White Rose Research Online
eprints@whiterose.ac.uk
https://eprints.whiterose.ac.uk
Universities of Leeds, Sheffield and York

Deposited via The University of Leeds.

White Rose Research Online URL for this paper:
https://eprints.whiterose.ac.uk/id/eprint/237913/

Version: Accepted Version

Article:

Walsh, G., South, J., Woodford, D.J. et al. (Accepted: 2026) Trophic diversity in aquatic food webs along a tropical coastal river continuum in west-central Africa. Freshwater Biology. ISSN: 1365-2427 (In Press)

This is an author produced version of an article accepted for publication in Freshwater Biology, made available via the University of Leeds Research Outputs Policy under the terms of the Creative Commons Attribution License (CC-BY), which permits unrestricted use, distribution and reproduction in any medium, provided the original work is properly cited.

Reuse
This article is distributed under the terms of the Creative Commons Attribution (CC BY) licence. This licence allows you to distribute, remix, tweak, and build upon the work, even commercially, as long as you credit the authors for the original work. More information and the full terms of the licence here: https://creativecommons.org/licenses/

Takedown
If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.

UNIVERSITY OF LEEDS
University of Sheffield
UNIVERSITY of York

Trophic diversity in aquatic food webs along a tropical coastal river continuum in west-central Africa

Gina Walsh[1,2,3], Josie South[4,5*], Darragh J. Woodford[1,4], Melanie L.J Stiassny[2], Jérôme Y. Gaugris[3,6], Valdie N. Boukaka-Mikembi[7], Allison A. Pease[8]

[1] School of Animal Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

[2] Division of Vertebrate Zoology, Department of Ichthyology, American Museum of Natural History, New York, New York, USA

[3] Flora Fauna & Man Ecological Services Ltd., Road Town, British Virgin Islands

[4] South African Institute for Aquatic Biodiversity, Makhanda, South Africa

[5] School of Biology, Faculty of Biological Sciences, University of Leeds, Leeds, UK

[6] Centre for African Ecology, School of Animal, Plant and Environmental Sciences, University of the Witwatersrand, Johannesburg, South Africa

[7] Institut National de Recherche en Sciences Exactes et Naturelles, Brazzaville, Republic of the Congo

[8] School of Natural Resources, University of Missouri, Columbia, Missouri, USA

[*]Correspondence: Josie South, j.south@leeds.ac.uk

Keywords: Stable isotopes, fish assemblages, river continuum, Congo Brazzaville, food webs

Abstract

1. In the understudied wet Afrotropics, information on trophic diversity and resource use in freshwaters is extremely limited.
2. Here we used stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) from food webs along the continuum of a coastal river in the Republic of the Congo to examine seasonal and spatial patterns of trophic diversity. We estimated assemblage-wide isotopic metrics and compared relative proportions of fish trophic positions along a fluvial gradient including a range of stream orders in the dry and wet seasons.
3. Headwater fish assemblages exhibited relatively high trophic niche breadths comparable to those in larger downstream sites, which deviates from the pattern expected based on widely accepted lotic models. Benthic algae was not visibly available at study sites across seasons, and fish, macroinvertebrate, and particulate organic matter $\delta^{13}C$ values were consistently depleted (< 25‰), suggesting predominance of forest C3 plants as a basal resource. However, we also observed $\delta^{13}C$ values in some fish and macroinvertebrate consumers which were well below riparian or particulate organic matter values, perhaps reflecting methane-derived sources from anoxic detritus.
4. Knowledge of trophic diversity and forest connections supporting stream food webs in this region is critical for conservation and maintenance of freshwater biodiversity and ecosystem services.

Introduction

Tropical stream ecosystems support highly diverse fish and macroinvertebrate assemblages with complex, seasonally shifting resources (Pease et al. 2020; Soto et al. 2019; Winemiller 1990). Understanding of the trophic diversity and resources fuelling these complex food webs is crucial for their long-term conservation, as alterations to food web resources can compromise healthy ecosystem functioning and provisioning of services (Cadotte et al. 2011; Wilkinson et al. 2021). Existing knowledge of trophic diversity and seasonal resource use in consumer communities of Afrotropical freshwaters is scarce (Sponseller et al. 2013; Thompson et al. 2012; Venarsky et al. 2020; Winemiller et al. 2011), and despite increasing knowledge of the diversity and functional ecology of fishes in the region (Cutler et al. 2019; Mamonekene and Stiassny 2012; Munene and Stiassny 2011; Stiassny et al. 2021; Walsh et al. 2014, 2022), literature on food web dynamics in freshwater ecosystems in the central African tropics is almost entirely absent, with just a single study of the Congo River published to date (Soto et al. 2019).

Established river ecosystem models such as the River Continuum Concept (Vannote et al. 1980), the Riverine Productivity Model (Thorp and Delong, 1994), and the Flood Pulse Concept (Junk et al. 1989) guide expectations of how longitudinal gradients and seasonality affect aquatic food webs through changes in availability of basal resources which subsequently shape consumer assemblages. Based on these concepts, it is possible to speculate on changes to trophic diversity along river networks (e.g., Power and Dietrich 2002, Hoeinghaus et al. 2007; Pease et al. 2019). In small headwater streams, less light penetrates the canopy of the riparian forest which results in relatively low in-stream productivity and large inputs of leaf litter and detritus. These low-order systems are also temporally variable (Coyle et al. 2014) with smaller habitat volumes and thus are expected to harbor more generalist consumers (lower trophic diversity) with shorter food chain lengths (Horwitz, 1978; Vannote et al. 1980; Woodward and Hildrew, 2002). Longitudinal studies in temperate streams have shown that headwaters have relatively low trophic diversity and higher trophic redundancy (Hette-Tronquart et al. 2016; Maitland and Rahel, 2023). Larger river systems have greater habitat volumes, higher productivity, and higher resource diversity, which is expected to result in higher trophic diversity, more trophic specialists (Thorp et al. 2008), and longer food chains (Power and Dietrich 2002; Thorp et al. 2006; Hette-Tronquart et al. 2016;).

In the tropics, distinct shifts in trophic habits of consumers can occur due to significant, predictable hydrological seasonality affecting fish assemblages through fluctuations in habitat and resource availability (Wantzen et al. 2002; Correa and Winemiller, 2014; Pease et al. 2020). Increased habitat volume in the wet season delivers subsidies of allochthonous detritus, macroinvertebrates, and nutrients to rivers, thus increasing primary production (Winemiller et al. 2004). As waters recede, predator-prey interactions are expected to intensify as fishes become increasingly concentrated in reduced habitat volumes (Jepsen et al. 1999). Eventually, piscivores may deplete prey during the dry season and systems may become resource limited during lowest water levels. Trophic positions of fishes in tropical food webs vary widely, with some instream consumer communities of large tropical systems functioning at lower trophic levels during the wet season due to increased availability of invertebrates causing a subsequent decrease in piscivory (Wantzen et al. 2002; McMeans et al. 2019). Other communities have a broader isotopic niche during the wet season, possibly due to a wider range of prey and increased access to inundated habitats (Pool et al. 2017).

Here we use carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) stable isotope ratios to provide insights into the trophic diversity patterns of freshwater fish and macroinvertebrate communities along a longitudinal and seasonal gradient of a coastal basin in the Republic of the Congo. We expected to see **(1)** lower isotopic niche diversity in lower-order streams with an increase in higher-order rivers as diversity of food resources increases along the continuum (Vannote et al.,1980; Thorp et al. 2008), **(2)** a shift in $\delta^{13}C$ values of consumers driven by use of more algae in the dry season and more terrestrial organic material in the wet season (Junk et al., 1989), and **(3)** a shift from higher trophic positions in fish communities during the dry season (due to higher piscivory) to lower trophic positions in the wet season (due to increased invertebrate resources and less constrained habitat). To address these expectations, we used an array of assemblage-wide isotopic metrics on data collected along a river continuum in the dry season and immediately following the wet season.

Methods

Study region and site selection

We sampled fishes, aquatic macroinvertebrates, and conspicuous basal resources in the Tintinda River, located in a small sub-basin of the Noumbi River near Conkouati-Douli National Park in the Republic of Congo. The Tintinda River, a tributary of the Noumbi, is an oligotrophic stream situated in the coastal plains of the Kouilou Department in the Lower Guinea forest (Error! Reference source not found.**)**. The basin land use consists of moderate logging around forested areas, harvesting of fruit and forbe species, with fishing a common practice in a sparsely populated landscape. The stream continuum transitions from *Annona senegalensis - Hyparrhenia diplandra* grassland-savannah in the first and second order headwaters, into *Manotes expansa - Millettia comosa* forest from the second order to fifth order, widening into *Elaeis guineensis - Hallea stipulosa* lowland swamp forest with a substantial lateral wetted area at the confluence of the Noumbi River (**Fig. 1**) (van Rooyen et al. 2016).

