sicydium* and *Sicyopterus*) are major periphyton grazers in streams of islands and coastal zones throughout the tropics (Barbee 2005, Covich & McDowell 1996, Inoue & Miyayoshi 2006, Winemiller 1983). In the absence of native predatory species from freshwater fish families, transient marine fishes, such as species of Centropomidae, Lutjanidae and Pomadasyidae, may assume the role of top predator (Pusey *et al.* 2004, Winemiller 1983).

This study examines food-web structure of coastal streams of the Caribbean and Pacific coasts of Costa Rica by comparing results from earlier dietary analyses performed by the first author with new results from stable isotope analyses. Stable isotope methods are useful for estimating production sources that support consumers and the relative trophic positions of food-web components (Peterson & Fry 1987, Post 2002). Stable isotope analysis yields estimates based on the organism's recent history of assimilation of elements from food (in the case of animals) or nutrient pools (in the case of plants). Dietary

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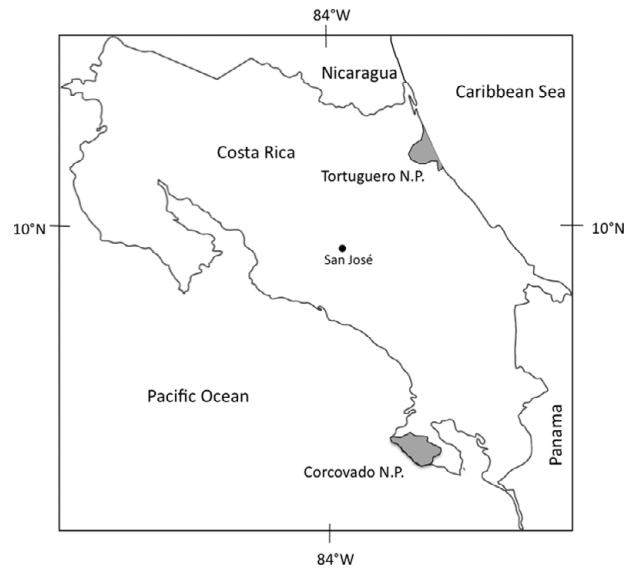
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analysis provides a more detailed estimation of feeding links, but large samples are required for reliable estimation of spatial, temporal and ontogenetic variation. Use of both methods not only allows for comparison of inferences, but also should yield a more accurate assessment of food-web ecology (Layman *et al.* 2005, Mantel *et al.* 2004, Rayner *et al.* 2010, Winemiller *et al.* 2007). Numerous studies analysing stable isotopes have identified algae as the principal source of organic carbon supporting food chains in tropical streams and wetlands (Brito *et al.* 2006, Bunn & Boon 1993, Douglas *et al.* 2005, Hamilton *et al.* 1992, Jepsen & Winemiller 2007, Lau *et al.* 2009). A few isotopic studies have shown allochthonous plant matter to be an important basal input for food webs in tropical streams (Lau *et al.* 2009), leading some to question whether tropical stream food webs are different from those of temperate streams (Dudgeon *et al.* 2010).

Here we examine several hypotheses related to food-web structure of tropical coastal streams by comparing findings from two alternative methodologies for investigating trophic ecology. First, we hypothesize that stable isotope analysis will support findings from dietary analysis indicating that consumer biomass in forested tropical streams is supported mostly by aquatic and terrestrial macrophytes (leaves, fruits, plant detritus) with a lesser contribution from benthic algae (Winemiller 1983, 1990; Winemiller & Morales 1989). Second, dietary analysis based on stomach contents and stable isotope analysis of consumer tissues will yield consistent estimates of species trophic positions that define vertical food web structure. Finally, isotopic signatures of obligate freshwater species versus diadromous species that move between fresh and marine habitats will be different, with diadromous species having heavier C and N ratios indicative of marine production sources. Resolution of the last hypothesis would provide insight regarding the potential influence of diadromy on food-web patterns and dynamics in tropical coastal streams.

## STUDY SITES

Food-web structure was investigated in two streams in Corcovado National Park (Pacific coast) and two streams in Tortuguero National Park (Caribbean coast) in Costa Rica, Central America (Figure 1). Corcovado sites were Quebrada Camaronal, a small, low-gradient stream draining low, densely forested terrain near the Sirena Station, and the Rio Claro, a larger stream draining forested ridges lying to the west of Sirena Station. Quebrada Camaronal empties into the lower estuary of the Rio Sirena, and the Rio Claro empties directly into the Pacific Ocean. The reaches of Quebrada Camaronal and Rio Claro surveyed for food-web components were located approximately 1 km upstream from the coast.



**Figure 1.** Map of Costa Rica showing locations of Corcovado National Park on the Osa Peninsula, Pacific coast, and Tortuguero National Park on the Caribbean coast.

More detailed descriptions of the two Corcovado survey sites and the surrounding region appear in Lyons & Schneider (1990), Winemiller (1983) and Winemiller & Morales (1989). The fish communities of these streams contain a few freshwater species, such as the characid *Astyanax fasciatus*, the poeciliid *Poecilia gilli* and the catfish *Rhamdia guatemalensis* (Pimelodidae), plus several diadromous species (e.g. *Centropomus nigrescens*, *Lutjanus argentiventris*, *Awaous transandeanus*, *Sicydium salvini*).

The Tortuguero sites were Quebrada Estacion, a small stream at the park's northern limit on the barrier island, and the Rio Tortuguero in the park's interior. Both Tortuguero streams drain dense lowland rain forest; however, deforestation and human settlement have occurred along the northern bank in the lowest reaches of Quebrada Estacion over the past 15 y. Both streams drain into the Laguna Tortuguero, a deep narrow estuary that receives water from several major rivers including the Rio Tortuguero. This estuary normally has fresh water with salinity <1‰ near the surface and a saline wedge at the bottom that can extend several kilometres upstream depending on fresh-water discharge and tides. Reaches of the two streams that were surveyed for food-web components are located more than 5 km from the mouth of the Laguna Tortuguero. The Rio Tortuguero samples include samples taken from Caño Agua Fria Viejo, a side channel containing dense mats of floating aquatic macrophytes and surrounded by dense swamp forest. Detailed descriptions of the two Tortuguero survey sites and the surrounding region appear in Winemiller (1990) and Winemiller & Leslie (1992). Like the streams at Corcovado, the streams at Tortuguero contain both

fresh-water adapted fish and macro-invertebrate species as well as diadromous species that, depending on species and life stage, spend variable periods of time in freshwater habitats (Gilbert & Kelso 1971, Winemiller & Leslie 1992). Compared with the stream fish assemblages at Corcovado, those at Tortuguero are comprised of larger percentages, in terms of population abundance, of species from freshwater families such as Characidae, Poeciliidae and Cichlidae.

## METHODS

### Dietary analyses

Dietary data for fishes at each of the four study reaches were compiled from published studies by the first author. Volumetric estimates of diet components were derived from stomach contents analysis. Corcovado samples were for the wet season (June–July) during 1982, 1983 and 1986. For fish species that occurred at both Quebrada Camaronal and Rio Claro, specimens from both study sites were combined in the calculation of volumetric proportions of diet items (Winemiller 1983, Winemiller & Morales 1989). For these nine species, the same dietary estimates were used for both sites. Tortuguero samples were taken monthly from February to December 1985, and data were aggregated into annual estimates of dietary composition (Winemiller 1990). For species that occurred at both sites, dietary estimates for each site were independent based on specimens collected *in situ*.

