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Functional Ecology of Afrotropical Freshwaters in the Republic of Congo, west-central Africa

A thesis submitted in accordance with the requirements for the degree of

Doctor of Philosophy in Ecology

In the School of Animal, Plant and Environmental Sciences

University of the Witwatersrand

by

Gina Walsh

November 2022



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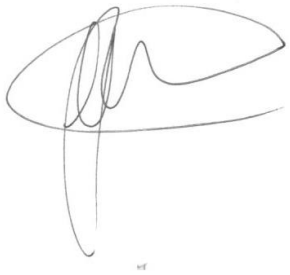
Declaration

I, Gina Walsh, submit this thesis to the Faculty of Science at the University of the Witwatersrand, Johannesburg in the fulfilment of the requirements for the degree of Doctor of Philosophy. I declare this thesis to be my own work that has not been submitted for examination before to any other university.



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Signed on this 1st day of November 2022

Abstract

Characterising functional freshwater biodiversity patterns and understanding community determinants can aid in predicting changes and prioritising specific conservation management actions. Afrotropical freshwater systems support diverse ecological communities, including many taxonomically undescribed species, yet few studies have been undertaken in this region and they remain poorly understood. Additionally, an overwhelming amount of research on north temperate streams has given rise to widely used lotic conceptual models which are superimposed over freshwaters globally, though the theory governing the structure of Afrotropical lotic communities remains largely unexplored. In this thesis I explored different aspects of the biodiversity, trophic ecology and functional diversity of an Afrotropical system in the Republic of Congo (west Central Africa) through a series of field studies. I formally describe two new to science fish species collected in the region, and use fish functional trait-based approaches to explore the functional diversity of fish assemblages and test selected expectations of riverine conceptual models in this regional setting. Following, I used carbon and nitrogen stable isotopes ($\delta^{13}C$, $\delta^{15}N$) from samples collected along a continuum of a coastal river in the Republic of the Congo to show temporal and spatial patterns of trophic diversity and basal carbon resource use. In addition to uncovering new fish diversity, I established that whilst tenets of certain conceptual models such as the River Continuum Concept and the River Habitat Template were supported by fish trait-environment relationships, unique patterns of functional diversity also exist which are scale dependent and result in different functional strategies driving communities between upper and lower positions in the basin. At sub-basin scale, headwater zones harbour substantial functional diversity in relation to upstream catchment size, and results suggest that historical forest refugia may play a role in the high functional richness at high elevations of the region. Isotopic analysis revealed that river order and season are important drivers in consumer trophic strategies along a river continuum in the wet Afrotropics, and that riparian forest sources are key basal carbon resources supporting consumers. Isotopic work reiterated that headwaters are distinctive in terms of trophic and functional diversity. I discovered potential evidence for methanogenic

pathways in lateral swamp forests that may subsidize instream food webs. The outcomes of this work demonstrate that existing river conceptual models only partially explain patterns of richness and functional diversity of river systems in this region, and highlights the importance of understanding mechanisms structuring regional functional diversity (e.g. nestedness and turnover) in efforts to conserve freshwater systems. It also emphasizes the need for swamp forest- and riparian specific policies in central Africa to be incorporated into freshwater conservation plans and strategies for forested regions of the Afrotropics.

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Publications

The following work from this thesis has been published in peer reviewed journals:

Chapter 2

Walsh, G., Pease, A., Woodford, D.J., Stiassny, M.L.J, Gaugris, J.Y. & South, J. (2022). *Functional diversity of Afrotropical fish communities across river gradients in the Republic of Congo, west Central Africa.* Frontiers in Environmental Science: Freshwater Science*.* https://doi.org/10.3389/fenvs.2022.981960

My contribution to the paper included design and coordination of field studies, collection of field data over seven sampling trips between 2012 and 2014 and collection of trait information from fish specimens lodged the American Museum of Natural History (AMNH). I statistically analysed and interpreted the data, and drafted the abstract, introduction, methods, results, discussion and conclusion sections of the paper. Co-authors read, edited, and provided comments to improve the overall paper.

Chapter 4

Walsh, G., Lamboj, A. & Stiassny M.L.J. (2019). *Review of the cichlid genus Thysochromis (Teleostei: Ovalentaria) with the description of a new species from the Kouilou Province in the Republic of Congo, west-Central Africa.* Journal of Fish Biology 96(5):1176-1185. https://doi.org/10.1111/jfb.14144

For this paper, I collected the specimens of the undescribed species over various field surveys between 2012 and 2017. I collected all associated environmental data, and performed all statistical computations on fish morphometric and environmental data. I led the writing of this manuscript, contributing to the writing, interpretation and review of all sections of the paper with other co-authors.

Chapter 5

Walsh, G., Bragança, P. H. & van der Zee, J. (2021) *Plataplochilus eliasi (Cyprinodontiformes: Procatopodidae) a new species from the upper Noumbi River in the Republic of the Congo.* Journal of Natural History 55 (45-46): 2849-2867. https://doi.org/10.1080/00222933.2022.2027035

My contribution to this paper included collection of fish specimens of the undescribed species over two field surveys in 2016 and 2017. I collected all environmental data, and contributed to the writing, interpretation and review of all sections of the paper with co-authors.

In preparation

The following data chapters have not been submitted for publication.

Chapter 3

Walsh, G., South, J., Woodford, D.J., Stiassny, M.L.J., Gaugris, J.Y. & Pease, A.A. *High-elevation rainforest headwaters harbour disproportionately high fish functional richness in the Afrotropics.*

I designed and coordinated field studies, collected field data over seven sampling trips between 2012 and 2014. I collected trait information from fish specimens at the AMNH, statistically analysed and interpreted the data for this chapter. I drafted the introduction, methods, results, discussion and conclusion sections. All co-authors read, edited, and provided comments to improve the overall chapter.

Chapter 6

Walsh, G., Pease, A.A., Woodford, D.J., Stiassny, M.L.J., Gaugris, J.Y., Boukaka-Mikembi, V.N. & South, J . *Riparian forest fuels river communities along the continuum of an Afrotropical coastal river in central Africa.*

I designed and coordinated field studies, and collected food web field data over two sampling trips in 2016 and 2017. I prepared the isotope samples for analysis, and cleaned, analysed and interpreted the data for this chapter. I drafted the introduction, methods, results, discussion and conclusion sections. All co-authors read, edited, and provided comments to improve the overall chapter.

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Chapter 1: General introduction

1.1 Tropical lotic ecology

Freshwater ecosystems provide substantial economic and social services to humans (Lynch et al., 2020) and are under major pressure globally. Recent publications have shown that approximately 28% of freshwater species are threatened (Tickner et al., 2020; IUCN, 2022) with an 87% decline in migratory freshwater fish species since 1970 (Deinet et al., 2020). A global analysis of river connectivity estimates that only 37% of rivers longer than 1000 km remain free-flowing and are primarily located in the inaccessible regions such as the Congo basin. Protection of these freshwater resources and associated services depends on the understanding of taxonomic diversity and ecosystem function, however only 5% of studies in the tropics have been undertaken in Sub Saharan Africa (Lo et al., 2021) which has perpetuated temperate bias in ecological theory of freshwaters (Dudgeon, 2008).

The term “temperate intellectual hegemony” was coined by Williams (1994) in papers focussing on the bias of long term, inland freshwater research to temperate regions. An issue often raised in freshwater ecology is the applicability of temperate theoretical concepts to tropical inland waters and the often impromptu and derivative approach such tropical studies utilise (Dudgeon, 2008; Kessler et al., 2011; di Marco et al., 2017; Titley et al., 2017). A synthesis on tropical stream ecology by Dudgeon (2008) highlighted that existing information on tropical streams is mostly from a small sub-set of well-studied localities in the Neotropics and north-eastern Australasia, but data from the Afrotropics is sparse for various reasons including political instability, economic disparity, geographical inaccessibility and poor dissemination of local research (Boyero et al., 2009; Collen et al., 2014; Lo et al., 2021).

An overwhelming amount of research on north temperate streams has given rise to widely used models such as the River Continuum Concept (Vannote et al., 1980) and the Riverine Productivity Model (Thorp and Delong, 1994) which are applied in freshwater research globally though the theory governing the structure of Afrotropical lotic communities still remains largely unexplored. Further confounding this issue, is the difficulty in defining an archetypal tropical stream due to variation in physical templates in the tropics across altitudes and latitudes

(Wantzen and Wagner, 2006; Dudgeon, 2008; Dudgeon et al., 2010). This gap in existing literature has implications for the conservation of Afrotropical freshwater ecosystems, where lack of understanding in the drivers of freshwater assemblages may ultimately lead to inappropriate conservation and management strategies being applied.

Contemporary genetic, functional and isotopic methods have allowed more in depth and informative studies and comparisons of gradients of biotic richness and processes of community assembly to be undertaken between regions. In this study we use a combination of the above approaches to explore the underlying drivers of fluvial assemblages at various scales in an Afrotropical setting in the lower Guinean ichthyofaunal province (Republic of the Congo), one of the most under studied regions in the world.

1.2 Diversity in the Afrotropics

Surface fresh waters cover about 0.8% of the earth's surface yet 25% of the world's known vertebrates are freshwater fish species (Lévêque et al., 2008). The diversity of freshwater species is confined to a relatively small extent of habitat area (Dudgeon et al., 2006), and thus freshwater habitats are considered to have the greatest spatial species richness of all ecosystems. Afrotropical freshwaters support particularly diverse freshwater communities with many species still unknown to science (Darwall et al., 2011; Reid et al., 2018). The Lower Guinean and Congo forests together hold over 1000 species of fish, which is likely an underestimation due to lack taxonomic surveys in many of these basins. More recently, the fish diversity of the region has received increased consideration (Munene and Stiassny, 2011; Mamonekene and Stiassny, 2012; Walsh et al., 2014, 2022b; Cutler et al., 2019; Stiassny et al., 2021) and new species are continually being discovered (Mamonekene and Stiassny, 2012; van der Zee et al., 2018; Walsh et al., 2019, 2021; Mipounga et al., 2020). From the 1970's an average of 309 new fish species are described or synonymised each year (Stiassny, 1999).

Widespread and taxonomically under-studied species in Africa are often resolved as divergent lineages with previously unrecognised taxa, underscoring the likely underestimation of freshwater diversity in Africa (Arroyave et al., 2019; Liyandja et al., 2022). Many recent collection surveys reflect the historical paucity of sampling in the Afrotropics, as reported species frequently represent new geographic distributions and significant range extensions (Stiassny et al., 2007; Darwall et al., 2011). Mamonekene & Stiassny (2012) found that approximately 10% of the total number of sampled fish species from a survey in the upper Ogowe and middle and upper Kouilou-Niari River basins in the Republic of Congo represented new to science species, which emphasizes the importance of documenting the biodiversity of this central African region before the potential loss of habitat from development and climate change. Specimens collected and described over the course of this project (**Chapters four and five**) contribute to this ongoing balancing of the region's taxonomic deficit.

1.3 Study area

The study area where the research for this thesis was conducted is situated in the Ogooue-Nyanga-Kouilou-Niari Freshwater Ecoregion in the Lower Guinea Basin (FEOW, 2022), which extends from northern Gabon through the eastern section of Congo, ending above the Congo River Basin in the Republic of Congo (Mamonekene, 2014). The boundaries of the ecoregion are defined by the Kouilou-Niari and Nyanga River basins, as well as the main stem of the Ogooué River (Mamonekene, 2014).

In a review of West African riverine biodiversity, Hugueny and Paugy (1995) found a strong correlation between species richness, watershed area and river discharge volume. Based on these relationships, the fish fauna of the Lower Guinea Ichthyological Province is disproportionately rich in relation to the size of the watersheds (Teugels et al., 1992; Darwall et al., 2011), possibly driven by the refuge offered by the forested West Coast Equatorial region during climatic fluctuations of the Pleistocene period (Lévêque, 1997). The high fish biodiversity in the region is hypothesized to be a result of the relative stability of the

hydrological regime and the large number of microhabitats created in these rivers by the forest element itself (Teugels et al., 1992).

Broadly, the study area encompasses a coastal (lower) and inland (upper) area in the Kouilou and the Niari administrative provinces of the Republic of Congo (**Figure 1-1**) and includes three consecutive large drainages in the southern Lower Guinea ichthyofaunal province (i.e. the Noumbi, Kouilou-Niari and Loemé basins).

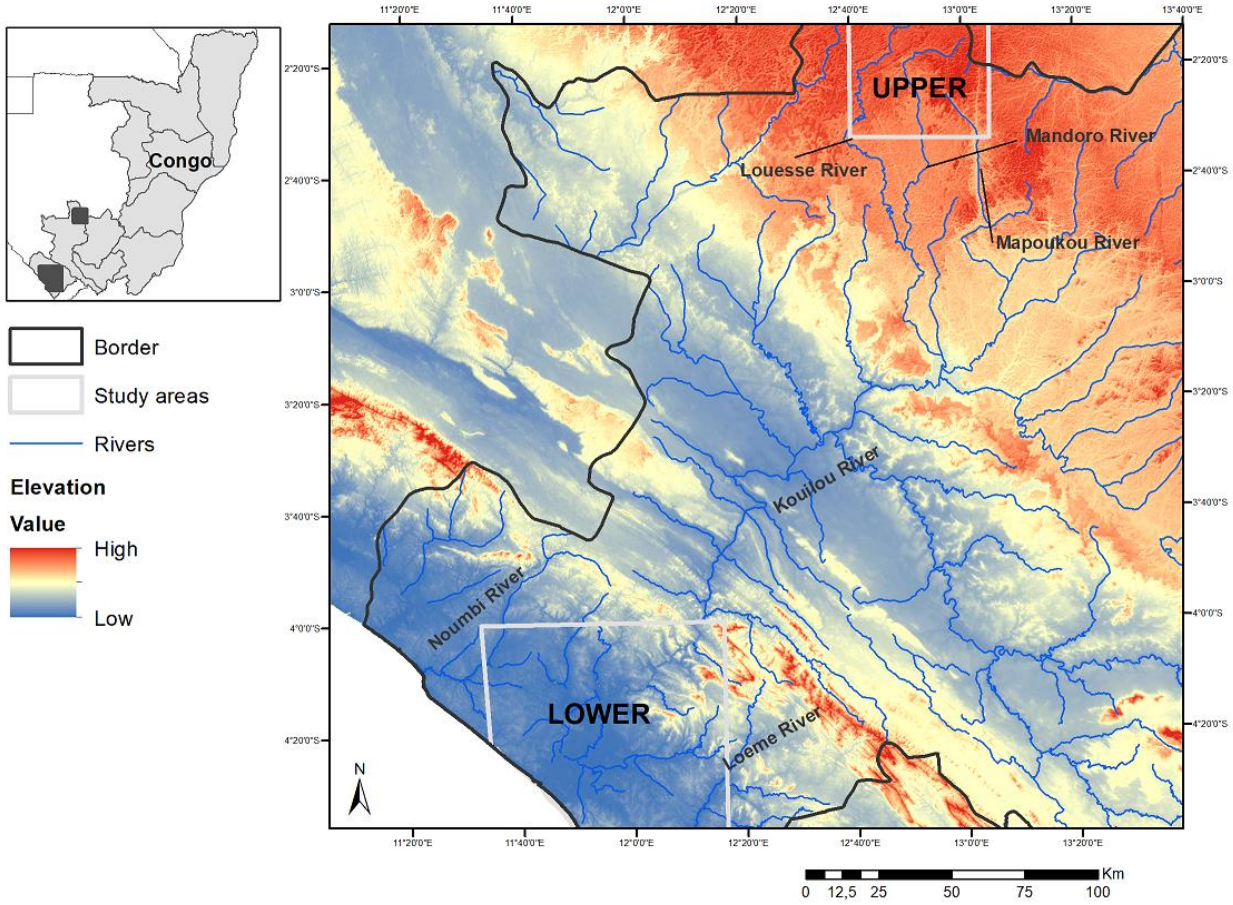

Figure 1-1: Upper and lower basins of the study area showing major drainages and elevations in the Lower Guinea, Republic of the Congo

The lower basin sites were located between 2 and 130 meters above sea level on the coastal plateau, draining the Kouilou, Loemé and Noumbi basins south-west into the Atlantic Ocean, with a series of small isolated coastal basins. The majority of the south-central part of the Congo is drained by the Kouilou and Niari and these two systems together have the second

largest catchment in the Republic of the Congo, with a surface area of approximately 50 000 km^2. The area experiences a bimodal flood regime (Mamonekene, 2014) with little or no rain falling between June and August (**Figure 1-2**).

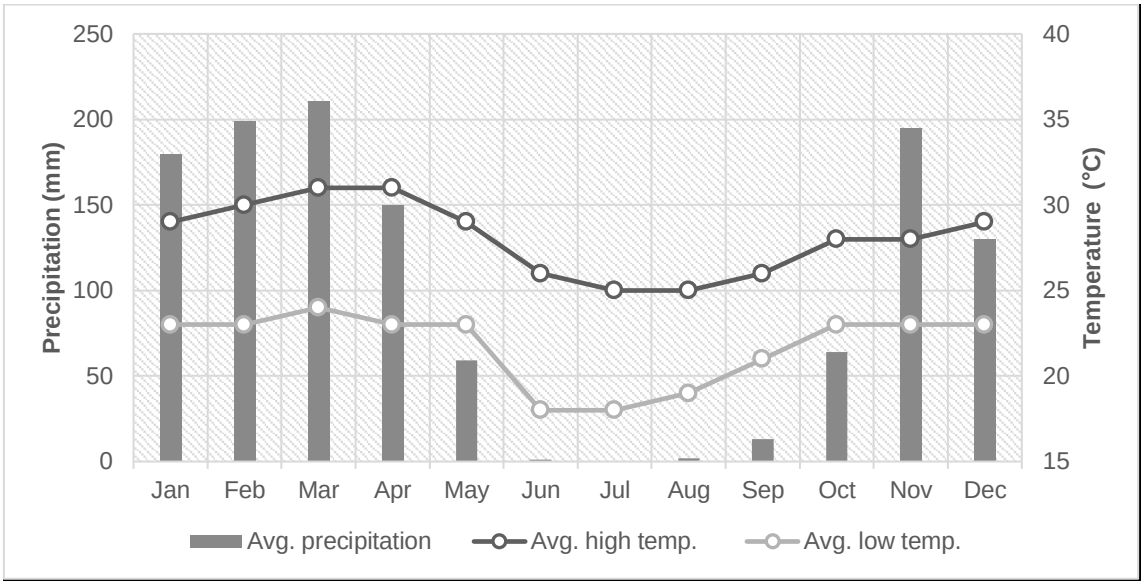

Figure 1-2: Average precipitation and minimum and maximum temperatures for the coastal study area (lower basin), Pointe Noire, Kouilou Province (http:www.climatedata.eu)

The upper basin is situated on the Du Chaillu Massif range, which consists of a metamorphic formation incised by steep-sloped hills and high mountainous regions (van Rooyen et al., 2019) situated between 550 and 750 meters above sea level. The main rivers of the study area are the Louessé and Mandoro rivers, which ultimately drain the Niari River basin toward the lower basin study area. The rainy season in this higher lying area extends from November to April, with the dry season spanning from May to October (**Figure 1-3**).

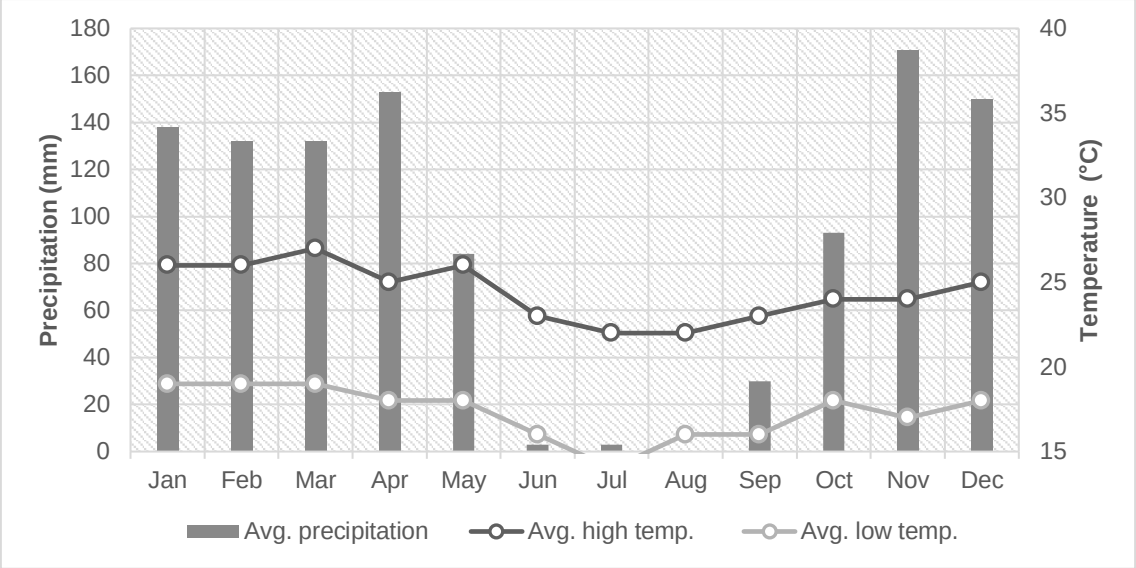

Figure 1-3: Average precipitation and minimum and maximum temperatures for the inland study area (upper basin), Mayoko, Niari Province (http://www.worldweatheronline.com)

1.4 Stream ecosystem conceptual models

The following section summarises the main conceptual fundamentals of fluvial ecology which were explored using various methods in this thesis. These concepts have stemmed from varied sources (Minshall, 1988; Ward et al., 2002) and have been highly influential in the development of the evolved lotic theory. Many studies have focused on the refinement of certain predictions of this theory (Ward et al., 2002; Melles et al., 2012 - **Figure 1-4**). Most of these major lotic conceptual frameworks on gradient, habitat filtering and hydrology have been superimposed on river ecology globally.

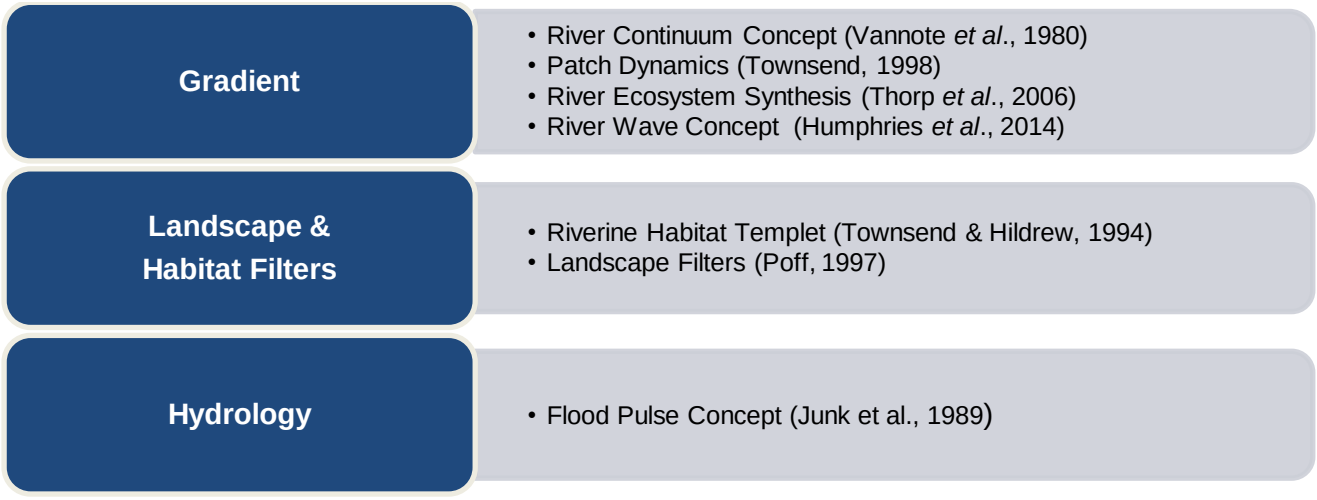

Figure 1-4: Major freshwater conceptual models explored within the context of this thesis, classified into relevant conceptual frameworks (adapted from Ward et al., 2002)**.**

1.4.1 Gradient

Contemporary concepts developed under the framework of lotic gradient evolved within the paradigm that fluvial systems were deterministic, homeostatic, spatially homogenous and in near-constant equilibrium (Ward et al., 2002). The River Continuum Concept (RCC - Vannote et al., 1980) was developed in this deterministic paradigm. Some research in river ecology now considers lotic systems as heterogeneous, non-deterministic systems in non-equilibrium, with highly scale dependent processes (Talbot, 1996; Ward et al., 2002; Melles et al., 2012).

The RCC (Vannote et al., 1980) is considered the biologic analogue of the Energy Equilibrium Theory of fluvial geomorphology (Leopold and Maddock, 1953) and assumes a continual gradient of physical conditions (loading, transport, utilisation, storage of organic matter) from headwaters to mouth that result in an ecological continuum within constituent lotic populations. Vannote *et al.* (1980) posited that producers and consumers of a river continuum conform to the manner in which the river system utilizes its kinetic energy to achieve dynamic equilibrium, and that downstream communities benefit from energy utilisation and inefficiencies upstream. The concept integrates predictable and observable biological and community features of lotic systems.

A central idea in the RCC is that species are replaced continuously rather than in discrete stages, and that aquatic biodiversity is lower in the headwaters and in lowlands of rivers and is highest in the middle reaches of streams, where habitat and flow heterogeneity and riparian cover are optimal and allow numerous different taxa to exploit habitat. Statzner & Higler (1985) refuted some of the concepts of the RCC for tropical systems and expressed a need for extension of the concept and modification, especially within the energy equilibrium analogue, temporal sequence of species replacement and patterns of biological diversity tenets for tropical systems.

In contrast to the RCC, the Patch Dynamics concept (PD - Townsend, 1989; Winemiller et al., 2010) operates within the new ecological paradigm of non-determinism. It hypothesizes that rivers are not a continuum as the deterministic view of the RCC suggests, but instead conform to patch dynamics principles in which disturbance over time creates patches in lotic systems generating heterogeneity and Functional Process Zones (FPZ). Patches are spatial units differing from their neighbours. They are not necessarily internally homogeneous, but may be further decomposable to smaller spatial scales that have distinctive disturbances, colonizers, colonist sources and species interactions (Townsend, 1989; Poole, 2002). Consequently, the concept of PD suggests that patches and FPZs are more important for the occurrence of a species than the position of the given site along a continuum (Wantzen and Junk, 2000). The PD concept may be more explanatory of gradient in tropical systems as the pattern of physical parameters in these systems may vary on a microscale and have additional base levels such as natural lakes within the stream continuum (Statzner and Higler, 1985).

Though both the RCC and PD concept have provided valuable insights into lotic community ecology, they are also both limited in their ability to explain community assembly. The Riverine Ecosystems Synthesis (RES - Thorp et al., 2006) and the River Wave Concept (RWC - Humphries et al., 2014) thus attempt to integrate various concepts to form unifying general lotic theories. The RES expands on the existing Hierarchical Patch Dynamics terrestrial landscape model (Wu and Loucks, 1995) and aspects of lotic models from 1980's to 2004 to

explain biocomplexity in lotic systems across a spatiotemporal scale from headwaters to large river mouths. The model contrasts with common view of rivers as continuous, longitudinal gradients in physical conditions, and rather portrays rivers as downstream arrays of large hydrogeomorphic patches formed by catchment geomorphology and climate. Some hydrogeomorphic patches re-occur in the downstream passage, and some are ecologically unique processing zones. The RWC presents a model that conceptualises the river as a series of longitudinal and seasonal waves in which different conceptual models dominate in terms of functionality depending on the position in the river continuum and the discharge (season).

1.4.2 Habitat and landscape filters

Some concepts posit that diversity of species assemblages are forged by physical templets at different spatial scales. The Riverine Habitat Template (RHT - Townsend & Hildrew, 1994) postulates that habitat provides the templet upon which evolution forges characteristic species traits. In this model, species traits in a stream community are driven by disturbance over time and spatial change in the stream template. The assumption of the RHT model is that species traits are determined by the organism's environment, where temporal heterogeneity refers to the frequency of disturbance, and spatial heterogeneity to the provision of refugia in space (Townsend and Hildrew, 1994). The two axes allow prediction of traits, for instance, in low order mountain streams on steep gradients with high temporal variability and high spatial homogeneity, the RHT expects a community which shows traits that infer resilience (e.g., physiological tolerance to desiccation, dorsoventrally flattened bodies, life histories that allow for rapid colonization). Conversely, middle reaches of higher order streams with lower temporal heterogeneity and high spatial heterogeneity will harbour species with a higher diversity of less resilient traits (i.e. long lifespans, diverse body forms, no potential for regeneration).

Poff (1997) proposed that habitat selective forces, which constitute filters occurring at hierarchical landscape scales (ranging from microhabitats to watersheds or basins) predict species occurrence. The Landscape Filters (LF) concept predicts a species' trait-environment

relationship and essentially constitutes a simple niche model to make hypothetical predictions about community composition at various habitat scales. Largescale filters are viewed as causal agents that constrain expression of local selective forces and biotic potential at lower scales (Poff, 1997). The model highlights a biotic approach to predicting species distribution and local community composition by considering environmental constraints at different scales. To join a local community, species in a regional pool must possess appropriate functional traits to pass through the nested filters at these various scales.

1.4.3 Hydrology

The Flood Pulse Concept (FPC - Junk et al., 1989) is interestingly one of few lotic models developed in a tropical setting of a large floodplain. The model considers hydrology as the main driving force for river-floodplain systems, as floodplains contribute significantly to nutrient cycling, distribution of suspended particles and provide habitat within a moving littoral zone. Because low order streams have short and unpredictable pulses, it is postulated that organisms have limited adaptations for using the Aquatic Terrestrial Transition Zone (ATTZ), instead organisms benefit indirectly due to inputs of nutrients from the ATTZ. Larger systems with predictable pulses have communities that are adapted to utilise the moving littoral ATTZ directly (Junk et al., 1989). The fringing ATTZ of streams provide additional habitats with different hydraulic and substrate conditions than the stream channel proper.

The FPC suggests that a large part of primary and secondary production occurs in the associated ATTZ, whereas the river is mainly a conduit for the transport of water, dissolved and suspended matter. In the FPC, the river is also considered as a refuge for aquatic organisms during the dry season and a route for dispersal. This concept is known as the “highway analogy” (Wantzen and Junk, 2000). The FPC incorporates the Intermediate Disturbance Hypothesis (Connell, 1978; Ward and Stanford, 1983) and the PD concept in that it expects that floodplain areas with a predictable, intermediate level of flooding will show the highest diversity, and that flooding will interrupt ecological succession in patches, causing a

mosaic of different successional stages at the same time on small spatial scales (Junk and Wantzen, 2004).

1.5 Functional trait diversity of fluvial biotic assemblages

Functional diversity of freshwater fish communities offers an excellent model for studying community assemblage patterns at various scales (Winemiller, 1991a) and has been increasingly used to understand the applicability of lotic theory to tropical community assembly (Poff, 1997; Mouillot et al., 2007; Pease et al., 2012a; Bower and Winemiller, 2019b; Walsh et al., 2022b). The functional diversity of stream communities allows for the measurement of organismal traits that ultimately influence performance and ecosystem functioning (Diaz and Cabido, 2001; Villéger et al., 2008). It has been proposed that functional diversity, as opposed to species diversity, enhances ecosystem functions such as productivity, resilience and nutrient cycling (Dukes, 2001; Bellwood et al., 2004; Petchey et al., 2004; Hooper et al., 2005). Functional diversity indices have been used to understand the processes governing community assembly and the impact of perturbations and environmental gradients on community structure (Mouillot et al., 2007) allowing comparison across regions and guiding region specific conservation (Sternberg and Kennard, 2013a).

The functional trait method has been used to study the influence of environmental filtering and limiting similarity (Bower and Winemiller, 2019c, 2019a), aquatic and riparian connectivity (Teresa and Casatti, 2012; Arantes et al., 2019) and environmental gradients (Ibanez et al., 2007; Pease et al., 2012b; López-Delgado et al., 2019b, 2019a). Certain studies found agreement with temperate gradient concepts of biotic richness and functional diversity in tropical systems (Greathouse and Pringle, 2006; Ibanez et al., 2007; Pease et al., 2012b) where others found that environmental filtering was the primary driver of community assemblage (Mouchet et al., 2013; Troia and Gido, 2015; Córdova-Tapia et al., 2018; Bower and Winemiller, 2019c, 2019a).

Pease et al. (2012) tested various conceptual models in a large Mesoamerican tropical basin using fish functional trait diversity and space, and environmental drivers. In comparison to purely taxonomic studies, the functional trait based approach allows data from the Neotropics to be compared with conceptual frameworks developed in other regions without the constraints of geography, climate or highly divergent taxa (Poff, 1997; Poff et al., 2006; Olden and Kennard, 2010; Pease et al., 2012b). Bower & Winemiller (2019b) found that water velocity, depth and substrate heterogeneity were the most significant factors confining fish occupation in some microhabitats across geographical scales. The establishment of functional trait-environment relationships also allows for prediction of responses to certain anthropogenically induced environmental changes (Pease et al., 2015). A periodic table of niches was developed from functional trait and performance data by (Winemiller et al., 2015) based on the concept of convergent evolution which shows repeated patterns among form-function relationships across divergent lineages.

1.6 Food webs and energy in Afrotropical systems

Ratios of stable isotopes of carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used widely to examine aquatic food webs and to test various river ecosystem theoretical concepts such as the RCC and FPC (Jepsen and Winemiller, 2002; Winemiller et al., 2010b; Pease et al., 2019, 2020; Machado-Silva et al., 2022). In Stable Isotope Analysis (SIA), ratios of carbon ($\delta^{13}C$) indicate energy flow between primary producers and consumers and elucidate links between consumers, whereas nitrogen isotope ratios ($\delta^{15}N$) indicate trophic position of organisms within a food web hierarchy (Adams and Sterner, 2000; Kraemer et al., 2012). Carbon pathways and nutrient transfer can expose important feeding links between consumers and their primary food sources and identify the ecological niche an organism occupies in a food web (Jepsen and Winemiller, 2002; Bearhop, 2004; Desta et al., 2006; Kraemer et al., 2012). Stable isotope analysis has been applied to explore lotic concepts in various geographical regions showing that the importance of basal carbon sources and composition of consumers of tropical systems are similar in some cases to temperate systems (Winemiller et al., 2010b).

Thorp et al. (1998) used SIA to test conformity of three contemporary lotic models to a large floodplain system in a temperate zone and found that certain concepts were more relevant than others to the ecosystem of interest.

Certain Neotropical and Australasian studies found that tropical systems are mainly supported by autochthonous production such as algae and biofilm (Jepsen and Winemiller, 2007; Dudgeon et al., 2010; Brett et al., 2017; Reis et al., 2020; Roussel et al., 2021). Other studies in forested basins of the tropics suggest that consumers are primarily fuelled by allochthonous riparian forest source (Coynel et al., 2005; Soto et al., 2019). Algae and biofilm were also found to be less important in supporting freshwater consumers in nutrient poor tropical systems (Correa and Winemiller, 2018a) due to limited light infiltration and productivity (Moreira-Turcq et al., 2003). In more recent literature of SIA studies in the Congo River, the authors suggest that methane oxidizing bacteria could be a potentially important producer resource subsidizing the food web (Soto et al., 2019). Existing knowledge of resources fuelling freshwater consumer communities of Afrotropical freshwaters in central Africa is rare (Thompson et al., 2012; Venarsky et al., 2020) with only a single study of the Congo River published to date (Soto et al., 2019).

Seasonality is also a driver of basal resource use in tropical habitats. Major shifts in food items can occur due to seasonality and changes in discharge which affect tropical fish assemblages (Wantzen et al., 2002; Correa and Winemiller, 2014; Pease et al., 2020). Trophic position of species in tropical food webs can vary seasonally, with instream consumers functioning at lower trophic levels in high flows due to increased availability of invertebrates from upstream drift and decreased piscivory (Wantzen et al., 2002; McMeans et al., 2019). Other communities have a wider feeding niche during the wet season due to a broader range of prey items and improved access to inundated lateral habitats such as floodplains and forests (Pool et al., 2017).

1.7 Relevance of this study

Historically the rate of exploitation of natural resources in Afrotropical forests of the Congo Basin has been markedly slower than in other tropical regions (Rudel, 2013; van Rooyen et al., 2016, 2019), but over the past decade this pattern has rapidly changed as many central African countries have stabilized politically, resulting in a sharp increase in development, resource extraction, and a range of associated environmental impacts (Megevand et al., 2013; Petrozzi et al., 2016). The rapidly increasing impacts felt in the region and the paucity of freshwater research on Afrotropical biodiversity is a growing obstacle for freshwater conservation (Grill et al., 2019; Leclère et al., 2020; Tickner et al., 2020). In the absence of a broad and data-rich understanding of ecosystems, characterizing functional diversity patterns in freshwater communities has aided in predicting changes, pinpointing vulnerabilities, and prioritizing regional conservation actions (Toussaint et al., 2016a). The research in this thesis intends to contribute to knowledge on the diversity (taxonomic descriptions of new fish species), ecosystem functionality (exploration of drivers of Afrotropical freshwater fish assemblages) and trophic ecology (food webs and resources in the coastal Afrotropics) of this under studied region to identify research gaps and implications for regional freshwater conservation.