Sampling was carried out during two field surveys to account for seasonality in August 2016 (the end of the approximately 3-month dry season) and May–June 2017 (the end of the wet season where the stream systems had been inundated by high water levels for several months and were receding). The Spatial Analyst Hydrology package for ArcGIS 10.5 was used to generate river networks and Strahler orders for site selection. We sampled aquatic food webs at five study sites along the continuum of the Tintinda River ranging from 2nd to 6th Strahler order in each season (Error! Reference source not found. **and 2**). Including consecutive stream orders allowed us to capture changes in trophic diversity along the longitudinal course of the river seasonally. First-order grassland-savannah headwaters of the stream network were dry and were therefore not included in the study. At an elevation of between 7 and 31 m.a.s.l, the study area drains Tertiary and Quaternary coastal sediments in a basin consisting of leached sandy soils, with Pliocene Cirque sand sheets occurring along the coastline (van de Weghe, 2004)

Field data collection

Environmental data collected at each site included landscape and local measures (**Table 1**). Catchment area (km^2) and distance (km) from source were calculated using the watershed module of the Spatial Analyst extension of the Hydrology package for ArcGIS 10.5 to generate catchments from digital elevation models. Channel width (m), depth (m), and flow velocity (m/s) profiles were measured for each site across a transect to calculate water discharge (m^3/s). Light (lux) was measured with a digital lux meter, and temperature (°C), pH, dissolved oxygen (mg/l) and conductivity (µs/cm) were measured using a YSI Professional Plus multiparameter water quality meter. Turbidity (NTU) was measured with a Hach 2100Q portable turbidimeter. Triplicate water samples were filtered through 0.45 µm glass-fibre filters (Whatman) and analysed for phosphorus (P), nitrate-N, ammonia (NH^3) and silica (S) concentrations using a Hach DR900 multiparameter portable colorimeter.

Fishes were collected at each site using seine nets, cast nets, dip-nets, experimental gill nets, fyke nets, and backpack electrofisher. Sampling was carried out until all common species were collected in sufficient quantities for tissue sampling, consulting Walsh et al. (2014, 2022) to confirm that expected species representing all families and all trophic levels captured in previous surveys in the region. Fishes were identified to species level, with taxonomic nomenclature following van der Laan et al. (2014). Specimens were collected according to the guidelines for the use of fishes in research (AFS/AIFRB/ASIH, 2004) with individuals euthanized with a lethal dose of isoeugenol anaesthetic before further processing.

Fish muscle tissue samples were taken from three to ten specimens of all common fish species at each site for each season by removing the skin and harvesting a muscle plug from the flanks (fish samples for dry season n = 366, wet season n = 348). For small-bodied fish species (e.g., *Aphyosemion* spp., *Epiplatys* spp., and *Neolebias ansorgii*), we removed heads and internal organs, fins, scales, and skin, and collected whole bodies.

Aquatic macroinvertebrates were sampled by kick sampling with a dipnet and hand collection from benthos at each site (dry season n = 105; wet season n = 104). Samples were collected from two consistently available basal resource categories for stable isotope analysis. Riparian and aquatic plants were sampled from the most abundant plant species including samples of riparian tree leaves and instream and emergent aquatic plants (dry season n = 102; wet season n = 167). Particulate organic matter (POM) samples included fine POM (FPOM) and coarse POM (CPOM) at each site (dry season n = 44; wet season n = 52). Triplicate CPOM was collected from detritus components, and FPOM was sampled in a plankton net with a 70 µm filter. Despite active searching, periphyton and algae resources were highly limited and thus could not be included in analyses. Three replicate samples were collected from all potential food sources. All samples of consumer and basal sources were preserved in the field using NaCl following Arrington and Winemiller (2002) .

Laboratory analysis of stable isotopes

We rinsed and soaked all samples with deionized water for 48 h, after which they were rinsed again. All tissues were dried in an oven at 60 °C for 48 hours to achieve a constant weight (O'Neill & Thorp, 2014). Dry samples were ground into a powder, placed into tin capsules and weighed on a five-place decimal Mettler Toledo XP6 balance. Animal material (composite aquatic macroinvertebrate samples and fish tissue) was weighed between 0.4 and 0.5 mg, while plant material (FPOM, CPOM and riparian and aquatic plants) was weighed between 0.8 and 1.0 mg (Symes et al. 2017). If adequate sample mass was not achievable with small fish species and macroinvertebrates, composite samples consisting of several individuals were used.

Samples were analysed for $\delta^{13}C$ and $\delta^{15}N$ stable isotopes at the DSI-NRF iThemba Laboratory for Accelerator-Based Sciences isotopic laboratory at University of the Witwatersrand (Johannesburg, South Africa) using a Flash HT Plus elemental analyser coupled to a Delta V Advantage isotope ratio mass spectrometer by a ConFloIV interface. The $\delta^{13}C$ and $\delta^{15}N$ values for each sample were run in triplicates and corrected using laboratory standards Merck Gel and the Urea Working Standard (IVA Analysentechnik e.K., Meerbusch, Germany). The results were referenced to atmospheric N_2 (Air) and Vienna PeeDee belemnite for the $\delta^{13}C$ and $\delta^{15}N$, respectively (Woodborne et al. 2012). The final isotope ratios were expressed in parts per thousand (‰) in standard delta notation (δ). The $\delta^{13}C$ and $\delta^{15}N$ values were calculated as $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{reference}) - 1] \times 10^3$, with $R = {}^{13}C/{}^{12}C$ for carbon and ${}^{15}N/{}^{14}N$ for nitrogen.

Statistical methods

To visualize the range of isotopic values for local food webs in this system, we created biplots of δ13C and δ15N values for basal production sources (CPOM and C3 plants), aquatic macroinvertebrates, and fishes across sites (each site representing a unique stream order) and seasons (wet and dry). We focused on fishes only for assemblage-wide isotopic metrics because they were sampled more exhaustively. We also used $\delta^{13}C$ and $\delta^{15}N$ for fish species to assess shifts in trophic positions in assemblages along the river continuum and between seasons. Preceding all isotopic analyses, arithmetic corrections were applied to adjust $\delta^{13}C$ values to account for $\delta^{13}C$-depleted signatures from lipids as per Post et al. (2007). Consumers with a C:N ratio of >3.5 were adjusted using the equation $\delta^{13}C = –3.32 + 0.99$ (C:N). Plants with C > 40% were adjusted using $\delta^{13}C = −5.83 + 0.14$ (%C).

We used the SIBER package (Jackson et al. 2011) in R version 4.1.3 (R Core Team, 2021) to calculate trophic structure of fish assemblages using assemblage-wide metrics (Jackson et al. 2011; Layman et al. 2007). The $\delta^{13}C$ range shows the extent of producer resources in the food web that support consumers. The $\delta^{15}N$ range expresses the vertical length of isotopic niche space, with higher values indicating a greater number of trophic positions. Total Area (TA) is the convex hull of consumers in the fish assemblage and represents the total amount of trophic niche space occupied. The mean distance to centroid is the average Euclidian distance of each consumer to the centroid in $\delta^{13}C$–$\delta^{15}N$ isotopic niche space (Layman et al. 2007). Mean nearest-neighbour distance (MNND) and standard deviation of nearest-neighbour distance (SDNND) are measures of density and clustering of species within a community and are indicative of trophic redundancy, where lower MNDD values signify higher trophic redundancy, and low SDNND more evenly distributed trophic niches (Layman et al. 2007). Bayesian standard ellipse areas (SEA_C, corrected for small sample sizes) were also calculated to give an estimate of the total trophic niche space occupied (Jackson et al. 2011). We performed linear regressions to understand the relationship between isotopic metrics and environmental variables along the river continuum (**Table 1**) using the car package (Fox and Weisberg, 2019) in the R version 4.1.3 (R Core Team 2021).