### Stable isotope analyses

During June 2004, samples of the most common plant and macrofaunal species were collected at all four of the stream survey sites for analysis of carbon and nitrogen isotope ratios. Submerged or floating leaves and seeds were collected by hand. Periphyton was collected from the surface of rocks by first rinsing to remove loose fine particulate matter, and then scraping to obtain attached algae and cyanobacteria. Fishes and macro-invertebrates were captured with a seine net, cast net, dip nets, or hook and line. Samples of periphyton, detritus, aquatic plants and terrestrial plants (including leaves and seeds) were crushed and preserved in salt. For fish and invertebrate specimens, a sample of muscle tissue (large specimens) or whole body minus viscera or shell (small specimens) was obtained and preserved in salt. Samples were subsequently stored in a freezer.

In the laboratory, tissue samples were thawed, soaked and rinsed in distilled water, then dried in an oven at 60 °C for 48 h. Dried samples were ground to a fine powder with pestle and mortar and stored in clean

glass vials. Subsamples of each sample were weighed to 1 µg and pressed into Ultra-Pure tin capsules (Costech, Valencia, CA). Samples were analysed for stable isotope ratios (<sup>13</sup>C/<sup>12</sup>C and <sup>15</sup>N/<sup>14</sup>N) at the Analytical Chemistry Laboratory, Institute of Ecology, University of Georgia, USA. Samples were dry combusted (micro Dumas technique) with a Carlo Erba CHN elemental analyser. Purified gases (CO<sub>2</sub> and N<sub>2</sub>) were introduced into a Finnigan Delta C mass spectrometer, and isotopic composition was quantified relative to standard reference materials. Results are reported as parts per thousand (‰) differences from the corresponding standard:

$$\delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] \times 10^3,$$

where  $R = {}^{15}\text{N}/{}^{14}\text{N}$  or  ${}^{13}\text{C}/{}^{12}\text{C}$ .

### Statistical analyses

Dietary data were used to estimate consumer taxon trophic position (TP) using the method presented in Winemiller (1990):

$$\text{TP}_i = \sum_{j=1}^n \text{TP}_j(p_{ij}) + 1,$$

where TP<sub>j</sub> is the trophic position of food category j, p<sub>ij</sub> is the volumetric proportion of food category j in the diet of species i, and n is the number of diet categories.

Biplots of δ<sup>15</sup>N and δ<sup>13</sup>C values of fishes, macro-invertebrates, periphyton and living and dead plant material were used to compare patterns of isotopic variation within and between sites. Given that δ<sup>13</sup>C values of diet items are usually conserved within 1‰ in consumer tissues (usually with consumer tissues shifting up to +1‰ δ<sup>13</sup>C in relation to their food, McCutchan *et al.* 2003), the relative importance of alternative sources of organic carbon assimilated is indicated by the relative positions of the consumer and the potential sources on the x-axis of the biplot. We did not attempt to perform quantitative estimates of the per cent assimilation of alternative primary production sources by consumers using a mixing model, because we were not able to obtain sufficient samples of either periphyton or the diverse terrestrial macrophytes in the riparian zone of these tropical wet forests. Therefore, our graphical analysis provides a general and preliminary assessment of source contributions to consumers in the food webs.

In contrast to carbon isotopes, nitrogen isotope ratios of consumer tissues typically are 2.5–3.4‰ higher than tissues of their food items (Vanderklift & Ponsard 2003), and therefore δ<sup>15</sup>N can serve as an indicator of trophic position in addition to refining estimates of source contributions based on δ<sup>13</sup>C values. The trophic position of each consumer taxon of each stream was estimated

based on fractionation of  $\delta^{15}\text{N}$  using the method of Vander Zanden & Rasmussen (1999):

$$\text{TP} = ((\delta^{15}\text{N}_{\text{consumer}} - \delta^{15}\text{N}_{\text{reference}})/3.4) + 2,$$

where  $\delta^{15}\text{N}_{\text{reference}}$  is the mean  $\delta^{15}\text{N}$  value for a representative primary consumer, and 3.4‰ is the mean trophic fractionation. In lake studies, molluscs have been used as the representative primary consumers – bivalves as consumers of pelagic primary production sources, and gastropods as consumers of benthic primary production sources. Because the four Costa Rican streams are in regions with high rainfall and run-off as well as dense forests that shade streams, we assumed relatively little availability of pelagic production sources. We, therefore, based our calculation of consumer TPs on gastropods (the neritid snail *Neritina latissima* Broderip, 1883 common at Corcovado, and ampullariid snails of the genus *Pomacea* common at Tortuguero) as reference primary consumers of periphyton and macrophytes. To the extent that these herbivores consume profitable sources of primary production available within their habitats, their  $\delta^{15}\text{N}$  signatures should provide a reliable index of the average trophic fractionation between the first and second trophic levels. The advantage of using primary consumers rather than primary producers as the trophic fractionation index is that primary consumers integrate the isotopic signal based on their proportional consumption of alternative production sources among the many encountered in the habitat (Anderson & Cabana 2007, Vander Zanden & Rasmussen 1999). In contrast, when the mean for primary production sources is employed as the reference for trophic fractionation, it is assumed that all sources are consumed by herbivores in equal proportions. To evaluate the influence of the choice of isotopic baseline on estimates of consumer TPs, we also calculated TP by using the mean  $\delta^{15}\text{N}$  of the primary producer samples obtained from each site and adding 1 instead of 2 to the right-hand side of the equation that calculates consumer TP.

To compare trophic position estimates based on isotopic and dietary methods, biplots were constructed with the mean TP of fish species based on isotopic data on x-axis and TP based on dietary data on the y-axis. To test for statistical significance of the linear relationship, we performed Pearson's correlation on untransformed TP values. To compare TP of obligate versus facultative (migratory) freshwater consumer taxa, we categorized each macroinvertebrate taxon and fish species as either freshwater or diadromous. All insect taxa, snails of the genus *Pomacea*, and fishes from freshwater families (e.g. Characidae, Cichlidae, Poeciliidae) were classified as freshwater, and all decapod crustaceans and fishes of estuarine and marine families (e.g. Gobiidae, Lutjanidae, Tetraodontidae) were classified as diadromous. Mean between-group differences

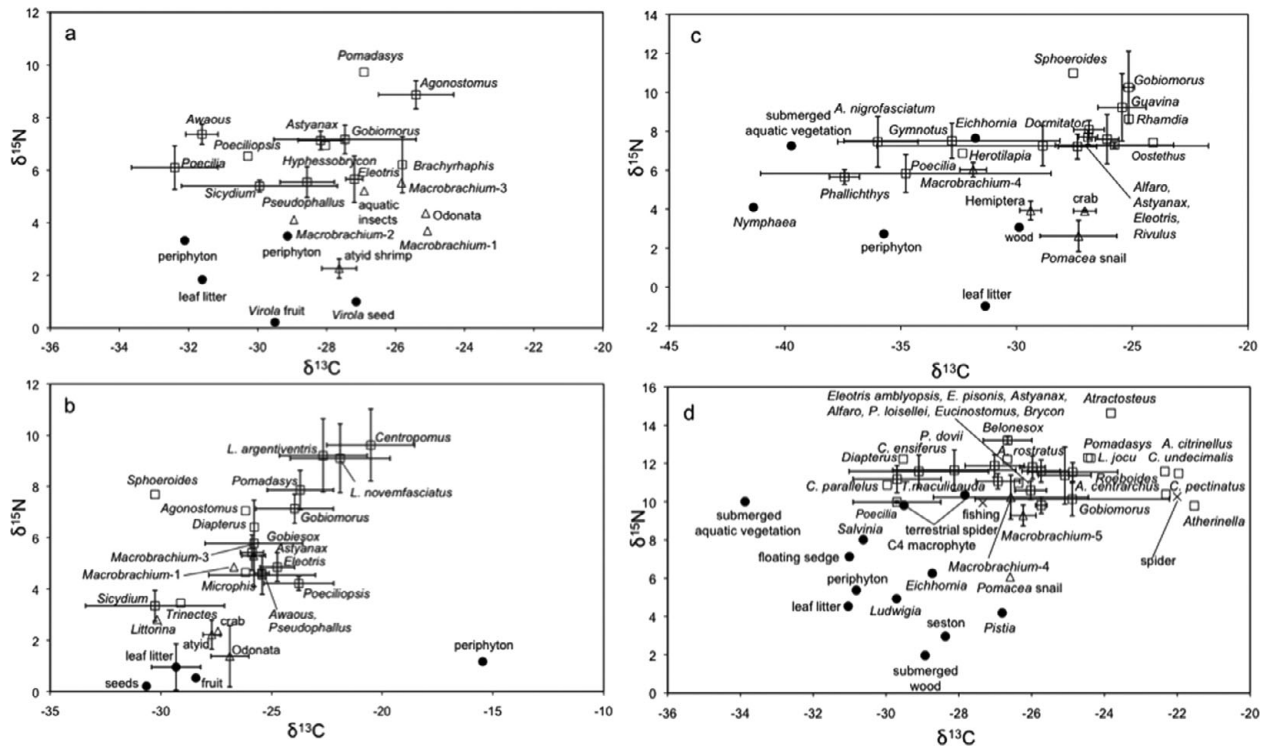
of stable isotope ratios (C, N) and TP were tested with a t-test (two-tailed, significance at  $\alpha = 0.05$  for each stream separately).