1.8 Thesis organisation

This thesis has been written as five separate scientific papers bookended by an introduction, and a conclusion and synthesis of key findings. **Chapters two, four** and **five** have been published in peer reviewed journals, and **Chapter three** and **Chapter six** are written as stand alone manuscripts which have not been submitted for publication.

The first two data chapters explore the functional diversity of fish assemblages in the Republic of the Congo along an elevational gradient from the upper to the lower basin in the study area. In **Chapter two** functional trait data of fish assemblages along the gradient of a large basin in the Republic of the Congo were used to describe potential freshwater community determinants and functional diversity along this fluvial gradient. Following from the results of **Chapter two**,

functional diversity metrics and linear mixed-effects models were used in **Chapter three** to interrogate conceptual expectations of ecological structuring along the same river gradient. Functional diversity in relation to various key physical and environmental drivers of community assembly were used to explicitly test and comment on the relevance of lotic freshwater models concerning gradient and environmental filtering in this region.

Chapters four and **five** focus on organismal diversity, taxonomically describing two fish species from the study region in the lower Guinean ichthyofaunal province. A combination of genetic and morphological techniques were used to resolve these new to science fish species. In **Chapter four** *Thysochromis emili* is described as a new species of chromidotilapiine cichlid from the lakes and rivers of the Kouilou and Noumbi basins in the Lower Guinea, and two species of *Thysochromis* from the upper Guinean ichthyofaunal province are synonymised. **Chapter five** describes a lampeye species (*Plataplochilus eliasi*) from streams in the upper reaches of the Bondo River (Noumbi Basin) in the Mayumbe Mountains of the Republic of the Congo. This genus belongs to the most ancient lineage of Procatopodidae (small African cyprinodontiform fishes) which diversified in the Pleistocene in the Last Glacial Maximum. This new species represents the first description of a new species of Plataplochilus since 1981.

In **Chapter six** I used stable isotope analysis along fluvial gradient of increasing river size between dry and wet seasons to describe the food web of a forested coastal river in Congo. A combination of various assemblage-wide metrics, isotopic metrics and mixing models were uses to identify the key basal carbon resources and seasonal shifts in feeding to understand which food resources are the most fundamental for fuelling and maintaining consumer (macroinvertebrate and fish) communities in this region.

Finally in **Chapter Seven** I synthesize the key findings of the thesis and the implications for the conservation of taxonomic and functional structure in the forested wet Afrotropics. I also highlight research needs and considerations for conservation of freshwater systems in the forested Afrotropics.

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Chapter 2: Functional diversity of Afrotropical fish communities across river gradients in the Republic of Congo, west central Africa

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2.1 Abstract

Characterizing functional freshwater biodiversity patterns and understanding community determinants can aid in predicting changes and prioritizing specific conservation management actions. Afrotropical freshwater systems support diverse ecological communities, including many taxonomically undescribed species, yet few studies have been undertaken in this region and they remain poorly understood. Here, we use functional trait-based approaches to describe the functional diversity of fish assemblages in the Republic of Congo (west Central Africa) from the headwaters of a large basin (upper basin) to the coastal plains (lower basin). Functional traits were assessed using multivariate ordination, and functional diversity was calculated using functional trait-space occupation. We investigated if fish β diversity is nested regionally and at a basin-scale to understand if species assemblages exhibit non-random or environmental filtering patterns. Trait-environment relationships were modelled to determine the most significant drivers of trait diversity, and functional richness (FRic) was calculated for communities in both basins to identify whether Afrotropical systems conform to expected patterns of biodiversity along a fluvial gradient. Moderate to weak community nestedness and various traits associated with resistance and feeding were significantly correlated with habitat variables in the upper and lower basins respectively. Whilst environmental filtering is an important process of community assembly at basin scale, non-random processes are evident in the upper basin, whereas a high turnover was noted in the lower basin. Trophic traits in larger coastal plain habitats of the lower basin suggest higher trophic diversity is associated with higher spatial heterogeneity and nutrient concentrations. High functional turnover between headwaters and coastal plains suggests different functional strategies in fish assemblages between localities along the fluvial gradient. In contrast to expectations derived from temperate lotic systems, functional richness was comparable across both basins which suggests that headwater zones in the region harbour substantial functional diversity, especially when considering upstream catchment size. Functional diversity and environment patterns documented show unique patterns of functional richness that may assist in improving understanding of responses, vulnerabilities and conservation needs in Afrotropical systems.

2.2 Introduction

General expectations for fish diversity patterns across large river basins are based on documented relationships with climate, physiography, flow conditions, and ecosystem size, but unique biogeographical characteristics in some regions may drive exceptions to predicted patterns (Heino et al., 2013). The conceptual fundamentals of lotic ecology were mainly derived from the study of small forested mesic streams in Europe and North America, (Horwitz, 1978; Vannote et al., 1980; Newbold et al., 1982) with studies in the tropics only appearing more recently (Ward et al., 2002; Minshall, 2007; Dudgeon, 2008). Lotic theory suggests that the environmental continuum changes in predictable ways across the gradient of larger basins, for example the River Continuum Concept suggests that changes in resources along a river continuum dictate the trophic structure of biotic assemblages (Vannote et al., 1980). A central tenet of the concept is that species are replaced along the continuum, and that aquatic biodiversity is lower in the headwaters and is highest in the middle and lower reaches where habitat, flow and riparian cover are optimal, allowing maximum exploitation of resources. In contrast, the Riverine Habitat Template (Townsend and Hildrew, 1994) emphasizes the importance of spatial and temporal heterogeneity for community structure, proposing that they provide the template upon which evolution forges functional species traits. Using this theory, one can predict that in the upper reaches of rivers with steep gradients, high temporal variability and homogenous habitat, a community would show traits that infer resilience and resistance such as firm attachment mechanisms, high mobility, streamlined and flexible bodies and/or dorsoventrally flattened bodies. Conversely, more stable habitats with more habitat heterogeneity such as in the lower parts of river basins will show species with a higher functional trait diversity of less resilient traits (i.e., long lifespans, diverse body forms).

Community ecology has increasingly incorporated the functional trait framework to provide a mechanistic understanding of the structure of communities, ecosystem processes and general patterns shaping communities making it a useful tool for exploring the adaptability and vulnerability of communities to environmental stressors (McGill et al., 2006; Poff et al., 2006;

Villéger et al., 2008, 2017; Olden and Kennard, 2010; Pease et al., 2012a, 2015; Nock et al., 2016). In comparison to purely taxonomic studies, a functional trait-based approach allows data to be compared without the constraints of geography, climate or highly divergent taxa (Poff, 1997; Poff et al., 2006; Pease et al., 2012a; Bower and Winemiller, 2019b, 2019a), can support understanding of processes governing regional community assembly (Mouillot et al., 2007; Pease et al., 2015) and guide regionally specific management and conservation of aquatic systems (Sternberg and Kennard, 2013b).

The functional trait approach has been used to understand community assemblages in tropical regions at different spatial scales, including environmental filtering and limiting similarity (Bower and Winemiller, 2019b, 2019a), aquatic-terrestrial linkages (Teresa and Casatti, 2012; Correa and Winemiller, 2014, 2018b; Arantes et al., 2019) and environmental gradients (Ibanez et al., 2007; Pease et al., 2012a; López-Delgado et al., 2019a, 2019b). Some studies found that gradients of biotic richness and functional diversity in tropical systems conformed with aspects of temperate gradient concepts (Greathouse and Pringle, 2006; Ibanez et al., 2007; Pease et al., 2012a). In contrast, numerous other findings suggested environmental filtering as the primary driver of community assemblage (Mouillot et al., 2007; Mouchet et al., 2013; Troia and Gido, 2015; Córdova-Tapia et al., 2018; Bower and Winemiller, 2019b). Boulton et al. (2008) noted in a review that published evidence for consistent differences in ecology and response of tropical streams was generally lacking in literature and argues that the term ‘tropical’ potentially has little significance when applied to stream ecology.

Afrotropical freshwaters support diverse ecological communities, with many species still unknown to science (Darwall et al., 2011; Reid et al., 2018) yet they are significantly under-represented in ecological literature (Dudgeon, 2008; Kessler et al., 2011; di Marco et al., 2017; Titley et al., 2017). Historically the rate of exploitation of natural resources in Afrotropical forests of the Congo Basin has been markedly slower than in other tropical regions (Malhi et al., 2013; Rudel, 2013; van Rooyen et al., 2016, 2019), but over the past decade this pattern has rapidly changed as many central African countries have stabilized politically, resulting in

a sharp increase in development, resource extraction, and a range of associated environmental impacts (Abernethy et al., 2013; Megevand et al., 2013; Petrozzi et al., 2016). The rapidly increasing impacts felt in the region and the paucity of freshwater research on Afrotropical biodiversity is a growing obstacle for freshwater conservation (Grill et al., 2019; Leclère et al., 2020; Tickner et al., 2020). In the absence of a broad and data-rich understanding of ecosystems, characterizing functional diversity patterns in freshwater communities has aided in predicting changes, pinpointing vulnerabilities, and prioritizing regional conservation actions (Toussaint et al., 2016).

In this study, we characterize the functional trait-environment and diversity relationships of fish assemblages along an environmental gradient in the Republic of Congo. We characterized functional trait-environment relationships in the upper and lower sections of the basin to understand what is likely driving community assemblage structure. We assessed nestedness of species assemblages at site level to understand if non-random or filtering process of species assemblage underlie patterns of community structure. Lastly, we calculated functional beta-diversity and the volume of functional trait space per basin to understand how functional diversity changes longitudinally. Based on the River Continuum Concept and previous functional studies of fish communities in large tropical basins (Ibanez et al., 2007; Pease et al., 2012a) we hypothesize that functional trait diversity would increase along the longitudinal gradient from the upper to lower basin and that feeding traits would reveal the change of food resources along the continuum. Based on the Riverine Habitat Template concept we expected to see evidence of environmental filtering and functional turnover where upper basin assemblages are dominated by traits that are adapted to resistance and hydrodynamics, with the lowland basin containing more diverse feeding traits representative of the increased resources as habitat becomes more heterogenous and stable.

2.3 Methods

2.3.1 Study region

The study area is situated in the Republic of Congo over two geographically diverse areas: the upper study area in the Kouilou-Niari River (the Louessé and Mandoro rivers), and the coastal plains of the Kouilou, Loémé and Noumbi basins (**Figure 2-1**). The headwaters of the Kouilou-Niari River system originate in the upper basin (**Figure 2 1**) and are situated on the Chaillu Massif which consists of incised formations with steep-sloped hills and mountains covered by rare forest types (Mamonekene and Stiassny, 2012; van Rooyen et al., 2019). The upper basin freshwater habitat comprises forest streams on steep slopes, and rivers with varying degrees of forest cover on moderately steep slopes. (**Figure 2-2A -H**). The lower basin landscape is crossed by a dense network of drainage channels flowing into larger rivers and natural lakes resulting in high freshwater habitat heterogeneity (Walsh et al., 2014, 2022a; van Rooyen et al., 2016) (**Figure 2-3A-H**). The lower basin is situated on gentle slopes and is covered by a mosaic of forest and grassland-savannah.

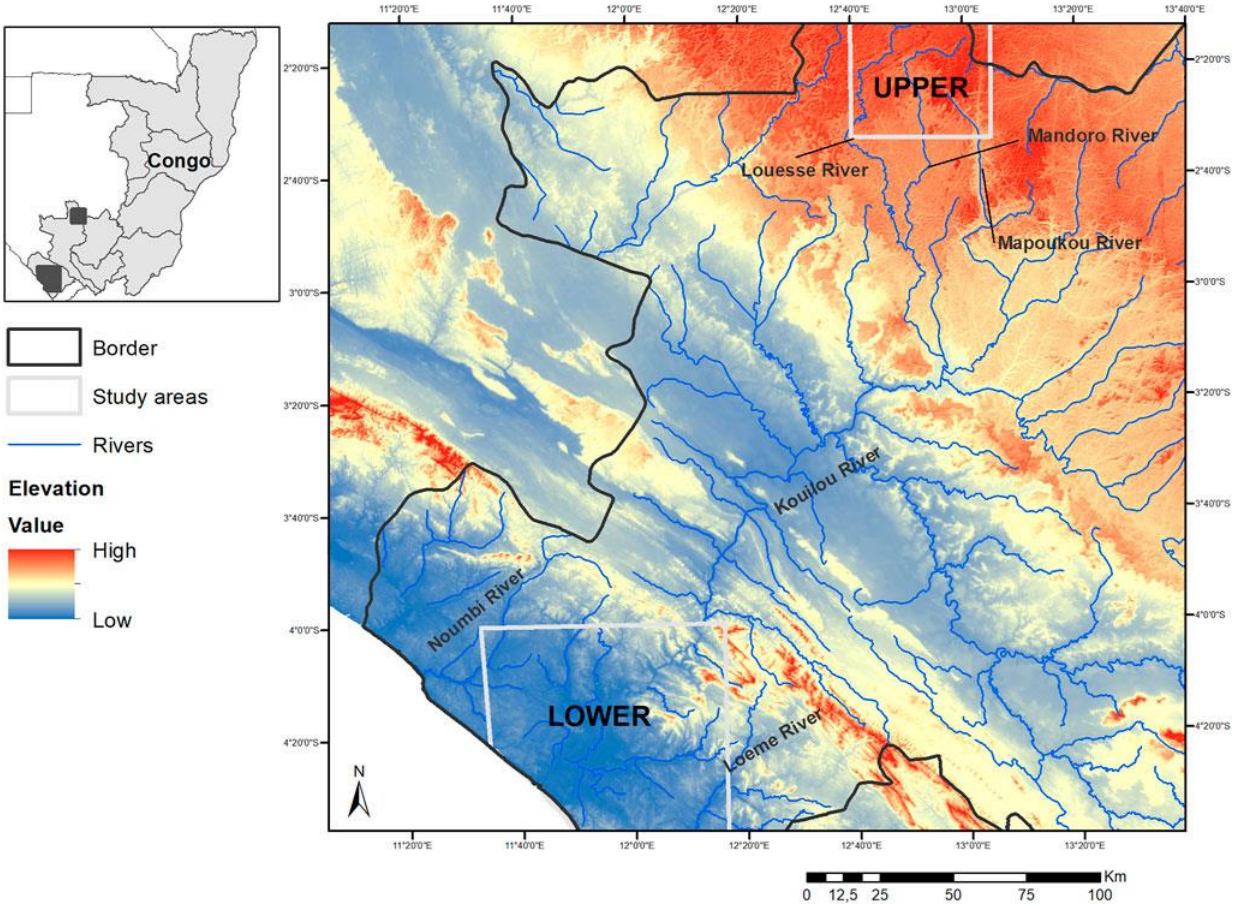

Figure 2-1: Upper and lower basin study areas in the Kouilou, Loémé and Noumbi basins in the Republic of Congo showing major drainages and elevations. The highest point regionally is approximately 750 m above sea level.

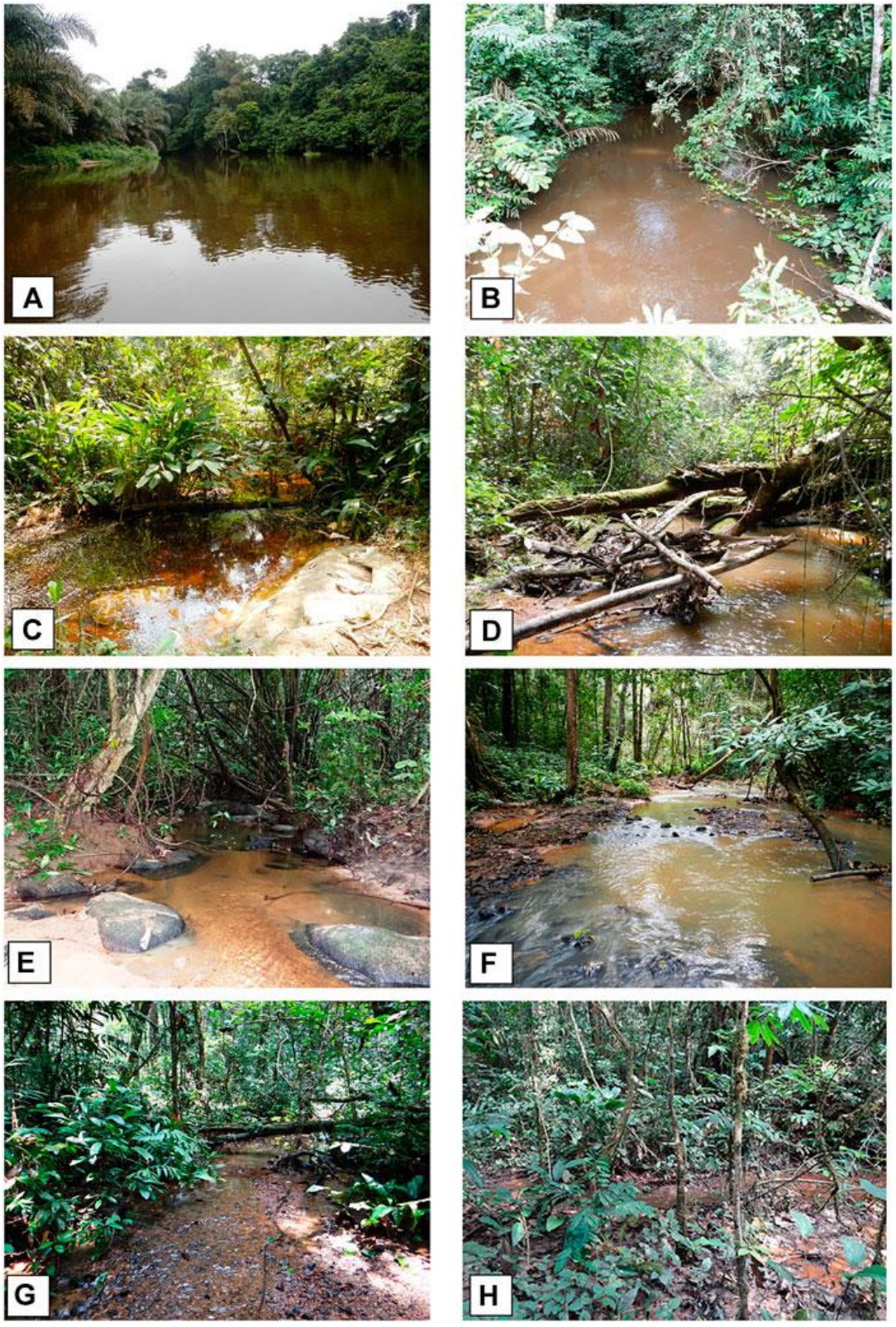

Figure 2-2: Major mesohabitat types sampled in the upper basin in the Louessé and Mandoro river drainages (A)–(B) Rivers; (C)–(G) forest streams, and (H) swamp forests.

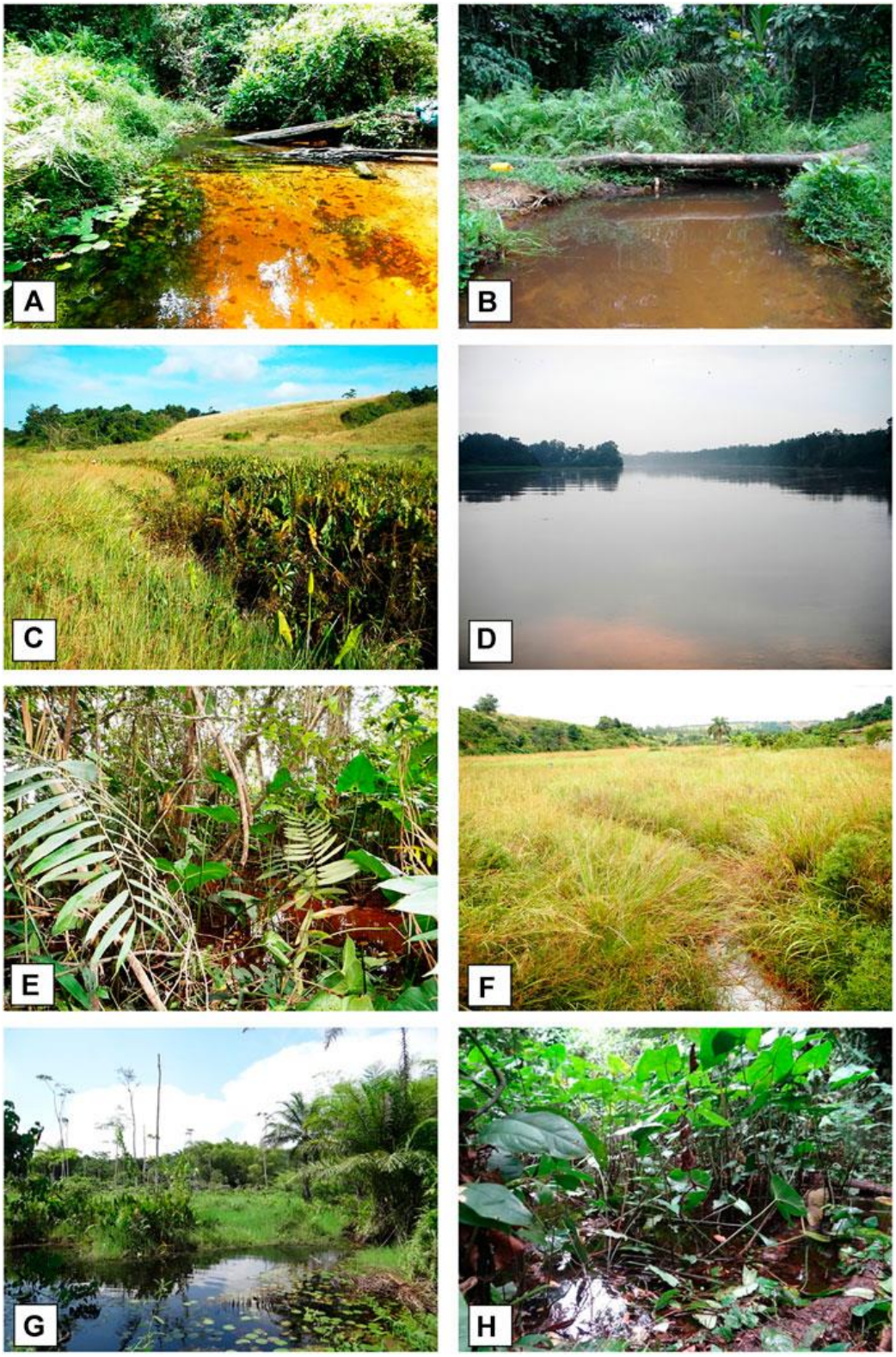

Figure 2-3: Major mesohabitat types sampled in the lower basin coastal study area in the Kouilou, Loémé and Noumbi basins (A)–(B) Forest streams; (C) coastal savannah stream; (D) rivers; (E) swamp forests; (F) savannah wetlands; (G) *Mitragyne stipulosa* swamp forest; and (H) tall swamp forest.

2.3.2 Field data collection

The study included a total of 90 sites (48 in the upper and 42 in the lower basin). Study sites included a range of stream orders encompassing swamp forests, headwater tributaries, larger tributaries, and main stem river channels (**Figure 2-2 & Figure 2-3**). Sites were sampled under base flow conditions in August 2012, October 2013, and October 2014 where fish capture was most efficient due to manageably low flows.

A 200 – 400 m stream reach was sampled to representatively include habitat and microhabitat units at each site in the upper and lower basins. Fishes were collected from all habitat and microhabitat types within each reach within each basin position (upper and lower) in a nested design (Smith et al., 2013; Bower and Winemiller, 2019b). Because of the diversity of habitat types in the study region, various fish sampling methods were applied depending on the habitat available at each site, including seine hauls, cast netting, dip-netting, gill netting, fyke netting and backpack electrofishing with a single pass moving in an upstream direction. We assume that survey methods and study design allow for the comparison of communities between sites as site sampling was applied consistently until no new species were collected at a site (Walsh et al., 2014, 2022a). Species accumulation curves were constructed for surveys to verify efficiency of sampling using the Observed richness (Sobs) and mean estimated richness by Chao 1 estimates in Primer 7 (Clarke and Gorley, 2015; Walsh et al., 2022a). Fishes were deposited in the Ichthyology Collection of the American Museum of Natural History in New York, USA, with all data accessible online s(https://emu-prod.amnh.org/db/emuwebamnh/).

Landscape and local environmental data were collected for all sites and the Spatial Analyst Hydrology package for ArcGIS 10.5 was used to generate river networks and Strahler orders. A total of 47 environmental parameters were measured including physical measures of width, depth and slope, *in situ* water quality and water samples for basic ion and nutrient analysis. Micro-habitat cover for 8 classes was estimated per reach and velocity-depth class according

to Kleynhans (2008). Environmental data were assessed for multicollinearity using the car and caret packages in the R environment (Fox., J. & Weisberg., 2019) where r values of >0.7 were removed from the environmental dataset reducing the total number of variables to 41 (**Table 2-1**).

Table 2-1: Landscape and local environmental and physical variables measured at sites in lower and upper basin of the Kouilou-Niari basin.

Category & Variable	Unit/Measure
Landscape scale	
Latitude	decimal degrees
Longitude	decimal degrees
Elevation	meters above sea level (m.a.s.l)
Precipitation	mm
Stream Order	Strahler Order
Average Basin Slope	%
Cumulative Upstream Basin Size	Hectares (Ha)
Local scale	
Site Slope	Measured percentage slope in site reach
Canopy Cover	Estimated percentage cover in site reach
Width	Average width (meters)
Depth	Average depth along Thalweg (meters)
Water quality	
Calcium	mg/l
Chloride	mg/l
Conductivity	µs/cm
P Alkalinity	ppm
M Alkalinity	ppm
Potassium	mg/l
Magnesium	mg/l
Sodium	mg/l
Nitrate as NO_3	mg/l
Nitrate as N	mg/l
Bicarbonate	mg/l
Carbonate	mg/l
pH	H^{1+} ions
Phosphate as PO4	mg/l
Sulphate as SO4	mg/l
Temperature	°C
Turbidity	NTU
Microhabitat	
No. of Riffles	Number of riffles in site reach
Leaf Litter	Leaf litter in site reach (scored abundant, common, rare, absent)
Woody Debris	Woody debris (scored abundant, common, rare, absent)

Category & Variable	Unit/Measure
Overhanging Vegetation	Overhanging bank vegetation (scored abundant, common, rare, absent)
Undercut Banks and Roots	Undercut banks and roots (scored abundant, common, rare, absent)
Substrate	Available substrate (scored abundant, common, rare, absent)
Aquatic Macrophytes	Aquatic macrophytes (scored abundant, common, rare, absent)
Water Column Availability	Water column for fish (scored abundant, common, rare, absent)
Substrate Diversity	Bedrock, boulders, cobbles gravel, sand, mud
Velocity-Depth	
Slow-Deep	Percent of site velocity-depth class (slow = <0.3m/s; deep = >0.5m)
Slow Shallow	Percent of site velocity-depth class (slow = <0.3m/s; deep = <0.5m)
Fast Deep	Percent of site velocity-depth class (fast = >0.3m/s; deep = >0.5m)
Fast Shallow	Percent of site velocity-depth class (fast = >0.3m/s; shallow = <0.5m)

2.3.3 Morphometric traits

Twenty-three functional traits were measured from fish specimens (**Table 2-2**). The traits chosen show relationships with habitat-use, locomotion, life history strategy and feeding (Winemiller, 1991b; Winemiller and Rose, 1992; Sibbing and Nagelkerke, 2000; Pease et al., 2012a). Five individual specimens of each species were measured on a Nikon SMZ800 microscope camera connected to a computer with the NIS-Elements D computer application and a 1× objective lens. Where we encountered less than five individuals of a species, available specimens were measured. Because depth could not be measured accurately using the software, head depth, body depth and oral gape were measured using callipers with measurements taken to the nearest 0.1mm (Winemiller, 1991b; Winemiller and Rose, 1992). To eliminate ontogenetic and sexual dimorphic biases, only adults of all species, and females of sexually dimorphic species (e.g., Alestidae, Cichlidae, and Nothobranchiidae) were included in the analysis. Before proceeding with the functional trait analyses, ANOVAs were performed on trait variance data to confirm that interspecific variation was greater than intraspecific variation ($P < 0.05$).

Table 2-2: Functional traits used for upper and lower basin fish assemblages with description of measurement from Pease et al. (2012). Trait definitions and functional categories follow (Gatz, 1979; Webb, 1984; Winemiller, 1991; Winemiller and Rose, 1992; Sibbing and Nagelkerke, 2000)

Trait	Description	Functional Category
Standard length	Length measured from the tip of the snout to the caudal flexion	Habitat use; Life history strategy
Head length	Distance from the tip of the upper jaw to the posterior edge of the operculum	Feeding
Head depth	Vertical distance from dorsum to ventrum passing through the pupil	Feeding
Oral gape	Vertical distance measured inside of fully open mouth at tallest point	Feeding
Mouth position	The angle between the line connecting the tips of the open jaws running between the centre of the pupil and the posterior-most vertebra	Feeding
Eye position	Vertical distance from the centre of the pupil to the ventrum	Habitat; Feeding
Eye diameter	Horizontal distance from eye margin to eye margin	Habitat; Feeding
Snout length	Distance from the pupil to the tip of the upper jaw with mouth shut	Habitat
Snout protrusion	Additional distance from the pupil to the tip of the upper jaw with mouth fully open and extended	Feeding
Body depth	Maximum vertical distance from dorsum to ventrum	Habitat; Feeding
Body width	Maximum horizontal distance from side to side	Habitat
Caudal peduncle length	Distance from the posterior proximal margin of the anal fin to the caudal margin of the ultimate vertebra	Habitat
Caudal peduncle depth	Minimum vertical distance from dorsum to ventrum of caudal peduncle	Habitat
Caudal peduncle width	Horizontal width of the caudal peduncle at mid-length	Habitat
Body depth below midline	Vertical distance from midline to ventrum	Habitat
Dorsal fin length	Distance from the anterior proximal margin to the posterior proximal margin of the dorsal fin	Habitat
Dorsal fin height	Maximum distance from the proximal to distal margin of the dorsal fin (excluding filaments)	Habitat
Anal fin length	Distance from the anterior proximal margin to the posterior proximal margin of the anal fin	Habitat
Anal fin height	Maximum distance from proximal to distal margin of the anal fin	Habitat
Caudal fin depth	Maximum vertical distance across the fully spread caudal fin	Habitat
Caudal fin length	Maximum distance from proximal to distal margin of the caudal fin (excluding filaments)	Habitat
Pectoral fin length	Maximum distance from proximal to distal margin of pectoral fin	Habitat
Pelvic fin length	Maximum distance from the proximal to distal margin of the pelvic fin	Habitat

2.3.4 Statistical methods

2.3.4.1 Environmental correlates of fish community structure

The environmental variables matrix was refined to include variables with Z-scores of Spearman's correlation at a significance of $p < 0.05$, which reduced the environmental dataset to 29 variables. To show the relationship between sites based on environmental variables, a Principal Component Analyses (PCA) was performed on the refined environmental dataset using normalized environmental data and a matrix based on Euclidean distance. Only variables with a Pearson's correlation of >0.5 were shown in the PCA.

A stepwise distance-based linear model permutation (DistLM) identified environmental variables which were predictors of fish community structure, considering possible interactions between environmental variables (Clarke and Gorley, 2015). The resemblance matrix was calculated using Log (X+1) Bray-Curtis similarity and transformed. An AIC (Akaike's Information Criterion) selection criterion was run using 999 permutations with a forward variable selection procedure. Results were represented using a distance-based redundancy analysis (dbRDA). The outcome of the analysis shows vectors representing the combination of environmental variables that were the best predictors of fish community structure at sites. All statistical analyses were completed in Primer 7 and R studio v 4.1.3 (Clarke and Gorley, 2015; RStudio Team, 2021).

Functional traits are phenotypic characteristics that influence ecosystem and community processes. A PCA was performed on the functional trait data collected in **Table 2-2** to show functional trait space and functional traits governing the placement of species in the upper and lower basins. Traits were regressed against standard length (SL), and residuals were used to generate the PCA from a matrix based on Euclidean distance of transformed values (Pease et al., 2012a). Mouth position was not correlated with the body size, therefore values for this trait were log transformed and used instead of a residual.

2.3.4.2 Taxonomic spatial nestedness and species turnover

Beta diversity can reflect either nestedness or spatial turnover. Nestedness of species assemblages occurs when the species at sites with lower richness are subsets of the species at sites with greater richness (Baselga, 2010) implying a non-random process of species assemblage and structural patterns (Gaston and Blackburn, 2000). Spatial turnover implies the opposite of nestedness and the replacement of some species by others due to environmental filtering and sorting (Qian et al., 2005).

We assessed whether fish β diversity was nested regionally and at a basin scale through the nestedness metric based on overlap and decreasing fill (NODF) (Ulrich and Almeida-Neto, 2012). The NODF quantifies the nestedness among sites or species independently. Values for NODF are between 0 (zero nestedness) and 100 (perfect nestedness). The significance of the NODF was assessed using the CE (proportional) null model which assigns a probability to each matrix cell to be occupied proportional to the corresponding row and column totals (Strona and Fattorini, 2014). To test for the existence of landscape and habitat filtering patterns of β diversity at the regional and basin scale (Townsend, 1989; Townsend and Hilldrew, 1994; Poff, 1997; Winemiller, Flecker and Hoeinghaus, 2010), assemblage data were split into upper and lower basin sets, and then combined for the final analysis. The NODF nestedness analysis was performed using NeD software (Strona and Fattorini, 2014).

2.3.4.3 Functional trait-environment relationships

Fourth-corner analysis was used to test relationships between species traits and environmental variables in the ade4 package in R Studio (Legendre et al., 1997; Dray and Dufour, 2007; Dray et al., 2014). The fourth-corner function measures the predictions by a Pearson correlation coefficient for two quantitative variables (i.e., trait and environmental variable), by Pearson χ^2 and G statistic for two qualitative variables and by a Pseudo-F and Pearson r for one quantitative variable and one qualitative variable. The significance of

relationships (p value = <0.05) was tested by a permutation of sites and species which avoids inflated Type 1 errors (Dray et al., 2014).

All functional diversity analyses were computed using the mFD package (Magneville et al., 2022). We compared upper and lower basin trait space using Functional richness (FRic - Cornwell et al., 2006; Villéger et al., 2008) which represents the volume of the convex hull defining the functional space present in each basin assemblage. Each trait was given equal weight in calculating the distance matrix and was computed in a 5-dimensional space which accounted for 91% of the variance.

Beta functional diversity was calculated for the two basins using *mFD: β.fd.multidim,* which computes pairwise functional β-diversity, using Jaccard dissimilarity, and resultant components of turnover and nestedness (Villéger et al., 2013). Jaccard dissimilarity indicates the functional dissimilarity between β diversity of the set of functional traits per basin.

2.4 Results

2.4.1 Environmental predictors and fish community

A total of 80 species in 22 families of the Teleostei were collected over the two study areas, with 41 species in the upper basin and 49 species in the lower basin. Family level classification followed van der Laan et al. (2014). Approximately 50% of the species from the upper and lower basins were members of the following four families: Cyprinidae (23.8%), Mormyridae (10.0%), Cichlidae (8.8%) and Clariidae (8.8%). Species richness ranged from three in small forest streams and swamp forests, to 19 in larger river systems and generally increased further downstream along the river gradient.

The first two axes of the environmental PCA explained 31.6% of the variation between sites (**Figure 2-4A**). Sites separated strongly between upper and lower basins on the PC1 axis (representing 17.1% of variance) as a function of mostly landscape-scale variables. Lower basin sites had highest variable loadings for water temperature and salt concentrations

(Chloride). The upper basin site placement was related to higher precipitation and steeper average catchment slopes according to the eigenvalues. The PC2 axis (representing 14.5% of variance) separated sites based on habitat type (**Figure 2-4B**), discriminating between fast velocity sites with larger catchments, diverse substrate and woody cover, and slow-deep sites with dense vegetation cover consisting of various streams and swamp forests.