Trophic positions (TP) of all fish species were estimated using the baseline $\delta^{15}N$ for each site per season by calculating the mean $\delta^{15}N$ of all basal production sources collected (TP = 1.0) following Zeug and Winemiller (2008). We used 2.54 as the estimated fractionation rate of $\delta^{15}N$ for each trophic level (Vanderklift and Ponsard, 2003) with the following calculation for each fish assemblage:

$$\text{Trophic Position}_{\text{fish consumer}} = [(\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{baseline}}) / 2.54] + 1$$

Results

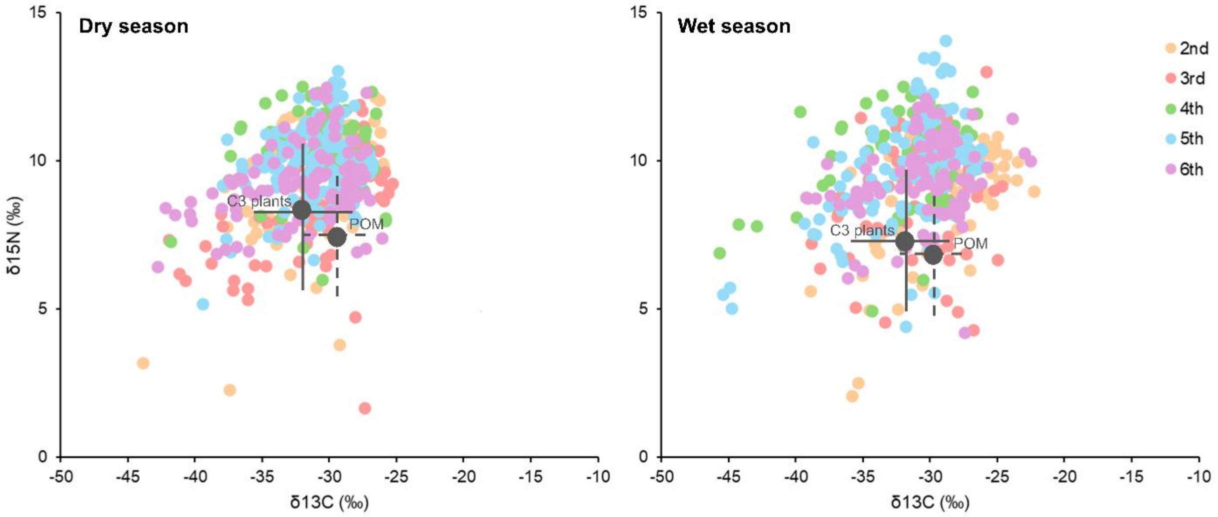
Environmental description of the continuum

The Tintinda River has a gentle gradient changing in elevation from 31 m.a.s.l at the smallest 2nd order site, to 7 m.a.s.l at the 6th order. River discharge did not show a substantial overall increase between dry and wet seasons due to sampling taking place in a receding river trough of the wet season (declining flows from a long wet season) with the maximum discharge ranging between 2.1 and 2.5 m^3/s between the dry and wet seasons, respectively (**Table 1**). Although measured discharge was comparable between sampling seasons, stable isotopes represent a measure of food consumption over a period of weeks (vander Zanden et al. 2015). During the wet season survey, river discharge had very recently declined following ~6 months of seasonal rains, and thus we consider the isotopic results of this study to represent two distinct seasons.

Sites from 2nd to 5th order had varying degrees of shade in both seasons with lux levels ranging from 77 to 490 lux, while the 6th order site received full sun (16,2100–18,890 lux). The river was acidic with decreasing pH in the wet season (dry season pH range: 5.2–5.9; wet season pH range: 4.2–4.8) and was nutrient and salt poor as indicated by the low values for conductivity, phosphate and nitrate which were consistent between seasons (**Table 1**). The water was clear in both seasons but showed a decrease in dissolved oxygen concentration in the wet season.

Trophic structure and assemblage-wide metrics

Biplots of $\delta^{13}C$ and $\delta^{15}N$ values of basal resources show an overlap between the standard deviations of the riparian and aquatic vegetation resource and the POM resource, indicating that POM was likely derived largely from broken down riparian and aquatic plant material (



). Some fish and macroinvertebrate consumers also had very depleted carbon values (δ^{13}C range between –45‰ and –38‰) that were not accounted for in our basal resource samples.

No significant linear relationships between assemblage-wide isotopic metrics and stream order, distance from source, or upstream catchment area were found. The δ^{13}C ranges for fish communities (reflecting the breadth of basal resources) were low (< 5.0) across all sites in both seasons (**Table 2**). However, we did observe some general patterns in relation to gradient and trophic structure. Ranges for δ^{15}N were highest in the 3rd order and 6th order sites, which both had higher δ^{15}N ranges in the dry season. In the dry season, sites showed a pattern of higher trophic diversity in δ^{13}C–δ^{15}N space in larger habitats, with an overall increase in TA, SEA*c*, and CD from 2nd to 6th order sites (**Table**). However, this pattern was largely driven by relatively low values for the 2nd order site, as 3rd and 4th order site values were comparable to the 6th order site (**Table**). In the wet season, TA and SEA*c* for small headwaters (2nd and 3rd order) were similar to those for 4th–6th order sites, indicating resource use breadth comparable to larger sites. Trophic redundancy generally decreased along the continuum in the dry season (i.e. lower MNND scores in 2nd and 3rd order sites compared to 6th order), whereas trophic redundancy remained relatively consistent along the continuum in the wet season survey. Trophic niche distribution was relatively even across sites and seasons, with low values for SDNND, except for the 6th order site in the dry season, which had a high value for SDNND. Overall ranges of assemblage-wide metrics across sites were similar between seasons, except for $\delta^{15}N$ range and SDNND, which had higher maximum values in the dry season (**Table 2**).

In general, fish assemblages had a larger proportion of individuals feeding at higher trophic levels in the wet season in comparison to the dry season, suggesting an increase in the consumption of predatory macroinvertebrates and fishes during the wet season (**Fig. 4**). We observed an increase in proportions of higher trophic positions in fish assemblages along the continuum in the wet season, with values generally indicating more carnivory in higher-order habitats. This was particularly pronounced in the 4th order stream, where several species typically expected to feed at intermediate levels, including some cichlids (*Chromidotilapia mamonekenei*, *Hemichromis elongatus*, *Pelvicachromis subocellatus*) and cyprinodontiforms (*Epiplatys ansorgii*, *Plataplochilus cabindae*), had higher trophic positions in the wet season (>4.5) compared to the dry season (2.9–4.3). The inverse trophic trend was seen at the small 2nd order headwater site, where the fish assemblages fed at higher trophic levels in the dry season. The small 2nd-order stream had a distribution of trophic positions comparable to larger 4th-6th order systems in the dry season.

Discussion

This study provides insights into food web ecology of an understudied Afrotropical freshwater region. Our findings support the importance of stream network position and season in consumer trophic strategies along a river continuum, where seasonal variation in flow influences the diversification of resources (Humphries et al. 2014; Pool et al. 2017; Cazzanelli et al. 2021). We found some unexpected patterns, however, with relatively high trophic diversity in small, forested headwaters. The consistently narrow ranges of very low δ^{13}C values in fish and macroinvertebrate consumers also contrasts with expectations for shifting basal resource contributions across seasons and along the river continuum. Our findings corroborate studies in other tropical freshwaters which have elucidated the significance of riparian forest resources in directly supporting consumer assemblages (Wootton and Oemke 1992; Pease et al. 2019) or through imported organic matter (Hoeinghaus et al. 2007).

Consumer δ^{13}C values aligned with forest resources

This study contributes to a growing body of work highlighting the variability in the role of terrestrial carbon in supporting tropical stream food webs (e.g. March and Pringle 2003; Hoeinghaus et al. 2007; Dudgeon et al. 2010; Correa and Winemiller, 2018), emphasizing that reliance on terrestrial resources may be more context-dependent than previously assumed. Across sites in both seasons, fish assemblages had low δ^{13}C values and very narrow δ^{13}C ranges, suggesting consistent predominance of C3 plants as a basal resource (Finlay, 2001). There was a large overlap of δ^{13}C signatures between C3 plants and POM resources (**Fig. 3**), which suggests that POM was derived from riparian sources, and was likely imported from upstream processed riparian material originating in the headwaters of the river (2nd and 3rd order systems in this study). Terrestrial riparian sources are typically ingested by consumers either indirectly in the process of feeding on aquatic macroinvertebrates in the benthos, or directly through consumption of riparian and POM material, although we did not examine gut data in this study to confirm this. The other known study of stream food webs in the central African tropics (Soto et al. 2019) found that riparian C3 plants were the predominant energy source for fish in the Congo River and tributaries. We did not collect sufficient samples of biofilm or algae to estimate their δ^{13}C values and contributions to local food webs because this resource was not visibly available even in the 6th order site of the Tintinda River where more sunlight reached the water. Correa and

Winemiller (2018) also documented this phenomenon, where blackwaters of a Neotropical river had limited production and growth of biofilm.