## RESULTS

### Sources of primary production supporting aquatic food webs

In all four of the coastal streams, many fish and invertebrate consumer taxa had C isotope ratios heavier (higher  $\delta^{13}\text{C}$  values) than the averages of basal source samples obtained during this study (Appendix 1, Figure 2). In Quebrada Camaronal (Corcovado Park, Pacific coast),  $\delta^{13}\text{C}$  of four primary producer samples ranged from  $-32.1\text{‰}$  (periphyton) to  $-27.1\text{‰}$  (seeds of *Virola* tree), and mean consumer values ranged from  $-32\text{‰}$  (poeciliid fish *Poecilia gilli*) to  $-25\text{‰}$  (prawn *Macrobrachium* sp. 1) (Figure 2). In the Rio Claro (Pacific coast),  $\delta^{13}\text{C}$  of four primary producer samples ranged from  $-30.6\text{‰}$  (seeds) to  $-15.5\text{‰}$  (periphyton), and mean consumer values ranged from  $-30\text{‰}$  (goby fish *Sicydium salvini*) to  $-20.5\text{‰}$  (juvenile black snook *Centropomus nigrescens*) (Figure 2). In Quebrada Estacion (Tortuguero Park, Caribbean coast),  $\delta^{13}\text{C}$  of six primary-producer samples ranged from  $-41.3\text{‰}$  (water lily *Nymphaea* sp.) to  $-29.9\text{‰}$  (submerged wood), and mean consumer values ranged from  $-37\text{‰}$  (poeciliid fish *Phallichthys amates*) to  $-24\text{‰}$  (pipefish *Oostethus lineatus*) (Figure 2). In the Rio Tortuguero (Caribbean coast),  $\delta^{13}\text{C}$  of 12 primary producer samples ranged from  $-33.9\text{‰}$  (unidentified submerged aquatic macrophyte) to  $-29.9\text{‰}$  (water lettuce *Pistia stratiotes*), and mean consumer values ranged from  $-30\text{‰}$  (juvenile fat snook *Centropomus parallelus*) to  $-21.5\text{‰}$  (silverside *Atherinella* sp.) (Figure 2). A comparison of the periphyton samples from the four streams reveals that  $\delta^{13}\text{C}$  values were similar for Quebrada Camaronal ( $-32.1\text{‰}$ ,  $-29.1\text{‰}$ ), Quebrada Estacion ( $-35.7\text{‰}$ ) and Rio Tortuguero ( $-35.7\text{‰}$ ), but the single periphyton sample scraped from rocks in the Rio Claro had a much heavier signature ( $-15.5\text{‰}$ ). Several fish species that were identified as benthic grazers based on observations of foraging behaviour and presence of algae and fine particulate detritus in stomachs (Winemiller 1983, 1990; Winemiller & Morales 1989) plotted in positions of two-dimensional isotope space that reflected periphyton as their likely principal food resource (aligned  $\pm 1\text{‰}$  on the x-axis and  $+3\text{--}3.5\text{‰}$  on the y-axis); these grazers included *Poecilia gilli* and *Sicydium salvini* at Quebrada Camaronal, *Phallichthys amates* and *P. gilli* at Quebrada Estacion, and *P. gilli* at Rio Tortuguero (Figure 2). *Poecilia* at Quebrada Estacion revealed large between-individual variation in carbon isotope ratios ( $-39.0\text{‰}$ ,  $-37.8\text{‰}$ ,  $-27.6\text{‰}$ ). All of these individuals





**Figure 2.** Biplots of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  (mean  $\pm$  1 SE) of plants (circles), invertebrates (triangles), and fishes (squares) sampled in Corcovado National Park streams: Quebrada Camaronal (a) and Rio Claro (b); and Tortuguero National Park streams: Quebrada Estacion (c) and Rio Tortuguero (d). Full taxon names appear in the Appendix 1.

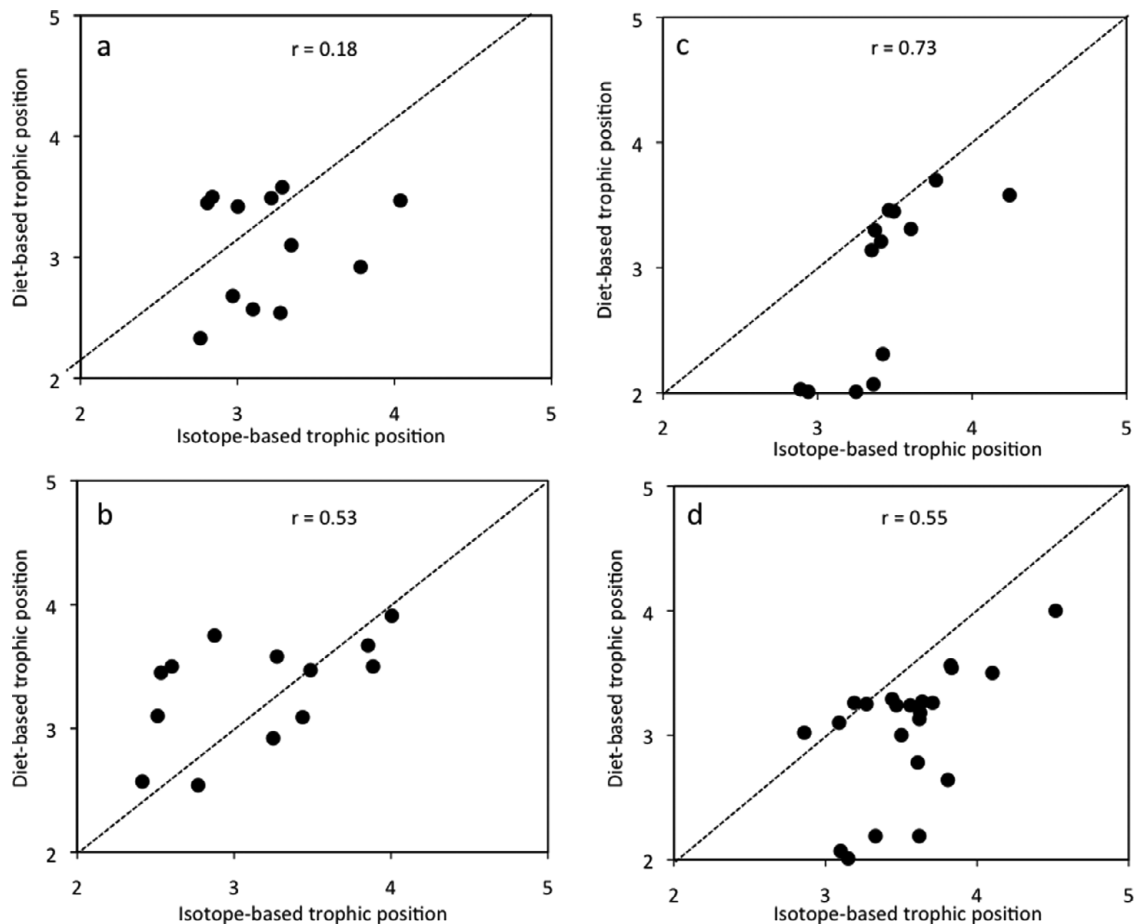
were subadults between 28–31 mm standard length (SL). *Poecilia* from the other streams varied less, and were a mixture of sizes (75–90 mm SL at Quebrada Camaronal, 25–53 mm SL at Rio Tortuguero). Another species at Quebrada Estacion showing large between-individual variation in carbon signatures was the omnivorous sleeper, *Dormitator maculatus*: 52 mm SL (−27.6‰), 54 mm (−22.4‰), and 110 mm (−36.6‰).