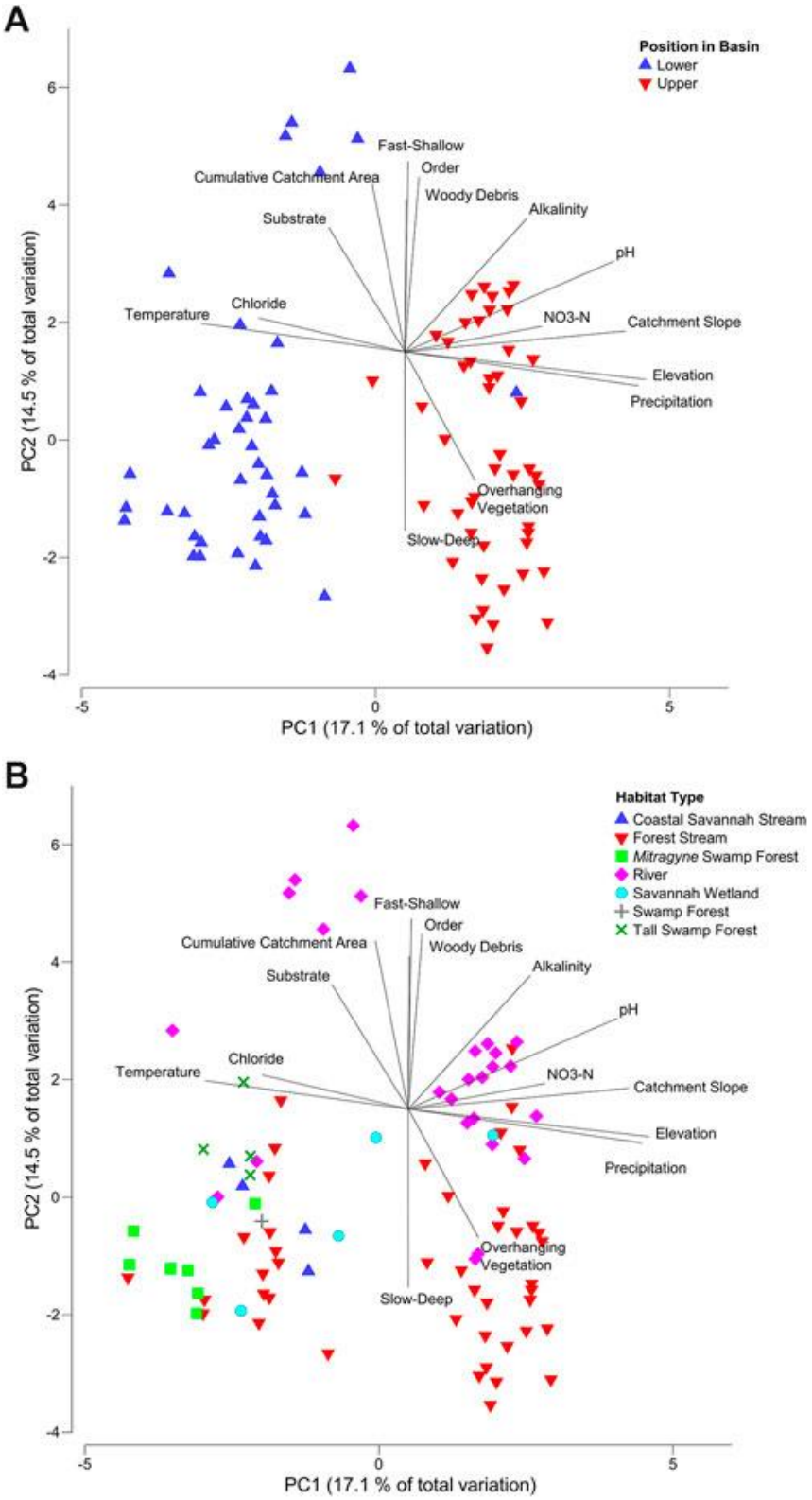

Figure 2-4: PCA with local and landscape scale normalized data using Euclidean distance (A) with position in basin, and (B) habitat type as factor. Vectors were included only from variables with Pearson's correlation of >0.5. Temp = temperature. M Alk = total alkalinity. Ave = Average.

The DistLM analyses showed patterns of fish community structure based on the combination of environmental predictors that contribute most significantly to the species composition of the fish assemblages at sites. The sites were separated by position in the basin (**Figure S1**). The first two dbRDA axes explained 78.96% of the variation out of the fitted model, and 29.59% of the total variation. The dbRDA1 axis explained 69.70% of fitted and 24.8% of total variation and split lower and upper basin fish assemblages based on landscape scale variables, with catchment size, average catchment slope and elevation ($p < 0.001$) gradients being the most important predictors on this axis. The dbRDA2 axis included site-scale variables along a hydrological ($p < 0.001$) and NO_3-N ($p < 0.002$) gradient. Most swamp forest and wetland habitat types were split from rivers on the dbRDA2 axis based on these local variables. Due to the clear separation of sites based on environmental and fish community data between basins, the remainder of the analyses were split such that the lower and upper basin data were analysed separately.

2.4.2 Taxonomic spatial nestedness

Fish assemblages showed varying degrees of nestedness between upper and lower basins, and across the entire basin (**Figure 2-5A** – **C**). Upper basin sites were moderately nested (NODF = 50.64, $z = 14.4$, $p < 0.001$) representing ordered ß diversity and species assemblage structure, where depauperate species assemblages constitute subsets of increasingly richer ones in the landscape (**Figure 2-5A**). The lower basin indicated a weak nestedness of assemblages, with NODF values that were lower than the upper basin (**Figure 2-5B** - NODF = 20.37, $z = 12.57$, $p < 0.001$). Combining the data from upper and lower basin to assess the continuum and turnover at a basin level, species assemblages were weakly nested indicating weak non randomness between ß diversity across the entire basin (**Figure 2-5C** - NODF= 24.122, $z = 12.57$, $p < 0.001$).

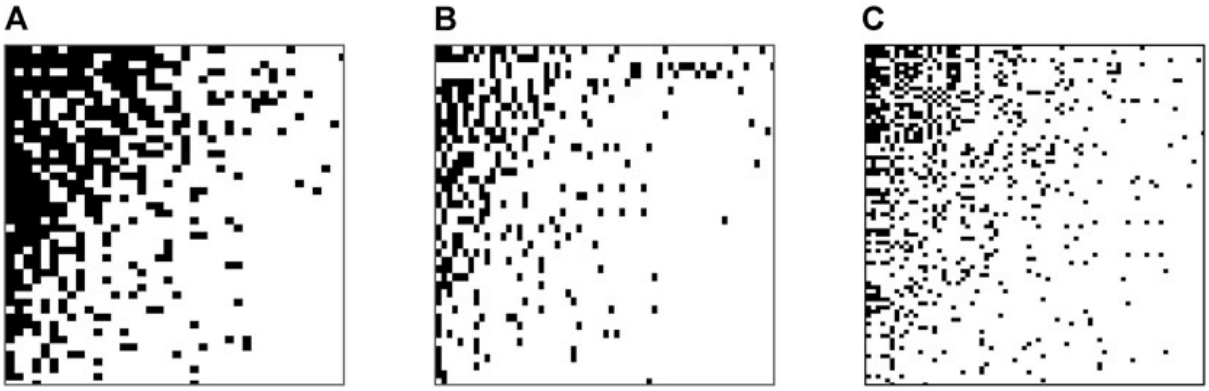

Figure 2-5: Packed nestedness showing distribution patterns of fish assemblages in (A) upper basin and (B) lower basin and (C) upper and lower basin using NODF metrics to overlap and decrease fills with the CE null model.

2.4.3 Functional trait-environment relationships

Functional trait composition of fish assemblages differed between upper and lower basins (**Figure 2-6A & B**); however, primary axes of trait variation were similar in both, with functional traits associated with locomotion strategies (manoeuvrability vs. hydrodynamism) and feeding (position in water column and maximization of food intake) noted as primary drivers of patterns in the fish communities. Phylogenetic influence on functional traits was stronger in the upper basin than in the lower basin.

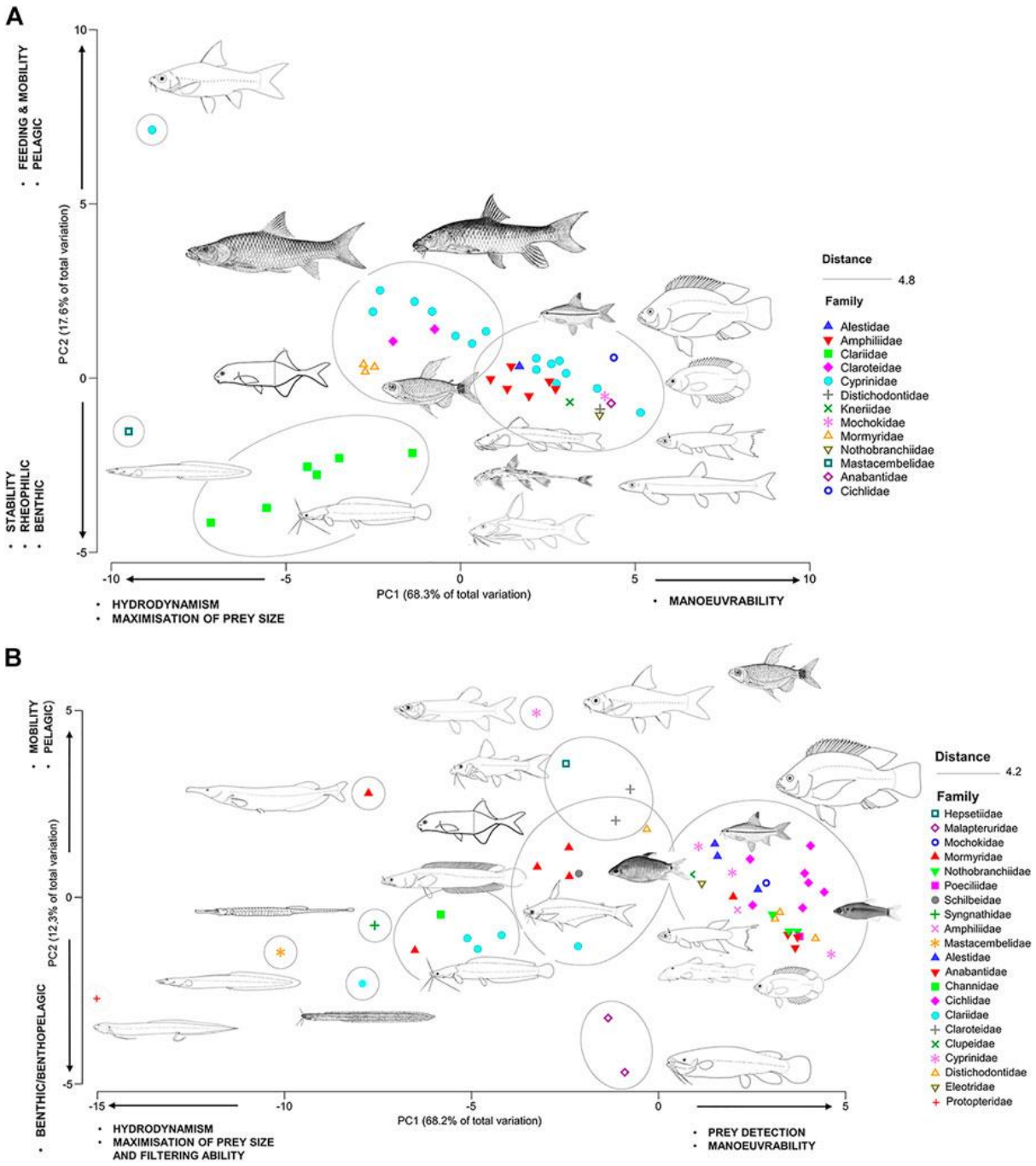

Figure 2-6: Functional traits PCA ordination for (A) Upper basin and (B) Lower basin species showing the position of the fish species in two dimensions of functional trait space. Traits used to characterize the two axes had correlation coefficients ≥0.25.

Upper basin sites with steep slopes contained higher abundances of fusiform and dorso-laterally flattened species and displayed a distinct phylogenetic signature. The PCA of functional traits of the upper basin species explained 85.9% of variation among species (**Figure 2-6A**). The PC1 axis (68.3% of variance) showed a gradient of manoeuvrability/

feeding strategy (pectoral fin length, body width, dorsal fin height and eye diameter; positive side of PC1) and hydrodynamics (mouth position, snout protrusion; negative side of PC1). The clariids (*Channallabes apus*, *Clariallabes longicauda*, *Clarias camerunensis*, *C. pachynema* and *C. gabonensis*), mastacembelids (*Mastacembelus niger*) and one algae scraping cyprinid (*Labeobarbus axelrodi*) had the most negative eigen loadings on PC1. These species are elongate with sub-terminal mouths. The second PC axis (PC2, explaining 17.6% of variance) for the upper basin functional traits separated traits related to water column position where benthopelagic traits (pectoral fin length, head length, snout length, and caudal fin height) showed negative eigen loadings and pelagic traits (eye diameter, dorsal fin height and snout protrusion) were less negative. The elongate clariids (*C. apus*, *C. longicauda*, *C. camerunensis, C. pachynema*, *C. longior* and C. *gabonensis*) had the lowest loadings on PC2, with the large-bodied and streamlined cyprinids (*Labeobarbus roylii*, *L. compinei*, *L. progenys*, *L. axelrodii* and *Labeo camerunensis*) exhibiting the highest loadings on this axis.

Lower basin sites with more gentle slopes, slow-deep habitat types, higher salt concentrations, and warmer temperatures contained more species with deep compressed and/or elongated bodies and a less distinct phylogenetic influence. The PCA of functional traits of the lower basin species explained 80.5% of variation among species (**Figure 2-6B**). The PC1 axis (68.2% of variance) showed a gradient of prey detection and manoeuvrability (head depth, eye position, pectoral fin length, and body depth) and hydrodynamics and maximization of prey size (mouth position, snout protrusion, and anal fin length). Species of the Cichlidae, Alestidae, Anabantidae, and Cyprinidae with relatively large heads, eyes and body depths had higher positive eigen loadings on PC1, while elongate species of Clariidae, Channidae, Protopteridae, and Syngnathidae had high negative values on PC1. The second PC axis (PC2, explaining 12.3% of variance) separated functional traits related to water column position with benthopelagic traits (head length, caudal fin depth and width and snout length) showing more negative eigen loadings and pelagic species (caudal peduncle width, anal fin height and eye diameter) showing positive loadings. The malapterurids and the elongate clariid *C. apus* had

the lowest loadings on PC2, while the large-bodied and streamlined *Hepsetus lineatus*, *Labeobarbus cardozoi* and *Oxymormyrus zanclirostris* had the highest loadings on this axis.

The fourth-corner analysis (**Figure 2-7**) for the upper basin showed two interesting patterns in the species-trait-environment data with a distinct splitting of rhithronic and potamonic species. Only significant trait-environment relationships are reported here. Firstly, inferior mouth position was positively correlated with slope and negatively correlated with Nitrate-Nitrogen (NO_3), inferring resistant traits capable of withstanding faster velocities, and benthic feeding patterns in oligotrophic habitat in lower-order systems. This fish group tends to mostly be represented by species in the lower right side of the upper-basin trait PCA from the Mochokidae, Amphilidae and Kneriidae (**Figure 2-6A**). Secondly, body width, caudal peduncle width, caudal peduncle length, dorsal fin length, pelvic fin length and anal fin length were all significantly positively correlated with variables related to instream structural cover (stones, algae, woody debris, substrate) and fast-flowing water. These traits were also negatively correlated with vegetation, aquatic macrophytes and slow-deep water which also suggests filtering out of laterally compressed species such as cichlids which generally prefer more lentic and vegetated habitat, and selection for fusiform fishes that require more mobility for feeding (e.g. Cyprinidae).

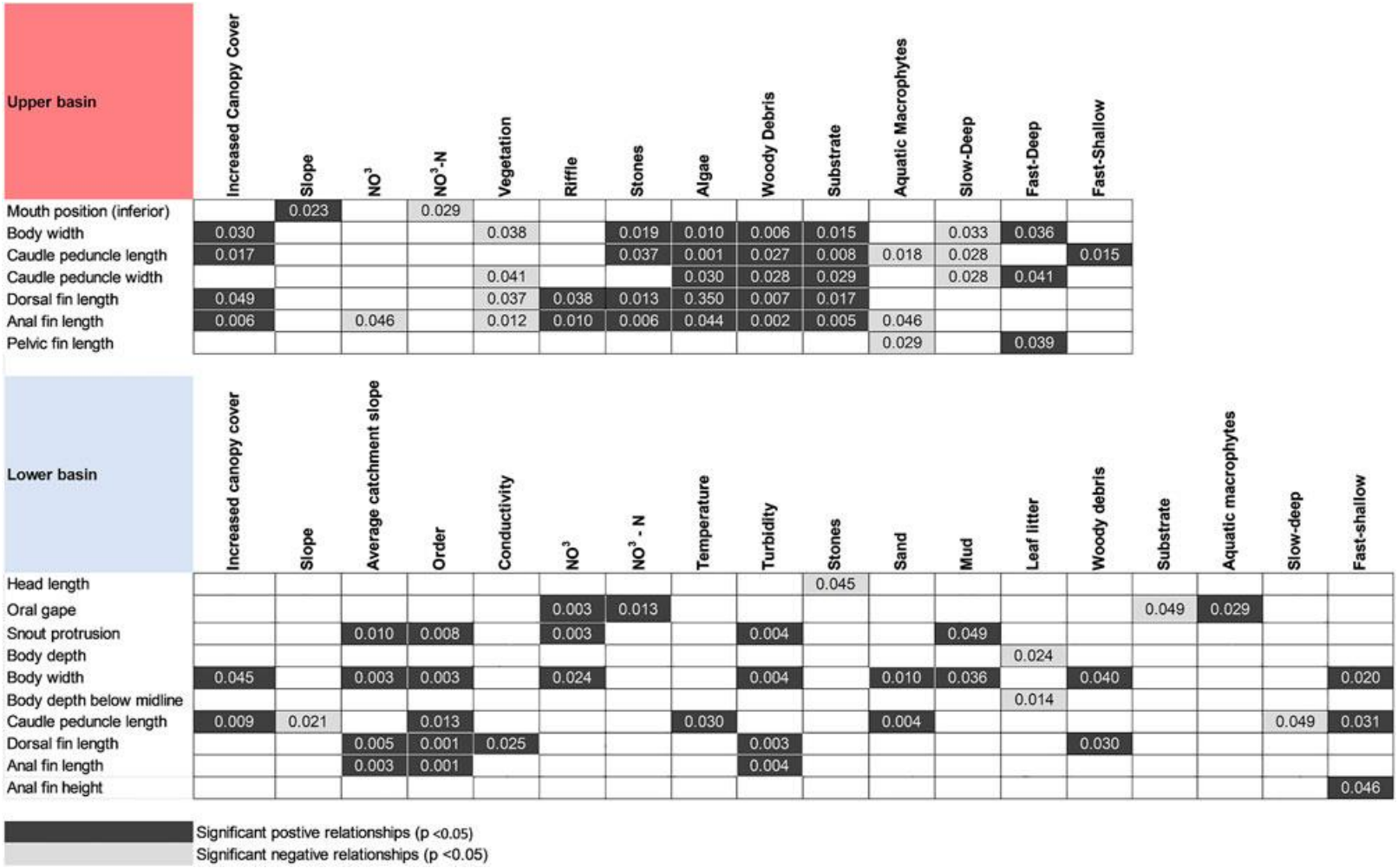

Figure 2-7: Results of the fourth-corner analyses for the upper and lower basin dataset. Black signifies a significant positive relationship (Pearson's correlation) between environmental variable and trait, and dark grey a significant negative relationship.

A divide associated largely with trophic ecology was noted in the lower basin based on trait-environment relationships (**Figure 2-6B & Figure 2-7**). Trophic traits in the lower basin suggest higher trophic diversity is associated with higher spatial heterogeneity and potentially nutrient concentrations. Manoeuvrable fish such as cichlids were negatively correlated with leaf litter benthos. Dorsal and anal fin lengths were associated with larger (higher order) systems and higher turbidity and habitat structure from woody debris. Longer head length was negatively correlated with stones, while increased head depth showed a negative correlation with leaf litter. Increased oral gape was positively correlated with increased Nitrate (NO_3), NO_3-N and aquatic macrophytes, and negatively correlated with substrate which suggests pursuit predators feeding on nekton in midwater habitat. Larger snout protrusion showed a strong positive correlation with catchment slope, order, NO_3, turbidity and mud indicating more piscivorous fishes in larger rivers with more nutrient richness. A similar pattern occurred with body width suggesting more mobile, fusiform species that generally consume nekton were strongly positively correlated with the same environmental drivers as snout protrusion, and additionally sand and woody debris. Increased caudal peduncle length and anal fin height, which are associated with mobility, were positively correlated with large, faster-flowing lotic systems with higher salt concentrations, warmer temperatures, and sandy substrate.

2.4.4 Functional trait space and β-diversity

Functional richness (convex hull trait space) was higher in the lower basin (0.52) than in the upper basin (0.26) (**Figure 2-8**) with a large overlap of in trait space between the upper and lower basin in the first two axes. There was also a high β-diversity due to a high turnover (**Figure S2**), with a low similarity of functional β-diversity between basins (Jaccard dissimilarity = 0.80) driven by a high functional turnover resultant (0.62) and a weak functional nestedness (0.17) suggesting that the upper and lower communities have different functional strategies (Villéger et al., 2013).

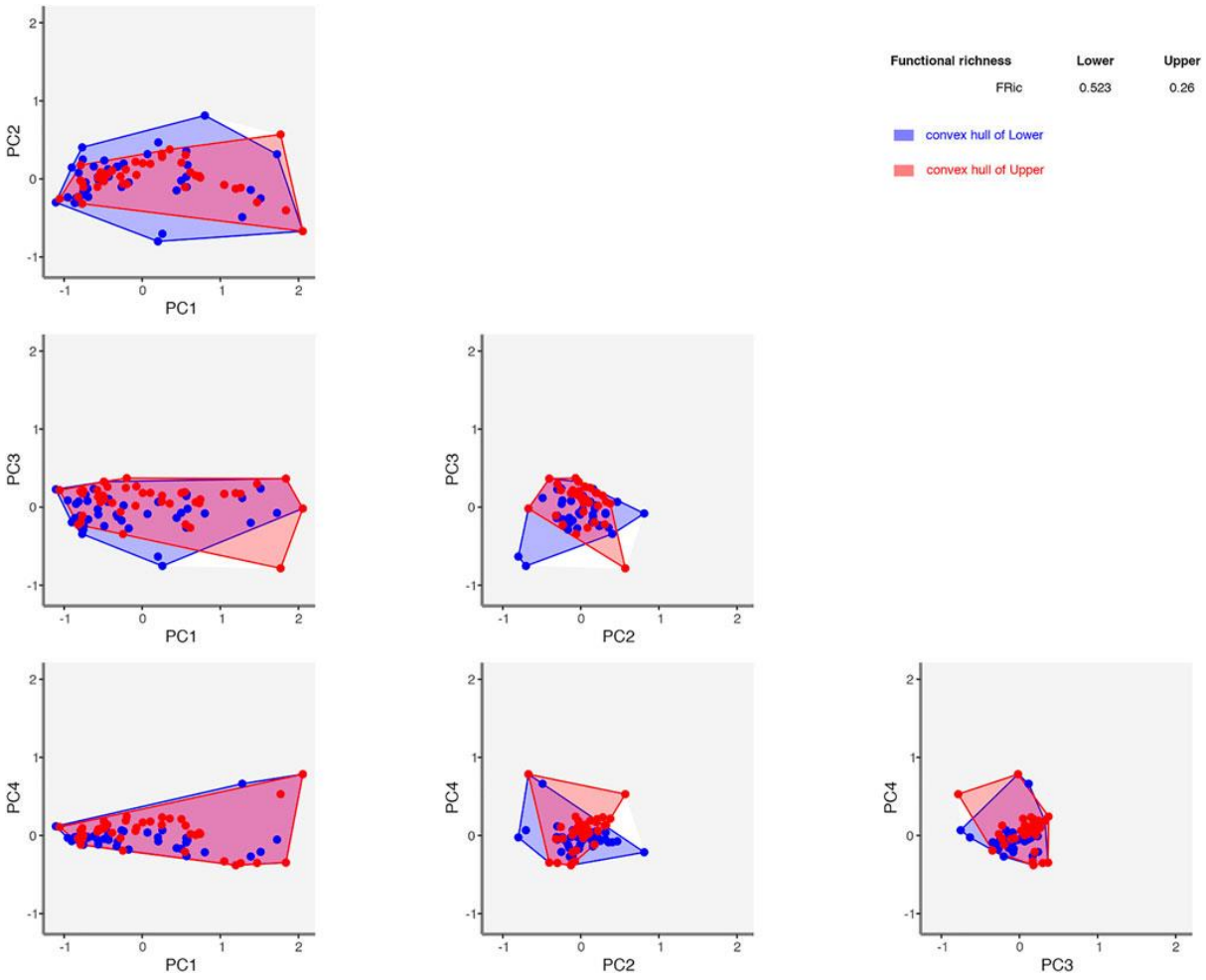

Figure 2-8: Functional richness (FRic) of upper (0.215) and lower basin (0.495) fish communities showing convex hulls and trait space per basin over 5 dimensions of space (accounting for 91% of observed variance in the data). Figure produced in mFD.

2.5 Discussion

2.5.1 Environmental variation and fish assemblage patterns

The structure of fish assemblages within the study area presented longitudinal patterns showing species turnover and increased richness from the upper basin in the Chaillu Massif to the lower coastal plains. This pattern has also been documented in large-scale regional studies of fish assemblages in the Neo- and Afrotropics (Kamden Toham and Teugels, 1998; Ibanez et al., 2007; Ibañez et al., 2009a; Pease et al., 2012a).

In this study, various environmental variables at different spatial scales were correlated with fish assemblage structure with distinct separation of fish assemblages between upper and

lower basins. The changes in fish assemblages between basins seem to be primarily predicted by a combination of temperature, hydrology, and geomorphology (driven by average catchment slope), increased productivity (NO_3-N), and habitat heterogeneity which are common patterns along fluvial gradients across climatic zones (Horwitz, 1978; Vannote et al., 1980; Ibarra and Stewart, 1989; Kamden Toham and Teugels, 1998; Pease et al., 2012). Fish species richness increased along the fluvial gradient from upper to lower basin; an expected pattern as increasing temperature/productivity, hydrological stability, and habitat structure generally support higher fish richness (Schlosser, 1987; Pease et al., 2012a). These observations conform with the River Continuum Concept in that turnover of species and increase in species richness appears to be primarily driven by a combination of temperature, resource availability and habitat heterogeneity (Vannote et al., 1980).

2.5.2 Taxonomic spatial nestedness

Our study highlights different patterns of taxonomic β diversity at a sub-basin and basin scale. We noted a high turnover component suggesting that environmental filtering and sorting is influential at a basin scale. López-Delgado et al. (2019a) found a similar pattern for a pristine Neotropical river, which is expected due to low dispersal rates at a basin scale where assemblages are strongly influenced by species sorting as organisms are excluded from habitats that reduce their fitness (Soininen, 2014). Our results mirror other studies where sorting and environmental filtering explained lotic fish assemblages at broad scales (Heino et al., 2015a, 2015b, Lopez-Delgado et al. 2019a).

Taxonomic β-diversity patterns differed based on position in the basin. The upper basin displayed a degree of nestedness implying a non-random process of species assemblage and structural patterns (Gaston and Blackburn, 2000). Caetano et al. (2021) showed that steep Neotropical stream assemblages were nested, containing a subset of fish species and traits of the fish metacommunity present in streams with gentle slopes. Slope affects fish diversity patterns by driving other abiotic features at larger scales, such as geomorphology, hydrology,

dispersal, and the persistence of species (Eros et al., 2011; Mozzaquattro et al., 2020). Steep average catchment gradients together with high rainfall in the upper basin in our study system influences stream geomorphology and hydrodynamics, often on a daily basis. Headwaters are also relatively isolated within river networks and species dispersal is often limited because of this. These dynamics likely affect the ability of certain species to remain in parts of the river under such conditions, thus reducing alpha diversity and producing nestedness patterns across a steepness gradient. On the contrary, habitats in lower basin reaches are generally less isolated with increased dispersal potential (Caetano et al., 2021). These environments facilitate species sorting and filtering, which is what we found in the lower basin, which had a higher turnover component.

2.5.3 Functional trait-environment relationships

Functional trait-environment associations showed signals of environmental filtering on functional traits at multiple levels, where habitat and landscape templates structured trait distributions within fish assemblages (Townsend and Hildrew, 1994; Poff, 1997). At regional and global scales, environmental filters appear to have the greatest influence on community structure over other factors such as limiting similarity (Poff, 1997; Blanchet et al., 2014).

Upper basin traits showed strong reach-scale environmental filtering patterns inferring resistance and hydrodynamics. High-energy environmental conditions such as those in fast-flowing streams with increased hydraulic forces and temporally variable hydrology increase the influence of environmental filtering on community assembly (Weiher and Keddy, 1995), and similar relationships have been described for other tropical and sub-tropical areas (Oliveira et al., 2010; Bower and Piller, 2015; Bower and Winemiller, 2019b). Lower basin traits separated out along a feeding and mobility trait gradient. The increased prevalence and diversity of manoeuvrable deep-bodied fish, such as cichlids and anabantids, indicates filtering of species for habitats that are structurally more complex (Helfman et al., 2009). At the basin scale, the shift from traits important for resistance and hydrodynamics in the upper basin, to

traits reflective of more diverse feeding strategies and manoeuvrability in the lower basin is consistent with the River Continuum Concept where trophic diversity is predicted to be lower in the headwaters, increasing in the lower reaches where a more diverse array of habitat types and food resources are available (Vannote et al., 1980).

Supporting the results of the traits PCA, trait-environment relationships from the 4th corner analysis for the upper basin show functional-trait associations driven by the physical habitat template and hydrology. Filtering of species with traits based on resistance and habitat strategies is consistent with predictions made by the Riverine Habitat Template (Townsend and Hildrew, 1994). Bower and Winemiller (2019b) also found that water velocity, depth and substrate complexity were most important in restricting fish occupation in certain microhabitats across geographical scales, and other studies have observed significant relationships between water velocity and fish assemblage structure (Lamouroux et al., 2002; Willis et al., 2005; Bower and Piller, 2015; Bower and Winemiller, 2019b).

Lower reaches of rivers tend to provide more diverse habitats, food resources and higher aquatic production because of their size and stable hydrology (Thorp et al., 2006). Functional-trait relationships in the lower basin were mostly driven by feeding and manoeuvrability which is likely driven by increased habitat diversity and increased autochthonous resources (Townsend and Hildrew, 1994). In comparison, trophic traits were not major drivers of the trait-environment associations for the upper basin. The reason for this could be that stochasticity from hydrological flashiness in headwaters may reduce fish populations below carry capacity thereby reducing resource competition (Poff, 1997).

The presence of fusiform, wide-bodied fish at steeper sites in the lower basin shows some conformance with environmental filtering concepts as such body forms are more resistant to variable hydrology and swifter flow velocities (Townsend and Hildrew, 1994). Although no strong relationships were elucidated for deep-bodied fish, diversity of the Cichlidae, Anabantidae, and Alestidae was much higher compared to the upper basin (**Figure S3**). While

some of this increased diversity could be linked to ancient taxonomic radiations of secondary freshwater fishes in the coastal regions, the present-day environment provides no evidence for salinity acting as a diversity filtering factor in the lower basin. Increased fin lengths are associated with woody debris allowing manoeuvrability in more structurally complex habitats such as those in the lower basin (Lamouroux et al., 2002; Wainwright et al., 2002; Dala-Corte et al., 2016). In the lentic microhabitats of the lower basin community there is less environmental filtering acting on body form and species with various body shapes often coexist (Winemiller, 1991b). The prominent environmental-feeding trait patterns in the lower basin suggest that more specialist feeders (pelagic pursuit predators and piscivores) are present in larger river assemblages (Vannote et al., 1980; Sibbing and Nagelkerke, 2000). Similarly, fish assemblages in larger, deeper, lower-velocity streams in the Amazon Basin were dominated by nektobenthic species that feed on fish and invertebrates and require cover (Santos et al., 2019)

Although phylogenetic relationships were not explicitly tested in this study, the morphospace of the upper basin assemblage showed a regional phylogenetic pattern in functional groups. Other communities assembled through habitat filtering have shown similar phylogenetic clustering containing closely related species (Cavender-Bares et al., 2009). The upper basin pool of species also shows patterns of phylogenetic underdispersion where assemblages are strongly driven by environmental filtering and have a narrower set of traits that are suited for their environmental conditions (Weiher and Keddy, 1995; Cornwell et al., 2006). Similar signals of phylogenetic underdispersion were prevalent in structuring regional fish assemblages in West Africa, South America, and Southeast Asia, with environmental filtering as the primary driver of regional community assemblage in these regions (Bower and Winemiller, 2019b). Weak phylogenetic patterns were seen in functional traits of the lower basin morphospace, except for the Cichlidae. This could possibly indicate underdispersion in the Cichlidae, and a higher degree of resource partitioning for other species in the lower basin

community. This pattern could also be attributed to the larger size of systems sampled in the lower basin, which contributed to more species being sampled locally.

2.5.4 Functional trait space and β-diversity

Functional richness increased along the longitudinal gradient from the upper to the lower parts of the basin. Longitudinal functional changes in fish assemblages are common and usually attributable to differences in local environments (Schlosser, 1991; Welcomme et al., 2006; Ferreira et al., 2019). The stress-dominance hypothesis (Coyle et al., 2014) posits that functional trait diversity decreases as environmental stress increases, whereas interspecific trait variation is expected to be greater in less stressful environments. The energy requirements of inhabiting high-velocity, temporally variable habitats restricts functional trait diversity and thus FRic is normally higher in more stable systems such as those in the lower basin (Webb, 1984). Relative to the lower basin, the upper basin's fish assemblages appear to be significantly richer in terms of functional diversity per unit of upstream catchment area.

The high β-diversity and functional turnover longitudinally suggest that different functional strategies characterize fish assemblages in the upper and lower portions of the basin. High functional turnover can occur if unique species from each community are functionally different, indicating niche differentiation between communities (Villéger et al., 2013). In our study, fish assemblages were characterized either by locomotion/resistance functional strategies likely driven by higher slopes and flashier hydrology in the upper basin, or by functional strategies reflecting more trophic diversity in the lower basin. This indicates that the upper basin community is driven more by environmental filtering and ability to deal with abiotic stress, where the lower basin functionality appears to be driven by resource availability. Small-bodied insectivorous species predominated in the upper basin and were replaced by an increase in large-bodied herbivores, detritivores and piscivores in the lower basin showing an increased trophic diversity along the river gradient. This agrees with other studies were lower basin assemblages are driven more by diversity of resources whereas the upper basin assemblages

seem to be more influenced by proximal environmental conditions (Dunning et al., 1992; Datry et al., 2016).

2.6 Conclusion

Whilst our results provide preliminary support for conceptual models such as the River Continuum Concept and the River Habitat Template in this Afrotropical system, we also found unique patterns of functional diversity. High β diversity turnover indicates that functional strategies driving communities are dependent on the position in the basin. At a sub-basin scale however, there is moderate nestedness in communities of the upper basin, and these headwater zones harbour substantial functional diversity, especially when considering upstream catchment size. Improved understanding of other mechanisms driving functional diversity in Afrotropical systems will be important for efforts to conserve freshwater systems, especially in light of increasing development and widespread loss of habitat in the region (van Rooyen et al., 2016). For instance, if the spatial distribution of fishes originates mainly from environmental filtering as is indicated in our study, then approaches to conserve fish diversity should focus on the conservation of habitat heterogeneity at suitable spatial and temporal scales (López-Delgado et al., 2019a). The distinction between the turnover and nestedness resultants is also important, as they require opposing conservation approaches (Wright and Reeves, 1992). Functionally nested communities such as those in the headwaters of our study would allow for the prioritization of a small amount of the functionally richest areas for conservation, whereas communities displaying high turnover resultants such as the lower basin would require conservation strategies that consider larger areas and connectivity (Baselga, 2010).