Notably, many fish and invertebrate consumers had δ^{13}C values ranging between –45‰ and –38‰, well below the δ^{13}C of our measured basal resources (riparian plants and POM) in both seasons. Among fishes, we recorded δ^{13}C values < 38‰ for *Microctenopoma nanum* (Anabantidae), *Pelvicachromis subocellatus* (Cichlidae), *Petrocephalus microphthalmus* (Mormyridae), *Aphyosemion australe* (Nothobranchiidae) and *Channallabes apus* (Clariidae) from the 6th-order site in the dry season. These species were generally captured in remaining wetted off-channel habitats. In the wet season, when more of the adjacent riparian swamp forest was inundated across the lower continuum from 4th–6th order sites, fish species including *M. nanum* (Anabantidae), *Neolebias ansorgii* (Distichodontidae), *Paramormyrops kingsleyae* (Mormyridae), *Marcusenius moori* (Mormyridae) and *Epiplatys ansorgii* (Nothobranchiidae) showed similarly depleted δ^{13}C values across those sites. This suggests that the inundated swamp forest may be providing an additional basal resource, perhaps from methane-derived C in decomposing detritus. Whiticar (1999) reported δ^{13}C of −47.5‰ ± 2‰ for methane oxidizing bacteria, and biogenic methane produced in anoxic detritus entering the food web and supporting benthic and pelagic consumers (Deines et al. 2007; Sanseverino et al. 2012). Soto et al. (2019) speculated that this could be the mechanism behind highly depleted δ^{13}C values (well below values for riparian and aquatic plants) for some consumers in Congo River tributaries. Similarly, some food web studies in the Neotropical Usumacinta River have suggested that methane oxidizers could be a seasonally important source that is mobilised from anoxic detritus in inundated habitats in the wet season to augment basal carbon resources (Sepúlveda-Lozada et al. 2015; Cazzanelli et al. 2021).

Trophic diversity along the longitudinal fluvial gradient and across seasons

Our expectation for increasing trophic diversity (higher TA and SEA*c*) in higher-order habitats was somewhat supported, and isotopic niche diversity was driven by vertical expansion along the δ^{15}N axis since δ^{13}C ranges were consistently low across sites. Trophic diversity in freshwater consumers is expected to increase along a fluvial gradient due to increased resource diversity associated with larger and more heterogeneous habitats (Vannote et al. 1980; Power and Dietrich, 2002; Thompson and Townsend, 2005; Thorp et al. 2006;) and in environments which receive resource subsidies from adjacent habitats (Polis et al. 1997). Consumers in small, low-order streams are often generalist feeders (Woodward and Hildrew, 2002; Pease et al. 2019). In the present study, the headwaters had tighter assemblage breadths and higher trophic redundancy in fishes in some cases. For example, in the dry season, the MNND was much lower for the 2nd order stream compared to the 6th order site where more specialized feeders, such as herbivorous *Distichodus notospilus* and piscivorous *Hepsetus lineatus* were present. This agrees with studies of trophic ecology in other tropical regions where fish trophic diversity increased with habitat size and associated resource expansion (Ibanez et al. 2007; Pease et al. 2019). Based on the concept of the flood pulse (Junk et al. 1989; Thompson and Townsend 2005; Humphries et al. 2014), we would expect to see this pattern more pronounced in the wet season.

Unexpectedly, in the wet season, assemblage-wide niche breadths of fish communities in small headwater systems (2nd and 3rd order) were comparable to the larger, downstream reaches suggesting that they support disproportionate trophic diversity given their size when forest connections are strengthened by flow connections. Because forest inputs were readily available in downstream reaches and δ^{13}C ranges were consistent across sites, this pattern was probably not driven by a decline in basal resource diversity in downstream reaches as observed in some large temperate streams (Thorp and Bowes, 2016; Maitland and Rahel, 2023). Instead, our results agree with findings from Walsh et al. (2022b) which reported uniquely high functional richness for fish assemblages in lotic sites with small upstream catchments in comparison to higher-order sites along river gradients in Congo coastal basins. That study suggested that paleo-biogeographic factors may drive the high functional richness of the region, preserving ancient trophic niche differentiation due to historic stability at longer geological timescales compared to other regions (Tedesco et al. 2005). Walsh et al. (2022b) also showed that relatively high trophic trait diversity in Congo coastal basins was associated with higher habitat heterogeneity and that ecomorphological diversity was strongly driven by trophic traits. Building on that work, the results of the current study underscore the importance of season and hydrology in supporting a broad array of trophic strategies, with seasonality potentially playing an important role in driving diversification of resources for freshwater consumer assemblages (Pool et al. 2017; Cazzanelli et al. 2021).

We also observed shifts in seasonal distribution of fish trophic positions in local food webs between small headwaters (2nd order) and other sites (3rd–6th order) along the continuum. Contrary to our expectations, we found a larger proportion of fishes feeding at higher trophic positions in the wet season compared to the dry season for the 3rd–6th order sites. Increased trophic position in the wet season could be due to mobilisation of resources through the system and increased habitat availability following flow pulses and forest inundation (Junk and Wantzen, 2004; Abrantes et al. 2014; Humphries et al. 2014). Some sites (3rd and 6th order) had relatively high values for δ^{15}N range in the dry season, but this appeared to be driven by fishes feeding at lower trophic levels (with lower δ^{15}N values) than observed in the wet season. The lower trophic positions of local fish assemblages in the dry season suggest more frequent consumption of plant and detrital material, perhaps because animal prey is less available with reduced habitat volume. Peterson and Winemiller (1997) found that a Neotropical stream fish species, *Roeboides dayi,* showed a dietary preference for aquatic invertebrates from drift in the wet season when that resource was abundant, moving their intake to less energy-rich items during the dry season when there was more competition for invertebrate prey due to habitat contraction.

We observed the opposite pattern (greater proportion of higher trophic positions in the dry season) for the 2nd order headwater site. Abundance and diversity of invertebrate prey resources typically decrease in the dry season, with increasing piscivory as fish density increases in shrinking habitats (Lowe-McConnell, 1987; Winemiller, 1989; Wantzen et al. 2002), and this could increase the overall trophic position of fish assemblages. Food resource constraints in the dry season may be more intense in hydrologically variable low-order streams compared to larger, more stable higher-order streams (Poff and Allan, 1995; Thorp et al. 2006; Pease et al. 2020).

Conservation implications

Our current and previous work (Walsh et al. 2022), has shown that Afrotropical headwaters in our study region are distinctive in terms of trophic and functional diversity, but are also constrained by habitat size which makes them particularly vulnerable to anthropogenic impact. Strong aquatic-terrestrial connectivity in low-order streams promotes diversity for many aquatic taxa (Besemer et al. 2013; Ferreira et al. 2023) and appears to support relatively high trophic diversity for fish assemblages in the Tintinda River headwaters, particularly in the wet season. Larger stream habitats further down the river continuum are likely fed by processed carbon sources that originate from riparian forests of these headwaters, and their disturbance would have knock on effects for large parts of the fluvial network. Reduction in allochthonous inputs can cause resource homogenisation and reduction in available niches, with severe effects for systems primarily fuelled by riparian forest (Wilkinson et al. 2021). We also saw that unique basal resources, perhaps involving methanogenic pathways in lateral swamp forests, contribute to aquatic food webs across the river network in this region (see Cazzanelli et al. 2021; Soto et al. 2019), highlighting the importance of swamp forest-river connectivity in subsidizing instream communities. Very little research on forest connections to food webs has been conducted to date in the Afrotropics, making it an important topic for future investigations (Lo et al. 2021). Deforestation is a primary threat to biodiversity in tropical streams, and a better understanding of forest–stream relationships is needed to design effective conservation policies (Ramírez et al. 2008).

Acknowledgments

This work was supported by the University of the Witwatersrand under the University of the Witwatersrand, Johannesburg National Research Foundation Rated Researchers Incentive Grant 103581 and the Axelrod Curatorship of the American Museum of Natural History. JS acknowledges funding from UKRI Future Leaders Fellowship [Grant/Award Number: MR/X035662/1]. We thank Jean de Dieu Mountou, Frédéric Avoukou, Gislain Nakoundzi Taty and the local authorities of Koutou Village for their support during our field collections. We thank Jonathan Levin, Vineet Katariya and Nicole Burri for statistical and GIS support. We are grateful to Azraa Ebrahim, Dinah Mukhari, Hima Rama and Michele Hall for their assistance in the preparation of the isotope samples. Mike Butler, Osbourne Malinga, Stephan Woodborne (iThemba Laboratories for Accelerator Based Sciences) and Grant Hall (University of Pretoria) kindly assisted with isotope analysis. All materials were collected and exported with permission of the Congolese Direction Générale de l'Economie Forestière, Direction dela Faune et des Aires Protégées (permits available on request from the corresponding author).