Two basal production sources at Quebrada Estacion (*Nymphaea* and an unidentified submerged macrophyte) and one at Rio Tortuguero (an unidentified submerged macrophyte) had  $\delta^{13}\text{C}$  values significantly lower than any of the consumers sampled at the same site. Tissue from living water hyacinth (*Eichhornia crassipes*) at Quebrada Estacion had a nitrogen signature higher than most consumers having a similar range of carbon values. Similarly, at Rio Tortuguero, the two samples of terrestrial plants had  $\delta^{15}\text{N}$  values as high or higher than taxa known to be herbivores. At Quebrada Estacion, half of the consumer taxa that were sampled had  $\delta^{13}\text{C}$  values significantly higher than any of the six *in situ* production sources. Likewise, about half of the 31 consumer taxa sampled at the Rio Tortuguero had carbon signatures that were significantly higher than any of the 12 *in situ* production source samples.

**Vertical food-web structure and consumer trophic positions**

Correlations between diet-based estimates of consumer trophic positions (TPs) and isotope-based estimates of TPs were as follows: Rio Claro:  $r = 0.53$ ,  $df = 11$ ,  $P > 0.05$ , Quebrada Camaronal:  $r = 0.18$ ,  $df = 10$ ,  $P > 0.05$ , Rio Tortuguero,  $r = 0.55$ ,  $df = 20$ ,  $P < 0.01$ ; Quebrada:  $r = 0.73$ ,  $df = 11$ ,  $P < 0.01$  (Figure 3). For the Tortuguero streams, isotope-based TP values tended to be higher than diet-based values, whereas there was no systematic pattern for the two Corcovado streams. Due to the manner by which TP is calculated from  $\delta^{15}\text{N}$  data, estimates based on snails as the reference and those based on the mean of primary producers as the reference were perfectly correlated (Appendix 1). TP values based on  $\delta^{15}\text{N}$  data and using primary consumers (snails) as the reference yielded estimates that were higher than those calculated using the mean of primary-producer samples as the reference (mean  $\pm$  SD difference =  $0.87 \pm 0.29$ ).

Primary consumer taxa other than snails, with TP values (based on  $\delta^{15}\text{N}$  with snails as reference) between 1.5 and 2.5, were as follows: Quebrada Camaronal: Odonata, unidentified aquatic insect, atyid prawn and *Macrobrachium* sp. 1 and sp. 2; Rio Claro: Odonata, crab, atyid shrimp, *Sicydium*, *Trinectes* and *Poeciliopsis*;



**Figure 3.** Comparison of consumer trophic position values estimated using dietary data with trophic position values estimated using stable isotope ratios in Corcovado National Park streams Quebrada Camaronal (a) and Rio Claro (b) and Tortuguero National Park streams Quebrada Estacion (c) and Rio Tortuguero (d). The diagonal line represents perfect one-to-one correspondence of the two estimates.

Quebrada Estacion: Hemiptera and crab; Rio Tortuguero: no taxon other than snails with TP < 2.5 (Appendix 1). Based on  $\delta^{15}\text{N}$  with snail references, top predators in Quebrada Camaronal were *Pomadasys bayanus* (TP = 4.0), *Agonostomus monticola* (3.8), *Awaous banana* (3.3), *Gobiomorus latifrons* (3.3) and *Astyanax fasciatus* (3.3). TP values of top predators in the Rio Claro, a larger stream than Q. Camaronal, were similar: *Centropomus nigrescens* (4.0), *Lutjanus argentiventris* (3.9), *L. novemfasciatus* (3.85), *Pomadasys bayanus* (3.5), *Sphoeroides annularis* (3.4) and *Gobiomorus latifrons* (3.3). TP values of top predators in the two Caribbean streams were as follows: Quebrada Estacion: *Sphoeroides testudineus* (4.5), *Gobiomorus dormitor* (4.2), *Guavina guavina* (3.9), *Rhamdia guatemalensis* (3.8) and *Parachromis loisellei* (3.6); R. Tortuguero: *Atractosteus tropicus* (4.5), *Belonesox belizanus* (4.1), *Lutjanus jocu* (3.8), *Centropomus ensiferus* (3.8), *Astatheros rostratus* (3.8), *Parachromis loisellei* (3.7), *Eucinostomus* sp. (3.7), *Parachromis dovii* (3.6), *Alfaro cultratus* (3.6), *Amphilophus citrinellus* (3.6), *Theraps maculicauda* (3.6), *Archocentrus centrarchus* (3.6),

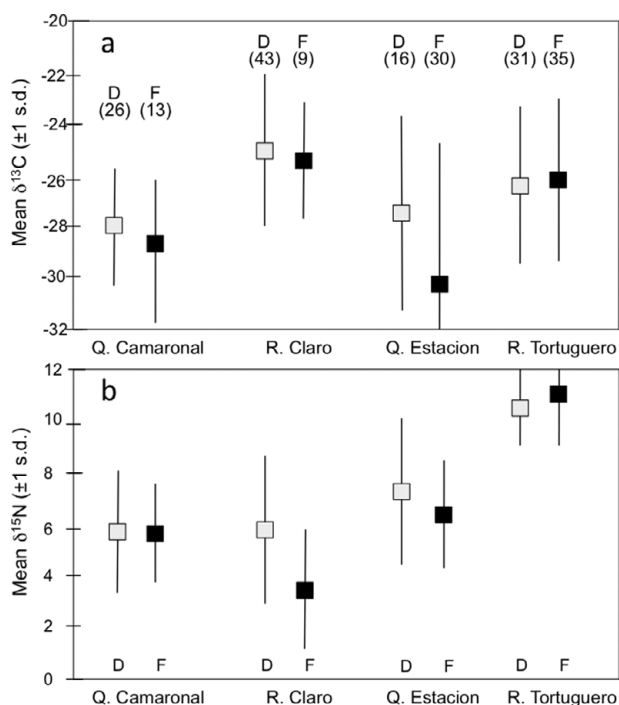
*Centropomus undecimalis* (3.6) and *Pomadasys crocro* (3.6). The mean TP of obligate freshwater taxa was not significantly different than the mean TP of diadromous taxa from the same local community for any of the four streams (two-tailed t test,  $P > 0.05$ ).

Mean  $\delta^{13}\text{C}$  of freshwater versus diadromous taxa was significantly different only for Quebrada Estacion at Tortuguero ( $t = 2.11$ ,  $df = 37$ ,  $P < 0.05$ ), with diadromous taxa having heavier signatures on average (Figure 4). Among the between-group comparisons of mean  $\delta^{15}\text{N}$ , only Rio Claro had a significant difference ( $t = 3.04$ ,  $df = 14$ ,  $P < 0.01$ ).