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2.9 Supplementary material

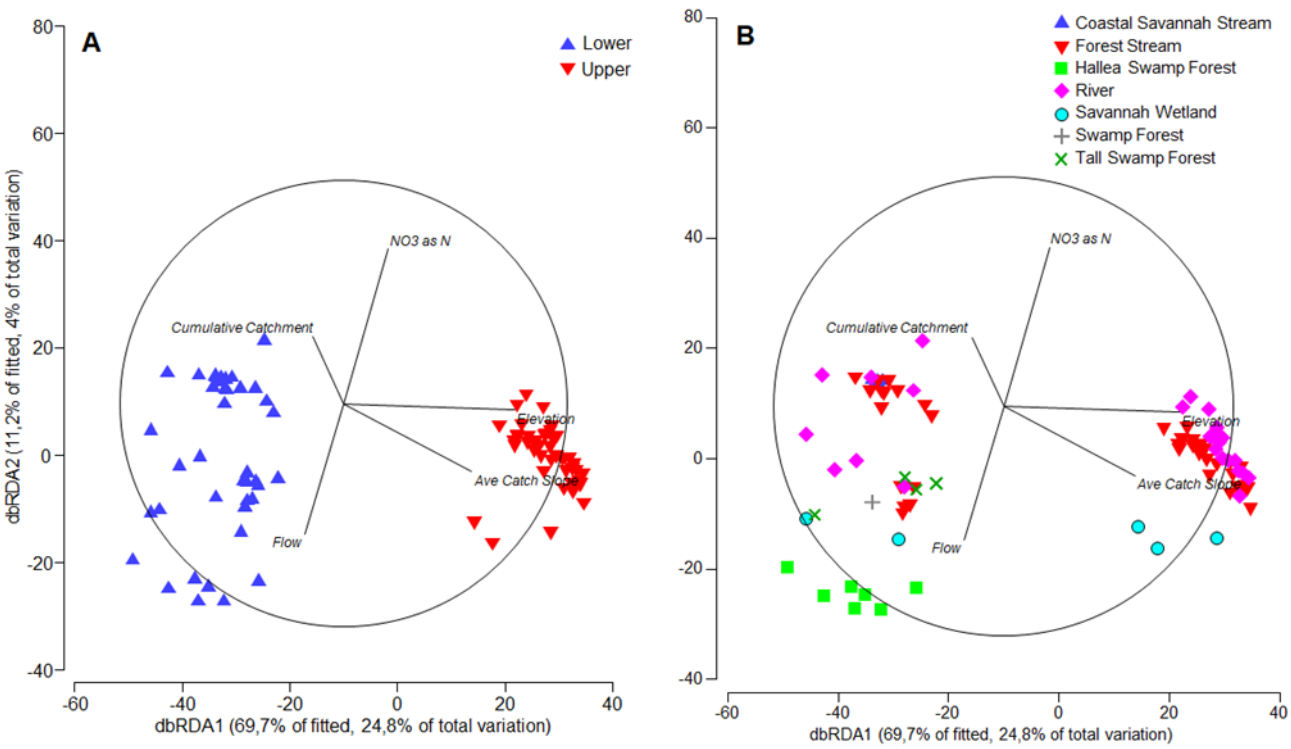

Figure S2: (A) DistLM analysis showing dbRDA of drivers of the fish community (upper and lower basins). The vectors shown are the combination of environmental variables that were the best predictors of fish community structure, and (B) dbRDA showing habitat type as a factor.

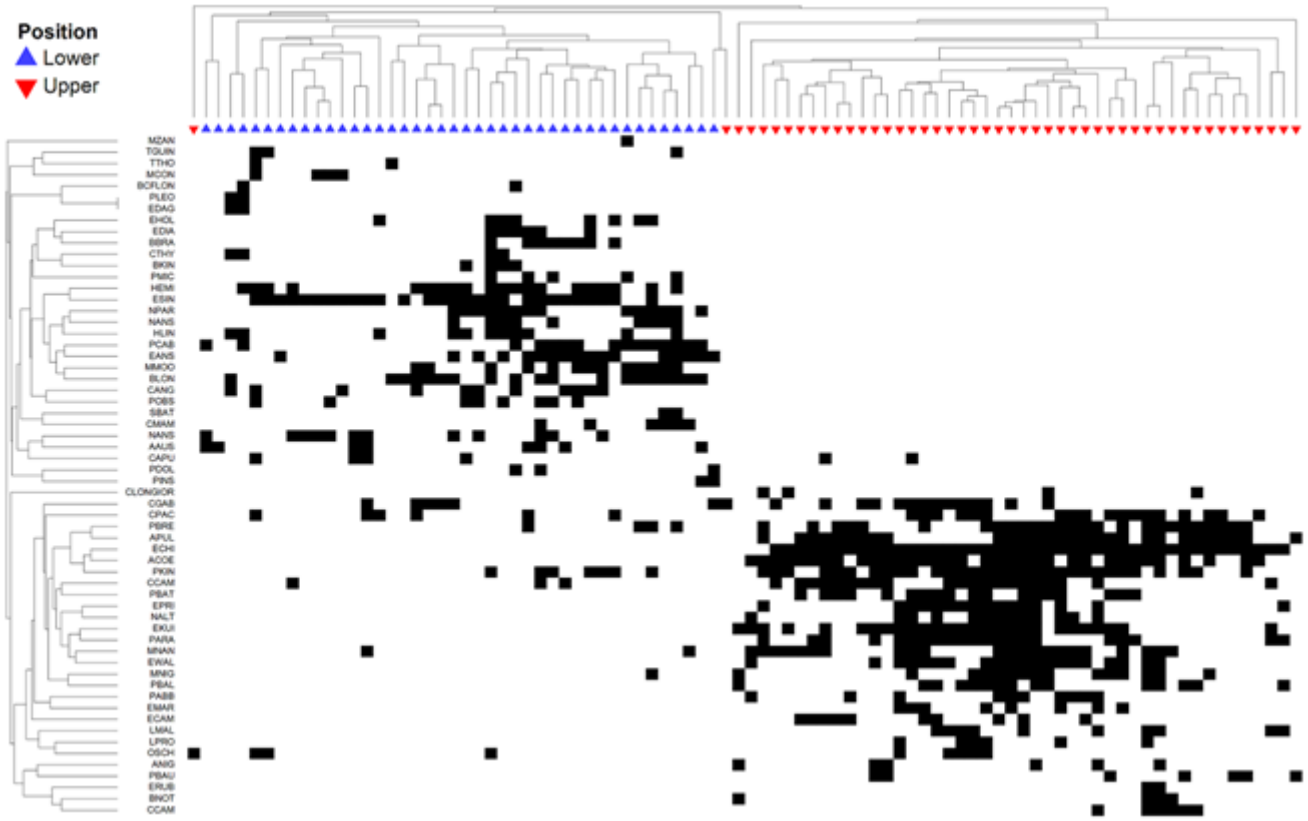

Figure S2: Shade plot showing fish species turnover between upper and lower basins from a multivariate species assemblage using Bray Curtis similarity matrix on presence-absence fish community data for sites.

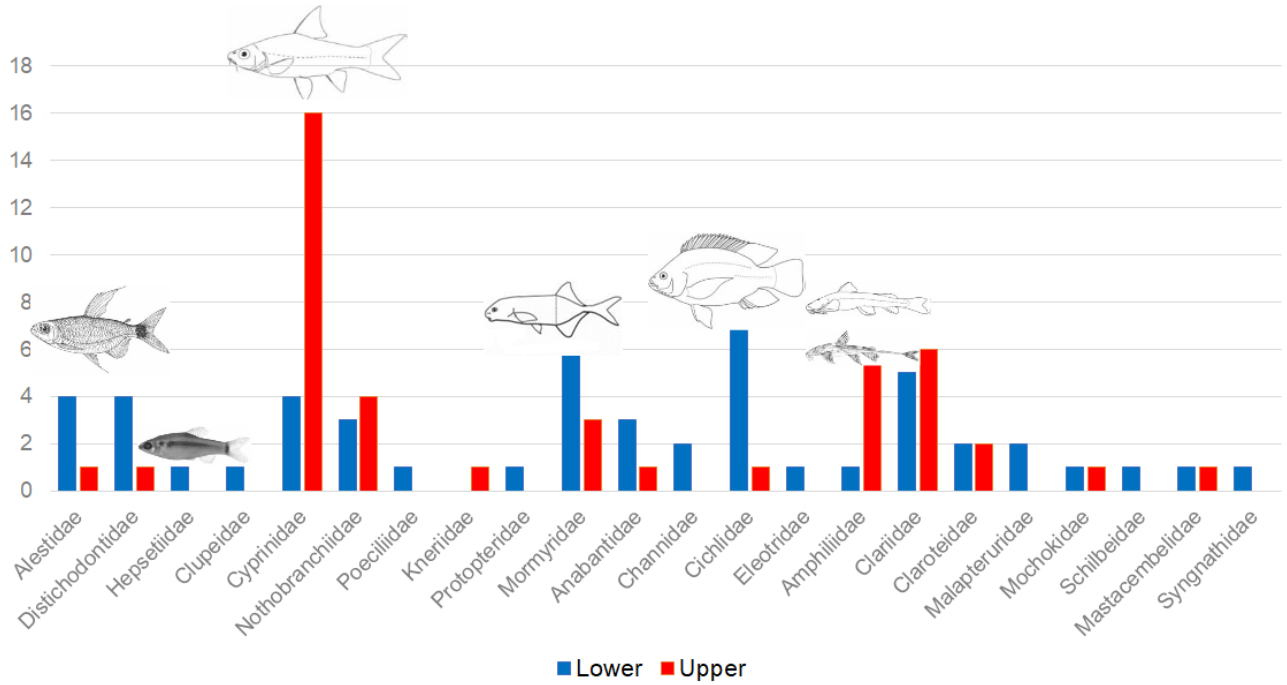

Figure S3: Species richness showing upper and lower basins per family and differences per basin.

Chapter 3: High-elevation rainforest headwaters harbour disproportionately high fish functional richness in the Afrotropics

3.1 Abstract

While tropical and temperate freshwater systems have many similarities, key environmental and biogeographical differences in environment and functionality may limit the applicability of temperate conceptual models to the tropics. Little is known about freshwater community assembly and diversity in the Afrotropics, and as a result region-specific policies and conservation approaches have lagged. Here we use functional diversity metrics and linear mixed-effects models to explore temperate expectations of ecological structuring in a large river system in the Republic of the Congo. Functional traits of fish communities in upper and lower sub-basins were used to show functional diversity gradients in relation to various key physical (catchment area) and environmental (slope and depth) drivers of community assembly allowing us to comment on the applicability of important temperate freshwater concepts related to gradients and environmental filtering. At the basin scale we found support for theories which suggest that environmental filtering is a strong driver of functional assemblage structure in this Afrotropical region and found general conformity to major gradient concepts which predict change in fish functional diversity associated with increasing resources downstream. However, unexpected patterns of functional richness between basins suggests historical Pleistocene forest refugia may have driven increased functional richness in higher elevations of the region. The high functional richness observed in the upper basin of our study suggests that conservation of freshwater diversity within the Lower Guinea ecoregion should consider both contemporary and historical phenomena when prioritising conservation areas, where the inclusion of higher elevation rainforest areas would maximize retention of functional richness in fish communities.

3.2 Introduction

Studies of environmental gradients and tropical freshwater fish communities are strongly skewed towards the Neotropics, with very few analyses undertaken in the Afrotropics. Debate continues as to the relevance of theoretical concepts with regards to freshwater tropical

systems in a wide range of topics including functional ecology, taxonomic diversity, terrestrial subsidies and nutrient inputs (Hoeinghaus et al., 2007b; Chang et al., 2012; Humphries et al., 2014b; Correa and Winemiller, 2018b). A number of ecological studies have explored various drivers in tropical assemblages and shown differences with their temperate counterparts in relation to taxonomic and functional community structure (Wootton and Oemke, 1992; Salas and Dudgeon, 2003; Chessman and Townsend, 2010; Dudgeon et al., 2010), primary and secondary productivity (Ortiz-zayas et al., 2005; Wantzen and Wagner, 2006; Yam and Dudgeon, 2006; Boyero et al., 2009, 2017), food web structure (Dudgeon, 1994; Dudgeon et al., 2010; Horn et al., 2011; Correa and Winemiller, 2014), organic matter processing (Covich, 1988), and nutrient dynamics (Roach et al., 2014). Other tropical studies have found support for lotic conceptual models developed in temperate zones (Ibañez et al., 2009b; Pease et al., 2012a; Bower and Winemiller, 2019b) and some have argued that consistent evidence for differences in tropical streams is generally lacking (Boulton et al. 2008).

Ecological processes such as environmental filters have a substantial influence on freshwater communities. Temperate lotic models such as the River Habitat Template model (Townsend and Hildrew, 1994) postulate that habitat provides the template upon which species traits are forged, shaping trait distributions by restricting establishment of organisms based on their trait suitability. The stress-dominance hypothesis posits that elevated environmental stress increases the influence of environmental filtering on community assembly (Weiher and Keddy, 1995; Swenson and Enquist, 2007), suggesting that functional diversity is lowered with increasing environmental stress, and trait variation is predicted to be greater in more stable environments (Weiher and Keddy, 1995; Coyle et al., 2014). In this way, abiotic stressors can behave as environmental filters that affect functional traits of assemblages. Based on the stress-dominance hypothesis, river slope steepness is a corollary factor predicting diversity, which should increase with decreasing slope. Various fish assemblage studies have found support for environmental filtering across different geographic regions and spatial scales

including tropical systems (Lamouroux et al., 2002; Bower and Piller, 2015; Haas et al., 2015; Bower and Winemiller, 2019b, 2019a). Though tropical and temperate systems have shown similarities, important differences in temperature regulation and habitat structure may limit the applicability of temperate models to tropical freshwaters, including the influence of forests on fish assemblages (Lo et al., 2021).

Other concepts related to lotic gradients such as the River Continuum Concept (Vannote et al., 1980) propose that species richness increases from the headwater to the lowlands of rivers where habitat heterogeneity and more diverse basal resources allow numerous different taxa to optimally exploit these conditions thereby increasing functional richness along the gradient. Spatial variability in species richness has also been explained prior to the development of the River Continuum Concept by area-based hypotheses, which propose that species richness increases as a function of surface area through increases in habitat diversity (Macarthur, 1964) and extinction or colonization rates (MacArthur and Wilson, 1967). In contrast to the River Continuum Concept, the Patch Dynamics Concept (Townsend, 1989; Winemiller et al., 2010a) hypothesizes that rivers are not a continuum, but instead consist of noncontinuous patches created by disturbance over time. Consequently, Patch Dynamics suggests that patches are more important for the occurrence of a species and its functional traits than the position of the given site along a continuum (Junk and Wantzen, 2004). Support for the River Continuum has been recorded in fish assemblages in the tropics (Ibañez et al., 2009b; Pease et al., 2012a) whereas other studies have shown disagreement with the concept of increasing fish diversity along the river continuum (Oberdorff et al., 2019).

Some of the differences in functional and species assemblages noted in the tropics have been interpreted as support for historical hypotheses such as the concept of Pleistocene forest refugia (Haffer, 1969). During the Last Glacial Maximum, forests were substantially reduced at all latitudes and many forest-restricted species survived only in localised forest refugia that provided relatively warm and moist climatic conditions (Hewitt, 2000). The hypothesis posits

that climatic changes caused rainforests to contract to isolated refugia separated by dry forests and savanna, and that this isolation promoted increased speciation. Buffering of climate change by cloud cover in these higher elevations is thought to have allowed vegetation to remain moist during dry periods when the forest contracted in the lowlands during the last glacial maximum, thereby creating a refuge for regional biodiversity (Maley, 1996). While the refugia hypothesis has been widely refuted in the Neotropics, there is good evidence of historical fragmentation in Lower Guinean central African rainforests (Hostetler and Mix, 1999; Born et al., 2011). As fish are physically constrained by their habitat, past events leave a discernible imprint on present day species diversity which have shown patterns of increased species richness in catchments with contact to Last Glacial Maximum rainforest refugia (Tedesco et al., 2005).

Given the potential influence of historical and contemporary factors on freshwaters (Haffer, 1969; Vannote et al., 1980; Townsend and Hildrew, 1994) it is possible to derive expectations of functional structure of fish assemblages along a gradient. We would expect that fish assemblages in more stressful upper basin headwaters where only a few resilient species occur result in decreased functional richness and show a higher signal of environmental filtering (Rahel and Hubert, 1991). Conversely, increased functional diversity would be expected in lowland streams where more resources and habitat stability prevail (Carvalho and Tejerina-Garro, 2015, 2018).

Functional diversity of freshwater fish communities offers an excellent model for studying community assemblage patterns at various scales (Winemiller, 1991) and has been increasingly used to understand the applicability of lotic theory to tropical community assembly (Bower & Winemiller, 2019b; Mouillot et al., 2007; Pease et al., 2012; Poff, 1997; Walsh et al., in press). Freshwater fish assemblages have been used to explore expectations in relation to environmental gradients (Ibanez et al., 2007; Pease et al., 2012a; Oberdorff et al., 2019), habitat structure (Hoeinghaus et al., 2007b; Teresa and Casatti, 2012) and environmental

filtering (Mouchet et al., 2013; Carvalho and Tejerina-Garro, 2015; Bower and Winemiller, 2019a). Whilst Ibanez et al. (2007) explored fish species assemblages along a gradient in Gabon, to our knowledge functional diversity of freshwater fish assemblages in west Central Africa has not been used to test these temperate-based assumptions at basin scale.

Here, we use functional diversity metrics of freshwater fish assemblages collected along an environmental gradient in the Republic of Congo to explore whether Afrotropical systems in this landscape meet assumptions of temperate lotic theory. Firstly, we anticipate an increase in functional richness from the upper- to lower basin along the fluvial gradient based on assumptions of the River Continuum Concept. Secondly, based on the stress-dominance hypothesis and the River Habitat Template we expect lower functional diversity and evenness with more stressful environments such as those on steeper slopes, with increased stochasticity and habitat diversity constraints, which would lend support to the concept of environmental filtering. Conversely, we hypothesize a positive relationship between functional diversity and niche space evenness, with increased habitat volume and hence increased stability.

3.3 Methods

3.3.1 Study region

Freshwater habitat and fish assemblages were surveyed in the upper Kouilou-Niari River (the Louessé and Mandoro rivers) and the coastal plains of the Kouilou, Loémé and Noumbi basins in the Republic of Congo (**Figure 3-1**). The upper basin study area originates in the Chaillu Massif which consists of incised geological formations with steep hills and mountains covered by various rare forest types (**Figure 3-1A**). The upper basin is situated at relatively high elevations in the region at approximately 700 m above sea level. It is thought that during the last Pleistocene glaciation, the Chaillu Massif was of major importance as a forest species refuge (Elenga et al., 2004; Born et al., 2011; van Rooyen et al., 2019). The upper basin lotic

habitat comprises of forest streams on steep slopes and rivers with forest fringes on moderate slopes. The lower basin study area (**Figure 3-1B**) is situated in the coastal plain and is traversed by a network of drainage channels flowing into larger rivers and natural lakes on gentle slopes covered by a mosaic of forest and grassland-savannah with a high freshwater habitat heterogeneity (Walsh et al., 2014, 2022a; van Rooyen et al., 2016). The lower basin study area does not fall within a postulated forest refuge.

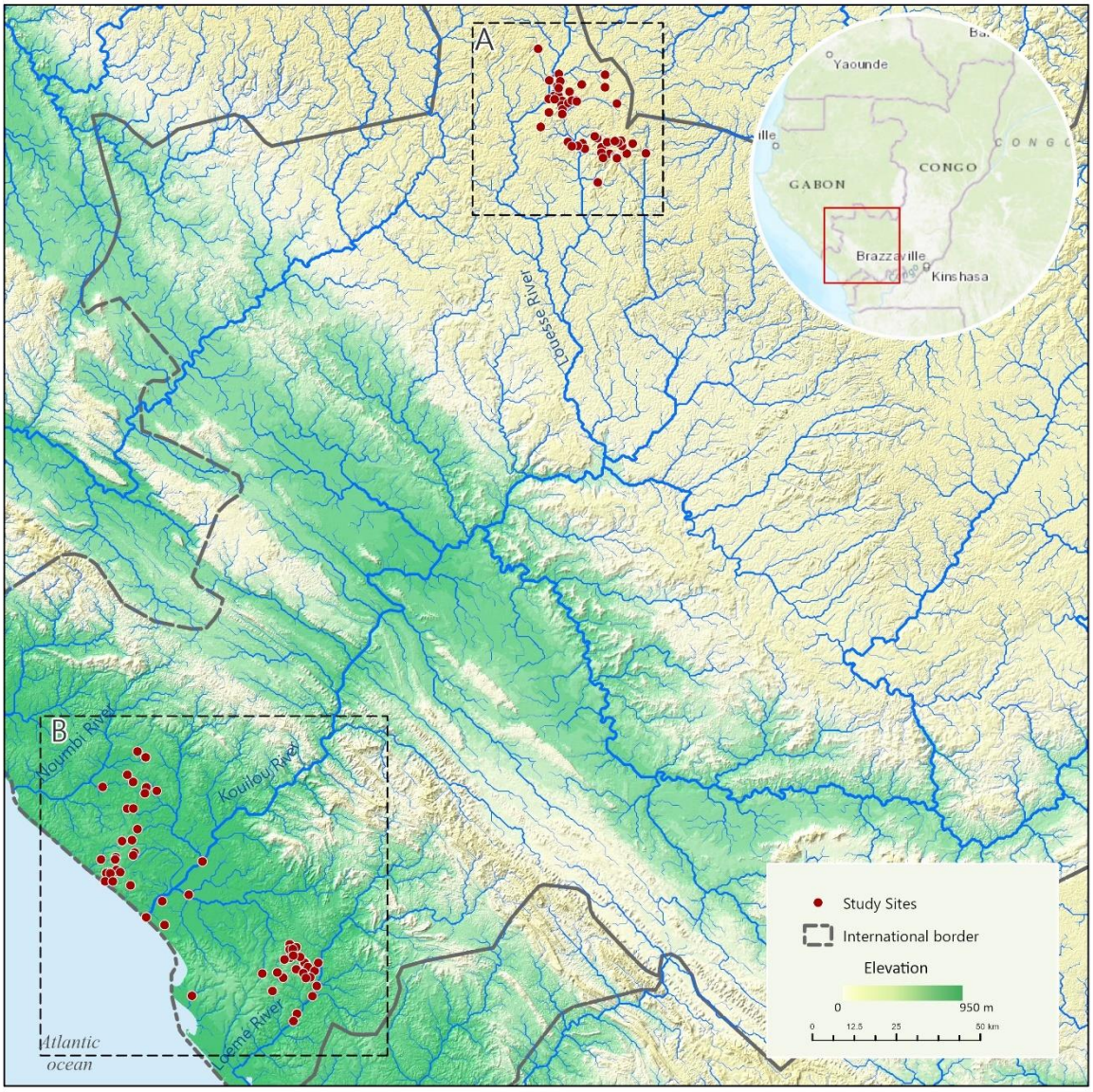

Figure 3-1: (A) Upper and (B) lower basin study areas in the Louessé, Kouilou, Loémé and Noumbi basins in the Republic of Congo showing major drainages and elevations. The highest point is approximately 750 meters above sea level.

3.3.2 Field data collection

Environmental data and fish assemblages were collected under base-flow conditions in 2012, 2013 and 2014 in the Kouilou Niari and adjacent coastal basins. Data were collected at 45 sites in the upper and 37 sites in the lower basin including a range of stream and river sizes. The inclusion of upper and lower basin localities allowed for the comparison of functional diversity relationships along an Afrotropical fluvial gradient.

A stream length of 200-400 m was sampled to representatively include all habitat units at each site and maximise species capture. Fishes were collected in a nested design from all units within each microhabitat unit in the sampled reach within basin position (Smith et al., 2013). Fish sampling methods applied were dependent on the available habitat and included seine hauls, cast netting, dip-netting, gill netting, fyke netting and electrofishing with a single pass moving in an upstream direction. We assume that standardisation of survey methods and the nested study design allow for the comparison of functional data between basin positions. Specimens were collected according to the guidelines for the use of fishes in research (AFS/AIFRB/ASIH, 2004) where individuals were euthanized with anaesthetic and preserved in 10% buffered formaldehyde. Fishes were deposited in the ichthyology collections of the American Museum of Natural History in New York, USA, with associated data accessible at https:/emu-prod.amnh.org/db/emuwebamnh/index.php.

3.3.3 Landscape and abiotic predictors

Landscape and local abiotic/environmental data collected for all sites included cumulative upstream catchment (km^2), distance from source of river (km), site slope (%), width (m), depth (m) and elevation (m above sea level) (**Table 3-1**). Slope was measured using a clinometer and depth and width were measured using a tape measure over 3 transects in a site reach and a sonar depth sounder (Vexilar LPS-1) to measure the thalweg at each site. The Spatial Analyst Hydrology package for ArcGIS 10.5 was used to produce river networks, watersheds

and Strahler orders using the hydrology toolbox. Digital elevation models were used to calculate flow accumulation and flow direction to define streams networks and boundaries of upstream watershed and the distance from source for each site.

Table 3-1: Landscape and local scale abiotic variables measured at sites in the lower and upper basin

Category & Variable	Unit/Measure
Landscape scale	
Latitude	decimal degrees
Longitude	decimal degrees
Stream Order	Strahler Order
Distance from source	Kilometres (km)
Cumulative upstream basin area	Square Kilometres (km^2)
Local scale	
Elevation	meters above sea level (m.a.s.l)
Site Slope	Measured percentage slope in site reach with clinometer
Width	Average width (meters)
Depth	Average depth along Thalweg (meters)

3.3.4 Morphometric traits

Twenty-three functional traits were measured from fish specimens (**Table 3-2**). The traits selected show relationships with habitat-use, locomotion, life history strategy, and feeding (Winemiller, 1991b; Winemiller and Rose, 1992; Sibbing and Nagelkerke, 2000; Pease et al., 2012a). Five individuals of common species and one to three individuals of uncommon species were measured on a Nikon SMZ800 microscope camera with NIS-Elements D computer. Some morphometrics could not be measured accurately using the computer software and therefore head depth, body depth and oral gape were measured using callipers taken to the nearest 0.1mm (Winemiller, 1991b; Winemiller and Rose, 1992). To reduce ontogenetic and sexual dimorphic biases, only adults of all species and females of sexually dimorphic species were included in the analysis. Prior to functional trait analyses, ANOVAs were performed on trait variance data to confirm that interspecific variation was greater than intraspecific variation ($P < 0.05$).

Table 3-2: Functional traits used for upper and lower basin fish assemblages with description of measurement from Pease et al. (2012). Trait definitions and functional categories follow Gatz, 1979; Webb, 1984; Winemiller, 1991; Winemiller & Rose, 1992; Sibbing & Nagelkerke, 2001

Trait	Description	Functional Category
Standard length	Length measured from the tip of the snout to caudal flexion	Habitat use; Life history strategy
Head length	Distance from the tip of the jaw to the posterior edge of the opercule	Feeding
Head depth	Vertical distance from dorsum to ventrum passing through the pupil	Feeding
Oral gape	Vertical distance measured inside of fully open mouth at tallest point	Feeding
Mouth position	The angle between the line connecting the tips of the open jaws running between the centre of the pupil and the posterior-most vertebra	Feeding
Eye position	Vertical distance from the centre of the pupil to the ventrum	Habitat; Feeding
Eye diameter	Horizontal distance from eye margin to eye margin	Habitat; Feeding
Snout length	Distance from the pupil to the tip of the upper jaw with mouth shut	Habitat
Snout protrusion	Additional distance from the pupil to the tip of the upper jaw with mouth fully open and extended	Feeding
Body depth	Maximum vertical distance from dorsum to ventrum	Habitat; Feeding
Body width	Maximum horizontal distance from side to side	Habitat
Caudal peduncle length	Distance from the posterior proximal margin of the anal fin to the caudal margin of caudal fin origin	Habitat
Caudal peduncle depth	Minimum vertical distance from dorsum to ventrum of caudal peduncle	Habitat
Caudal peduncle width	Horizontal width of the caudal peduncle at mid-length	Habitat
Body depth below midline	Vertical distance from midline to ventrum	Habitat
Dorsal fin length	Distance from the anterior proximal margin to the posterior proximal margin of the dorsal fin	Habitat
Dorsal fin height	Maximum distance from the proximal to distal margin of the dorsal fin (excluding filaments)	Habitat
Anal fin length	Distance from the anterior proximal margin to the posterior proximal margin of the anal fin	Habitat
Anal fin height	Maximum distance from proximal to distal margin of the anal fin	Habitat
Caudal fin depth	Maximum vertical distance across the fully spread caudal fin	Habitat
Caudal fin length	Maximum distance from proximal to distal margin of the caudal fin (excluding filaments)	Habitat
Pectoral fin length	Maximum distance from proximal to distal margin of pectoral fin	Habitat
Pelvic fin length	Maximum distance from the proximal to distal margin of the pelvic fin	Habitat

3.3.5 Statistical methods

3.3.5.1 Functional diversity indices

Functional trait data (**Table 3-2**) were used to calculate functional measures for each fish community in the upper and lower basins to determine trait diversity metrics, namely functional richness, functional evenness and functional divergence (FRic, FEve and FDiv – Villéger et al., 2008), functional dispersion (FDis - Laliberte & Legendre, 2010) and Rao's quadratic entropy RaoQ - (Rao, 1982). Analyses were performed in R studio v 4.1.3 (RStudio Team, 2021), using the FD (Laliberte & Legendre, 2010) and fundiv packages (Bartomeus, 2013) in the R environment. We spilt the analysis between upper and lower basins due the high turnover in the fish assemblages and the change in elevational gradient.

The FRic metric conveys the volume of trait space occupied by a set of species and is calculated as the convex hull volume (Villéger et al., 2008). FRic is often positively correlated with species richness and therefore is not fully informative for species distribution within the functional space as it primarily considers the species with the most extreme traits in the assemblage (Villéger et al., 2008). Functional metrics such as FDis, FEve and FDiv which complement FRic are required to provide a more thorough description of the functional diversity patterns of species assemblages in a functional space. The FDis metric defines the mean distance in trait space of a species within a community to the centroid and is not affected by species richness (Laliberte and Legendre, 2010), and high FDis normally contain assemblages with diverse functional trait combinations. FEve measures the uniformity of the distribution of species abundances and dissimilarities in functional space, and only applies to the distribution of abundance in a niche space (Mason et al., 2005a). Functional evenness represents the degree to which the biomass of a community is distributed in niche space to effectively utilise the full range of resources available. Lower FEve values indicate that some

parts of niche space are under-utilised, which will decrease productivity and reliability, and increase opportunity for invaders (Mason et al., 2005).

The FDiv metric indicates a species divergence from the mean distance to the centre of gravity weighted by relative abundance within a multidimensional trait space and tends to the maximum when a single species dominates in abundance and therefore traits (Villéger et al., 2008; Kondratyeva et al., 2019). High FDiv indicates a high degree of niche separation, and thus low resource competition. Communities with high FDiv values may have higher ecosystem function due to more efficient resource use (Mason et al., 2013).

Rao's Q defines the mean functional distance between two randomly chosen species in a community (Lamothe et al., 2018). In this study RaoQ was significantly correlated with FDis (R^2 = 0.895; P <0.001) and therefore we excluded it from further analysis of functional trends. We performed a linear regression to understand the relationship between species richness and FRic along the upper, lower, and combined basins. Functional measures were used as response variables, while species richness was used as the predictor variable.

To test our expectations, linear mixed-effects models (LMM) were fitted to each functional trait index (FRic, FDis, FEve and FDiv) based on maximum likelihood. Each model contained continuous fixed factors to test assumptions of lotic models (cumulative upstream catchment, site slope, width, depth, elevation) and a categorical fixed factor: “position in basin”, with two levels (lower and upper). Habitat type was included in these models as a random factor; due to once off sampling of sites at a single reach in the dataset we could not nest “reach” into the model. This, however, allows the model to account for variability in the data caused by potential habitat filtering. Cumulative catchment, width and elevation were log transformed to standardise the variable scales. Functional richness was log transformed as it was not normally distributed.

For each functional index, a full model was fitted containing all fixed factors without interaction terms. Non-significant terms were removed stepwise from the model using the drop1 function in the R package car (Fox and Weisberg, 2019). Model selection was assessed using the Multi-Model Inference (MuMIn) with Akaike Information Criterion corrected for small sample sizes (AICc), where the AIC is adjusted for sample size and k is held at two (Barton, 2022). The final models were checked for residual distribution using QQ plots. All analyses were performed in an R environment Click or tap here to enter text. using the package nlme (Pinheiro & Bates, 20222).

3.4 Results

Linear regressions indicated that species richness was positively correlated with FRic in the upper, lower, and combined basins (**Figure 3-2 -** upper basin $R^2 = 0.768$, P = <0.001; lower basin adjusted $R^2 = 0.575$, P = <0.001; combined basin adjusted $R^2 = 0.657$, P = <0.001). The positive linear relationship between species richness and FRic indicate that supplementary species contribute proportionately to FRic at different spatial scales, and we therefore expect FRic to increase with species richness in each basin and in the entire study area.

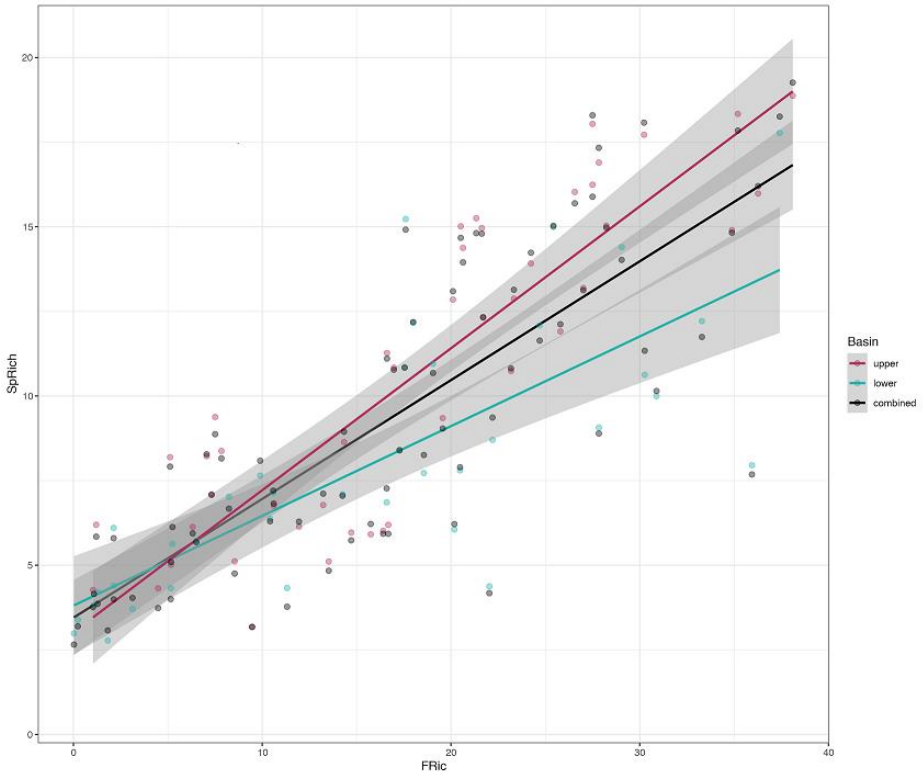

Figure 3-2: Linear regression showing the relationship between fish species richness and fish functional richness per site in the (A) upper and lower basins combined R2 = 0.657, slope = 0.351, P = <0.001; (B) upper basin R2 = 0.768, Slope = 0.420, P = <0.001, (C) lower basin R2 = 0.575, Slope = 0.265, P = <0.001. The grey shaded areas represents 95% confidence intervals.

Cumulative catchment size, slope and depth were significantly correlated with functional diversity metrics in the LMM analyses. At the landscape scale, FRic was positively correlated with cumulative upstream catchment size (b = 0.12, t (74) = 2.21, p <0.05 – **Figure 3-3A**), indicating an increased volume of functional trait space occupied by species as upstream catchment size increases (**Figure 3-3A; Table 3-3**). FRic was also significantly different between basin positions where sites in the upper basin had an average FRic value 1.85 times higher than in the lower basin (b =0.10, t (74) = 2.35, p <0.05) in relation to upstream catchment size. Cumulative catchment size was correlated with distance from the source (R^2 = 0.723, p <0.0001), and therefore we assume the same positive relationship for FRic and distance from source.

Table 3-3: Final LMM model terms and significant predictors for functional indices. Asterisk denotes significant values (p < 0.05)

Predictors	*Value*	*SE*	*t-value*	*p*
			FRic	
(Intercept)	1.17	0.49	2.36	**0.021***
Position in Basin	0.626	0.26	2.35	**0.021***
log (Cumulative Catchment)	0.129	0.058	2.21	**0.032***
AIC				264.5
Log Likelihood				-127.25
			FDis	
(Intercept)	2.44	0.217	11.21	**0.001***
Site Slope	-0.098	0.537	-1.84	**0.047***
Depth	0.159	0.066	2.397	**0.019***
AIC				240.9
Log Likelihood				-115.498
N_{Site}				82
			FEve	
(Intercept)	0.566	0.034	16.32	**0.001***
Site Slope	-0.023	0.008	-2.859	**0.005***
Depth	0.026	0.011	2.1841	**0.014***
AIC				-67.99
Log Likelihood				39.274
N_{Site}				82

A positive relationship was noted for FDis which increased significantly with depth of habitat (b = 0.15, t (74) = 2.39, p <0.05) but also had a significant negative trend with site slope (b = -0.09, t (74) = -1.84, p <0.05 -**Figure 3-3B & C; Table 3-3**). The positive relationship of FDis

and depth infers more diverse species trait combinations in deeper systems such as those in the lower basin, and less diverse combinations in shallow habitats on steeper slopes in the upper basin. Because RaoQ was significantly correlated with FDis (R^2 = 0.895; P <0.001), we assume comparable patterns for RaoQ.