Data Availability Statement

Data are available from the authors upon reasonable request and archived within The IsoFresh database available at Zenodo (https://doi.org/10.5281/zenodo.14982323).

Author contribution statement: Conceptualisation: GW, JS, DJW, MLJS, JYG, AAP. Developing methods: GW, JS, DJW, AAP, VNBM. Conducting the research: GW, VNBM, MLJS, JYG. Data analysis: GW, AAP. Data interpretation: GW, JS, DJW, AAP, MLJS. Preparation of figures & tables: GW, AAP. Writing: AAP, GW, JS, DJW, MLJS, JYG, VNBM.

Competing Interests

Authors Gina Walsh and Jérôme Gaugris are employed by Flora Fauna & Man Ecological Services Ltd**.** The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics statement

All research was undertaken according to the ethics protocol of the University of the Witwatersrand (Wits - Johannesburg, South Africa). Research methods were approved by the Wits Animal Ethics Screening Committee under the permit numbers 2015/03/08B, 2017/02/06/B. Fishes were collected according to the guidelines for the use of fishes in research (AFS/AIFRB/ASIH 2003). All fishes were collected and exported with permission of the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES permit numbers CG 1125774 and CG 1125818), and Institut National de Recherche en Sciences Exactes et Naturelles (IRSEN Permit numbers 205/MRSIT/IRSEN/DEO and 328/MRSIT/IRSEN/DO) on file at American Museum of Natural History (AMNH). All collected vouchers were deposited at the Ichthyology Department of the AMNH in New York, USA. Data are accessible online at the AMNH vertebrate zoology database (https://emu-prod.amnh.org/db/emuwebamnh/).

References

Abrantes, K. G., A. Barnett, and S. Bouillon. 2014. “Stable Isotope-Based Community Metrics as a Tool to Identify Patterns in Food Web Structure in East African Estuaries.” *Functional Ecology* 28: 270–282. https://doi.org/10.1111/1365-2435.12155.

AFS/AIFRB/ASIH. 2004. “Guidelines for the Use of Fishes in Research.” https://research.fiu.edu/documents/iacuc/documents/guidelines-fish-research.pdf

Arrington, D. A., and K. O. Winemiller. 2002. “Preservation Effects on Stable Isotope Analysis of Fish Muscle.” *Transactions of the American Fisheries Society* 131: 337–342. https://doi.org/10.1577/1548-8659(2002)131<0337.

Cadotte, M. W., K. Carscadden, and N. Mirotchnick. 2011. “Beyond Species: Functional Diversity and the Maintenance of Ecological Processes and Services.” *Journal of Applied Ecology* 48: 1079–1087. https://doi.org/10.1111/j.1365-2664.2011.02048.x.

Cazzanelli, M., M. Soria-Barreto, M. M. Castillo, and R. Rodiles-Hernández. 2021. “Seasonal Variations in Food Web Dynamics of Floodplain Lakes with Contrasting Hydrological Connectivity in the Southern Gulf of Mexico.” *Hydrobiologia* 848: 773–797. https://doi.org/10.1007/s10750-020-04468-8.

Correa, S. B., and K. O. Winemiller. 2018. “Terrestrial–Aquatic Trophic Linkages Support Fish Production in a Tropical Oligotrophic River.” *Oecologia* 186: 1069–1078. https://doi.org/10.1007/s00442-018-4093-7

Correa, S. B., and K. O. Winemiller. 2014. “Niche Partitioning among Frugivorous Fishes in Response to Fluctuating Resources in the Amazonian Floodplain Forest.” *Ecology* 95: 210–224. https://doi.org/10.1890/13-0393.1.

Coyle, J. R., F. W. Halliday, B. E. Lopez, K. A. Palmquist, P. A. Wilfahrt, and A. H. Hurlbert. 2014. “Using Trait and Phylogenetic Diversity to Evaluate the Generality of the Stress-Dominance Hypothesis in Eastern North America Tree Communities.” *Ecography* 37: 814–826.

Fox, J., and S. Weisberg. 2019. An R Companion to Applied Regression, Third Edition. https://socialsciences.mcmaster.ca/jfox/Books/Companion/

Hette-Tronquart, N., J. Belliard, E. Tales, and T. Oberdorff. 2016. “Stable Isotopes Reveal Food Web Modifications along the Upstream–Downstream Gradient of a Temperate Stream.” *Aquatic Sciences* 78: 255–265. https://doi.org/10.1007/s00027-015-0421-8.

Hoeinghaus, D. J., K. O. Winemiller, and A. A. Agostinho. 2007. “Landscape-Scale Hydrologic Characteristics Differentiate Patterns of Carbon Flow in Large-River Food Webs.” *Ecosystems* 10: 1019–1033. https://doi.org/10.1007/s10021-007-9075-2.

Horwitz, R. J. 1978. “Temporal Variability Patterns and the Distributional Patterns of Stream Fishes.” *Ecological Monographs* 48: 307–321. https://doi.org/https://doi.org/10.2307/2937233.

Humphries, P., H. Keckeis, and B. Finlayson. 2014. “The River Wave Concept: Integrating River Ecosystem Models.” *BioScience* 64: 870–882. https://doi.org/10.1093/biosci/biu130.

Ibanez, C., T. Oberdorff, G. Teugels, V. Mamononekene, S. Lavoué, Y. Fermon, D. Paugy, and A. K. Toham. 2007. “Fish Assemblages Structure and Function along Environmental Gradients in Rivers of Gabon (Africa).” *Ecology of Freshwater Fish* 16: 315–334. https://doi.org/10.1111/j.1600-0633.2006.00222.x

Jackson, A. L., R. Inger, A. C. Parnell, and S. Bearhop. 2011. “Comparing Isotopic Niche Widths among and within Communities: SIBER - Stable Isotope Bayesian Ellipses in R.” *Journal of Animal Ecology* 80: 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x.

Jepsen, D. B., K. O. Winemiller, D. C. Taphorn, and D. R. Olarte. 1999. “Age Structure and Growth of Peacock Cichlids from Rivers and Reservoirs of Venezuela.” *Journal of Fish Biology* 55: 433–450. https://doi.org/10.1006/jfbi.1999.1009.

Junk, W. J., P. B. Bayley, and R. E. Sparks. 1989. “The Flood Pulse Concept in River-Floodplain Systems.” Canadian Journal of Fisheries and Aquatic Sciences, 106, 110–127. https://doi.org/10.1371/journal.pone.0028909

Junk, W. J., and K. M. Wantzen. (2004). “The Flood Pulse Concept: New Aspects, Approaches and Applications - An Update.” In R. L. Welcomme and T. Petr (Eds.), Proceedings of the Second International Symposium on the Management of Large Rivers for Fisheries. Bangkok: Food and Agriculture Organization and Mekong River Commission, FAO Regional Office for Asia and the Pacific.

Layman, C. A., D. A. Arrington, C. G. Montaña, and D. M. Post. 2007. “Can Stable Isotope Ratios Provide for Community-Wide Measures of Trophic Structure?” *Ecology* 88: 42–48.

Maitland, B. M., and F. J. Rahel. 2023. “Aquatic Food Web Expansion and Trophic Redundancy along the Rocky Mountain – Great Plains Ecotone.” *Ecology* 104: e4103. https://doi.org/10.1002/ecy.4103.

McMeans, B. C., T. Kadoya, T. K. Pool, G. W. Holtgrieve, S. Lek, H. Kong, K. O. Winemiller, V. Elliott, N. Rooney, P. Laffaille, and K. S. McCann. 2019. “Consumer Trophic Positions Respond Variably to Seasonally Fluctuating Environments.” *Ecology* 100: e02570. https://doi.org/10.1002/ecy.2570.

O’Neill, B. J., and J. H. Thorp. 2014. Untangling Food-Web Structure in an Ephemeral Ecosystem.” *Freshwater Biology* 59: 1462–1473.