## DISCUSSION

### Basal production sources

Based on the positions of consumers relative to periphyton samples in biplots (Figure 2), periphyton could have been a significant source of carbon supporting many



**Figure 4.** Comparison of mean ( $\pm 1$  SD) standardized isotopic ratios of diadromous (D) and freshwater (F) consumer taxa from the four tropical streams:  $\delta^{13}\text{C}$  (a) and  $\delta^{15}\text{N}$  (b). Sample sizes appear in parentheses.

and perhaps most primary consumers in every stream. This inference for the Rio Claro is based on a single periphyton sample with a high  $\delta^{13}\text{C}$  value that greatly exceeded values for periphyton from the other three streams in this study. Mean carbon isotope signatures of benthic scrapers in the Rio Claro (e.g. *Neritina* snails and *Sicydium* gobies) were much lower ( $-30\text{‰}$ ) than this periphyton sample, which suggests there could have been error associated with this periphyton sample. Standing biomass of periphyton was extremely low in the Rio Claro, and periphyton scraped from rocks could have been contaminated with calcium carbonate, which could have elevated the carbon isotopic signature. Even so, high  $\delta^{13}\text{C}$  values for periphyton samples ( $-10\text{‰}$  to  $-18\text{‰}$ ) have been recorded by other studies of tropical streams (March & Pringle 2003, Mendoza-Carranza *et al.* 2010), although lower values ( $-30\text{‰}$  to  $-25\text{‰}$ ) seem to be more commonly reported (Kilham & Pringle 2000, Mantel *et al.* 2004, Verburg *et al.* 2007).

Based on relative positions in the isotopic biplots, leaf litter, fruits, seeds and certain aquatic macrophytes are potentially important sources of carbon supporting macro-invertebrates and fishes in all four streams. For example, atyid shrimp in Quebrada Camaronal plotted approximately  $+2\text{‰}$  above *Virola* fruits and seeds on the y-axis, and aligned with them on the x-axis. Nitrogen isotope signatures of periphyton were  $1.5\text{--}3\text{‰}$  higher than that of atyid shrimp, making it an unlikely resource

used by these grazers. Using C and N stable isotope data, Yam & Dudgeon (2005) and Lau *et al.* (2009) estimated that periphyton, fine particulate organic matter, and lesser fractions of leaf litter supported atyid shrimp biomass in both shaded and unshaded streams in Hong Kong. In forest streams in Puerto Rico, shrimp (*Atya*, *Macrobrachium* and *Xiphocaris* spp.) were estimated to have assimilated most of their carbon from periphyton ( $\delta^{13}\text{C}$ : c.  $-18\text{‰}$ ) and biofilm ( $\delta^{13}\text{C}$ :  $-25\text{‰}$  to  $-20\text{‰}$ ), and leaves ( $\delta^{13}\text{C}$ :  $-30\text{‰}$  to  $-29\text{‰}$ ) seemed to contribute little (March & Pringle 2003). Lau *et al.* (2009) reported that leaf litter had generally lower C and N isotopic signatures compared with periphyton in Hong Kong streams, and isotopic ratios of both basal production sources and consumers differed with regard to season and stream shading. Robust conclusions about the importance of local production sources for the food web cannot be made, because our samples were collected over a period of a few days with little replication in space. Aquatic production sources, periphyton (microalgae) in particular, may undergo temporal shifts in isotopic ratios in response to changing environmental conditions, or may vary depending on physical conditions in specific microhabitats (Boon & Bunn 1994, Finlay 2004, Finlay *et al.* 1999). Both Corcovado and Tortuguero national parks have variable monthly rainfall but lack strong wet/dry seasonality, and the 2004 samples were collected during a relatively dry period with stable stream discharges at both locations.

Based on positions in the isotope biplots, most consumer taxa of the two coastal streams at Tortuguero National Park (Caribbean drainage) probably are supported by variable fractions of material derived from periphyton, terrestrial leaf litter, and certain aquatic plants, including water hyacinth (*Eichhornia crassipes*), water lettuce (*Pistia stratiotes*) and water primrose (*Ludwigia* sp.). Seston, including phytoplankton, also could be a significant basal production source supporting consumers in the Rio Tortuguero. The lower reaches of both the Rio Tortuguero and Quebrada Estacion have very low gradients with slow velocities during periods of low discharge. During the driest periods, these lower reaches actually reverse flow during high tides, and these lentic conditions would be conducive for phytoplankton growth and retention. Two of the aquatic macrophytes sampled at Tortuguero (water lily, *Nymphaea* sp., and an unidentified submerged plant) had extreme negative  $\delta^{13}\text{C}$  values and apparently contributed little or no carbon to consumer taxa, however it should be noted that, in a manner similar to benthic microalgae, aquatic macrophytes can undergo temporal shifts in isotopic signatures (Boon & Bunn 1994).

In all four of the coastal streams we examined, many consumers had heavier carbon isotopic signatures than any of the local primary production sources that were sampled. Because stable isotopes of carbon usually do

not fractionate more than approximately 1‰ during transfer between trophic levels (McCutchan *et al.* 2003), the carbon isotope ratio of a consumer should reflect the proportional assimilation of carbon from primary production sources supporting food chains leading to a given consumer. Limited samples of the most conspicuous fishes, aquatic macro-invertebrates, periphyton, aquatic plants and terrestrial vegetation, including seeds and fruits that had fallen into or near the water, were collected from each of the study streams. At least four non-mutually exclusive hypotheses could account for the disparity between consumers with carbon isotopic signatures that were heavier than those of all basal production sources collected from the habitat. First, our study could have failed to sample at each location one or more important plants that were enriched in  $^{13}\text{C}$ . Given the limited number of periphyton, aquatic macrophyte and terrestrial plant material sampled during the study, this hypothesis cannot be falsified without analysis of additional samples. It seems unlikely that more samples of the same basal resource categories, including primary producer taxa that were rare in these habitats, would produce very many, or perhaps any, heavy  $\delta^{13}\text{C}$  values. Tropical C4 grasses typically have heavy  $\delta^{13}\text{C}$  ( $-20\%$  to  $-10\%$ , Jepsen & Winemiller 2007), but these grasses were essentially absent from the riparian habitats of the wet forests of Corcovado and Tortuguero. Shifts in the abundance of primary production sources associated with hydrological variation was documented in a North American temperate river (Zeug & Winemiller 2008), and this possibility cannot be ruled out given the very limited time interval in which our tissue samples were collected.

A second possible explanation for the presence of many consumers with heavier  $\delta^{13}\text{C}$  values than any of the local sources that were collected was already mentioned. Benthic microalgae, aquatic macrophytes and/or terrestrial vegetation entering the streams could have undergone temporal shifts in their isotopic ratios. Studies have documented such shifts in algae and aquatic plants in both temperate and tropical lotic ecosystems (Boon & Bunn 1994, Finlay 2001, 2004; Finlay *et al.* 1999). These isotopic shifts apparently are caused by physico-chemical environmental factors, including water velocity and pressure, changes in relative availability of organic and inorganic carbon molecules, and other factors. In addition, bulk periphyton isotopic ratios could be influenced by dynamics of cell growth and community dynamics in response to changing nutrients, light or grazing pressure. Our samples were collected during a relatively dry period with relatively stable stream discharges at both locations.

A third hypothesis is that migratory invertebrates and fishes in each coastal stream could have assimilated carbon derived from primary producers in the adjacent marine ecosystem before entering the freshwater habitat.