Similar patterns were recorded for FEve with significant increases in evenness noted with increased water depth (b=0.02, t(74) = 2.51, p<0.01), decreasing significantly with slope (b= -0.02, t(74) = -2.85, p<0.01 – **Figure 3-4A & B; Table 3-3**). Increased depths are thus associated with uniformity in the distribution of abundances of species with a range of traits, and decreased uniformity in distribution for steeper slopes. There were no significant relationships for FDiv with any of the environmental variables tested in the LMM analyses.

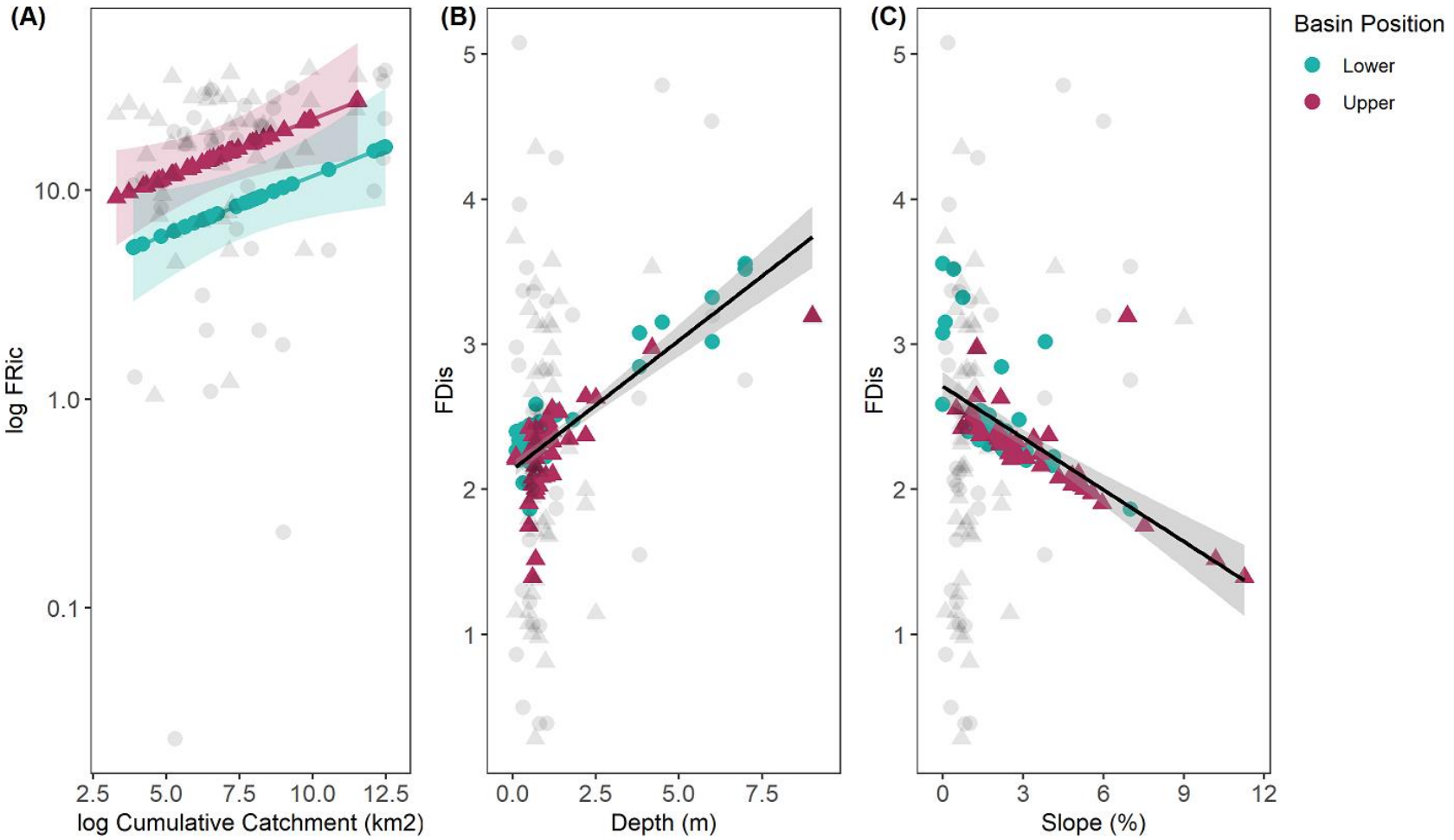

Figure 3-3: Linear mixed-effects models (LMM) of predicted (red and blue points) and observed (grey points) data showing significant ($p < 0.05$) relationships between gradient variables (log cumulative catchment size, slope and depth) and functional metrics (log FRic and FDis)

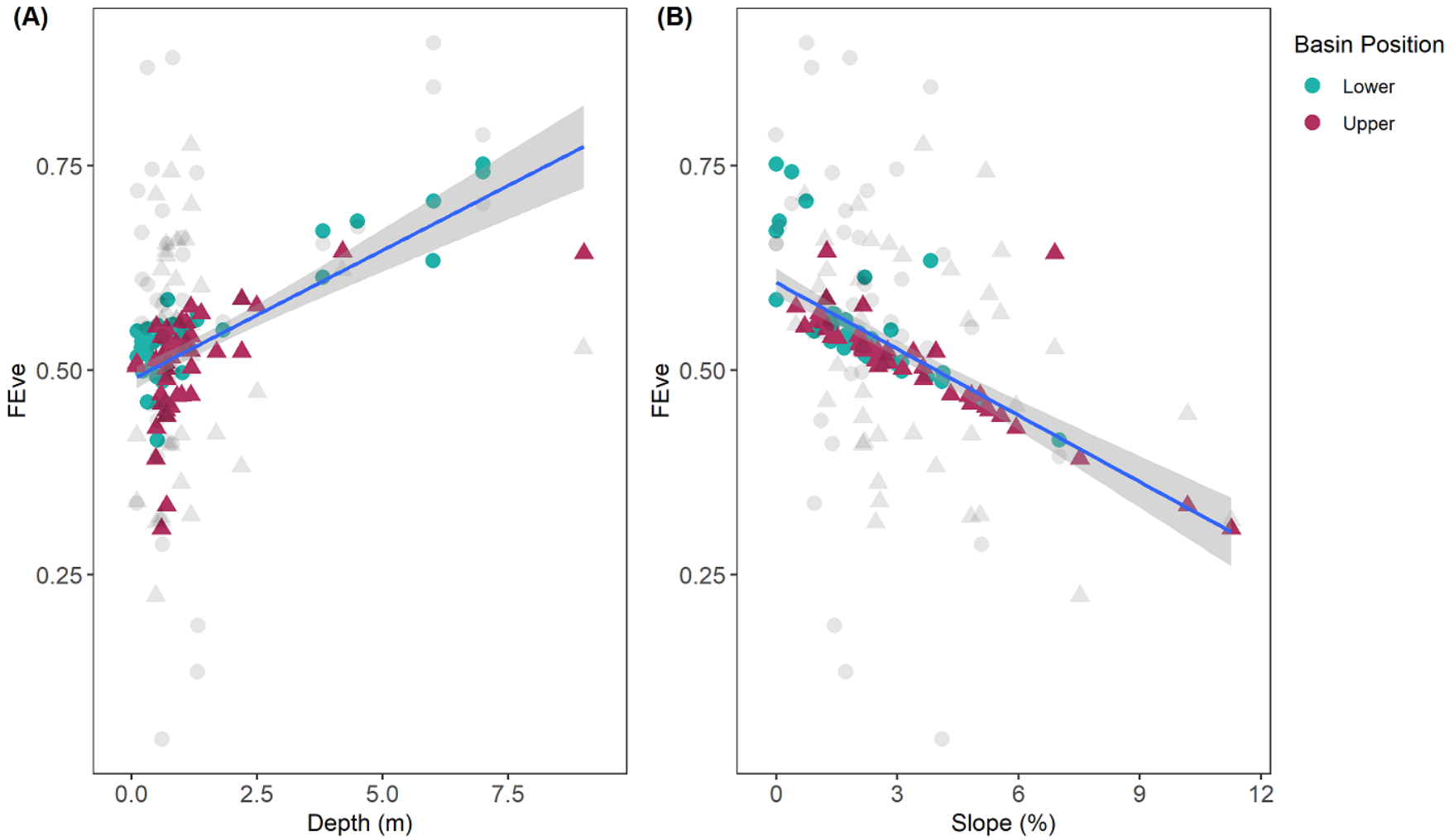

Figure 3-4: Linear mixed-effects models (LMM) of predicted (red and blue points) and observed (grey points) data showing significant ($p < 0.05$) relationships between physical variables (slope, depth) and FDis.

3.5 Discussion

3.5.1 Functional richness vs. species richness

We found partial conformity to our first expectation that functional richness increases from the upper to lower basin along a fluvial gradient in this Central African system. We observed positive functional richness and species richness relationships at the sub-basin scale, a pattern that has been shown in fish assemblages at various scales across biogeographical realms (Winemiller, 1991a; Hoagstrom and Berry, 2008; Oberdorff et al., 2011; Pease et al., 2012a). The relatively stronger positive relationship between FRic and species richness in the upper basin was, however, unexpected as we assumed that FRic would be more constrained in more stressful environments resulting in a lower number of functional traits in the assemblage (Weiher and Keddy, 1995).

3.5.2 Functional richness-area relationship

Diversity of functional traits is expected to increase along a fluvial gradient according to the River Habitat Template concept, as streams become more heterogeneous and hydrologically stable (Townsend and Hildrew, 1994). Fish community richness is consequently expected to increase with catchment area according to the species–area theory which has been shown to be strongly correlated with species richness (Hugueny, 1989; Oberdorff et al., 1995). The sub-basin scale FRic and upstream catchment area relationship shows agreement with the River Habitat Template and also supports expectations of the River Continuum Concept (Vannote et al., 1980) which posits that functional diversity increases in lower reaches as growing diversity of resources promote increased trophic diversity in assemblages (Thorp et al., 2006). Patterns of increasing richness in relation to upstream catchment size have been recorded in previous studies in the tropics (Hugueny, 1989; Ibanez et al., 2007; Pease et al., 2012; Tedesco et al., 2005).

Unexpected patterns for FRic were however noted between sub-basins, where FRic values in the upper basin were significantly higher than the lower basin when considering upstream catchment size. This is unusual, as we would expect to see an overall increase in richness from the upper to lower sub-basins (Matthews, 1998; Thorp et al., 2006; Ibañez et al., 2009b). Increases in longitudinal species richness along fluvial gradients have been ascribed to higher extinction and lower colonisation rates in upper sections of river networks, and to lower extinction rates in lower sections due to increased habitat diversity, stability, productivity and connectivity (Hitt and Roberts, 2012). Though increased richness may be driven by greater habitat heterogeneity, temporal stability may allow higher FRic because of the consistency of resource availability over an extended period of time (Tedesco et al., 2012).

In an intercontinental study on fish species richness, Tedesco et al. (2005) found that freshwater fish species richness was highest in drainages that were connected to rainforest refuges. Both river basin area and contact with Last Glacial Maximum rain forest refugia explained the greatest proportions of variance in the geographical pattern of riverine species richness (Oberdorff et al., 2011). Tedesco et al. (2005) showed that historical events give a strong signal in relation to species richness independent of contemporary factors. Similar atypical patterns of richness to those seen in our study were recorded at a larger scale in the Neotropics, where a linear decrease of species richness was observed along the gradient of a large basin (Oberdorff et al., 2019). This phenomenon was partially explained by paleogeographic history where the upstream portion of the basin experienced a more stable climate during the Last Glacial Maximum in comparison to drier conditions in the lowlands which caused forest contraction. The genetic structure of the forest-restricted tree *Aucoumea klaineana* in Lower Guinean forests provided evidence that the Chaillu Massif was a postulated rainforest refugia (Born et al., 2011). The climatic history of the upper basin suggest that it was likely a Pleistocene refugium, and the atypical patterns of functional richness observed in this study could be explained by paleo-biogeographic factors.

However, the relatively high FRic in the upper basin could also be related to contemporary factors such as ecological and climatic gradient conditions in tropical mountain streams, which drive increased ecotonal speciation (Smith & Kraft, 2005). Because forested habitats have high allochthonous riparian input, they can support increased trophic diversity including specialised and rare species, such as terrestrial-input dependent fish like the hyper-diverse *Aphyosemion* species (Zeni and Casatti, 2014; Zeni et al., 2019). The FRIc pattern observed at sub-basin scale appears to be an uncommon trend in literature, and does not agree with widely held theories. This observation may lend support to historical theories of Afrotropical rainforest refugia in shaping species and functional diversity.

3.5.3 Functional diversity and slope

Confirming our expectations, we found decreased functional dispersion and evenness at sites with steeper slopes providing support for the stress-dominance hypothesis and the River Habitat Template frameworks (Townsend and Hildrew, 1994; Weiher and Keddy, 1995). Increased slope in fluvial systems affects instream communities by increasing water velocity and shaping geomorphology (Gorman et al., 2011; Caetano et al., 2021), thus limiting functional diversity by excluding species with unsuitable traits (decreasing functional dispersion) resulting in fish assemblages with high trait similarity and low functional evenness (Weiher and Keddy, 1995; Camana et al., 2016). Low functional dispersion and evenness values are indicative of assemblages with a low diversity of functional trait combinations and a limited niche space (Mason et al., 2005a; Laliberte and Legendre, 2010). Various studies have shown a negative relationship between different functional diversity metrics and increased velocity, having a strong influence on fish traits across spatial scales (Bower & Piller, 2015; Bower & Winemiller, 2019b; Caetano et al., 2021; Lujan & Conway, 2015). In an intercontinental study Bower et al. (2021) found that fish species inhabiting high-velocity habitats with limited microhabitats occupied limited functional space suggesting that water velocity partly selects for a confined distribution of traits globally. The functional diversity-trait

relationship indicates that at a basin scale, slope and water velocity have a strong influence on filtering of species traits.

3.5.4 Functional diversity and depth

Functional dispersion and evenness increased with depth in our study, a result consistent with other studies (Bower & Winemiller, 2019b; Carvalho & Tejerina-Garro, 2015, 2018; Willis et al., 2005). Increased functional dispersion in deeper fluvial environments is thought to be a result of a higher habitat heterogeneity often associated with larger rivers, which usually results in an increase in species trait diversity and subsequent over-dispersion (Willis et al., 2005; Súarez et al., 2007). Increased habitat heterogeneity and niche space associated with deeper environments also support functional trait differentiation and more efficient use of numerous resources which is likely to underlie the higher functional evenness in our study (Mason et al., 2005c; Suárez-Castro et al., 2022). Whilst environmental filtering appears to be a significant ecological process in controlling functional patterns in the upper basin, increased evenness and dispersion in habitats with deeper waters appear to be shaped by more stability and heterogeneity of habitat resulting in more trait variation for increased resource utilisation (Carvalho and Tejerina-Garro, 2015). The associated increase in channel width as depth increases often exposes rivers to an increase in sunlight, providing resources for grazers and algivorous species (Carvalho and Tejerina-Garro, 2018). This increase in trophic resource availability and higher productivity also supports increased functional diversity by the addition of carnivores to fish assemblages due to increased prey size and resource availability (Scharf et al., 2000).

3.6 Conclusion

In summary, we demonstrate support for some widely held theories such as the River Habitat Template and the stress-dominance hypothesis which suggests that environmental filtering is a strong driver of functional assemblage in this Afrotropical region. While we also noted a

general conformity to the River Continuum Concept, we observed surprisingly different patterns of functional richness between basins which suggests the Pleistocene forest refugia of this region may have driven substantial functional richness in higher elevations such as those in the Chaillu Massif. Welcomme (1976) estimates that in Africa the combined total length of low-order rainforest streams equates to the length of approximately half of all watercourses on the continent, making them the largest single freshwater ecosystem. This gives further perspective to the high functional richness recorded in the upper basin of our study and the significance of tropical rainforest mountain streams to the contribution of regional functional diversity. Limited understanding of community assembly and diversity in Afrotropical freshwaters has resulted in a lack of policies and conservation approaches that include riparian areas (Luke et al., 2019a), and attempts to conserve freshwater diversity in the region should thus consider paleoclimatic and consequent biogeographic phenomena and prioritise the inclusion of elevated rainforest areas which would allow for the prioritisation of the functionally richest areas for conservation (Born et al., 2011).

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Chapter 4: Review of the cichlid genus *Thysochromis* (Teleostei: Ovalentaria) with the description of a new species from the Kouilou Province in the Republic of Congo, west–Central Africa

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4.1 Abstract

A new species of the chromidotilapiine genus *Thysochromis*, is described from the Noumbi and Kouilou River drainages in the Republic of Congo. Based on the current investigation, *Thysochromis* is resolved as containing two geographically disjunct species, *T. ansorgii* from localities in the upper Guinean ichthyofaunal province (Ivory Coast, Ghana, Benin and Nigeria) and *Thysochromis emili* sp. nov. restricted to coastal regions of the Republic of Congo in the lower Guinean province.

4.2 Introduction

In a report on the fishes of the lower Kouilou River in the Republic of Congo, Teugels *et al*. (1991) recorded the presence in Lake Koubambi of two specimens (MRAC 90-57-P-5288-89) of the cichlid *Thysochromis ansorgii* (Boulenger 1901). They noted that this record represented a significant range extension for a species that was otherwise known only from the upper Guinean ichthyological province in south-eastern Ivory Coast, south-western Ghana, Benin and Nigeria (**Figure 4-1**). Later, Lamboj and Stiassny (2007) commented on the questionable composition and taxonomy of the genus, originally named *Thysia* by Loiselle and Welcomme (1972) but renamed due to preoccupation as *Thysochromis* by Daget (1988). While the synonymy of *Pelmatochromis* (*Thysochromis*) *arnoldi* Boulenger 1912 with *T. ansorgii* has generally been accepted (Fricke *et al*., 2018; Loiselle & Welcomme, 1972), the status of *Pelmatochromis* (*Thysochromis*) *annectens* Boulenger 1913 has remained uncertain (Froese & Pauly, 2019; Lamboj, 2004), as Loiselle and Welcomme (1972) considered the only differences between *T. annectens* (type locality, lower Niger River) and *T. ansorgii* (type locality, Ethiop River in Niger Basin) were slight variants of colour patterning. Beyond these they were unable to separate the two and concluded that it was best to accept *T. annectens* as a species *inquirenda* (Froese & Pauly, 2019). A fourth species, *Tilapia maculifer* Ahl 1939 (type locality, Lagos, Nigeria) was synonymised with *T. ansorgii* by Daget (1991), but unfortunately the holotype and unique specimen, originally housed in the Zoologisches

Museum Berlin, is lost and unavailable for study (Fricke *et al*., 2018). Pending a more detailed review, Lamboj and Stiassny (2007) accepted the identification of the two Lake Koubambi specimens as *T. ansorgii* thereby recognizing a range extension for the species of well over 1000 km from the upper Guinean ichthyofaunal province into the southern portion of the lower Guinean province.

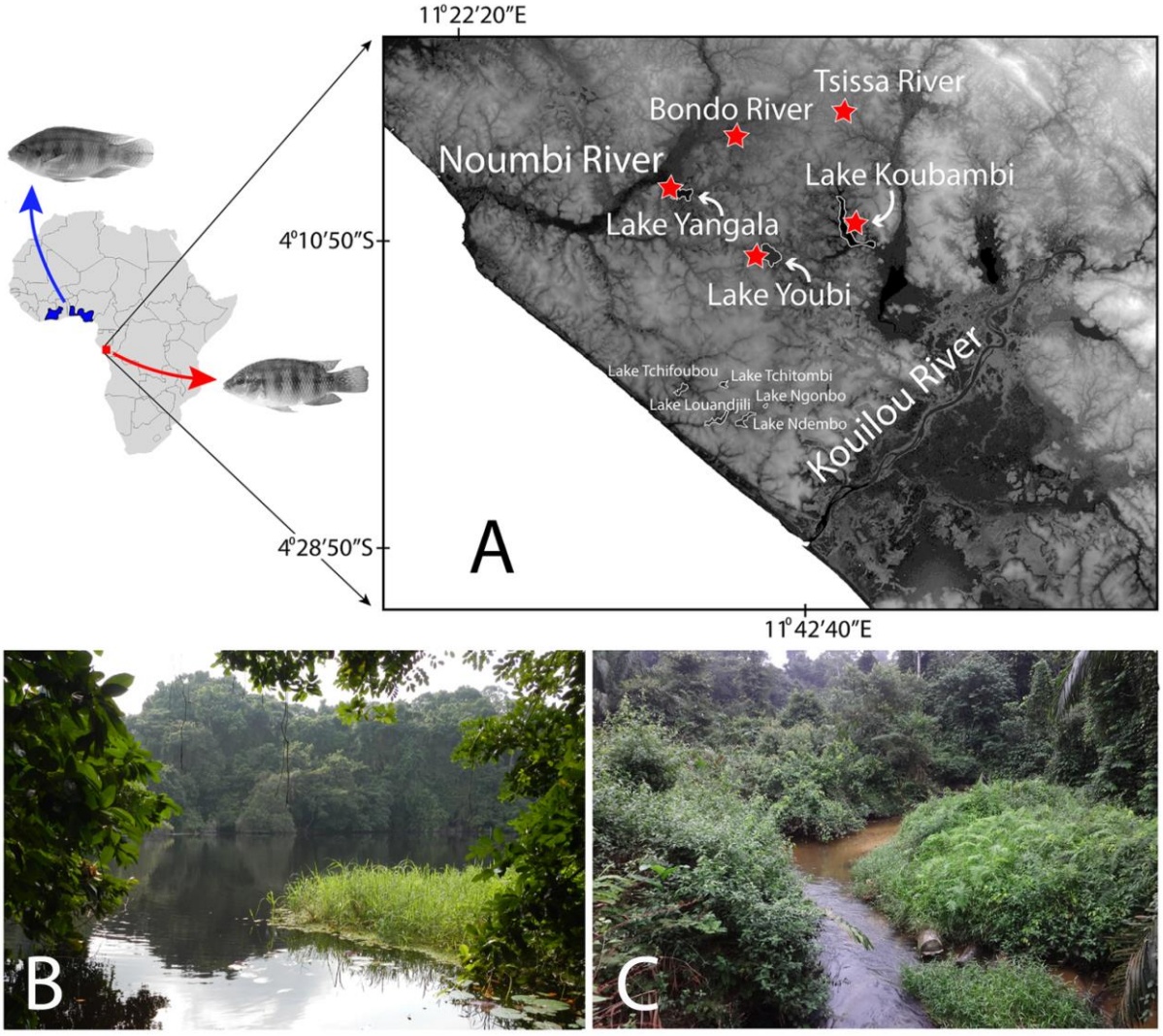

Figure 4-1: Distribution of *Thysochromis* species (*Thysochromis ansorgii* in blue, *Thysochromis emili* sp. nov. in red). (A) Inset map indicating sampled localities, red stars denote localities from which *Thysochromis emili* sp. nov. has been collected. Typical habitat in (B) Lake Youbi, (C) in Tsissa River

Walsh *et al*. (2014) reported on a series of collections made throughout the lower Kouilou, Noumbi and nearby coastal basins where numerous additional samples of *Thysochromis* spp. were recovered from Lakes Youbi and Yangala. In 2017, G.W. collected additional specimens in both black water (Bondo River, Noumbi Basin) and clear water (Tsissa River, Kouilou Basin) rivers in the same region (**Figure 4-1**). Examination of these materials and comparison of the available type materials of the putative West African species, indicates that although phenetically similar, the Republic of Congo specimens are diagnostically distinct from their West African congener and in the current paper we describe the Congolese populations of *Thysochromis* as a new species.

4.3 Methods

Collection permits were issued by the National Institute for Research in Exact and Natural Sciences (IRSEN Permit 205 and 328) and CITES permits for exportation are CG1125774 and CG1125818.

4.3.1 Field sampling

Various lentic (lake and swamp) and lotic (river and stream) habitats were sampled in 2012 and 2017 in the lower Kouilou and Noumbi River basins, as well as in numerous coastal lakes and associated drainages (**Figure 4-1**). Fish sampling effort was standardized according to habitat type and fishes collected according to AFS/AIFRB/ASIH (2003) guidelines. Specimens are deposited in the ichthyology collections of the American Museum of Natural History (AMNH) in New York, USA, with all data accessible online (http://sci-web-001.amnh.org/db/emuwebamnh/).

4.3.2 Morphology

Twenty-five standard morphometric measurements and 14 meristic counts (**Table 4-1 &**

Table 4-2) were recorded following Barel et al. (1977). Vertebral and fin-ray counts were taken from radiographed or cleared and stained (c&s) specimens. Total gill raker counts for the first arch include the raker at the junction of ceratobranchial and epibranchial elements. Lateral line counts exclude any small pored scales distal to the hypural plate.

Table 4-1: Morphometric measurements and meristic data for the holotype and 18 paratypes of *Thysochromis emili* sp. nov

	Holotype	**Paratypes**				
Morphometric measurements		**min**	**max**	**n**	**mean**	**SD**
Standard length (mm)	69.4	36.0	69.4	18		
% SL						
Head length	35.9	34.2	38.9	18	36.8	1.4
Body depth	38.4	38.4	47.8	18	43.4	2.4
Caudal peduncle length	10.9	7.7	11.3	18	9.9	1
Caudal peduncle depth	15.8	15.6	18.5	18	17.4	1
Dorsal-fin base	61.7	58.0	62.7	18	60.4	1.4
Anal-fin base	21.2	18.6	21.3	18	19.4	0.8
Predorsal length	30.1	29.7	34.3	18	31.9	1.5
Preanal length	69.0	69.0	75.8	18	73.6	1.8
Prepectoral length	37.0	36.7	41.0	18	38.8	1.2
Prepelvic length	40.7	40.7	47.0	18	43.6	1.7
Longest dorsal fin-ray	19.4	15.6	26.6	18	19.2	1.8
Longest anal fin-ray	23.2	15.6	23.8	18	24.4	2.2
Longest pectoral fin-ray	23.1	22.6	26.3	18	24.2	2.1
Longest pelvic fin-ray	35.7	29.7	37.8	18	31.5	2
% HL						
Head depth	69.4	65	76.8	18	68.7	3.2
Snout length	31.0	28.8	34.6	18	31.2	1.6
Eye diameter	31.0	27.0	30.1	18	28.6	1.1
Postorbital length	40.0	38.2	41.9	18	40.2	1.1
Interorbital distance	27.6	25.4	33.2	18	29.1	2.3
Cheek depth	28.5	26.2	31.2	18	29.4	1.8
Premaxillary length	28.2	23.1	28.2	18	26.1	1.9
Lower jaw length	33.9	30.2	38.1	18	32.3	2.2
Preorbital length	19.2	17.2	24	18	20.3	1.7
Meristic counts	**Holotype**	**Paratypes**				
Lateral line scales (upper row)	19		17(2), 18(2), 19(9), 20(1)			
Lateral line scales (lower row)	7		6(1), 7(9), 8(2), 9(1)			
Lateral line scales (total)	28		26(2), 27(10), 28(2)			
Circumpeduncular scales	16		15(1), 16(13)			
Dorsal-fin spine	XVI		XV(6), XVI(7), XVII(1)			
Dorsal-fin rays	10		9(2), 10(10), 11(3)			

	Holotype	Paratypes
Anal-fin spine	III	III(14)
Anal-fin rays	9	7(1), 8(12), 9(1)
Pectoral-fin rays	13	13(14)
Gill rakers (lower limb)	9	8(2), 9(8), 10(4)
Total gill rakers	14	14(3), 15(8), 16(2), 17(1)
Abdominal vertebrae	13	13(14)
Caudal vertebrae	13	11(1), 12(110), 13(3)
Total vertebrae	26	24(1), 25(9), 26(4)

Table 4-2: Morphometric measurements and meristic data for the syntypes *Thysochromis ansorgii*, *T. annectens* and *T. arnoldi*

	Syntypes								
	T. ansorgii			***T. annectens***			***T. arnoldi***		
	BMNH 1901.1.28:9–			BMNH 1913.7.15:8–9			BMNH 1912.2.2:10–		
Morphometric	min	max	mean	min	max	mean	min	max	mean
Standard length (mm)	40.7	67.5		63.6	52.8		56.1	65.4	
% SL									
Head length	31.7	33.3	33.1	35.1	37.7	36.4	34.0	35.6	34.9
Body depth	43.1	46.0	44.1	38.2	37.1	37.7	35.8	39.9	37.9
Caudal peduncle length	11.4	12.7	12.3	11.6	13.8	12.7	12.5	13.3	12.8
Caudal peduncle depth	16.8	17.4	17.2	16.1	17.5	16.8	16.1	17.5	15.5
Dorsal-fin base	60.5	64.5	62.0	60.2	60.1	60.2	58.4	63.9	60.9
Anal-fin base	18.6	20.1	18.9	19.4	19.2	19.3	19.9	21.3	20.5
Predorsal length	26.5	29.3	28.7	30.7	31.6	31.2	26.2	31.0	28.7
Preanal length	69	72.1	69.4	69.4	68.2	68.8	65.4	68.6	66.8
Prepectoral length	33.8	38.2	35.9	36.8	38	37.4	34.6	38.3	35.9
Prepelvic length	37.9	40.7	40.2	42.4	42.4	42.4	38.5	40.6	39.5
Longest dorsal fin-ray	17.8	25.9	21.1	23.1	18.6	20.9	26.1	26.6	24.1
Longest anal fin-ray	22.1	23.8	22.7	24.4	20.2	22.3	31.4	28.4	29.8
Longest pectoral fin-ray	22.9	39.1	29.7	27.0	25.6	26.3	24.6	30.7	27.7
Longest pelvic fin-ray	29.4	33.3	31.4	38.8	26.7	32.8	25.0	44.5	33.3
% HL									
Head depth	63.1	71.6	64.0	60.9	65.0	63.0	70.7	78.7	75.5
Snout length	24.2	30.6	27.1	26.5	29.2	27.9	25.4	30.1	28.5
Eye diameter	25.8	33.0	29.5	27.4	29.3	28.4	24.5	28.9	27.0
Postorbital length	42.8	46.7	43.5	43.5	44.2	43.9	41.3	47.2	44.6
Interorbital width	28.5	32.6	30.7	27.6	29.4	28.5	27.1	30.1	28.8
Cheek depth	31.0	33.2	31.8	27.7	30.0	28.6	25.7	33.8	31.0
Premaxillary length	25.3	29.2	27.0	27.2	29.2	28.2	28.9	33.4	30.8
Lower jaw length	28.5	34.1	30.7	29.9	31.3	30.6	33.1	34.4	33.8
Preorbital length	18.4	19.6	18.9	16.9	17.2	17.1	17.0	18.8	17.8
Meristic counts									

	Syntypes		
	T. ansorgii BMNH 1901.1.28:9–	***T. annectens*** BMNH 1913.7.15:8–9	***T. arnoldi*** BMNH 1912.2.2:10–
Lateral line scales (upper row)	18(1), 19(2)	18(1), 19(1)	19(3)
Lateral line scales (lower row)	6(1), 7(3)	7(1), 8(1)	7(2), 8(1)
Lateral line scales (total)	26(1), 27(1), 28 (1)	26(1), 27(1)	27(1), 28(2)
Circumpeduncular scales	16(4)	16(2)	16(3)
Dorsal-fin spines	XV(2), XVI(2)	XV(2)	XV(1), XVI(2)
Dorsal-fin rays	10(3), 11(1)	9(1), 10(1)	9(1), 10(2)
Anal-fin spines	III(4)	III(2)	III(3)
Anal-fin rays	7(1), 8(3)	7(1), 8(1)	8(1), 9(2)
Pectoral-fin rays	12 (2), 13(2)	13(2)	13(3)
Gill rakers (lower limb)	9(3), 10(1)	10(2)	9(3)
Total gill rakers	13(3), 14(1)	15(1), 16(1)	12(1), 13(2)

For detailed anatomical investigation two specimens (*T. ansorgii*, AMNH 235838 and *T. emili* sp. nov., AMNH 258135) were scanned using a Phoenix v/tome/x M scanner (GE Measurements and Control; GR Digital; www.ge.com), processed using datos/x software 2.3 and segmented and visualized using VG StudioMax 3.3 (Volume Graphics; www.heidelberg.com). Institutional abbreviations follow Sabaj (2016), L_S is standard length and L_H, head length.

The smatr package for (standardized) major axis estimation and testing routines (Warton et al., 2012) was used in RStudio (www.rstudio.com) to calculate allometric coefficients for morphometric data (Mipounga et al., 2019; Sidlauskas et al., 2011). Allometric analyses were factorized using two groups to simultaneously test morphometric similarity between the upper Guinean type materials and dissimilarity between those and lower Guinean specimens. Factor groupings included (1) *Thysochromis emili* sp. nov. and (2) the type series of *T. ansorgii*, *T. annectens* and *T. arnoldi*. Allometric coefficients for each factor were calculated using standardized major axis regression of natural-log transformed morphometric traits against the natural log of L_S.

Although morphometric allometries were parallel between species, size-standardized multivariate principal component analysis (PCA) could not be applied due to marked variation in life stage structure among samples and limited among-group patterns on PC2–PC5 (Sidlauskas et al., 2011). Bivariate plots from the regressions with parallel allometries and significant offset intercepts ($P < 0.05$) were used to investigate morphological differences between the upper and lower Guinean samples.

4.3.3 Mitochondrial DNA

DNA extraction, cytochrome oxidase subunit I gene (*coI*) amplification and sequencing were undertaken by Inqaba Biotechnical Industries (Pty) Ltd. (www.inqababiotec.co.za). Contig assembly and sequence editing were performed using Geneious 11.1.5. (Kearse et al., 2012) and GenBank accession numbers for the sequences generated in this study are given in **Table 4-3**.

Table 4-3: Taxa, voucher catalogue numbers and GenBank accession numbers for *coI* sequences obtained in this study

Taxon	AMNH catalog	Tissue code	GenBank	Locality
Thysochromis emili	AMNH 258106	AMCC 211381	MN160147	Lake Youbi, Republic of
Thysochromis emili	AMNH 258135	AMCC 211386	MN160148	Lake Yangala, Republic of
Thysochromis	ZCUV 2018/003	NIG-001	MN160149	Niger River, Nigeria
Thysochromis	Uncatalogued Photo	NIG-002	MN160150	Niger River, Nigeria

4.3.4 Habitat association

Abiotic (landscape scale, physico-chemical and qualitative habitat) data were collected at 15 surveyed sites. Fish habitat assessment was undertaken following Kleynhans (2007) to estimate the occurrence of broad fish-habitat cover types in various depth classes. Site slope, average catchment slope, catchment size and stream order were modelled using Shuttle

Radar Topography Mission (SRTM) imagery at a 30 m resolution (METI & NASA, 2018) processed in ESRI ArcMap10.5 (www.resources.esri.com).

For the purpose of illustrating habitat associations of *T. emili* sp. nov., abiotic and fish community data were subjected to canonical correspondence analysis (CCA) using Canoco 4.5 (ter Braak & Smilauer, 2002). The CCA was carried out on log-transformed species and environmental data. Forward selection with partial Monte Carlo permutation testing was used to assess the significance of each environmental predictor variable for extending the subset of explanatory variables used in the ordination model. The significance of axes was tested using unrestricted Monte Carlo permutation testing (499 permutations' $P < 0.05$).

4.4 Results

4.4.1 Group separation

Standardized major axis regressions indicated that the two geographically disjunct groups of specimens differ significantly ($P < 0.05$) in allometric trajectories of caudal-peduncle length ($P < 0.05$) and postorbital length (pol) ($P < 0.05$; **Figure 4-2**). Caudal-peduncle length (cpl) showed a weak negative allometry for upper Guinean samples (*T. ansorgii* group), while lower Guinean samples (*T. emili*) exhibited a strong positive allometry (0.872 and 1.477, respectively). The postorbital length was weakly positive for the upper Guinean samples (1.129) while the lower Guinean samples showed a weak negative allometry (0.857).