Pease, A. A., K. A. Capps, R. Rodiles-Hernández, M. M. Castillo, M. Mendoza-Carranza, M. Soria-Barreto, and A. A. González-Díaz. 2019. “Trophic Structure of Fish Assemblages Varies across a Mesoamerican River Network with Contrasting Climate and Flow Conditions.” *Food Webs* 18: e00113. https://doi.org/10.1016/j.fooweb.2019.e00113.

Pease, A. A., M. Soria-Barreto, A. A. González-Díaz, and R. Rodiles-Hernández. 2020. "Seasonal Variation in Trophic Diversity and Relative Importance of Basal Resources Supporting Tropical River Fish Assemblages in Chiapas, Mexico." *Transactions of the American Fisheries Society* 149: 753–769. https://doi.org/10.1002/tafs.10269.

Poff, N. L., and J. D. Allan. 1995. "Functional Organization of Stream Fish Assemblages in Relation to Hydrological Variability." 76: 606–627. https://doi.org/10.2307/1941217.

Polis, G. A., W. B. Anderson, and R. D. Holt. 1997. "Toward an Integration of Landscape and Food Web Ecology: The Dynamics of Spatially Subsidized Food Webs*." Annual Review of Ecology and Systematics* 28: 289–316. https://doi.org/10.1146/annurev.ecolsys.28.1.289.

Pool, T., G. Holtgrieve, V. Elliott, K. McCann, B. McMeans, N. Rooney, A. Smits, T. Phanara, M. Cooperman, S. Clark, C. Phen, and S. Chhuoy. 2017. "Seasonal Increases in Fish Trophic Niche Plasticity within a Flood-Pulse River Ecosystem (Tonle Sap Lake, Cambodia)." *Ecosphere* 8: e01881. https://doi.org/10.1002/ecs2.1881

Power, M. E., and W. E. Dietrich. 2002. "Food Webs in River Networks." *Ecological Research* 17: 451–471.

Ramírez, A., C. M. Pringle, and K. M. Wantzen. 2008. "Tropical Stream Conservation." In D. Dudgeon (Ed.), Tropical Stream Ecology (pp. 285–304). Elsevier Academic Press.

R Core Team. (2021). R: A language and environment for statistical computing. R Foundation for Statistical Computing.

Sepúlveda-Lozada, A., M. Mendoza-Carranza, M. Wolff, U. Saint-Paul, and A. Ponce-Mendoza. 2015. "Differences in Food Web Structure of Mangroves and Freshwater Marshes: Evidence from Stable Isotope Studies in the Southern Gulf of Mexico." *Wetlands Ecology and Management* 23: 293–314. https://doi.org/10.1007/s11273-014-9382-2.

Soto, D. X., E. Decru, J. Snoeks, E. Verheyen, L. van de Walle, J. Bamps, T. Mambo, and S. Bouillon. 2019. "Terrestrial Contributions to Afrotropical Aquatic Food Webs: The Congo River Case." *Ecology and Evolution* 9: 10746–10757. https://doi.org/10.1002/ece3.5594.

Sponseller, R. A., J. B. Heffernan, and S. G. Fisher. 2013. "On the Multiple Ecological Roles of Water in River Networks." *Ecosphere* 4: 1-14. https://doi.org/10.1890/ES12-00225.1

Symes, C., F. Skhosana, M. Butler, B. Gardner, and S. Woodborne. 2017. "Isotope (δ 13C, δ 15N, δ 2H) Diet–Tissue Discrimination in African Grey Parrot *Psittacus erithacus*: Implications for Forensic Studies." *Isotopes in Environmental and Health Studies* 53: 580–596.

Tedesco, P. A., T. Oberdorff, C. A. Lasso, M. Zapata, and B. Hugueny. 2005. "Evidence of History in Explaining Diversity Patterns in Tropical Riverine Fish." *Journal of Biogeography* 32: 1899–1907. https://doi.org/10.1111/j.1365-2699.2005.01345.x.

Thompson, R. M., J. A. Dunne, and G. Woodward. 2012. "Freshwater Food Webs: Towards a More Fundamental Understanding of Biodiversity and Community Dynamics." *Freshwater Biology* 57: 1329–1341. https://doi.org/10.1111/j.1365-2427.2012.02808.x.

Thompson, R. M., and C. R. Townsend. 2005. “Food-Web Topology Varies with Spatial Scale in a Patchy Environment.” *Ecology* 86: 1916–1925. https://doi.org/10.1890/04-1352.

Thorp, J. H., & Bowes, R. E. (2016). Carbon sources in riverine food webs: New evidence from amino acid isotope techniques. Ecosystems, 20, 1–13.

Thorp, J. H., M. C. Thoms, and M. D. Delong. 2006. “The Riverine Ecosystem Synthesis: Biocomplexity in River Networks across Space and Time.” *River Research and Applications* 22: 123–147. https://doi.org/10.1002/rra.901.

van der Laan, R., W. N. Eschmeyer, and R. Fricke. 2014. “Family-Group Names of Recent Fishes.” *Zootaxa* 3882: 1–230. http://biotaxa.org/Zootaxa/article/view/zootaxa.3882.1.1

van Rooyen, M. W., N. van Rooyen, B. Orban, G. Nsongola, E. S. Miabangana, and J. Gaugris. 2016. “Floristic Composition, Diversity and Structure of the Forest Communities in the Kouilou Département, Republic of Congo.” *Tropical Ecology* 57: 805–824.

Vande weghe, J. P. 2004. Forests of Central Africa: Nature and Man. Protea Book House.

Vander Zanden, M. J., M. Clayton, E. Moody, C. Solomon, and B. Weidel. 2015. “Stable Isotope Turnover and Half-Life in Animal Tissues: A Literature Synthesis.” *PLoS ONE* 10: 1–16. https://doi.org/10.1371/journal.pone.0116182.

Vanderklift, M. A., and S. Ponsard. 2003. “Sources of Variation in Consumer-Diet d15N Enrichment: A Meta-Analysis.” *Oecologia* 136: 169–182.

Vannote, R. L., G. W. Minshall, K. W. Cummins, J. R. Sedell, and C. E. Cushing. 1980. “The River Continuum Concept.” *Canadian Journal of Fisheries and Aquatic Sciences* 37: 130–137. https://doi.org/10.1139/f80-017.

Venarsky, M. P., B. Stewart-Koster, R. J. Hunt, T. D. Jardine, and S. E. Bunn. 2020. “Spatial and Temporal Variation of Fish Community Biomass and Energy Flow Throughout a Tropical River Network.” *Freshwater Biology* 65: 1782–1792. https://doi.org/10.1111/fwb.13581.

Walsh, G., M. P. Alexandre, V. N. Boukaka Mikembi, M. N. Jonker, V. Mamonekene, A. J. Henri, and J. Y. Gaugris. 2022. “Fishes of the Loémé River System, Republic of the Congo, Lower Guinea, West-Central Africa.” *African Journal of Ecology* 60: 848-856. https://doi.org/10.1111/aje.13016

Walsh, G., M. N. Jonker, and V. Mamonekene. 2014. “A Collection of Fishes from Tributaries of the Lower Kouilou, Noumbi and Smaller Coastal Basin Systems, Republic of the Congo, Lower Guinea, West-Central Africa.” *Check List* 10: 900–912. https://doi.org/10.15560/10.4.900.

Walsh, G., A. A. Pease, D. J. Woodford, M. L. Stiassny, J. Y. Gaugris, and J. South. 2022. “Functional Diversity of Afrotropical Fish Communities across River Rradients in the Republic of Congo, West Central Africa.” *Frontier in Environmental Science* 10: 981960. https://doi.org/10.3389/fenvs.2022.981960.

Wantzen, K. M., F. de Arruda Machado, M. Voss, H. Boriss, and W. J. Junk. 2002. “Seasonal Isotopic Shifts in Fish of the Pantanal Wetland, Brazil.” *Aquatic Sciences* 64: 239–251. https://doi.org/10.1007/PL00013196.

Wilkinson, C. L., K. W. J. Chua, R. Fiala, J. H. Liew, V. Kemp, A. Hadi Fikri, R. M. Ewers, P. Kratina, and D. C. J. Yeo. 2021. “Forest Conversion to Oil Palm Compresses Food Chain Length in Tropical Streams. *Ecology* 102: e03199. https://doi.org/10.1002/ecy.3199.

Winemiller, K. O., D. J. Hoeinghaus, A. A. Pease, P. C. Esselman, R. L. Honeycutt, D. Gbanaador, E. Carrera, and J. Payne. 2011. “Stable Isotope Analysis Reveals Food Web Structure and Watershed Impacts along the Fluvial Gradient of a Mesoamerican Coastal River.” *River Research and Applications* 27: 791–803. https://doi.org/10.1002/rra.1396.