Marine ecosystems tend to be associated with more  $^{13}\text{C}$ - and  $^{15}\text{N}$ -enriched plants and animals when compared with freshwater ecosystems (Garcia *et al.* 2007, Winemiller *et al.* 2007, 2011). Some of the predatory fishes of these streams, such as juvenile and adult snooks (*Centropomus* spp.) and snappers (*Lutjanus* spp.), migrate between freshwater streams and coastal brackish or marine waters. Thus, their relatively heavy isotopic signatures could reflect recent histories of feeding on a mixture of food resources from marine and freshwater ecosystems. According to Lyons & Schneider (1990), all but three or four of the fish species in the Rio Claro spend a portion of their lives in the estuary or Pacific Ocean, and fish community structure was correlated more with distance from the coast than with local habitat variables. Snails of the genus *Neritina* migrate seasonally for several kilometres in Rio Claro (Schneider & Frost 1986), and presumably the Quebrada Camaronal as well. Palaemonid shrimp of the genus *Macrobrachium* are amphidromous, with juveniles migrating upstream, sometimes for hundreds of kilometres and crawling over numerous natural obstructions (Benstead *et al.* 1999).

Fourth, many of the resident freshwater macrocrustaceans and fishes could have preyed upon invertebrates and juvenile fishes that immigrate into streams from coastal marine waters. For example, the 'tismische' at Tortuguero is a mass immigration of post-larval shrimp and fishes (dominated by *Eleotris* spp. and other Gobiiformes) from marine coastal waters that occurs several times per year (Gilbert & Kelso 1971, Winemiller & Ponwith 1998), thus providing a massive influx of food resources for resident freshwater consumers. The tismische is an example of a spatial food-web subsidy involving transfer of biomass originating from the marine ecosystem into coastal streams, but other examples can be cited. Upstream migrations of *Neritina* snails in the Rio Claro provide a marine-derived subsidy for predators in the freshwater food web. Although they do not typically migrate in large numbers in synchrony, many juvenile marine fishes enter freshwater streams where some of them ultimately fall prey to larger fishes.

The isotopic data currently available cannot directly test these four alternative hypotheses for consumers with carbon isotope signatures heavier than *in situ* primary production sources, but the second and third seem most likely. Nonetheless, insight may be gained from the comparison isotopic ratios of freshwater versus diadromous taxa (Figure 4). As noted previously, studies of coastal zones have consistently documented heavier carbon and nitrogen ratios of both plants and consumers from marine habitats compared to nearby freshwater ecosystems (Garcia *et al.* 2007, Mendoza-Carranza *et al.* 2010, Winemiller *et al.* 2007, 2011). Diadromous species tended to have heavier carbon and nitrogen signatures



than their freshwater taxa at every stream except for Rio Tortuguero, however only two of these six cases were statistically significant (Quebrada Estacion for  $\delta^{13}\text{C}$ ; Rio Claro for  $\delta^{15}\text{N}$ ). Quebrada Estacion is a small stream that empties into the Laguna Tortuguero (estuary) and thus was easily colonized by crustaceans and estuarine fishes such as pufferfish (*Sphoeroides testudineus*). The Rio Claro empties directly into the Pacific Ocean, and its community was dominated by decapod crustaceans and fishes from marine families, including pufferfish, snappers (Lutjanidae), snooks (Centropomidae) and pipefish (Syngnathidae).

### Trophic position

Differences between dietary and isotopic estimates of consumer trophic positions were likely due to three factors: (1) biases associated with small sample sizes for the diet estimates of several species (affecting TP estimates from stomach-contents data), (2) error in estimating contributions of basal sources supporting consumers (affecting TP estimates based on isotopic data) and (3) error in the assumed trophic fractionation of  $\delta^{15}\text{N}$  (Caut *et al.* 2009). Nonetheless, there were moderate to high correlations between TP values derived from the two methods for three of the four streams; the only low correlation was for Quebrada Camaronal. There are obvious strengths and limitations of each method. Dietary analysis provides a short-term feeding history of the individual organism (minutes to hours), and its precision and accuracy are highly dependent on resolution of food items identified within gut contents, sample size (number of guts examined), and heterogeneity of the sample (e.g. temporal, spatial and body-size variation within the consumer population). Stable isotope analysis provides a time-integrated estimate of feeding history (weeks to months), and thus may not reflect recent feeding history, especially for organisms that move between ecosystems. Using the isotopic method, Lau *et al.* (2009) found that maximum TP estimated for shaded streams in Hong Kong remained fairly constant between the dry and wet seasons, whereas TP values for unshaded streams were higher and showed seasonal fluctuations. Clearly, the temporal scale of sampling for both methods influences TP estimates. In the present study, all Corcovado samples were obtained during June–July. The Tortuguero diet samples were integrated over an entire annual cycle and isotope samples were obtained during June, and yet Tortuguero TP estimates had highest correlations for the two methods.

Isotope-based TP values of certain species were clearly underestimated, e.g. atyid shrimp, odonate nymph and pseudoscorpion crab all with values  $< 2$  (strict herbivore = 2.0). Other isotope-based TP values appear

to be overestimates, e.g. herbivorous *Poecilia gilli*, *Phallichthys amates* and *Brycon guatemalensis* with  $\text{TP} \sim 3$ ; the dietary method estimated  $\text{TP} \sim 2$  for these species. The dietary method probably underestimated TP for *Awaous banana* (2.75), a goby that engulfs and winnows sand and other fine substrates to sift out aquatic invertebrates. These fish have relatively short guts consistent with carnivory, which was reflected by the isotope-based TP (3.34). Gut-contents analysis of the *Awaous* diet included a minor fraction of detritus, but this material apparently is not assimilated. Similarly, the cichlids *Archocentrus centrarchus* and *Theraps maculicauda* had large disparities between isotopic and diet-based TP estimates, and this appears to have been due to ingestion of large fractions of detritus but with low assimilation.

The maximum trophic position (TP of the top predator) can be interpreted as an indicator of the mean food-chain length of the food web (Hoeinghaus *et al.* 2008, Post *et al.* 2000). Comparing the four Costa Rican streams, the maximum TPs were: Q. Camaronal 4.04 – *Pomadasys bayanus*, R. Claro 4.01 – *Centropomus nigrescens*, Q. Estacion 4.46 – *Sphoeroides testudineus* and R. Tortuguero 4.52 – *Atractosteus tropicus*. Thus, there was no difference in maximum TP between the small and large stream at each location, but the Tortuguero streams had higher maximum TP (+0.5). Ecosystem size was not correlated with maximum food-chain length as was found for temperate lakes (Post *et al.* 2000), because stream sizes were fairly evenly matched between locations (Q. Camaronal = Q. Estacion, R. Claro = R. Tortuguero). Another potential determinant of maximum TP is species richness, but this relationship is weak: Q. Camaronal = 16 fish spp. and R. Claro = 18 fish spp. (Winemiller & Morales 1989); Q. Estacion = 22 fish spp., R. Tortuguero = 59 fish spp. (Winemiller 1990). Aquatic ecosystem productivity (i.e. net aquatic productivity) was not measured in these streams, but it seems likely that they would rank as R. Tortuguero  $>$  Q. Estacion  $>$  R. Claro  $>$  Q. Camaronal. This ranking is inferred based on hydrology, nutrient inputs and degrees of shading. Both the Rio Tortuguero and Quebrada Estacion receive water from both local watershed and the estuary via tidal fluxes, and therefore should receive seston inputs from relatively lentic habitats downstream. Whereas Quebrada Estacion is heavily shaded and supports few aquatic macrophytes, the Rio Tortuguero has a broader channel with more exposure to sunlight that supports dense beds of floating and submerged aquatic macrophytes. No aquatic macrophytes are found in either of the Corcovado streams, but the wider channel of the Rio Claro is more exposed to sunlight than the narrow channel of Quebrada Camaronal that is almost entirely covered by rain-forest canopy. Thus, maximum TP in these coastal streams is probably influenced most strongly by ecosystem productivity, with species richness and other factors

having secondary influences, and ecosystem size having little apparent correlation.