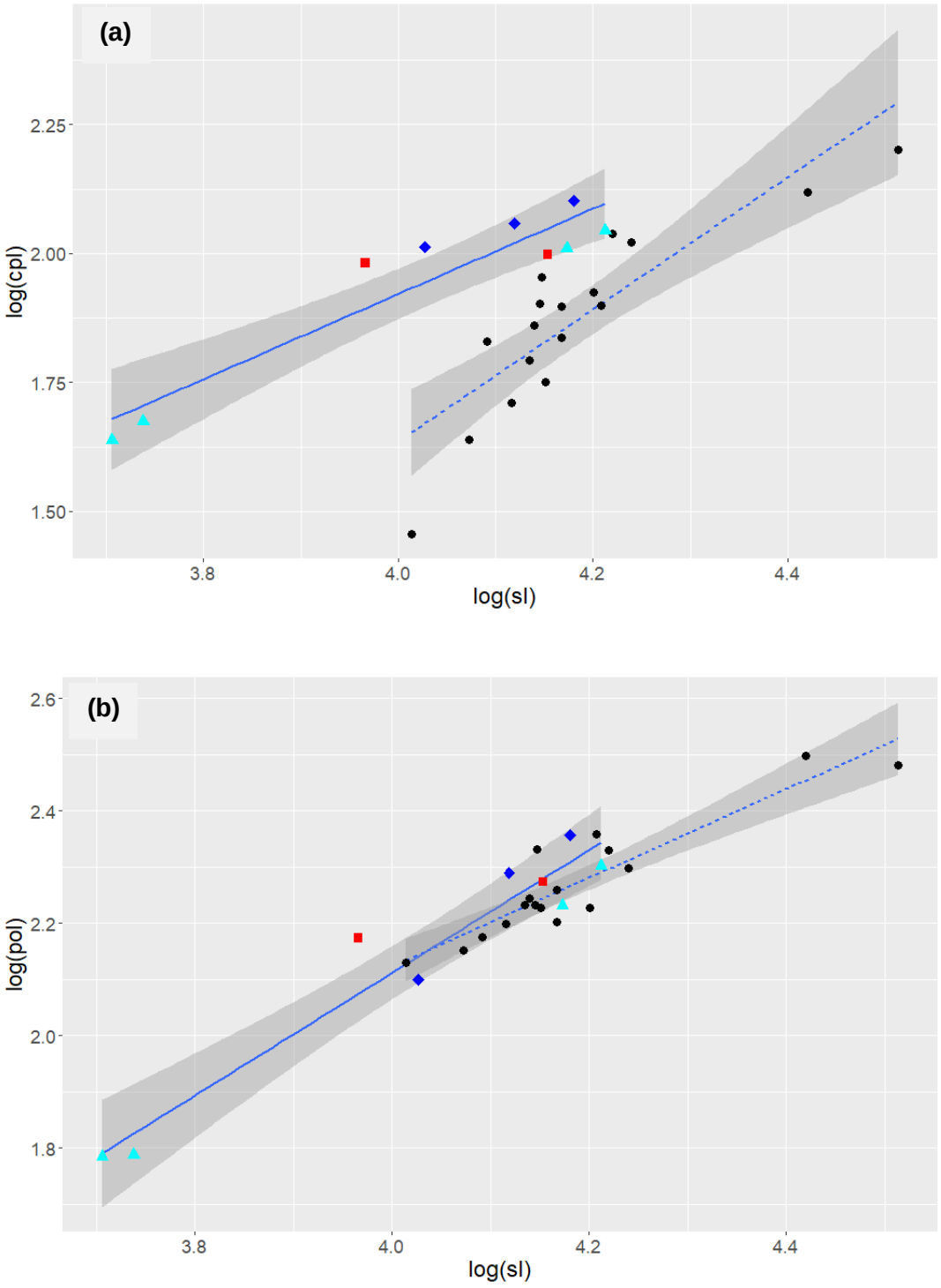

Figure 4-2: Standardized major axis regressions of the natural log of (a) caudal peduncle length (cpl), and (b) postorbital length (pol) against the natural log of standard length (L_S) for: ●*Thysochromis emili* sp. nov.; ▲ *T. ansorgii*; ◆*T. arnoldi*; ■*T. annectens*. Area of 95% CI

Separation of upper and lower Guinean samples is further supported by the degree of sequence divergence in the bar-code col gene (**Table 4-3**) between exemplars from these two regions. These data are represented here as a distance matrix (**Figure 4-3**) and divergence between exemplars from the two geographically separated populations exceeds the standard heuristic threshold of 3% commonly used as an indicator of species-level differentiation (Arroyave et al., 2019; Herbert et al., 2003). While exceptions to this heuristic threshold certainly exist (Joyce et al., 2011; van der Walt et al., 2017) these molecular data, taken in combination with the morphometric separation highlighted above and a few qualitative anatomical differences (discussed below), reveal a clear separation between upper and lower Guinean populations. Based on these combined data we describe here the lower Guinean populations of *Thysochromis* as a new species.

	T. emili n.sp. (Lake Youbi)	*T. emili n.sp.* (Lake Yangala)	*T. ansorgii* (Nigeria 1)	*T. ansorgii* (Nigeria 2)
T. emili n.sp. (Lake Youbi)		99.8%	96.2%	96.2%
T. emili n.sp. (Lake Yangala)	99.8%		96.1%	96.1%
T. ansorgii (Nigeria 1)	96.2%	96.1%		100%
T. ansorgii (Nigeria 2)	96.2%	96.1%	100%	

Figure 4-3: Matrix of cytochrome oxidase subunit I gene (col) distance between *Thysochromis* emili sp. nov. and *T. ansorgii* from different locations. Inset images are of voucher specimens photographed immediately post mortem

It is worth noting that a few specimens previously identified as *Thysochromis* from intervening regions are housed in museum collections. None are reported from Cameroon and the few from Gabon have been examined in the present study. With the single exception of a juvenile collected near Mayumba, in a coastal basin just north of the Kouilou in the Republic of Congo

(MRAC A1-88-P-2497, photograph examined), all others are misidenfications of the chromidotilapiine species *Chromidotilapia kingsleyae* Boulenger 1898 and *Divandu albimarginatus* Lamboj & Snoeks 2000 (pers. obs).

4.4.2 *Thysochromis emili* sp. nov

Figure 4-4, **Figure 4-5** and **Table 4-1**; urn:lsid:zoobank.org:pub:1E60CEA4-3DAE-45b7-AF12-04EB6250E9D7.



Figure 4-4: (a) Lateral view of preserved male *Thysochromis* emili sp. nov. general habitus (holotype, AMNH 271740) and (b) female (paratype, AMNH 271742). Scale bar = 1 cm

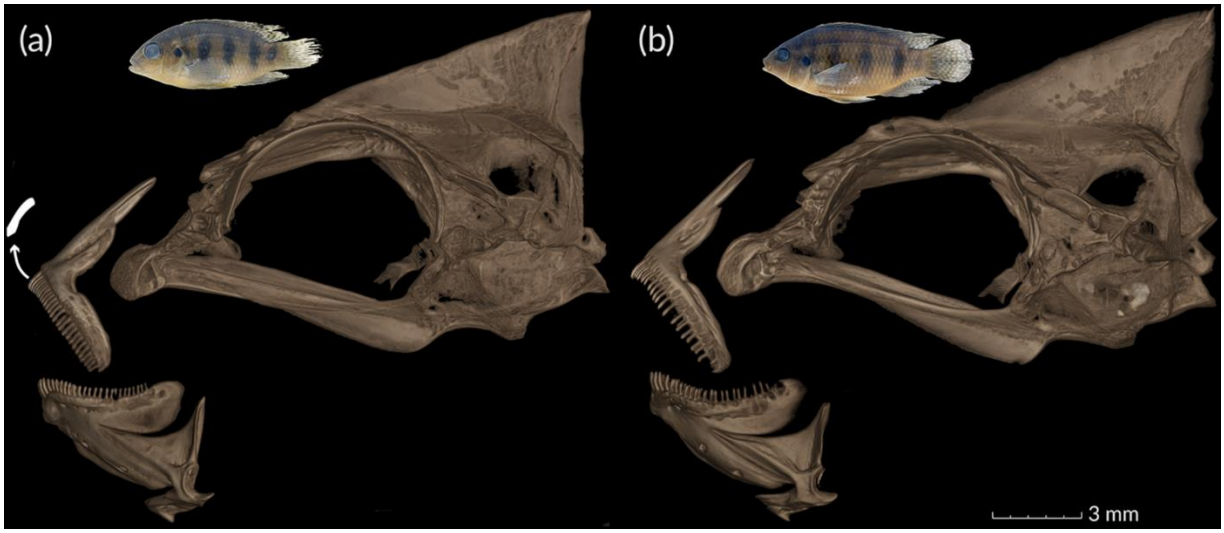

Figure 4-5: Whole specimen and microCT scanned lateral views of neurocranium and premaxilla, of (a) *Thysochromis* emili sp. nov. (AMNH 258135) and (b) *Thysochromis* ansorgii (AMNH 235838). Scale bar = 3 mm

4.4.2.1 Holotype

AMNH 271740, male, 69.4 mm L_S, Republic of Congo, Kouilou Province, Lake Youbi, 04° 11′ 30.4″ S, 11° 40′ 12.8″ E, M.N. Jonker, V. Mamonekene, V. Boukaka Mikembi, 17 July 2012.

4.4.2.2 Paratypes

AMNH 271741, six specimens, 1 c&s, 55.4–66.7 mm L_S, same data as holotype. MRAC 2018-027-P-0003-0004, two specimens, 60.7–61.2 mm L_S., same data as holotype. NMW 99244, two specimens, 58.2–62.4 mm L_S, same data as holotype. AMNH 271742, three specimens, 63.1–67.2 mm L_S, Republic of Congo, Kouilou Province, Lake Yangala, 04 ° 07′ 26.1″ S, 11° 35′ 48.9″ E, M.N. Jonker, V. Mamonekene, V. Boukaka Mikembi, 19 July 2012. ZSM 47495, two specimens, 63.4–65.0 mm L_S, same data as AMNH 271742. AMNH 271747, two specimens. 36.0–54.0 mm LS, Republic of Congo, Kouilou Province, Bondo River, 04° 4′ 7.04” S, 11° 38′ 3.08″ E, G. Walsh, V. Boukaka Mikembi, J. Mountou, 6 September 2017. AMNH 271748, one specimen, 61.0 mm L_S, Republic of Congo, Kouilou Province, Tsissa River, 04° 0′ 57.63” S, 11° 42′ 4.09″ E, G. Walsh, V. Boukaka Mikembi, J. Mountou, 7 September 2017.

4.4.2.3 Differential diagnosis

While no unambiguous morphological autapomorphies have been located to diagnose *T. emili* sp. nov., it is distinguished from its West African congener, *T. ansorgii*, by the following combination of characters (**Table 4-1 &**

Table 4-2). Fewer scale rows between the pectoral and pelvic-fin origins (3–4 v. 5–6), jaw teeth closely spaced and evenly implanted over entire length of both upper and lower jaws (v. widely space and unevenly implanted along distal portions) and a supraoccipital crest that is continuous with the frontal ridge (v. terminating posterior to frontal ridge). Shorter postorbital length (38.2–41.9% v. 42.8–46.7% L_H) and a shorter caudal peduncle length (7.7–11.3% v. 11.4–12.7% L_S).

4.4.2.4 Description

Medium-sized cichlid, maximum recorded L_S 91.3 mm (mature male, MRAC 90-57-P-5288; **Figure 4-4**). Counts and proportional measurements for holotype and 18 paratypes given in **Table 4-1**. Deep-bodied (body depth, DB 38.4–47.8% L_S), greatest depth at level of fourth or fifth dorsal-fin spine. Head small (L_H 34.2–38.9% L_S, depth 65.0–76.8% L_H), short postorbital length (38.2–41.9% L_H), snout short, mouth small with well-developed, fleshy lips. Dorsal head profile 40–50° to mid-orbit, rising steeply to nape. Dorsal and ventral body profiles convex to short (7.7–11.3% L_S), deep (15.6–18.5% L_S) caudal peduncle.

Dorsal fin XV–XVII (mode XVI) 9–11 (mode 10). Anal fin III 7–9 (mode 8). Dorsal spines increase in length posteriorly. Soft dorsal and anal fins extending over caudal-fin base. Caudal fin large, sub-truncate to rounded, with 14 branched rays. Pectoral fins short and rounded, extending to mid-body, not reaching anus. Pelvic fins reaching just short of, or to anus in females, extending beyond anal-fin origin in mature males. First branched ray of pelvic fin longest in both sexes.

Jaws short and isognathus. Outer row teeth in both jaws, somewhat recurved unicuspids, with distally flattened, bilaterally shouldered crowns (**Figure 4-5a**). Outer row premaxillary and dentary teeth evenly implanted, closely spaced over entire length of dentigerous arms (**Figure 4-5a**) v. widely spaced and unevenly implanted along distal portions in *T. ansorgii* (**Figure 4-5b**). Two to four inner rows of small, similarly shaped, unicuspid teeth restricted to anterior third of both jaws. Infraorbital series with broad, plate-like first infraorbital (lachrymal) bearing four small, sensory-canal pores. Second to fifth infraorbital elements narrow and tubular, forming complete suborbital ring.

Lower pharyngeal jaw wider than long, with slightly convoluted ventral suture. Twenty-four to 28 teeth along posterior row, symphysial teeth moderately robust, becoming slender laterally. Eight to ten tuberculate ceratobranchial rakers along first arch, often short raker in angle of arch, 4–7 simple epibranchial rakers. Microbranchiospines lacking on inner faces of second to fourth arches. Pharyngeal hanging pad moderately developed, extending just anterior to first epibranchial.

Scales cycloid. Flank scales large, uniformly sized onto chest, 3–4 between pectoral and pelvic-fin origins. Cheek with three scale rows, four horizontal scale rows on opercle. Prominent dark opercular blotch unscaled. Pored lateral-line scales 26–28, upper and lower lateral lines not overlapping. Upper line separated from dorsal-fin base at highest point (eighth pored scale) by 2–2.5 scales, last pored scale separated by 1–1.5. Proximal third of caudal fin covered with small interradial scales. Sixteen (rarely 15) circumpeduncular scales.

Total number of vertebrae: 24–26, comprising 13 abdominal and 11–13 caudal centra.

Supraoccipital crest contiguous with frontal ridge (**Figure 4-5a**) v. crest terminating posterior to frontal ridge (**Figure 4-5b**).

4.4.2.5 Colouration in life

Head and body yellowish or yellowish-brown, paler ventrally (**Figure 4-6**). Scaleless, dark opercular spot often extending posteriorly over one or two scale rows. Three short dark bars along mid-flank, not extending to ventrum. Single dark bar or rounded spot in midline or over dorsal half of caudal peduncle. Flank scales ringed with dark-brown pigment contrasting with paler central field, more strongly marked in males (**Figure 4-6a**) than females (**Figure 4-6b**). Ventral portions of cheek, operculum and chest silvery with a greenish or turquoise flush. Cluster of silvery scales located lateroventrally around vent, reduced in number and less clearly marked in males (**Figure 4-6a**), prominent in mature females (**Figure 4-6b**). Belly flushed pale rosy pink in mature females. Leading edge of pelvic fin darkly pigmented. Soft dorsal and anal fins with alternating pale and dark maculae variously evident, but always present. Caudal-fin membranes with conspicuous rows of maculae, strongest proximally. All fin spotting more prominent in mature males than in females and juveniles. In preservation (**Figure 4-4**), ground coloration of body brownish yellow, darker dorsally. Opercular spot and bars on flank and caudal peduncle clearly visible, fin maculae variously evident.



Figure 4-6: Immediately post mortem coloration of *Thysochromis* emili sp. nov. (a) male from Lake Youbi and (b) female from Lake Yangala

4.4.2.6 Distribution

Despite extensive collections made during a number of surveys throughout the region, *T. emili* sp. nov.is known from only five localities (**Figure 4-1**). Currently it has been recorded in the Noumbi and Kouilou River drainages in the Republic of Congo and appears to be restricted to larger systems in the coastal plain of the Republic of Congo. The species has been recorded from Lakes Youbi, Yangala and Koubambi and in the Bondo and Tsissa Rivers. The record of *T. emili* in Lake Koubambi is based on specimens collected by Teugels *et al.* (1991), but despite extensive sampling of suitable habitat in that lake during the 2012 and 2017 expeditions, the species was not re-collected there. Vreven *et al.* (2007) recorded multiple introductions of the Nilo-Sudanic species, *Heterotis niloticus* (Cuvier 1829), throughout the region and Walsh *et al*. (2014) reported on the dominance of *H. niloticus* in catches from Lake

Koubambi. We suggest that the current absence, or rarity, of *T. emili* sp. nov. in Lake Koubambi may be the result of competition with introduced *H. niloticus* for habitat and resource partitions.

As noted above, examination of a photograph of a single juvenile *Thysochromis* specimen (MRAC A1-88-P-2497, specimen lost) from a coastal basin just north of the Kouilou, in Gabon, suggests that the distribution of *T. emili* sp. nov. probably extends into suitable habitats in southern coastal Gabon.

4.4.2.7 Habitat preferences

The species–environment correlations showed a cumulative percentage variance of species data of 49.1% explained on the first two axes. Environmental variables significantly ($P \leq 0.05$) contributing towards the measured variance in the data include catchment size ($P < 0.01$) conductivity ($P < 0.01$), turbidity ($P < 0.01$), stream order ($P < 0.01$) and aquatic macrophytes ($P < 0.05$; **Figure 4-7**).

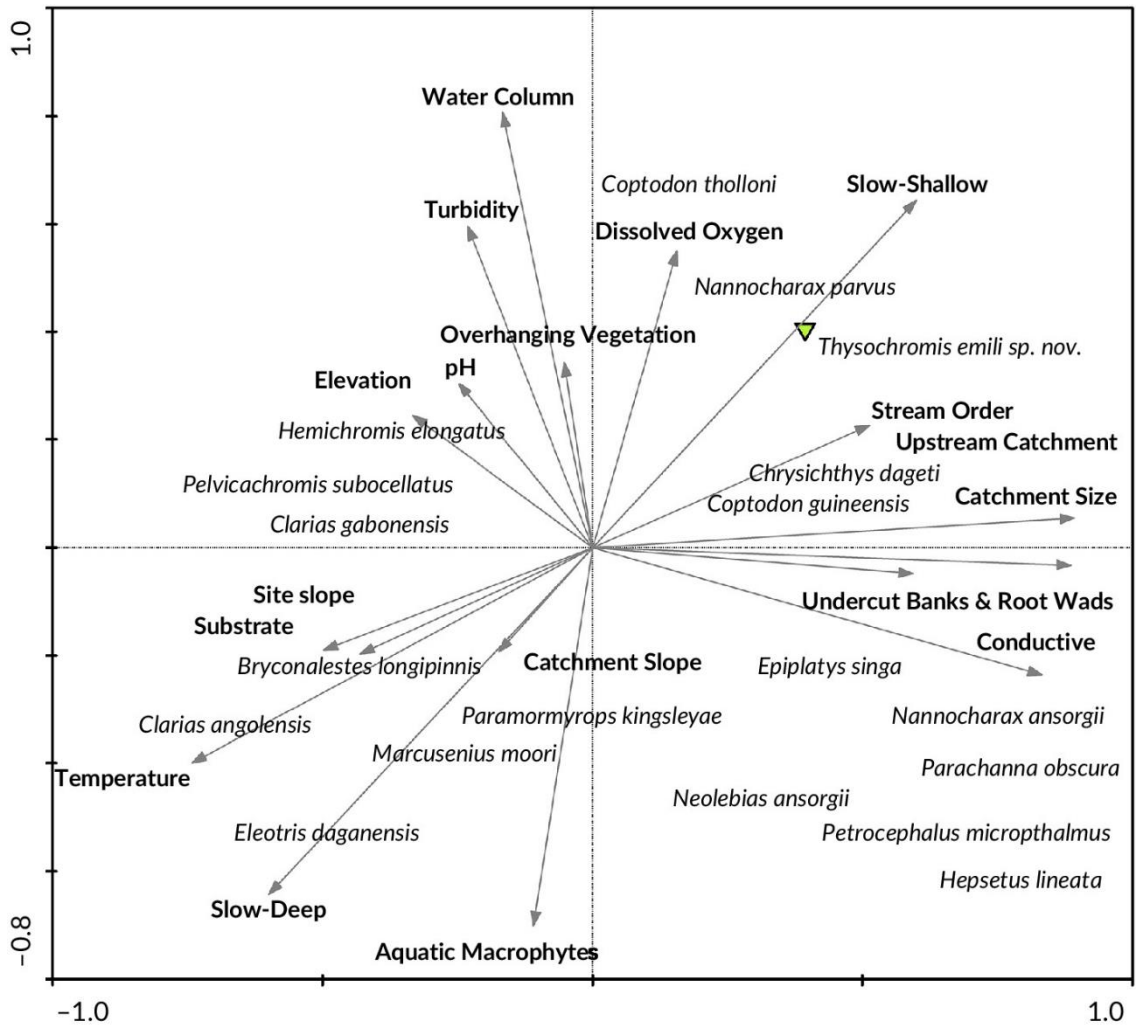

Figure 4-7: Canonical correspondence analysis of the fish community data in relation to various environmental attributes. ← and bold text, abiotic variables; ▽ position of *Thysochromis emili* sp. nov

Thysochromis emili sp. nov. were collected exclusively in well oxygenated lakes and rivers situated in higher order systems (> fourth order) of the Kouilou and Noumbi River basins. The species was sampled over sandy substrate in shallow (< 0.5 m), clear, shoreline waters fringed by abundant overhanging vegetation, deeply undercut banks and root wads. It was not found in other coastal lake or river basins despite efforts to collect it in these localities. The new species showed a positive correlation and co-occurrence with the distichodontid *Nannocharax parvus* Pellegrin 1906 and two other omnivorous or herbivorous substrate-brooding cichlids *Coptodon guineensis* (Günther 1862) and *Coptodon tholloni* (Sauvage 1884).

4.4.2.8 Breeding behaviour

Not known, but as compared with its west African congener and its preferred habitat, it is probably a pair-bonding, cave brooder.

4.4.2.9 Etymology

The species is named for Emil Woolf Kentridge-Young, grandson of William Kentridge and Anne Stanwix, in recognition of their support of research in Africa.

4.4.3 Additional material examined

AMNH 258106, 12 specimens, Republic of Congo, Kouilou Province, Lake Youbi, 04° 11′ 30.4" S, 11 ° 40′ 12.8″ E, M.N. Jonker, V. Mamonekene, V. Boukaka Mikembi, 17 July 2012. AMNH 258135, eight specimens, 1 c&s, Republic of Congo, Kouilou Province, Lake Yangala, 04 ° 07′ 26.1" S, 11° 35′ 48.9″ E, M.N. Jonker, V. Mamonekene, V. Boukaka Mikembi, 19 July 2012. AMNH 261035, two specimens, Republic of Congo, Kouilou Province, Lake Yangala, 04 ° 07′ 26.1" S, 11° 35′ 48.9″, Mamonekene and Walsh, 19 July 2012. MRAC 90-57-P-5288-89, two specimens, Republic of Congo, Kouilou Province, Lake Koubambi, G. Teugels et al., 1990. MRAC A1-088-P-2497, 1 specimen (photograph examined), Gabon, Petit ruisseau sur route forestière aprés Doumvou, Mamonekene, 24 July 2001.

4.4.4 Comparative material examined

4.4.4.1 Thysochromis annectens

BMNH 1913.7.15:8–9, syntypes, two specimens, 52.8–83.4 mm L_S, Nigeria, Lower Niger River, J.P. Arnold.

4.4.4.2 Thysochromis arnoldi

BMNH 1912.2.2:10–12, syntypes of *T. arnoldi*, three specimens, 56.1–65.4 mm L_S, Nigeria, lower Niger River, J.P. Arnold.

4.4.4.3 Thysochromis ansorgii

BMNH 1901.1.28:9–12, syntypes, four specimens, 40.7–67.5 mm L_S, Nigeria, Niger River system, Ethiop River at Sapele station, W.J. Ansorge.–MRAC 91-10-P-661–671, 11 specimens, 35.8–54.0 mm L_S, Nigeria, Niger River system, New Calabar River, Akpor, C.B. Powell, December 1990.–AMNH 225991, two specimens, 1 c&s, 54.5–61.5 mm L_S, Nigeria, New Calabar River system, stream 3 km southeast of Emuhoa, C.B. Powell, 5 January 1991.–AMNH 235838, two specimens, 1 c&s, 62.9–71.6 mm L_S, Benin, Oueme River, small stream near game village, R. Schelly et al., 13 November 2003.

4.5 Discussion

Lamboj and Stiassny (2007) accepted the identification of Teugels et al. (1991) for two specimens from Lake Koubambi in the Republic of Congo as *T. ansorgii* thereby recognizing a 1000+ km range extension spanning two ichthyofaunal regions. However, based on the current study, *Thysochromis* is resolved as containing two geographically disjunct species: *T. ansorgii* (including T. annectens and *T. arnoldi* as synonyms) from the upper Guinean ichthyofaunal province and *T. emili* sp. nov. restricted to the lower Guinean province, specifically the Kouilou Department of the Republic of Congo. The Kouilou Department is located along the Atlantic coastal plain in the southern portion of the lower Guinean ichthyofaunal province. The region consists of low-altitude savanna and forest ranging between Gabon to the north and Cabinda to the south (van Rooyen et al., 2016; FEOW, 2019). The lowland coastal area has a continuum of beaches and dunes in the west, transitioning into undulating hills on the coastal plains and the Mayumbe Escarpment in the east. The landscape is crossed by a dense network of drainage channels flowing into larger rivers and

natural lakes, resulting in a high freshwater habitat heterogeneity (Vande Weghe, 2004; Walsh et al., 2014; van Rooyen et al., 2016). The high richness in freshwater fish in the lower Guinean province (Stiassny et al., 2007) was probably driven by the aquatic refugium offered by the forested west coast equatorial region during climatic fluctuations during the Pleistocene (Leveque, 1997; Brooks et al., 2011). It appears that the lower and upper Guinean ichthyological provinces have more recently experienced recurrent isolation during dry phases of the climatic cycle that reduced the rainforest between the Late Pleistocene and present day and which created optimal conditions for allopatric speciation (Leveque, 1997; Teugels et al., 1991).

The current study highlights the importance of including multiple data sources when considering geographically widespread and taxonomically under-studied species where failure to recognize divergent lineages has often resulted in the underestimation of diversity (Arroyave et al., 2019).

4.6 Acknowledgements

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Chapter 5: *Plataplochilus eliasi* (Cyprinodontiformes: Procatopodidae) a new species from the upper Noumbi River in the Republic of the Congo

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5.1 Abstract

Forty years after the last *Plataplochilus* description was published, a new species, *P. eliasi*, is described from streams in the upper reaches of the Bondo River tributary (Noumbi Basin) in the Mayumbe Mountains of the Republic of the Congo. The new species is distinguished from all other congeners by the males' colouration pattern, which consists of a crescent shaped dark blue band on the lower half of the body, and the larger body depth (38.4–42.4 % of SL). All other described species of which the colour pattern in life specimens is known are lacking this band and have a silvery lower body with (depending on the species) a light blueish, greenish, or brownish iridescence. In addition, *Plataplochilus miltotaenia* and *P. pulcher* have a narrow mid longitudinal red line on the flanks that is absent in all other described species, including *P. eliasi*, new species. The body depth (presented as % of standard length) is also useful in distinguishing the new species (body depth 38.4–42.4 % of SL) from its congeners: *P. cabindae* (25.3–27.8 % of SL) *P. chalcopyrus* (23.3–27.0 % of SL), *P. micrurus* (25,5 % of SL in the single type), *P. mimus* (29.7– 32.5 % of SL), *P. ngaensis* (28.4–31.5 % of SL), *P. pulcher* (27.4–33.5 % of SL), *P. terveri* (28.7–30.9 % of SL), and *P. loemensis* (30.7–33 % of SL). The taxonomic status of the genus is discussed, and for the first time osteology of the genus is examined revealing the following likely diagnostic characters shared by all examined species: anterior portion of alveolar arm of premaxilla developed in a squared process, rectangular and anteroposteriorly elongated lachrymal, dorsoposterior process of autopalatine directed upwards, elliptical supracleithrum, and dorsoposterior border of cleithrum broad and posteriorly elongated. Detailed habitat, ecological and distribution data for this new species are also provided.

5.2 Introduction

The genus *Plataplochilus* Ahl, 1928 belongs to the most ancient lineage of Procatopodidae, a family of small African Cyprinodontiform fishes, popularly known as lampeyes, due to the presence of a bright colouration in the dorsal region of eye (Bragança & Costa, 2019).

According to a recent molecular based phylogenetic analysis of the Procatopodidae (Bragança & Costa, 2019), *Plataplochilus* split from a clade containing all other procatopodids in the late Oligocene, approximately 30 million years ago, but diversification within the genus only happened more recently in the Pleistocene (Bragança & Costa, 2019). Despite the ancient origin, when compared to the other procatopodids, species of the genus are only known from the Lower Guinea ichthyological Province, being widely distributed in the coastal plains of Equatorial Guinea, Gabon, Republic of Congo and in the Angolan territory of Cabinda (Van der Zee, Woeltjes, & Wildekamp, 2007). Recent expeditions, however, reported undescribed species occurring at high altitude streams in the Monts de Cristal in northern Gabon and southern Equatorial Guinea (personal communication L, Chirio, 2019; F. Malumbres, 2020; R. Schmidt, 2019 and R. Sonnenberg, 2018).

Ahl (1928) erected *Plataplochilus* and designated *Haplochilichthys ngaensis* Ahl, 1924 as the genus type species, which was defined by its dentition type and the lack of scales in the caudal fin base. Later, Lambert & Clausen (1967) recognized the need to redefine the genus, because of a series of inconsistencies when examining the types of *P. ngaensis* and the other species assigned to the genus such as *P. cabindae* (Boulenger, 1911), *P. chalcopyrus* Lambert, 1963, *P. loemensis* (Pellegrin, 1924), and *P. miltotaenia* Lambert, 1963. The presence of slightly larger and more robust outer teeth in the outer row of both premaxila and dentary was confirmed for all *Plataplochilus* species, but it was not considered as diagnostic because a similar condition is also present in species of *Hylopanchax* Poll & Lambert, 1965, *Hypsopanchax* Myers, 1924 and *Procatopus* Boulenger, 1904 (Lambert & Clausen, 1967). On the other hand, contrasting to Ahl (1928), well developed scales were found in the base of the caudal fin. The genus *Plataplochilus* was then defined by the presence of ctenoid scales in the supraorbital region and on the sides of head and nuchal region; a wide preorbital region, due to an enlarged lachrymal bone, covered by two rows of ctenoid scales flanking the preorbital sensory canal; a distinct chain of minute ctenoid suborbital scales bordering the

lower eye orbit, and a closed anterior and posterior sections of the supraorbital canal (Lambert & Clausen, 1967). In the same publication, similarities and differences between *Plataplochilus, Lamprichthys* Regan, 1911, and *Procatopus* were detailed.

Huber (1982) considered *Plataplochilus* a junior synonym of *Procatopus*, because it was considered to represent a well-defined and single lineage, to replace each other ecologically and geographically, and according to Huber (1982) both share highly inserted pectoral fins. In addition, Huber (1982), despite recognizing the presence of an open supraorbital cephalic system in *Procatopus* and the presence of closed canal with pores in *Plataplochilus*, attributed this difference to a gradation from one state to the other. According to him, the fleshy lobes bordering the open grooves in *Procatopus* are fused in *Plataplochilus*, and this difference should not be considered to distinguish them. Further, Huber (1982) reported some variation related to the supraorbital cephalic system in *Plataplochilus*. The ctenoidy in the head was confirmed in all *Plataplochilus* species, but Huber (1982) argued that there was considerable interspecific variability in this trait and that ctenoid scales were described in the flank scales of *Procatopus*. Also, Huber (1982) mentioned the presence of the typical *Procatopus* branchiostegal appendages in some *Plataplochilus* species, and that the lachrymal pore size, the pelvic-fin position and the number of expanded haemal arches vary only slightly between both genera.

Since Huber (1982), few studies mentioned or included species of *Plataplochilus*, and the genus remained largely neglected. However, recent expeditions to the coastal drainages of Lower Guinea are revealing a high number of species with restricted ranges and endemics that still await description (Eberl et al., 2020). Currently, *Plataplochilus* includes nine species: *P. cabindae*, *P. loemensis*, *P. ngaensis*, *P. micrurus* (Ahl, 1928), *P. chalcopyrus*, *P. miltotaenia*, *P. mimus* Lambert, 1967, *P. pulcher* Lambert, 1967 and *P, terveri* (Huber, 1981). Until now, many phenotypes were assigned to one of these species due to the scarce information on *Plataplochilus* systematics.

A new species was collected by the first author in the Bondo River catchment (Noumbi River basin) in the Mayumbe mountains near the coast of the Republic of the Congo (**Figure 5-1**). The new species could initially was distinguished from its congeners by differences in the colouration pattern and external morphology. Additionally, for the first time the osteology of *Platalochilus* species is analysed herein, and an updated definition for the genus is provided as well as detailed aspects of its habitat presented.

5.3 Material and Methods

The description of the new species is based on the collections made by the first author in the Bondo River catchment (Republic of the Congo) in 2016 and 2017. The Bondo River (Noumbi Basin) was sampled in the wet and dry seasons as part of a greater ecological study of the area (**Figure 5-1**). Fish sampling effort was standardized according to habitat type, and fish were collected according to the guidelines for the Use of Fishes in Research (AFS/AIFRB/ASIH, 2003) and preserved in 96% ethanol. Specimens and comparative specimens were deposited in RMCA, Tervuren (Belgium), RBINS , Brussels (Belgium), AMNH, New York (USA), SAIAB, Makhanda/Grahamstown (South Africa), MNHN, Paris (France), MFN, Berlin (Germany) and SNSB, München (Germany). Collection permits were issued by the National Institute for Research in Exact and Natural Sciences from Republic of Congo (IRSEN Permit 205 and 328) and CITES permits for exportation are CG1125774 and CG1125818.

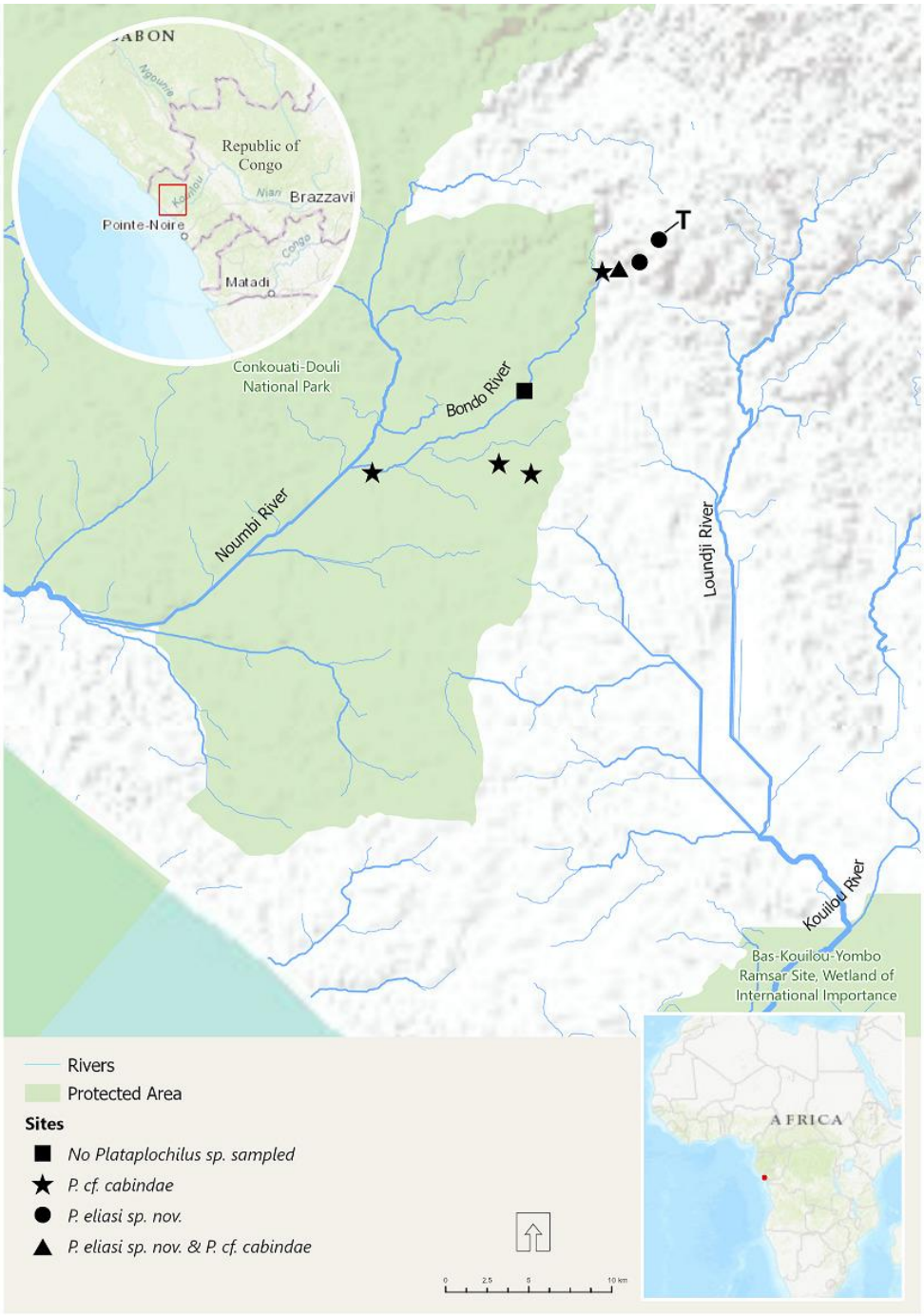

Figure 5-1: Distribution of Plataplochilus species in the Bondo River catchment, Republic of Congo. T = type locality.