Winemiller, K. O., R. L. Welcomme, and T. Petr. 2004. “Floodplain River Food Webs: Generalizations and Implications.” *2nd International Symposium on the Management of Large Rivers for Fisheries* 2: 285–309.

Woodborne, S., K. D. A. Huchzermeyer, D. Govender, D. J. Pienaar, G. Hall, J. G. Myburgh, A. R. Deacon, J. Venter, and N. Lübcker. 2012. “Ecosystem Change and the Olifants River Crocodile Mass Mortality Events.” *Ecosphere* 3(10): 1-17. https://doi.org/10.1890/ES12-00170.1.

Woodward, G., and A. G. Hildrew. 2002. “Food Web Structure in Riverine Landscapes.” *Freshwater Biology* 47: 777–798. https://doi.org/10.1046/j.1365-2427.2002.00908.x.

Wootton, J. T., and M. P. Oemke. 1992. Latitudinal Differences in Fish Community Trophic Structure and the Role of Fish Herbivory in a Costa Rican Stream.” *Environmental Biology of Fishes* 35: 311–319. https://doi.org/10.1007/BF00001899.

Zeug, S. C., and K. O. Winemiller. 2008. “Evidence Supporting the Importance of Terrestrial Carbon in a Large-River Food Web.” *Ecology* 89: 1733–1743.

Table 1 Landscape and local environmental and physical variables measured at each site in the Tintinda Basin in the dry and wet seasons

Site no. (Strahler Order)	**2**	**3**	**4**	**5**	**6**	**2**	**3**	**4**	**5**	**6**
Season	**Dry**					**Wet**				
Elevation (masl)	31	30	16	14	7	31	30	16	14	7
Catchment area (km^2)	3.2	3.5	14.7	40.1	119.4	3.2	3.5	14.7	40.1	119.4
Distance from source (km)	3.0	4.1	7.5	11.0	21.7	3.0	4.1	7.5	11.0	21.7
Discharge (m^3/s)	0.01	0.26	0.31	0.9	2.1	0.03	0.31	0.44	1.1	2.5
Depth (m)	0.15	0.30	0.43	0.56	4.00	0.12	0.26	0.29	0.44	4.5
Channel Width (m)	1	3	3.8	5.5	8	1	2	3.5	4.5	9
Light (Lux)	347	420	117	77	16210	180	490	260	91	18890
pH	5.8	5.9	5.5	5.8	5.9	4.8	4.7	4.7	4.2	4.8
Conductivity (µs/cm)	12.4	16.8	20.2	22.5	39.7	11.7	16.8	19.3	21.0	43.3
Temperature (C)	24.1	24.0	23.9	23.9	23.0	24.8	24.2	24.7	24.2	24.6
Dissolved Oxygen (mg/l)	8.5	5.8	7.8	9.3	8.8	3.32	4.48	6.67	6.81	4.27
Turbidity (NTU)	7	4	5	8	11	0	4	5	8	11
Ammonia (mg/l)	0	0	0.04	0	0.02	0	0	0.03	0.01	0.01
Phosphate (mg/l)	0.08	0.05	0.2	0.15	0.25	0.09	0.06	0.25	0.08	0.31
Nitrate-N (mg/l)	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3	<0.3
Silica (mg/l)	9	10	11	11	12	8	8	9	10	13

Table 2 Assemblage-wide metrics (for fish communities) calculated using isotope data at each study site along the river continuum for each season. TA = total area of convex hull, SEA$_C$ = Bayesian standard ellipse area (corrected for small sample sizes), CD = mean distance to centroid, MNND = mean nearest-neighbour distance, SDNND = standard deviation of nearest-neighbour distance

Site stream order and season	δ^{13}C Range	δ^{15}N Range	TA	SEA$_C$	CD	MNND	SDNND
2nd Dry	3.11	7.31	34.09	6.69	1.99	0.62	0.39
3rd Dry	4.25	12.84	53.32	14.09	3.30	0.87	0.33
4th Dry	2.70	8.43	64.81	14.24	2.85	1.57	0.71
5th Dry	3.45	6.59	38.21	7.99	1.94	0.63	0.43
6th Dry	3.47	14.43	59.29	14.54	3.20	1.35	1.73
2nd Wet	1.73	8.02	51.58	9.52	1.83	0.94	0.40
3rd Wet	3.61	9.73	38.94	11.57	2.64	1.49	0.68
4th Wet	3.39	8.06	33.94	10.82	2.16	0.94	0.71
5th Wet	4.69	8.90	65.68	13.75	2.51	0.85	0.35
6th Wet	2.94	9.49	38.94	9.94	2.63	0.98	0.36

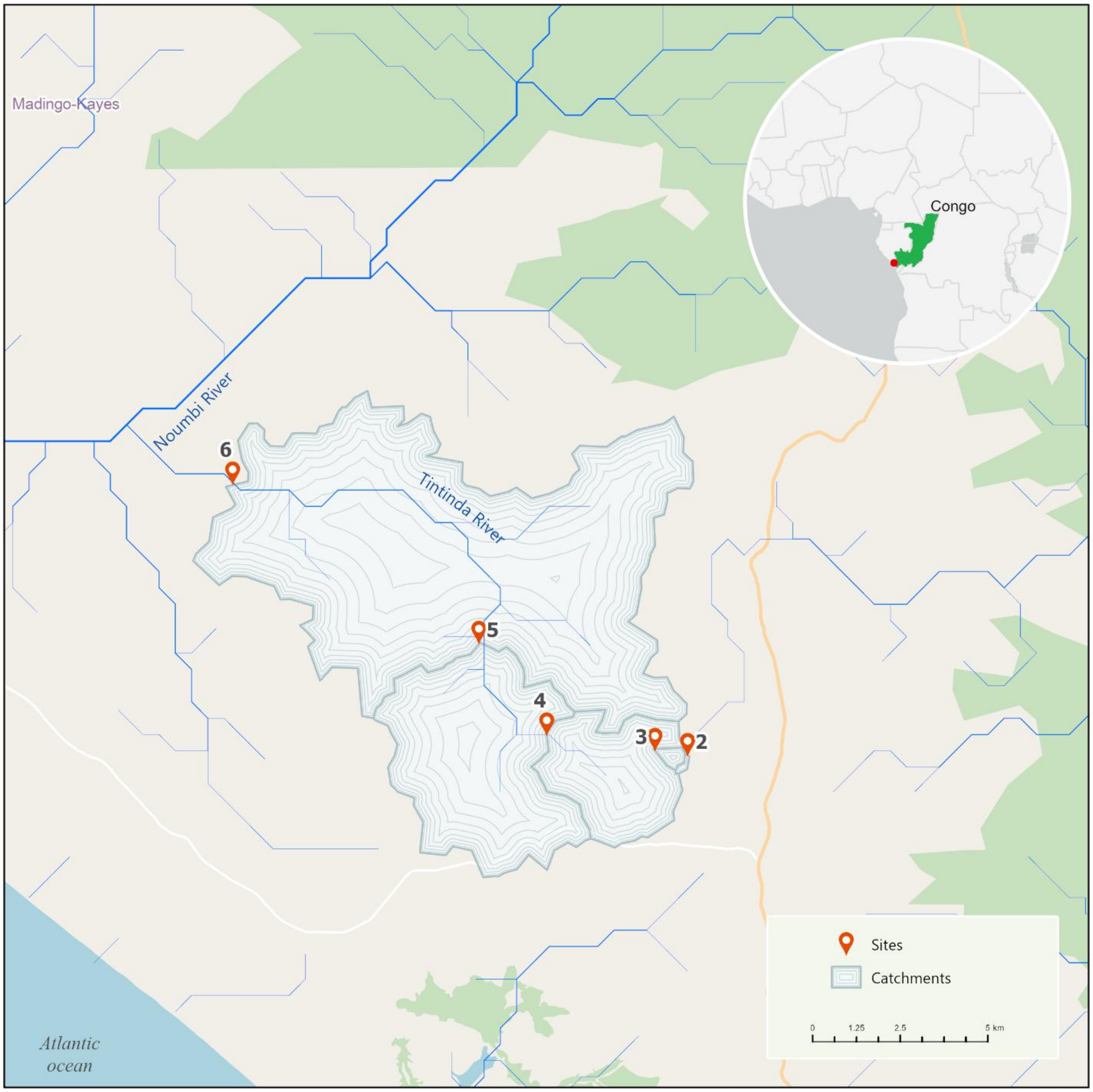
Figure Captions

Fig. 1 Study area showing study sites (numbers reflect Strahler order, from second to sixth) along the continuum of the Tintinda River in the Republic of the Congo.