## Conclusions

Overall, the two methods revealed similar patterns of resource utilization and vertical trophic structure in the four coastal streams, and differences could be attributed to data deficiencies and/or potential sources of bias associated with either method. These limitations notwithstanding, it appears probable that both periphyton and terrestrial vegetation, including fruits, are important basal production sources supporting biomass of aquatic organisms in all four streams. For three of the four streams, isotopic signatures of substrate grazing fishes were consistent with observed diets that were dominated by periphyton and fine particulate matter that likely is derived from a combination of periphyton and terrestrial vegetation.

Marine fishes and invertebrates enter coastal streams where they take up residence for variable periods of time (Kinzie 1988, Nordlie 1981, Pender & Griffin 1996) and become integral parts of the stream food web. Certain decapod crustaceans and fishes have life cycles whereby adults spawn within freshwater streams, and their eggs and larvae drift to the estuary (McDowall 1988), providing food resources for consumers along the route (March *et al.* 1998). Post-larval and juvenile decapods and fishes actively migrate from coastal waters into freshwater streams, sometimes *en masse* as with the tismische phenomenon at Tortuguero. Several studies have shown marine primary producers with heavier C and N isotopic ratios than producers from regional freshwater ecosystems, and diadromous species in the four Costa Rican streams tended to have heavier isotopic signatures than obligate freshwater species, although distributions were broadly overlapping in most cases. Thus, evidence for a trophic contribution of migratory organisms was weak with the exception of two comparisons that yielded statistically significant differences. It is important to note that diadromous fishes can influence coastal stream food webs in ways besides influencing predator–prey interactions and providing subsidies. Marine immigrants sometimes influence ecosystem processes, such as sediment accumulation and benthic net primary production (Pringle 1996, Pringle & Hamazaki 1998, Pringle *et al.* 1993), in tropical coastal streams.

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**Appendix 1.**  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  values, estimated trophic positions (TP) based on stable isotope data using either snails or plants as the baseline, trophic positions based on dietary data, and sample sizes for dietary and isotopic analyses of organisms (F = obligate freshwater; D = diadromous) comprising the food webs of four tropical streams. Fish species names follow Bussing (1998).

Taxon	Mean $\delta^{13}\text{C}$	Mean $\delta^{14}\text{N}$	TP-iso (snail)	TP-iso (plants)	TP-diet	$N_{\text{diet}}, N_{\text{iso}}$
Quebrada Camaronal, Corcovado National Park, Costa Rica						
Periphyton	-30.6	3.4	—	—	—	—, 2
Leaf litter	-31.6	1.8	—	—	—	—, 1
Fruit ( <i>Virola</i> )	-29.5	0.2	—	—	—	—, 1
Seed ( <i>Virola</i> )	-27.1	1.0	—	—	—	—, 1
Aquatic insect (Odonata) (F)	-28.1	2.8	2.0	1.4	—	—, 2
Aquatic insect (Unidentified) (F)	-26.9	5.2	2.7	2.1	—	—, 1
Prawn (Atyidae) (D)	-27.6	2.3	1.8	1.2	—	—, 2
Prawn ( <i>Macrobrachium</i> sp. 1) (D)	-25.1	3.7	2.3	1.6	—	—, 1
Prawn ( <i>Macrobrachium</i> sp. 2) (D)	-28.9	4.1	2.4	1.7	—	—, 1
Prawn ( <i>Macrobrachium</i> sp. 3) (D)	-25.8	5.5	2.8	2.1	—	—, 1
<i>Sicydium salvini</i> (D)	-29.9	5.4	2.8	2.1	2.2	15, 3
<i>Pseudophallus starksi</i> (D)	-28.6	5.5	2.8	2.1	3.0	23, 3
<i>Eleotris pictus</i> (D)	-27.2	5.7	2.8	2.2	3.2	39, 3
<i>Poecilia gilli</i> (F)	-32.4	6.1	3.0	2.2	2.3	13, 3
<i>Brachyrhaphis rhabdophora</i> (F)	-25.8	6.2	3.0	2.3	3.4	37, 2
<i>Poeciliopsis turrubarensis</i> (F)	-30.3	6.5	3.1	2.3	2.4	15, 1
<i>Hyphessobrycon savaaei</i> (F)	-28.0	6.9	3.2	2.4	3.5	45, 1
<i>Astyanax fasciatus</i> (F)	-28.2	7.1	3.3	2.6	2.5	41, 3
<i>Gobiomorus latifrons</i> (D)	-27.5	7.2	3.3	2.6	3.3	20, 4
<i>Awaous banana</i> (D)	-31.6	7.4	3.3	2.6	2.7	8, 2
<i>Agonostomus monticola</i> (D)	-25.4	8.9	3.8	2.7	2.8	3, 3
<i>Pomadasys bayanus</i> (D)	-26.9	9.7	4.0	3.2	3.0	5, 1
Rio Claro, Corcovado National Park, Costa Rica						
Periphyton	-15.5	0.5	—	—	—	—, 1
Leaf litter	-29.3	1.4	—	—	—	—, 3
Fruit	-28.4	1.0	—	—	—	—, 1
Seeds	-30.7	1.6	—	—	—	—, 1
Snail ( <i>Neritina latissima</i> ) (D)	-30.2	2.8	2.0	1.5	—	—, 1
Aquatic insect (Odonata) (F)	-26.9	1.4	1.6	1.1	—	—, 3
Crab (Pseudothelphusidae) (D)	-27.4	2.3	1.9	1.4	—	—, 1
Shrimp (Atyidae) (D)	-27.0	3.9	2.4	1.4	—	—, 3
Prawn ( <i>Macrobrachium</i> sp. 1) (D)	-26.7	4.9	2.6	2.1	—	—, 1
Prawn ( <i>Macrobrachium</i> sp. 3) (D)	-25.8	5.3	2.7	2.3	—	—, 2
<i>Sicydium salvini</i> (D)	-30.3	3.3	2.2	1.7	2.2	15, 4
<i>Trinectes paulistanus</i> (D)	-29.1	3.4	2.2	1.7	—	—, 1
<i>Poeciliopsis turrubarensis</i> (F)	-23.8	4.2	2.4	2.0	2.4	15, 3
<i>Awaous banana</i> (D)	-25.4	4.5	2.7	2.0	2.7	8, 3
<i>Pseudophallus starksi</i> (D)	-25.4	4.6	2.5	2.1	3.0	23, 2
<i>Microphis brachyurus</i> (D)	-26.2	4.6	2.5	2.1	—	—, 1
<i>Eleotris pictus</i> (D)	-24.7	4.8	2.6	2.1	3.2	39, 3
<i>Astyanax fasciatus</i> (F)	-25.9	5.4	2.8	2.3	2.5	41, 3
<i>Gobiesox potamius</i> (D)	-25.8	5.8	2.9	2.4	3.9	1, 2
<i>Diapterus</i> sp. (D)	-25.8	6.4	3.1	2.6	—	—, 1
<i>Agonostomus monticola</i> (D)	-26.2	7.0	3.2	2.8	2.8	3, 1
<i>Gobiomorus latifrons</i> (D)	-24.0	7.1	3.3	2.8	3.3	20, 3
<i>Sphoeroides annularis</i> (D)	-22.8	7.7	3.4	3.0	3.0	9, 1
<i>Pomadasys bayanus</i> (D)	-23.7	7.9	3.5	3.0	3.0	5, 4
<i>Lutjanus novemfasciatus</i> (D)	-21.9	9.1	3.8	3.4	3.5	11, 3
<i>Lutjanus argentiventris</i> (D)	-22.7	9.2	3.9	3.4	3.1	17, 3
<i>Centropomus nigrescens</i> (D)	-20.5	9.6	4.0	3.5	4.1	8, 3
Quebrada Estacion, Tortuguero National Park, Costa Rica						
Periphyton	-35.7	2.7	—	—	—	—, 1
Leaf litter	-31.3	1.0	—	—	—	—, 1
Wood	-29.9	3.1	—	—	—	—, 1
Water lily ( <i>Nymphaea</i> )	-41.4	4.1	—	—	—	—, 1
Aquatic macrophyte	-39.7	7.2	—	—	—	—, 1
Water hyacinth ( <i>Eichhornia</i> )	-31.8	7.6	—	—	—	—, 1
Snail ( <i>Pomacea</i> sp.) (F)	-27.3	2.6	2.0	0.8	—	—, 3
Aquatic insect (Hemiptera) (F)	-29.4	3.9	2.4	1.2	—	—, 2
Crab (Pseudothelphusidae) (D)	-27.0	3.9	2.4	1.2	—	—, 2
Prawn ( <i>Macrobrachium</i> sp. 4) (D)	-31.9	6.0	3.0	1.8	—	—, 3
<i>Phallichthys amates</i> (F)	-37.4	5.6	2.9	1.7	2.0	168, 3
<i>Poecilia gilli</i> (F)	-34.8	5.8	2.9	1.8	2.0	160, 3