5.3.1 Morphometrics and meristics

Seventeen morphometrics measurements were taken using a dissection microscope with a digital calliper and rounded to the nearest 0.1 mm. Nine meristic counts were made. Measurements of bilaterally symmetrical features were taken on the left side of specimens. Counts and morphometrics follow Bragança et al. (2020) except for the following characters that were not considered in that paper. Head depth and head width were measured along the posterior orbit rim, snout length was measured from tip of snout to anterior orbit rim, length of pelvic and pectoral fin were measured from the insertion of these fins. The relative position of first dorsal-fin ray in relation to the anal-fin rays (D/A) was estimated as in Sonnenberg and Schunke (2010). Nomenclature for the cephalic lateral line system follows Clausen (1967) and Van Bergeijk and Alexander (1962), and that for the supraorbital (frontal) squamation follows Hoedeman (1958). Osteological studies were made on cleared and stained specimens (c&s) prepared according to Taylor & Van Dyke (1985), after keeping the specimens in formalin for two months. Osteological nomenclature follows Costa (2006). The osteological structures presented in **Figure 5-3** most likely represent unique character states shared by all examined *Plataplochilus* species. The species concept followed in this study is the unified species concept, as proposed by De Queiroz (2007).

5.3.2 Comparative material

Plataplochilus cabindae, BMNH 1912.4.1.459–460, syntypes , Angola, Lucola river near Cabinda, Ansorge, no year; MRAC 91-068-P-1567–1598, 33 specimens, Republic of the Congo, about 11 km from Dolisie, direction Pointe-Noire, Mbamba River, affluent of the Lombi River, a tributary of the Loubomo, 04°13'S, 12°33'E; Teugels, Mamonekene, 15 August 1991; MRAC 91-068-P-1599–1601, 3 specimens (c&s), Republic of Congo, Mbamba river, affluent of the Lombi river, a tributary of the Loubomo, about 11 km from Dolisie, direction Pointe-Noire, Republic of Congo, 04°13' S-12°33' E; Teugels & Mamonekene, 15 Aug 1991. - MRAC

164750 –791, 42; Riviére Tissala, PK 45 de la route Pointe Noire-Dolisie, Rep. Congo (Brazzaville); J. Lambert, 18 April 1962.

Plataplochilus loemensis, MNHN 1924-0086–0087, syntypes, Republic of the Congo, Lombo River, tributary of Loémé River, Baudon, no year.

Plataplochilus aff. *loemensis*, MNHN 2003–0860, 6 specimens (1 c&s); Gabon, petite riviére en amont du pont, Echira drainage, Rabi, Ogooue Maritime, 1°55'1.2''S 9°58'1.2''E; 14 Jul 2002.

Plataplochilus aff. *loemensis* 2. MRAC 69319–69323, 5 specimens Democratic Republic of Congo, Maduda, riviére Pemba (Bassin, Shiloanga), 04°55'S 13°06'E; Dartevelle, 1946. MRAC 68140, 1 specimen, Democratic Republic of Congo, Maduda, riviére Pemba (Bassin Shiloanga), 04°55'S 13°06'E; Dartevelle, 1946.

Plataplochilus micrurus, ZMB Pisces 21063, 1 specimen. Chinchoxo, Mündung des Quillu, Republic of the Congo.

Plataplochilus miltotaenia, MRAC 140312, Holotype, Gabon, 19 km on the road from Lambaréné to Mouila, J. Lambert, 02-05-1962; MRAC 164735–742, 8 paratypes, Gabon, secondary road near PK 40 road Libreville-Kango, 00°22' N, 09°47' E, Lambert, 22 October 1964; MRAC 140314 –333, 22 specimens, Gabon, PK 18 road between Lambaréné-Mouila, Lambert, 02 May 1965; MRAC

140334–335, 2 specimens (c&s), Gabon, PK 18 road between Lambaréné-Mouila, Lambert, 02 May 1965; RBINS 26553 –26560, 8 specimens, Gabon, Moutéki, 0°46'56.21''S, 10°22'2.28''E, probably type locality, L. Chirio, 01 June 2013; RBINS 26564 –26577, 14 specimens, Gabon, small left bank Ogowe tributary, Zilé River mission I, 9 km north-east of Lambaréné, 0°42'32.58''S, 10°20'39.88''E, L. Chirio, 26 February 2004 and 21 March 2004;

RBINS 26561 –26563, 3 specimens, Gabon, Ngouni basin, Biweni River, 0°50'11.87"S, 10°22'37.42"E, L. Chirio, 20 July 2009.

Plataplochilus mimus, MRAC 153265, Holotype, Gabon, marigot de Nkoltang (PK 28 de la route Libreville-Kango), J. G. Lambert, 29-10-1964; RBINS 26578 –26585, 8 specimens, Gabon, upper reaches of small river emptying in Gabon Estuary, 8 km South of Nkoltang, 0°18'13.21"N, 9°39'2.56"E; L. Chirio, 20 December 2005.

Plataplochilus ngaensis, ZMB 2104, lectotype, Gabon, "Nga-Zuflüssen bei Attogondema", Cameroon (possibly Noya basin, Gabon); RBINS 26586 –26612, 27 specimens, Noya basin, 11 km south of Atogondema, L. Chirio, 11 August 2010.

Plataplochilus sp. MNHN 2003 –0861, 75 (4 C&S); Gabon, localité 30km au sud de l'aéroport de Rabi, Echira Basin, Ogooue-Maritime, 2°7'40.8"S 9°50'45.6"E; Lavoué, Mamonekene, Pawels, 10 Jul 2002.

Plataplochilus pulcher, MRAC 164734, Holotype, Gabon, marigot du PK 40 de la route Libreville-Kango, J. G. Lambert, 22/10/1964; RBINS 26613-26617, 5 specimens, Gabon, Mbé basin, rivière 212, small tributary of Avebe River, between Jauzein and Essia Rivers, 0°31'27.30"N, 10°12'29.23"E, L. Chirio, 27 April 2004; RBINS 26618 –26626, 9 specimens, Gabon, Mbé basin, small tributary of Avebe River, Engong Kouamé, 0°29'55.61"N, 10° 8'58.92"E, L. Chirio, 27 April 2014 and 22 July 2006.

Plataplochilus chalcopyrus, MRAC 140288, holotype, Gabon, Diala River, Lambaréné-Mouila;RBINS 26627 –26656, 30 specimens, Gabon, small left bank Ogowe tributary, Zilé River mission I, 9 km north-east of Lambaréné, 0°39'32.83"S, 10°19'29.42"E, L. Chirio, 26 February 2004 and 21 March 2004.

Plataplochilus terveri, MRAC 80-054-P-0011– 0018, Gabon, 21 km nach Mvengue richtung Moanda, E. Pürzl, 1980; MNHN 2000 –1693, 8 (1 c&s), Gabon, route de Bakoumba, a 5 km

du rond point des douanes de Moanda, Doumay, Haut-Ogooue, 1°34'58.8"S 13°10'1.2"E; Auguste, 27 Mars 2000; RBINS 26657 –6667, 11 specimens, Gabon, Konda, 12 km south-west of Moanda, 1°37'48.90"S, 13° 8'7.94"E L. Chirio, 23 February 2005 and 10 July 2009.

Procatopus aberrans: MRAC 75-38-P-2886–2894, 9 specimens, Cameroon, 17 miles west of Mamfe, Clausen, 31 December 1957; MRAC 75-38-P-2753–2772, 19 specimens (3 c&S), Cameroon, water course just east of the crossroad Ikom-Mamfe, Calabar, Clausen, 22 December 1957.

Procatopus nototaenia, MRAC 73-39-P-2835–2846, 13 specimens, Cameroon, Kribi towards Ebolowa, Pongo, Scheel, 29 January 1969; MRAC 73-39-P-2847–2848, 2 specimens (c&s), Cameroon, Kribi towards Ebolowa, Pongo, Scheel, 29 January 1969; MRAC 143201–143207, 7 specimens, Equatorial Guinea, Fernando Po, Thys van den Audenaerde, 12 November 1964; MRAC 143208, 1 specimen (c&s), Equatorial Guinea, Fernando Po, Thys van den Audenaerde, 12 November 1964; MRAC 75-38-P-641–655, 25 specimens, Cameroon, Kienke River, Clausen & Scheel, 11 January 1966; UFRJ 4104, 1 specimen (c&s), aquarium material.

Procatopus similis: MRAC 95-063-P-0084–0087, 4 specimens, Cameroon, Lake Mevia, 10 km to Edea, Van den Bergh et al., 28 February 1995; MRAC 90-19-P-418–454, 36 specimens, Nigeria, Isiokpo, Powell; MRAC 97-004-P-0010–0078, 69 specimens, Cameroon, Yabassi, Levy, 1–31 January 1997; MRAC 97-004-P-0079–0084, 6 specimens (c&s), Cameroon, Yabassi, Levy, 1–31 January 1997.

Additional comparative material belonging to all other Procatopodidae genera are listed in Supplementary file 1.

5.4 Results

Plataplochilus eliasi, new species (**Figure 5-2, Figure 5-3, Figure 5-4 & Figure 5-5; Table 5-1 & Table 5-2**)

Zoobank id: urn:lsid:zoobank.org:pub: 4EA2F636-0B53-4B59-9EEE-6C90AF9E0B47

Holotype. MRAC 2020.010.P.0001, male, 41.0 mm SL, Republic of the Congo, Noumbi River basin, Bondo River catchment, small unnamed first order tributary, just south of Bamba village, 3º57´04.14´´S, 11º45´58.58´´E, Field code BFVT4-1, Collected 1st June 2017 by G. Walsh, V.N Boukaka Mikembi and J. Mountou.

Paratypes. MRAC 2020.010.P.0002–0008, 4 males (24.7–34.6 mm SL) and 3 females (25.0–28.9 mm SL), collected with the holotype; MRAC 2020.010.P.0009–0010, 2 females (30.0–33.4 mm SL), Republic of the Congo, Noumbi River basin, Bondo River catchment, small unnamed first order tributary, south of Bamba village, 3º57´50.00´´S, 11º45´20.56´´E, Field code BFVT4-2, Collected 3rd August 2016 by G. Walsh, V.N Boukaka Mikembi and J Mountou.

Not measured type material: AMNH 276997, 11 specimens (16.8–27.5 mm SL), collected with the holotype; AMNH 276998, 5 specimens (23.1–41.1 mm SL), Republic of the Congo, Noumbi River, Bondo River catchment, small unnamed first order tributary, south of Bamba village, 3º57´50.00´´S, 11º45´20.56´´E, Field code BFVT4-2, Collected 4th August 2016 by G. Walsh, V.N Boukaka Mikembi and J. Mountou; SAIAB 209956, 2 specimens (30.3–30.9 mm SL), Republic of the Congo, Noumbi River basin, Bondo River catchment, small unnamed first order tributary, just south of Bamba village, 3º57´04.14´´S, 11º45´58.58´´E, Field code BFVT4-1, Collected 3rd August 2016 by G. Walsh, V.N Boukaka Mikembi and J. Mountou; SAIAB 209928, 1 specimen (c&s) (38.5 mm SL); Republic of the Congo, Noumbi River basin, Bondo River catchment, small unnamed first order tributary, south of Bamba village, 3º57´50.00´´S, 11º45´20.56´´E, Field code BFVT4-2, Collected 3rd August 2016 by G. Walsh ,V.N Boukaka Mikembi and J Mountou; SAIAB 209929, 10 specimens (2 c&s) (19.7–35.0 mm SL), collected

with the holotype; ZMB 37000–37002, 3 specimens (27.4–34.5 mm SL), Republic of the Congo, Bondo River basin near Bamba village, 3º57'58.16"S 11º44'40.00"E, Field code BTVT4-3-WET, Collected 3rd June 2017 by G. Walsh, V.N Boukaka Mikembi and J. Mountou.; ZSM 48222, 6 specimens (18.7–25.1 mm SL), collected with the holotype.

Table 5-1: Morphometrics of *Plataplochilus eliasi,* sp. nov. Based on 13 specimens. Left 8 males including holotype, right 5 females, M = mean, SD = standard deviation, MED = median. All measurements in percentages of standard length (SL), except standard length in mm. BD = body depth, HL = head length, PD = pre dorsal length, PA = pre anal length, PP = pre pelvic length, DB = dorsal base, AB = anal base, PL = pelvic length, CPL= caudal peduncle length, CPD = caudal peduncle depth, CR = caudal peduncle ratio, HD = head depth, HW = head width, HD = head depth, ED = eye diameter, IO = interorbital width expressed as percentages of head length

	Holo Type male	Mean (SD) Males	Median Males	Range Males	Mean (SD) Females	Median Females	Range Females
SL	41.0	33.3 (5.1)	33.2	24.7–41.0	29.0 (3.2)	28.9	25.0–33.4
BD	42.4	39.6 (1.9)	39.8	36.6–42.4	35.2 (1.2)	36.0	33.6–36.2
HL	24.9	25.2 (0.8)	24.9	24.6–26.7	25.3 (1.3)	24.8	23.7–27,0
PD	58.3	60.5 (2.3)	60.4	58.3–62.3	61.0 (0.2)	61.1	60.7–61.2
PA	52.0	55.5 (2.8)	55.3	52.0–59.5	59.1 (1.0)	59.3	57.8–60.4
PP	36.3	40.5 (3.9)	39.0	36.3–39.2	39.5 (1.8)	39.5	36.4–41.3
DB	24.4	22.5 (1.1)	22.6	20.8–24.4	19.4 (1.2)	19.7	**17.6–20.7**
AB	25.0	23.1 (1.3)	22.3	22.0–25.0	19.7 (2.0)	19.7	17.4–22.0
PL	15.3	16.1 (0.8)	16.5	15.3–17.8	14.6 (2.4)	15,0	11.6–17.4
CL	26.0	27.5 (1.0)	28.2	26.0-28.6	27.0 (0.9)	27,0	25.9-28.0
CPL	21.2	21.5 (0.7)	21.9	20.5-22.0	23.1 (0.9)	23,4	21.8–23.6
CPD	17.3	17.0 (0.6)	17.2	16.5–17.8	16.1 (0.6)	15.7	15.6–17.0
CR	1.2	1.2 (0.1)	1.2	1.2–1.3	1.4 (0.1)	1,4	1.4–1.5

HD	88.4	90.1 (5.3)	88.4	88.3 –	90.1 (5.3)	88.4	77.6 –89.6
HW	61.8	65.8 (3.7)	65.4	6.,8 –69.7	65.8 (3.7)	65.4	61.5 –74.0
SN	15.7	18,8 (2.4)	17.3	15.7 –	18.8 (2.4)	17.3	15.2 –19.0
ED	39.4	41.9 (2.2)	42.3	39.4 –	41.9 (2.2)	42.3	43,0 –46.8
IO	51.8	51.7 (1.9)	51.8	49.8 –	51.7 (1.9)	51.8	47.4 –54.4

Diagnosis. *Plataplochilus eliasi* belongs to *Plataplochilus* by the presence of the anterior portion of alveolar arm of premaxilla developed in a squared process (**Figure 5-3A**) (polymorphic in *P. ngaensis* that presents also a small indented process), the dorsoposterior process of autolapalatine directed upwards (**Figure 5-3B**), an elliptical supracleithrum (**Figure 5-3C**), the dorsoposterior border of cleithrum broad and posteriorly elongated (**Figure 5-3C**), a rectangular and anteroposteriorly elongated lachrymal (**Figure 5-3D**), and the presence of an upper lobe extension in caudal fin of males (**Figure 5-2A**) (absent in *P. terveri*, *P. loemensis,* and *P. cabindae*). It differs from all described *Plataplochilus* species by the unique colour pattern of adult males: flank above the mid longitudinal line is brownish to olive green whereas the lower half of flank and the operculum are bright dark blue (Figure 5-4**A & B**); a large dark blue to black round spot is present on the operculum; a broad submarginal black band is present from the origin of anal fin to end of the caudal peduncle (Figure 5-4**A & B**)**.** *Plataplochilus chalcopyrus*, *P. mimus P. ngaensis* and *P. terveri* are lacking this band and opercular spot, and have a silvery lower flank with (depending on the species) a blueish, greenish or brownish iridescence. *Plataplochilus eliasi* differs from *P. pulcher* and *P. miltotaenia* by lacking a narrow longitudinal red line on the flank (Figure 5-4**D**). The colour pattern of *P. cabindae*, *P. micrurus* and *P. loemensis* is not known. *Plataplochilus micrurus* has the largest predorsal distance of all *Plataplochilus* species (73% of SL vs. less than 64% of SL), and a strikingly low number of dorsal and anal-fin rays (respectively 6 and 12 vs. 9–16 and 14–18 in all other species (12–13 and 16–18 in *P. eliasi*). Furthermore, the new species differs also from all described *Plataplochilus* species by having a deeper body. Adult males are the deepest of all species in the genus (38.4–42.4 % of SL vs. less than 36.2% of SL). All other described species have more slender bodies (**Figure 5-5**). The species with more slender bodies are *P. micrurus* (25.5% of SL measured by third author on the single type), *P. chalcopyrus* (23.3–27.0% of SL), *P. ngaensis* (28.4–31.5% of SL) and *P. cabindae* (35% of SL according to the description, but measurements by the third author on the two syntypes resulted in 25.3 and 27.8% of SL). Intermediate body depth is found in *P. terveri* (28.7–30.9

% of SL), *P. mimus* (29.7– 32.5% of SL), *P. pulcher* (27.4–33.5% of SL) and *P. loemensis* (30.7–33.0 % of SL). In addition *Plataplochilus eliasi* differs from all other described species except *P. miltotaenia* by having 12–13 dorsal fin rays (vs. 13-16 in *P. miltotaenia*; less than 13 rays in all other species). .*Plataplochilus eliasi*, is similar to *P. miltotaenia*, *P. terveri* and *P. loemensis* by having 7–8 scales in transversal row, and distinguishes from all other congeners that have only 6 scales. Additionally, *Plataplochilus eliasi* is also distinguished from *P. miltotaenia* by having the first ray of the dorsal fin inserted above anal-fin rays 4–5 vs. 1–3 in *P. miltotaenia*. *Plataplochilus eliasi* adult females have a rather similar colour pattern to other species of the genus, but can be easily distinguished from *P. miltotaenia* females by lacking a narrow mid lateral longitudinal red line.



Figure 5-2: *Plataplochilus eliasi*, preserved specimens: A, MRAC 2020.010.P.0001, Holotype, male, 41.0 mm SL; B, MRAC 2020.010.P.0009-010, Paratype, female, 33.4 mm SL; Republic of the Congo, Noumbi River basin, Bondo River catchment.

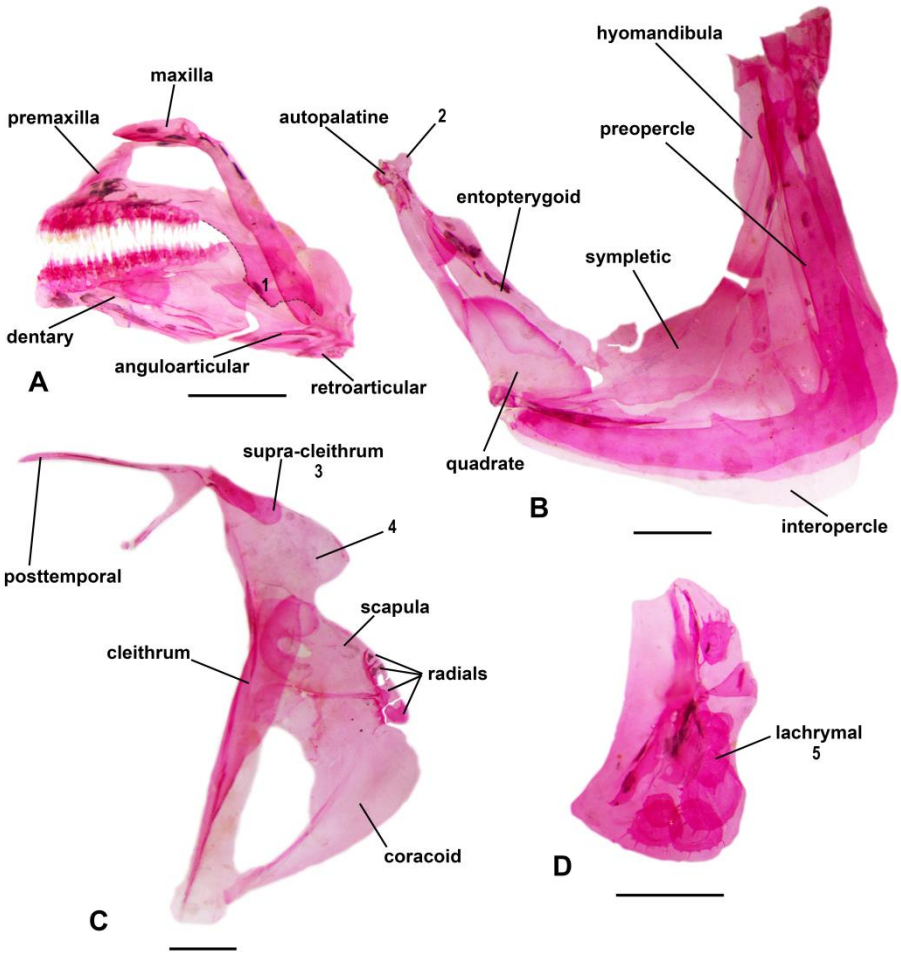

Figure 5-3: ***Plataplochilus eliasi*** **(SAIAB 209929), osteology plate: A, jaws (dashed line indicates the alveolar arm of premaxilla); B, jaws suspensorium apparatus; C, pectoral girdle; D, lachrymal. Numbers indicate** ***Plataplochilus*** **diagnostic characters: 1, anterior portion of alveolar arm of premaxilla developed in a squared process; 2, dorsoposterior process of autopalatine directed upwards; 3, elliptical supracleithrum; 4, dorsoposterior border of cleithrum broad and posteriorly elongated; 5, rectangular and anteroposteriorly elongated lachrymal. Scale bars = 1.00 mm.**

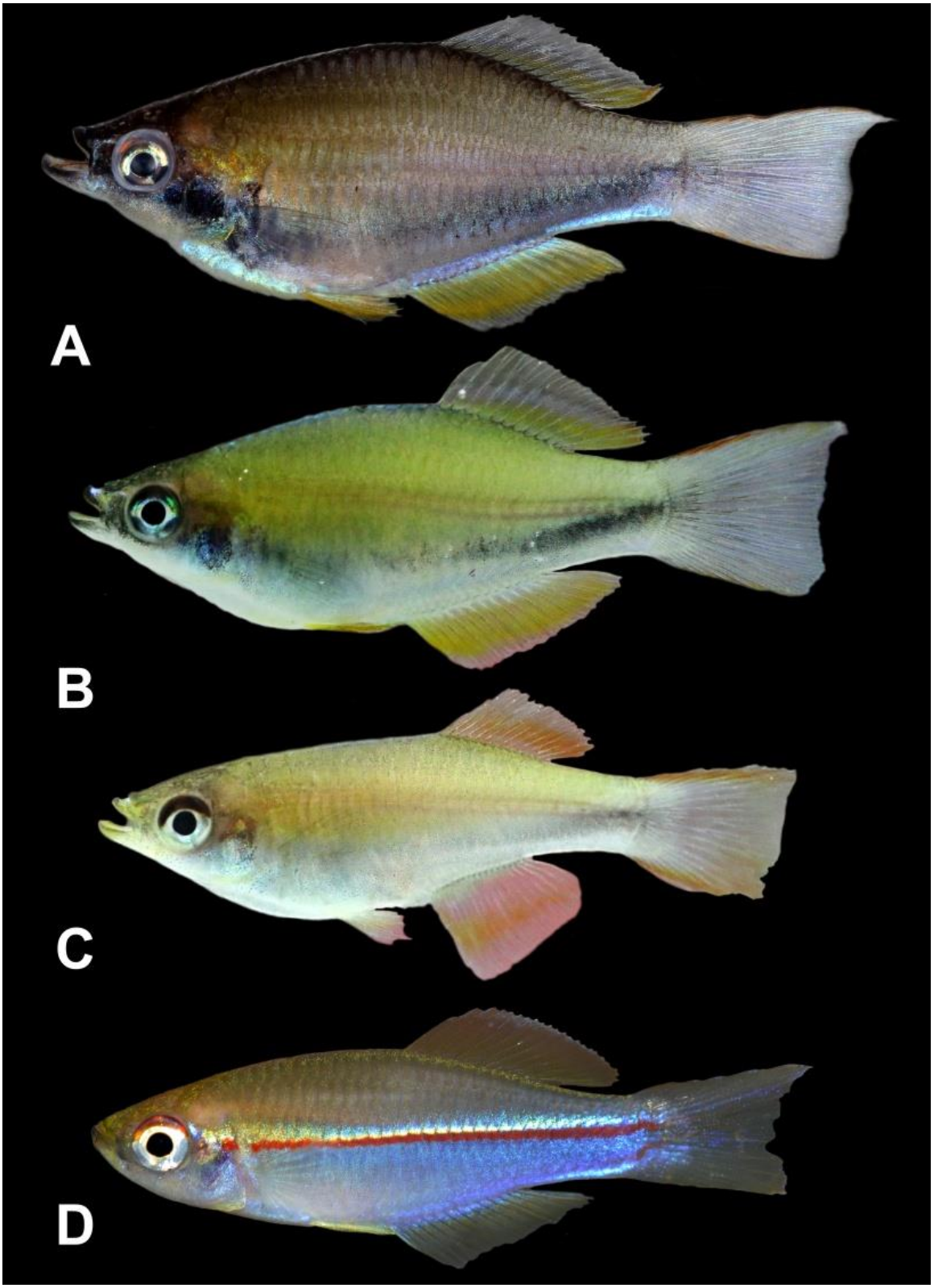

Figure 5-4: Life colouration of *P. eliasi* and *P. miltotaenia*: A, MRAC 2020.010.P.0001, *P. eliasi*, Holotype, male, 41.0 mm SL; B, SAIAB 209956, *P. eliasi*, paratype, male, 31.8 mm SL; C, SAIAB 209956, *P. eliasi*, paratype, female 31.3 mm SL; D. *P. miltotaenia*, from a small left bank tributary of the Ogowe, Zilé River mission I, 9 km north-east of Lambaréné, Gabon, collected by L. Chirio, 26 February 2004 (Photo L. Chirio).

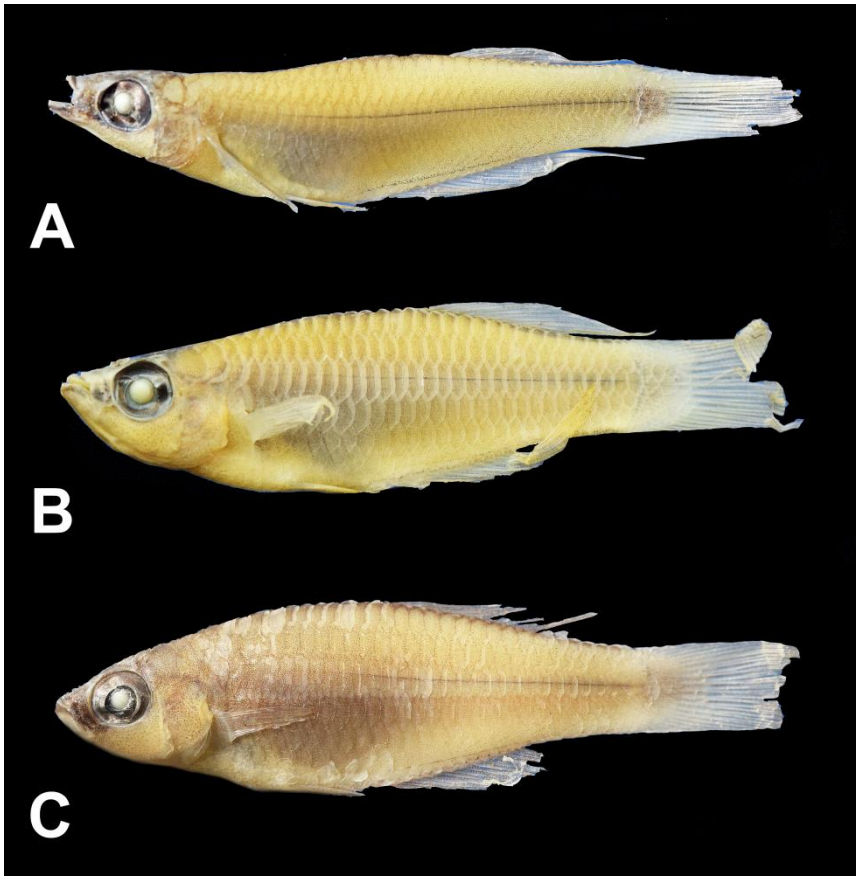

Figure 5-5:Body shape in *Plataplochilus* species. A, slender, *P. chalcopyrus*, male, Zile, Ngounie basin, NW Gabon, 23.4 mm SL; B. intermediate, *P. mimus*, male, Mikolongo Nkoltang, NW Gabon, 27.1 mm; C, deep, *P. miltotaenia*, male, Mouteki, Ngounie basin, NW Gabon, 26,3 mm SL.

Description. Morphometric data presented in **Table 5-1**. Maximum recorded adult size 41.0 mm SL. Dorsal profile of body deeply convex from snout tip to the end of dorsal-fin base; slightly concave to straight from the end of dorsal-fin base to end of the caudal peduncle. Ventral profile deeply convex from lower jaw to end of anal-fin base; slightly concave to nearly straight on caudal peduncle. Anterior portion of body laterally compressed, becoming more compressed behind anal-fin origin. Snout pointed, mouth directed upwards; lower jaw longer than upper jaw; posterior end of mouth just below ventral edge of eye and at the level of the midline of the lachrymal bone in the pre-orbital region (Figs. 2&4).

Males anal and dorsal fins slightly pointed with respectively straight ventral and dorsal margin. Caudal fin about truncate, with upper lobe rays slightly extended in males. Pectoral fin elliptical in both males and females. Females' unpaired fins with straight ventral and dorsal margins. Dorsal fin inserted posterior to mid-body in both sexes, anal fin inserted at mid-body in males and posterior to mid-body in females. Dorsal fin origin at a vertical through the 4th anal-fin ray in males. Pelvic fin reaching between urogenital papilla and origin of anal fin in males, and to the urogenital papilla in females. Female with enlarged left and right scale at the origin of anal fin, covering urogenital opening (Figs 2&4). Meristic data is presented in **Table 5-2**.

Anterior portion of supra orbital system with four large pores, the central pores elongated, posterior portion trapezoid shaped with four small, round to ovoid pores (**Figure 5-6**). Pre-opercular system with seven pores. Pre-orbital system with four to five pores. Post-orbital system closed with two pores and connected dorsally with the posterior portion of supraorbital system. Mandibular canal closed with five pores.

Table 5-2: Meristics of *Plataplochilus eliasi,* sp. nov*.* Based on 10 specimens. SD = standard deviation. D = number of dorsal fin rays. A = number of anal fin rays. D/A = dorsal / anal fin position. C = number of caudal fin rays, PL = number of pelvic fin rays, PC = number of pectoral fin rays, LS = number of lateral line scales + number of scales on caudal fin base. TS = number transversal scales. CS = number of scales around caudal peduncle, PS = number of pre-dorsal scales

	Holotype	All types mean (SD)	Median	All types range
D	13	13.0 (0.4)	13	12–14
A	16	17.0 (0.8)	17	16–18
D/A	5	4.2 (0.8)	4	3–5
C	27	26.9 (0.9)	27	26–28
PL	6	6.0 (0.0)	6	6
PC	13	12.8 (0.7)	13	12–15
LS	28	28.5 (1.4)	28.5	26–30
TS	7	7.7 (0.5)	8	7– 8
CS	12	11.7 (0.5)	12	11–12
PS	14	15.6 (1.0)	16	14–17

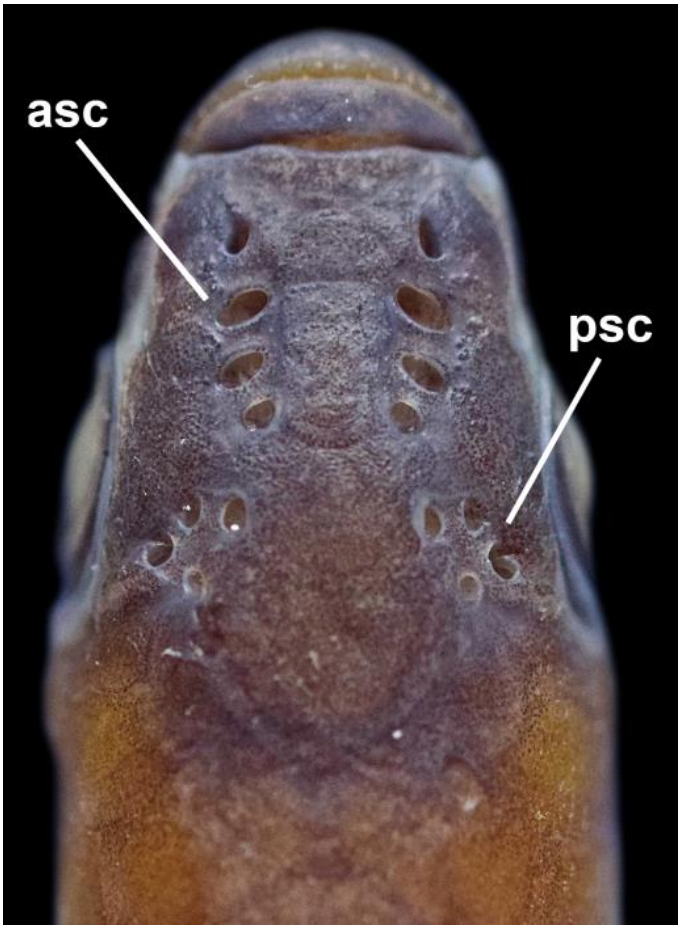

Figure 5-6:*Plataplochilus eliasi*, head dorsal view. Abbreviations: asc, anterior portion of supraorbital canal; psc, posterior portion of supraorbital canal.

Osteology. Only informative characters illustrated (**Figure 5-3**). Mesethmoid absent. Vomer V-shaped, overlapping with parasphenoid anterior region ventrally, and with the lateral ethmoids laterally. Epiotic process short, not extending beyond supraoccipital process. Premaxilla alveolar arm, anterior portion forming a squared process. Maxilla dorsal and ventral processes developed overlapping premaxilla ascending processes. Dentary robust. On both dentary and premaxilla 3 to 4 rows of unicuspid, posteriorly directed small teeth. Teeth of outer row slightly larger. Anguloarticular anterior process pointed. Retroarticular triangular. Sympletic deep. Autopalatine dorsoposterior process directed upwards. Supracleithrum elliptical. Cleithrum dorsoposterior border broad and posteriorly elongated. Lachrymal rectangular and anteroposteriorly elongated. Second pharyngobranchial 5-7 teeth. Third

pharyngobranchial wide, with well-developed dentigerous plate containing large claw-shaped teeth. Fourth pharyngobranchial tooth plate with few small conical teeth. Anterior portion of fifth ceratobranchial about the same width of anterior portion of fourth ceratobranchial. Fourth ceratobranchial anterior third or fourth covered with teeth. Hypurals fused but with a median longitudinal aperture in the anterior region of the compound caudal centrum. Total vertebrae 30, 12 precaudal and 18 caudal. First proximal radial of dorsal fin in a vertical to neural spine of vertebrae 11 and 12. First proximal radial of anal fin in a vertical to pleural rib of vertebrae 10 and 11. Gill rakers on first branchiostegal arch 9–11. Branchiostegal rays 5

Colouration after 4 years in Ethanol. Males (**Figure 5-2A**). Dorsal region of body brownish, top two scale rows with dark edges. Flanks below these rows light greyish brown with a prominent crescent shaped dark grey to black band reaching from lower jaw to pectoral fin insertion; grey along the middle portion of body, just after pectoral fin insertion to end of anal fin base; and black from region posterior to anal fin base to caudal peduncle. Pre-orbital area and sub-orbital rim black. Centre of operculum black. Head ventral region and throat light yellowish brown. Unpaired fins hyaline, with minute black chromatophores scattered over fins membranes. Dorsal fin, posterior rays tip black. Pectoral fin hyaline. Pectoral fin base, dark brown to black. Pelvic fins yellowish brown.