Fig. 1 Photographs of sites on the Tintinda River from second (2) to sixth (6) Strahler order along the fluvial gradient. First-order sites (1) situated in the grassland-savannah vegetation zones of the Tintinda River were dry in both seasons.

Fig. 3 Biplot of $\delta^{13}C$ and $\delta^{15}N$ values for consumer assemblages (fishes and aquatic macroinvertebrates) and two basal resource categories (C3 riparian and aquatic vegetation and POM) from each site along the continuum of the Tintinda River for dry (n = 471 consumer tissue samples) and wet seasons (n = 452 consumer tissue samples). Different colours represent Strahler order of the sites along the river continuum. There was one site representing each Strahler order. Values for consumers were adjusted for trophic fractionation.

Fig. 4 Percentages of trophic positions occupied by fish assemblages along the continuum of the Tintinda River in the dry (top panel) and wet (bottom panel) seasons with examples of fish species that typically occupied low, medium and high trophic positions.


Madingo-Kayes
Congo
Noumbi River
Tintinda River
6
5
4
3
2
Atlantic ocean
Sites
Catchments
0
1.25
2.5
5 km


1

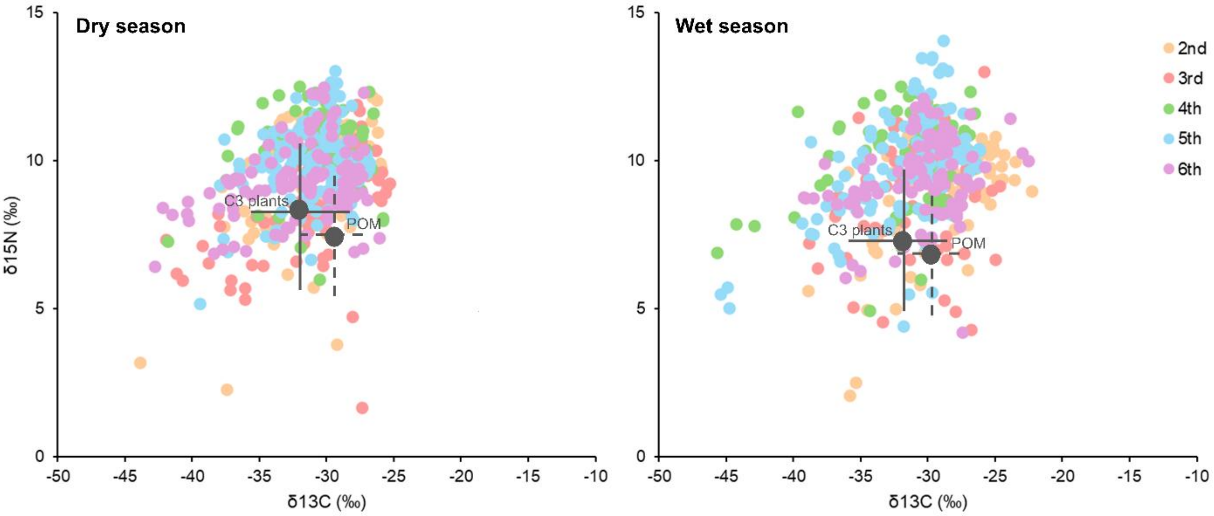

2

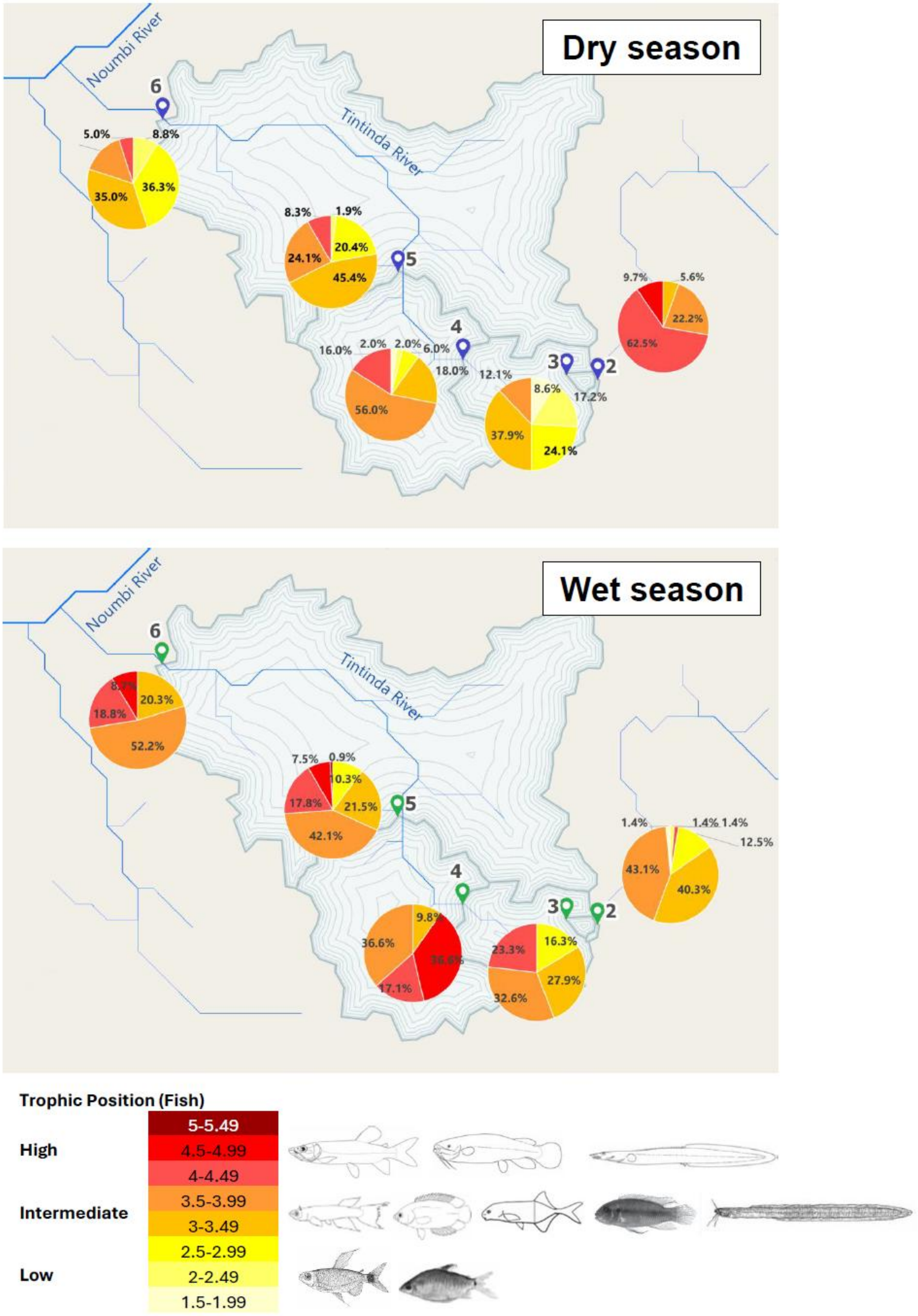

3


4


5


6


Dry season
Wet season
δ15N (‰)
δ13C (‰)
δ13C (‰)
15
10
5
0
-50
-45
-40
-35
-30
-25
-20
-15
-10
C3 plants
POM
2nd
3rd
4th
5th
6th


Dry season
Noumbi River
Tintinda River
6
5
4
3
2
5.0%
8.8%
35.0%
36.3%
8.3%
1.9%
24.1%
20.4%
45.4%
16.0%
2.0%
2.0%
6.0%
18.0%
56.0%
12.1%
8.6%
17.2%
37.9%
24.1%
9.7%
5.6%
22.2%
62.5%
Wet season
Noumbi River
Tintinda River
6
5
4
3
2
8.7%
18.8%
20.3%
52.2%
7.5%
0.9%
10.3%
17.8%
21.5%
42.1%
9.8%
36.6%
36.6%
17.1%
23.3%
16.3%
27.9%
32.6%
1.4%
1.4%
1.4%
12.5%
43.1%
40.3%
Trophic Position (Fish)
High
Intermediate
Low
5-5.49
4.5-4.99
4-4.49
3.5-3.99
3-3.49
2.5-2.99
2-2.49
1.5-1.99