## Appendix 1. Continued.

Taxon	Mean $\delta^{13}\text{C}$	Mean $\delta^{14}\text{N}$	TP-iso (snail)	TP-iso (plants)	TP-diet	$N_{\text{diet}}, N_{\text{iso}}$
<i>Herotilapia multispinosa</i> (F)	-32.3	6.9	3.2	2.1	2.0	18, 1
<i>Rivulus isthmensis</i> (F)	-27.4	7.2	3.3	2.2	3.1	76, 3
<i>Dormitor maculatus</i> (D)	-28.9	7.2	3.4	2.2	2.1	81, 3
<i>Eleotris amblyopsis</i> (D)	-25.8	7.3	3.4	2.2	3.3	299, 2
<i>Oostethus lineatus</i> (D)	-24.1	7.4	3.4	2.2	3.2	57, 1
<i>Amatitlania nigrofasciata</i> (F)	-36.0	7.5	3.4	2.2	2.3	232, 2
<i>Gymnotus cylindricus</i> (F)	-32.8	7.5	3.4	2.3	—	—, 3
<i>Astyanax fasciatus</i> (F)	-26.1	7.6	3.5	2.3	2.6	245, 3
<i>Alfaro cultratus</i> (F)	-26.9	7.7	3.5	2.3	3.4	255, 3
<i>Parachromis loisellei</i> (F)	-26.9	8.1	3.6	2.3	3.3	148, 3
<i>Rhamdia guatemalensis</i> (F)	-25.1	8.6	3.8	2.4	3.7	182, 1
<i>Guavina guavina</i> (D)	-25.4	9.2	3.9	2.6	—	—, 2
<i>Gobiomorus dormitor</i> (D)	-25.2	10.2	4.3	2.8	3.6	58, 2
<i>Sphoeroides testudineus</i> (D)	-27.5	11.0	4.5	3.1	—	—, 1
Rio Tortuguero, Tortuguero National Park, Costa Rica						
Periphyton	-35.7	2.7	—	—	—	—, 1
Seston	-28.4	3.0	—	—	—	—, 1
Leaf litter	-31.0	4.5	—	—	—	—, 1
Wood	-28.9	2.0	—	—	—	—, 1
Aq. macrophyte ( <i>Pistia</i> )	-26.8	4.2	—	—	—	—, 1
Aq. macrophyte ( <i>Ludwigia</i> )	-29.7	4.9	—	—	—	—, 1
Aq. macrophyte ( <i>Salvinia</i> )	-30.6	8.0	—	—	—	—, 1
Water hyacinth ( <i>Eichhornia</i> )	-28.7	6.3	—	—	—	—, 1
Floating sedge	-31.0	7.1	—	—	—	—, 1
Submerged aq. macrophyte	-33.9	10.0	—	—	—	—, 1
Terrestrial plant leaf sp. 1	-29.5	9.8	—	—	—	—, 1
Terrestrial plant leaf sp. 2	-27.8	10.3	—	—	—	—, 1
Snail ( <i>Pomacea</i> sp.) (F)	-26.6	6.1	2.0	1.0	—	—, 1
Prawn ( <i>Macrobrachium</i> sp. 4) (D)	-24.9	10.1	3.2	2.2	—	—, 3
Prawn ( <i>Macrobrachium</i> sp. 5) (D)	-26.2	9.3	2.9	1.9	—	—, 2
Fishing spider ( <i>Dolomedes</i> ) (F)	-27.3	9.9	3.1	2.1	—	—, 1
Spider (unidentified) (F)	-22.0	10.3	3.2	2.2	—	—, 1
<i>Oostethus lineatus</i> (D)	-26.8	9.0	2.9	1.8	3.0	191, 3
<i>Atherinella</i> sp. (F)	-21.5	9.8	3.1	2.1	3.1	172, 3
<i>Brycon guatemalensis</i> (F)	-25.7	9.8	3.1	2.1	2.1	115, 3
<i>Poecilia gilli</i> (F)	-29.7	10.0	3.1	2.1	2.0	166, 3
<i>Gobiomorus dormitor</i> (D)	-24.9	10.1	3.2	2.2	3.3	129, 4
<i>Centropomus pectinatus</i> (D)	-22.3	10.4	3.3	2.2	3.2	27, 1
<i>Astyanax fasciatus</i> (F)	-26.0	10.6	3.3	2.3	2.2	304, 3
<i>Centropomus parallelus</i> (D)	-30.0	10.9	3.4	2.4	—	—, 1
<i>Eleotris pisonis</i> (D)	-26.4	11.0	3.4	2.4	3.3	146, 1
<i>Eleotris amblyopsis</i> (D)	-26.9	11.1	3.5	2.4	3.2	326, 3
<i>Diapterus</i> sp. (D)	-29.7	11.2	3.5	2.5	3.0	4, 3
<i>Pomadasys crocro</i> (D)	-25.1	11.4	3.6	2.5	3.2	97, 2
<i>Centropomus undecimalis</i> (D)	-22.0	11.5	3.6	2.6	—	—, 2
<i>Archocentrus centrarchus</i> (F)	-24.9	11.5	3.6	2.6	2.8	182, 2
<i>Theraps maculicauda</i> (F)	-29.1	11.6	3.6	2.6	2.2	160, 3
<i>Amphilophus citrinellus</i> (F)	-22.3	11.6	3.6	2.6	3.1	88, 1
<i>Alfaro cultratus</i> (F)	-25.7	11.6	3.6	2.6	3.2	158, 3
<i>Parachromis dovii</i> (F)	-28.1	11.6	3.6	2.6	3.3	156, 3
<i>Eucinostomus</i> sp. (D)	-26.0	11.8	3.7	2.7	—	—, 3
<i>Parachromis loisellei</i> (F)	-27.0	11.9	3.7	2.7	3.3	345, 3
<i>Astatheros rostratus</i> (F)	-26.7	12.2	3.8	2.8	2.6	11, 1
<i>Centropomus ensiferus</i> (D)	-29.5	12.2	3.8	2.8	—	—, 1
<i>Roeboides guatemalensis</i> (F)	-24.4	12.3	3.8	2.8	3.6	98, 1
<i>Lutjanus jocu</i> (D)	-24.4	12.3	3.8	2.8	3.5	57, 1
<i>Belonesox belizanus</i> (F)	-26.7	13.2	4.1	3.1	3.5	137, 3
<i>Atractosteus tropicus</i> (F)	-23.8	14.6	4.5	3.5	4.0	8, 1