Females (**Figure 5-2B**). Body predominantly light brown with darker dorsum. The crescent shaped dark pigmentation is shorter and inconspicuous, extending from region posterior to pectoral fin insertion to caudal peduncle. Lower jaw and pre-orbital region dark black, and sub-orbital rim, opercle and pectoral fin insertion yellowish brown. Unpaired fins with small black spots, lacking at base of fin. Paired fins hyaline.

Colouration of live specimens. Males (Figs. 4A&B). Dorsum and anterior dorsal part of flank, from region just posterior to head to dorsal fin origin, brownish to olive green; posterior region of flank silver. Head dorsal region dark brown, posterior region light brown and with a bright

cupric blotch. Opercle dark black with metallic bright blue chromatophores. Snout, pre-orbital region and sub-orbital rim dark black, scattered with metallic bright blue minute chromatophores. Branchiostegal appendages bright yellow. Ventral region of head whitish grey. Ventrum bright blue from opercle posterior margin to caudal peduncle. Ventral portion of flank dark metallic blue with a broad submarginal black band from origin of anal fin to end of the caudal peduncle. Pectoral fins hyaline, pelvic fins yellow to orange. Dorsal fin base with a yellowish-orange band, narrow anteriorly becoming wider posteriorly, followed by a blue-grey band edged distally with a narrow black band, distal edge of fin hyaline. Anal fin with narrow light bright blue-grey band at base, followed by a wide yellow band, edge of fin grey. Caudal fin grey with blue to black centre inter-radials, narrow red to orange ventral and dorsal edges. Pelvic fins yellow. Pectoral fin hyaline.

Females (Figure 5-4**C**). Dorsum dark grey. Dorsal part of flanks brownish, ventrally whitish with vague dark grey band anteriorly. Dorsal fin orange-red with narrow hyaline band at base and broad hyaline outer edge. Anal fin hyaline with orange-red band just distally of midline of fin. Caudal fin hyaline with orange-red dorsal and ventral edges. Pelvic and pectoral fins hyaline. Females have less distinctly darkened scale margins compared to males, or darkened scale margins even absent.

5.4.1 Distribution

Plataplochilus eliasi, is only known from the Bondo River catchment, a left bank tributary of the Noumbi River basin (**Figure 5-1**), itself part of the Ogooue-Nyanga-Kouilou-Niari Freshwater Ecoregion in the Lower Guinean ichthyofaunal province (Freshwater Ecoregions of the World, 2020; Stiassny et al., 2007). Despite extensive collections made during a number of surveys throughout the region, the new species is known from only three localities (**Figure 5-1**) south of the village of Bamba in the upper Bondo River catchment, in the Republic of the Congo. The first author collected *P. eliasi* only in the upper and middle Bondo catchment in

August 2016 and June 2017. Other expeditions were carried out at many locations south of this area in May 2012 and April and May 2014, however, without sampling the new species. Records from RMCA show that G. Teugels and V. Mamonekene collected east and south-east of Bamba in August 1991, whereas L. Chirio (personal communication, 2020) collected north and south of the range of *P. eliasi* in March 2019, sampling several *Plataplochilus* species but not *P. eliasi.* The area west of the Bondo catchment has never before been sampled.

5.4.2 Habitat and ecological notes.

Plataplochilus eliasi occurs in very shallow clear water creeks (<0.2m) over diverse substrate types consisting of boulders, cobbles and pebbles with *Anubias* sp. and overhanging vegetation (**Figure 5-7**). The species appears to have a strong preference for fast flowing stream sections on steep slopes. *Plataplochilus eliasi* specimens were only collected in 1st to 3rd order streams, despite many sampling attempts undertaken at similar longitudinal intervals down the course of the system to its coastal plain. *Plataplochilus eliasi* was replaced by *P.* cf. *cabindae* more downstream, after the river becomes a 4th order system with a lower slope and sandier substrate.

The two above mentioned species have a small syntopic overlap between the transition of 3rd to 4th order reaches of the Bondo River, however, they appear to have a distinct habitat partitioning between the various longitudinal zones of the river, with *P. eliasi* occurring at slightly higher elevations in the basin, between 67–207 m asl. In the wet season, *P. eliasi* was sampled downstream in the system, in a 3rd order section of the Bondo River, whereas it was only sampled in typical 1st and 2nd order creeks in the dry season. Despite sampling similar environments in a neighbouring headwater tributary of the Bondo River, the species was not found. This suggests *Plataplochilus eliasi* is distributed over a small area that is restricted to

headwater tributaries of the Noumbi River, which mostly occur outside of the Conkouati-Douli National Park.

The following syntopic species were collected with *Plataplochilus eliasi*: *Phractura brevicauda* Boulenger, 1911, *Aphyosemion escherichi* (Ahl, 1924), *Epiplatys* aff. *ansorgii* (Boulenger, 1911), and *Chromidotilapia* cf. *mamonekenei* Lamboj, 1999. The fish, as well as the freshwater shrimps, prawns and tadpoles, are housed in the American Museum of Natural History collections. The system had a high diversity of Odonata and Ephemeroptera larvae.

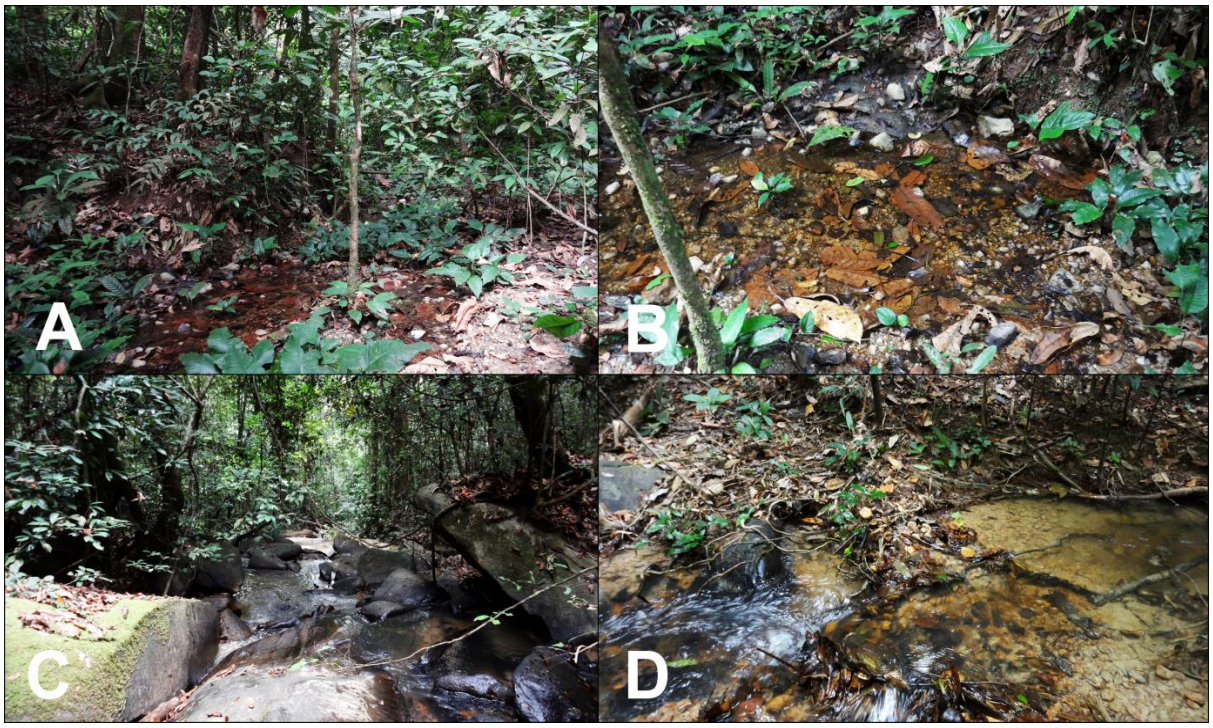

Figure 5-7: *Plataplochilus eliasi* habitat: A,B, 1st order shallow creek within dense forest at 207 m asl, Bondo River system, Republic of Congo (Field number - BVT4-1), 3 August 2016; C,D, 2nd to 3rd order stream at 130 m asl, Bondo River, Republic of Congo (Field number - BVT4-2), 4 August 2016.

Etymology. In the naming of this species, we extend the sentiment of the Congolese tradition of the burying of a new-born baby's placenta under a large tree in the child's village of birth, representing a wish for growth, wisdom and strength for the child's soul. The species is named for Elias Charly Arnaud Robert de Blic, a child no longer physically here, but remembered in this small fish in a forest stream, a space that is very much living and alive.

5.5 Discussion

Until now, only nine *Plataplochilus* species have been described, and although many expeditions were conducted by scientists and aquarists in Gabon, no new *Plataplochilus* species were described since 1981, probably due to a lack of interest in this particular genus because of its complexity and the difficulty in keeping living strains in aquaria. Another main reason for the long period without the description of new *Plataplochilus* species, a genus from which the colour pattern is highly informative and critical for its taxonomy, is the poor fixation of pigments after preservation. In preserved *Plataplochilus* only melanophores remain for longer periods and the red pigments disappear within months, leading to many misidentified individuals in ichthyological collections.

In the past two decades, however, expeditions were often well documented with field pictures, specimens being sampled for scientific purposes, and a greater success achieved in transporting specimens alive to Europe and the US. This has allowed the recognition of a much greater and still undescribed diversity for the genus. Recently, *P. eliasi*, was sampled within a much broader expedition focusing on the ecology of coastal Congolese systems, specimens were photographed alive in the field and preserved for research. Through the analysis of external morphology and colouration pattern it was possible to find evidence supporting it as belonging to a new species. *Plataplochilus eliasi* was distinguished from all other congeners by the male's colouration pattern that consist of a brown to olive green anterodorsal region of the flank, and by the presence of a distinct crescent shaped dark grey to black band reaching from lower jaw to pectoral fin insertion; grey along the middle portion of body, just after pectoral fin insertion to end of anal fin base, and black from region posterior to anal fin base to caudal peduncle (Figure 5-4).

In the present study, for the first time, osteological information from *Plataplochilus* is presented revealing characters apparently uniquely shared by *Plataplochilus* species. These characters are: anterior portion of alveolar arm of premaxilla developed in a squared process

(polymorphic in *P. ngaensis* that presents also a small indented process); rectangular and anteroposteriorly elongated lachrymal; dorsoposterior process of autolapalatine directed upwards; elliptical supracleithrum; dorsoposterior border of cleithrum broad and posteriorly elongated; presence of upper lobe extension in the caudal fin of males (absent in *P. terveri*, *P. loemensis* and *P. cabindae*) (**Figure 5-3**).

Within all examined *Plataplochilus* species, the pectoral fins are inserted low on the flank, contrasting with Huber's (1982) statement to the contrary. In addition, the presence of closed anterior and posterior portions of the supraorbital system is shared by all *Plataplochilus* species and absent in *Procatopus*, and is here considered as a useful character in distinguishing both genera. Further, the ctenoidy in the head scales of all *Plataplochilus* species was confirmed, and a similar condition was not confirmed in *Procatopus*. Here we agree with Huber (1982) on the presence of branchiostegal appendages in some *Plataplochilus* species, such as *P. eliasi*, *P. cabindae*, *P. mimus* and *P. pulcher*. However, the branchiostegal appendage is much more elongated and pointed in *Procatopus*, whereas in the aforementioned species it is rounded and only slightly longer than the opercle. Thus, the examination of external morphology, osteology and genetics (Bragança & Costa, 2019), supports *Plataplochilus* as a valid genus.

We expect that in the following years, new *Plataplochilus* species will be described. Recently Eberl, Litz & Pfleiderer (2020) conducted a detailed study of *Plataplochilus* species in north-west Gabon and discovered several undescribed species. Further, recent expeditions suggests a much more diverse *Plataplochilus* with new species to be described from the lowlands of western Gabon and Congo (Chirio, pers com). Ongoing research in the taxonomy and systematics of *Plataplochilus* will provide relevant data for species conservation and in understanding its biogeographical and diversification patterns.

5.6 Acknowledgements

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Chapter 6: Riparian forest fuels aquatic food webs along the continuum of an Afrotropical coastal river in central Africa

6.1 Abstract

Knowledge of trophic diversity and key resources driving consumer communities across natural river gradients is critical for the conservation and maintenance of freshwater biodiversity and ecosystem provisioning services. In under-studied regions such as the wet Afrotropics in central Africa, information on trophic diversity and freshwater consumer resource use is extremely limited. Here we used carbon and nitrogen stable isotope ratios ($\delta^{13}C$, $\delta^{15}N$) from samples collected along a continuum of a coastal river in the Republic of the Congo to show temporal and spatial patterns of trophic diversity and basal carbon resource use. We populated assemblage-wide Bayesian metrics, scaled isotopic metrics, and mixing models along a fluvial gradient which included Strahler orders in dry and wet seasons to understand patterns in trophic diversity and basal carbon resource. Our results showed patterns deviating from widely accepted lotic models where headwaters exhibited relatively high trophic niche spaces in comparison to larger downstream sites. At a assemblage level, riparian C3 vegetation is primarily fuelling the riverine food web in this forested, seasonal Afrotropical system. However, we also noted highly depleted carbons signatures in some fish and macroinvertebrate consumers which were not accounted for in the resource contribution of the mixing models, and which potentially originates from methane-derived sources. Further research is required to identify the key source and importance of this methane derived resource to food webs in the Afrotropics.

6.2 Introduction

Freshwater ecosystems produce substantial resources for human use (Lynch et al., 2020) and are under major pressure globally, with 28% of the freshwater species threatened (Tickner et al., 2020; IUCN, 2022). Tropical ecosystems support highly diverse fish and macroinvertebrate assemblages with complex, seasonally shifting resources, supporting their food webs (Pease et al., 2019, 2020; Soto et al., 2019). Understanding of the trophic diversity and basal resources fuelling these complex food webs is crucial to 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., 2020). Existing knowledge of trophic diversity and seasonal resource use in consumer communities of Afrotropical freshwaters is scarce (Winemiller et al., 2011; Thompson et al., 2012; Sponseller et al., 2013; Venarsky et al., 2020), and despite an increasing knowledge on the fish diversity and functional ecology of the region (Munene and Stiassny, 2011; Mamonekene and Stiassny, 2012; Walsh et al., 2014, 2022; Cutler et al., 2019; Stiassny et al., 2021), literature on food web dynamics in freshwater ecosystems in the central African tropics is almost entirely absent in literature, with just a single study of the Congo River published to date (Soto et al., 2019).

River gradients and seasonality affect aquatic food webs through changes in availability of basal carbon resources which subsequently shape consumer assemblages (Thorp et al., 2006; Winemiller et al., 2011). Various conceptual models have been developed to explain flows of carbon in riverine food webs. For example, the River Continuum Concept (Vannote et al., 1980) proposes a longitudinal import of carbon from the upstream processing of allochthonous sources in higher order rivers, and the Flood Pulse Concept (Junk et al., 1989) suggests significant lateral carbon inputs from floodplains after seasonal hydrological pulses. In contrast, the Riverine Productivity Model (Thorp and Delong, 1994) proposes that primary carbon sources stem mostly from local algal and riparian primary producers as opposed to processed material imports from upstream.

More recently theories such as the Riverine Ecosystem Synthesis (Thorp et al., 2006) and the River Wave Concept (Humphries et al., 2014a) incorporate spatial and seasonal considerations, proposing that different basal resources are important at different positions in the river continuum and hydrograph. According to the River Wave Concept, during the dry season local inputs from riparian and aquatic plants are likely to support the rivers (Thorp and Delong, 1994), where longitudinal import of basal carbon are increasingly important under decreasing flows (Vannote

et al., 1980). At high flows the river is likely to function under the principals of the Flood Pulse Concept, receiving lateral inputs from adjacent floodplains and swamps (Humphries et al., 2014a).

Except for the Flood Pulse Concept, these theories have largely been modelled on large, temperate lotic ecosystems, and their ability to predict basal carbon resource importance in tropical systems is a topic of continuing debate. Some studies of tropical rivers in the Neotropics and Australasia argue that tropical systems are primarily fuelled by autochthonous production such as algae and biofilm due to ease of enzymatic digestion (Jepsen and Winemiller, 2007; Dudgeon et al., 2010; Brett et al., 2017; Reis et al., 2020; Roussel et al., 2021) and lower availability of stream macroinvertebrates which grow slowly due to feeding primarily on leaf litter (Guo et al., 2016). Other work in forested basins of the Neo- and Afrotropics indicate that consumers obtain the majority of food resources from allochthonous production associated with abundant riparian C3 sources, conforming more the River Continuum Concept (Coynel et al., 2005; Soto et al., 2019; Roussel et al., 2021), and that coastal basin communities in Congo are strongly shaped by resource availability (Walsh et al., 2022b). Algal and biofilm resources are also noted as relatively inconspicuous and less important in supporting the consumer assemblages in nutrient poor tropical systems (Correa and Winemiller, 2018c) due to leaching tannins from riparian leaves limiting light infiltration and productivity (Moreira-Turcq et al., 2003). More recently methane oxidizing bacteria have been identified as a potentially significant producer source (Soto et al., 2019).

Based on these concepts it is possible to speculate on changes to trophic diversity along fluvial continuum (Vannote et al., 1980; Power and Dietrich, 2002; Hoeinghaus et al., 2007a; Pease et al., 2019). In small headwater streams less light penetrates the canopy of the riparian forest which results in a decrease in productivity and large inputs of homogenous food resources 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 harbour more generalist consumers (lower

trophic diversity) with shorter food chain lengths (Horwitz, 1978; Vannote et al., 1980; Woodward and Hildrew, 2002). Larger river systems have increased habitat volumes, productivity, and higher resource diversity which is expected to result in higher trophic diversity, more consumer specialists (Thorp et al., 2008) and longer food chains (Power and Dietrich, 2002; Thorp et al., 2006).

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 increased allochthonous detritus, macroinvertebrates, and nutrients to rivers thus increasing primary production (Winemiller et al., 2004). Densities of aquatic consumers increase over the wet period due to increased productivity and resource diversity, causing an increase in omnivorous aquatic species which reach their highest densities at the end of the wet season (Junk et al., 1989). As waters recede, predator-prey interactions are expected to increase 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 level causing a reduction in the condition of consumers. Conversely, optimal foraging theory predicts that species will have more restricted and specialised diets when increased resources are available and consumers are able to choose their food, but will expand the diversity of food types during periods where food is scarce (Perry and Pianka, 1997). Trophic position 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 likely from upstream drift 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 communities, and the relative contribution/importance of basal carbon resources for consumers along a longitudinal and seasonal gradient of a coastal basin in the Republic of the Congo. We expected to see **(1)** more generalists and shorter food chains in lower order streams than in higher order rivers as diversity of food resources increases along the continuum (Vannote et al., 1980), **(2)** a shift from high trophic positions in the consumer communities during the dry season to a lower trophic positions in the wet season due to increased resources and less constrained habitat in high flows, and **(3)** a shift in consumer resource use from local riparian material in the dry season to imported upstream organic material in the wet season. To address these questions, we used a combination of assemblage-wide Bayesian metrics, scaled isotopic metrics, and mixing models on data collected along a river continuum in dry and immediately post wet seasons.

6.3 Materials and Methods

6.3.1 Study region and site selection

We sampled fishes, aquatic macroinvertebrates, and conspicuous food 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 left bank tributary of the Noumbi, is an oligotrophic stream situated in the coastal plains of the Kouilou Department in the Lower Guinea forest (**Figure 6-1**). 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 (**Figure 6-2**) (van Rooyen et al., 2016).

Sampling was carried out during two field surveys to account for seasonality in August 2016 (the end of the c.3 month long dry season) and May/June 2017 (the end of the wet season where the river 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 (**Figure 6-1 & Figure 6-2**) to include consecutive stream orders to capture changes in food webs and basal carbon resources along the longitudinal course of the river and 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 - 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).

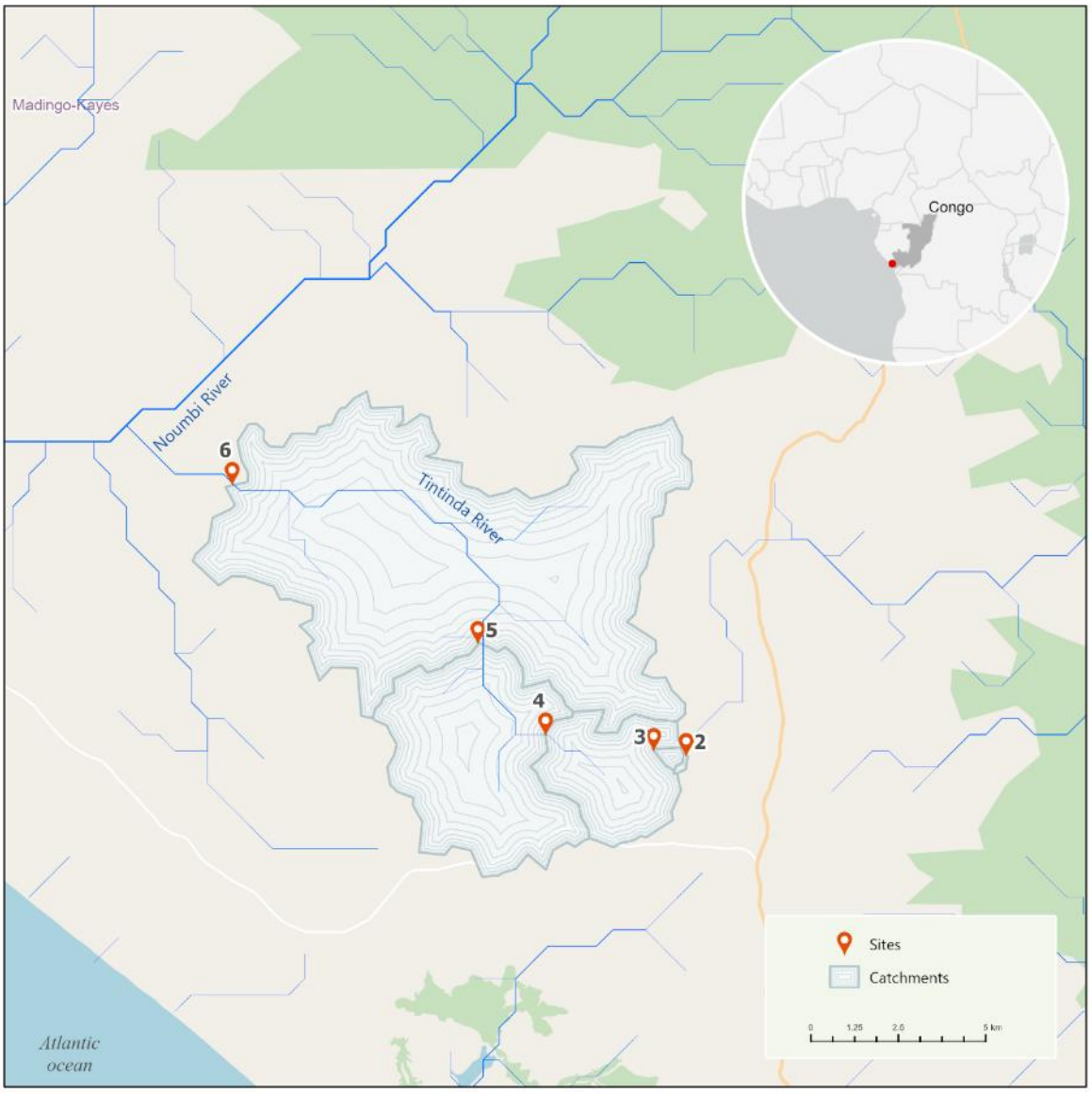

Figure 6-1: Study area from second (Site 2) to sixth (Site 6) Strahler Order along the continuum of the Tintinda River in the Republic of Congo.

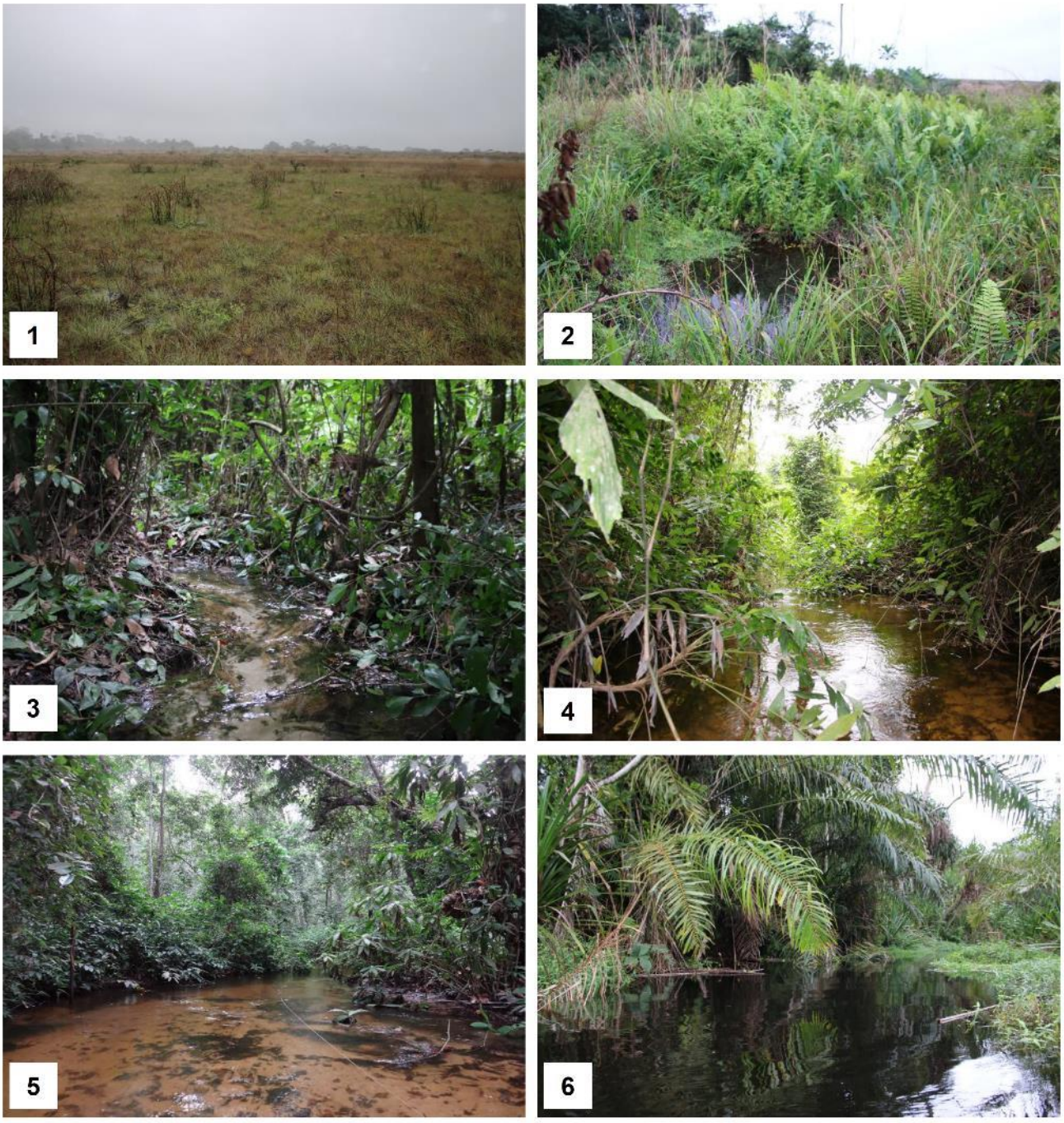

Figure 6-2: Sites on the Tintinda River from second (2) to sixth (6) Strahler Order along the continuum fluvial gradient. First order sites (1) situated in the grassland-savannah vegetation zones of the Tintinda River were dry in both seasons.

6.3.2 Sample collection

Environmental data at each site included landscape and local measures shown in **Table 6-1**. Catchment area (km^2) which include the polygons corresponding to all areas that drain to the studied sites (nested) 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, depth (m) and flow velocity 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 hauls, cast nets, dip-nets, experimental gill nets, fyke nets, and backpack electro fisher. 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 species representing all trophic levels were collected. Fish 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 MS-222 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 (dry season n = 105; wet season n = 104). Samples were collected from two basal carbon resources for stable isotope analysis: (1) Riparian and aquatic plants (C3 Riparian and Aquatic) were sampled from the most abundant plant species including samples of riparian tree leaves and instream and 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 inconspicuous and highly limited and were thus excluded in analyses. Three replicate samples were collected from all potential food sources. All isotopic samples of consumer and food sources were preserved in the field using NaCl following Arrington and Winemiller (2002) .

6.3.3 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 and Thorp, 2014). Dry samples were ground into a powder and placed into Ultra-Pure elemental tin cans and weighed on a five-place decimal Mettler Toledo - XP6 balance. Animal material (aquatic macroinvertebrates and fish) was weighed between 0.4 – 0.5 mg, while plant material (FPOM, CPOM and vegetation) was weighed between 0.8 – 1 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 iThemba Laboratory for Accelerator-Based Sciences isotopic laboratory 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 replicates of 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 the $R = {}^{13}C/{}^{12}C$ for carbon and ${}^{15}N/{}^{14}N$ respectively.

6.3.4 Statistical methods

6.3.4.1 Trophic structure

We first used stable isotope ratios of $\delta^{13}C$ and $\delta^{15}N$ for fish species to calculate isotopic community metrics to assess shifts in trophic position of fish assemblages along the river continuum for each season. Preceding all isotopic analyses arithmetic corrections were applied to adjust $\delta^{13}C$ values to account for $\delta^{13}C$-depleted signatures from lipids. 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) as per Post et al. (2007).

We used the SIBER package (Jackson et al., 2011a) in R studio v 4.1.3 (RStudio Team, 2021) to calculate trophic structure of fish assemblages using assemblage-wide metrics (Layman et al., 2007; Jackson et al., 2011b). The $\delta^{13}C$ range shows the extent of producer resources in the food web that support aquatic consumers (fish and macroinvertebrates). Total Area 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 $\delta^{13}C$-$\delta^{15}N$ isospace (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) were also calculated to give an estimate of the total trophic niche space occupied (Jackson et al., 2011a).

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:

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

6.3.5 Isotopic indices

Isotopic diversity indices differ from isotopic community metrics calculated above are mathematically independent of the number of organisms analysed and account for the abundance of organisms. Isotopic indices are complementary to stable isotope metrics previously developed in that they add multidimensional, abundance-weighted and unitless indices to the toolbox of stable isotope ecologists. Isotopic diversity metrics were calculated per site based on stable isotope values of fish using scaled $\delta^{13}C$ and $\delta^{15}N$ data (corrected by centroid values) to allow for comparisons between sampling sites with different isotopic baselines (Cucherousset and Villéger, 2015). The convex hull of the isoplot in scaled isotopic space was calculated to measure isotopic functional diversity or isotopic richness (IRic). Low IRic can reflect a reduced number of species or a loss of functional positions in the fish assemblage. Stable isotope metrics do not usually take species abundance into account, weighting rare and common species equally (Rigolet et al.,

2015). We therefore also calculated abundance-weighted isotope metrics for fish using the Isotopic Dispersion (IDis), Isotopic Divergence (IDiv) and Isotopic Uniqueness metrics (IUni) - (Cucherousset and Villéger, 2015).

These metric outputs range from 0 to 1, where low values for IDis suggests packing of organisms in a small isotopic space near the centre of gravity. Higher IDiv values indicate increased variation in trophic ecology in the food web when organisms with the most extreme stable isotope values dominate the food web (Villéger et al., 2008; Cucherousset and Villéger, 2015). Isotopic uniqueness is zero when communities are composed of species that have similar diets and tends toward one when most have isotopic values that are different from other species.

We performed linear regressions to understand the relationship between isotopic metrics and environmental metrics along the river continuum using the car package (Fox and Weisberg, 2019)in the R studio v 4.1.3 (RStudio Team, 2021). Functional measures were used as response variables, while environmental variables (**Table 6-1**) were used as the predictor variable.

6.3.6 Mixing models

Finally, we calculated the relative contribution of resources to the consumer assemblage (fish and macroinvertebrates) by using $\delta^{13}C$ and $\delta^{15}N$ values of consumer tissues. We used individual mixing models per site and season in the R package MixSIAR (Stock and Semmens, 2016) to ascertain how the proportion of **(1)** C3 Riparian and Aquatic vegetation and **(2)** instream POM (FPOM and CPOM) is consumed by fish and macroinvertebrate communities. MixSIAR is a Bayesian mixing model used to estimate the probability of the contribution of each resource to consumer diet, whilst incorporating variation in the isotopic signatures of consumers and sources and trophic fractionation (Parnell et al., 2013; Correa and Winemiller, 2018c). Sample size was sufficient for model convergence using a “normal” run (100 000 chain length, 50 000 burn, 50 thin with 3 chains) based on Gelman-Rubin and Geweke tests (Stock and Semmens, 2016).

Trophic enrichment factors (TEF) for herbivorous fish were used to incorporate differences in tissue concentrations of $\delta^{13}C$ and $\delta^{15}N$ caused by variation in the protein content of resources consumed. We used a TEFs of 4.08 ± 0.14 for $\delta^{15}N$ and 0.1.75 ± 0.15 for $\delta^{13}C$ for plant-based resources (German and Miles, 2010; Correa and Winemiller, 2018c).

6.4 Results

6.4.1 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 major 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 m3/s between the dry and wet seasons, respectively (**Table 6-1**). Although measured discharge is 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 declined due to cessation of c. 6 month long 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 - 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. The water was clear in both seasons but showed a decrease in dissolved oxygen concentration in the wet season.

Table 6-1: 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 (m3/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

6.4.2 Trophic structure and community-wide metrics

Overall fish assemblages contained an increased 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 macroinvertebrates and fish resources during the wet season (**Table 6-2**). We recorded an increase in high trophic level consumers in the fish assemblage along the continuum in the wet season, indicating an increased use of animal sources in fish diets from 3rd to 6th order sites. An inversed trophic trend was seen at the small 2nd order headwater site, where the fish assemblages fed at higher trophic levels in the dry season, likely consuming more animal material

in the dry season than in the wet season. The small 2nd order stream presented proportions of trophic positions that were comparable to larger 4th-6th order systems in the dry season.

Table 6-2: Percentages of trophic positions for fish assemblages along the continuum of the Tintinda River in the dry and wet seasons.

	Trophic Position (Fish)	2nd Order Dry	2nd Order Wet	3rd Order Dry	3rd Order Wet	4th Order Dry	4th Order Wet	5th Order Dry	5th Order Wet	6th Order Dry	6th Order Wet
Low	1.5-1.99		1.4%	8.6%		2.0%					
	2-2.49		1.4%	17.2%		2.0%		1.9%		8.8%	
	2.5-2.99		12.5%	24.1%	16.3%	6.0%		20.4%	10.3%	36.3%	
	3-3.49	5.6%	40.3%	37.9%	27.9%	18.0%	9.8%	45.4%	21.5%	35.0%	20.3%
	3.5-3.99	22.2%	43.1%	12.1%	32.6%	56.0%	36.6%	24.1%	42.1%	15.0%	52.2%
	4-4.49	62.5%	1.4%		23.3%	16.0%	17.1%	8.3%	17.8%	5.0%	18.8%
	4.5-4.99	9.7%					36.6%		7.5%		8.7%
High	5-5.49								0.9%		

No significant linear relationships between order, distance from source and upstream catchment area in relations to the assemblage-wide metrics were noted, however we did observe some general patterns in relation to gradient and trophic structure. Dry season sites showed an overall linear increase in resource diversity based on assemblage-wide metrics measured in $\delta^{13}C$-$\delta^{15}N$ space with an overall linear increase in TA, SEAc and CD from 2nd – 6th order sites– **Table 6-3** and **Figure 6-3**. Wet season sites were comparable in TA, SEAc and CD values along the river continuum, with similar use of resources at all sites. Small headwater sites (2nd and 3rd order) had very similar values for these metrics to 4th-6th order, indicating comparable resource use breadth comparable to larger sized sites. Assemblage-wide metrics across seasons were comparable between seasons, with similar overall ranges.

Trophic redundancy decreased marginally along the continuum in the dry season from (lower MNND scores in 2nd and 3rd order sites compared to 6th order), where trophic redundancy remained relatively consistent along the continuum in the wet season survey. An overall decrease in the distribution of trophic niches (SDNND) was found along the continuum in the dry season,

with an increase in the wet season suggesting that fish diets are more constrained at lower discharges with fish feeding on similar food types, broadening their diets, and decreasing overlap of resource use in the wet season. Total Area followed similar patterns with a longitudinal increase in the dry season, and a decrease in the wet season with high TAs at 2nd order sites in comparison to larger sites (5th order).

Table 6-3: Assemblage-wide metrics (fish community) calculated using isotope data at each study site along the river continuum for each season. TA = total area convex hull, SEA_C = Bayesian standard ellipse area, CD = mean distance to centroid, MNND=mean nearest-neighbour distance, SDNND = standard deviation of nearest-neighbour distance.

Site/Season	$\delta^{13}C$ Range	$\delta^{15}N$ 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

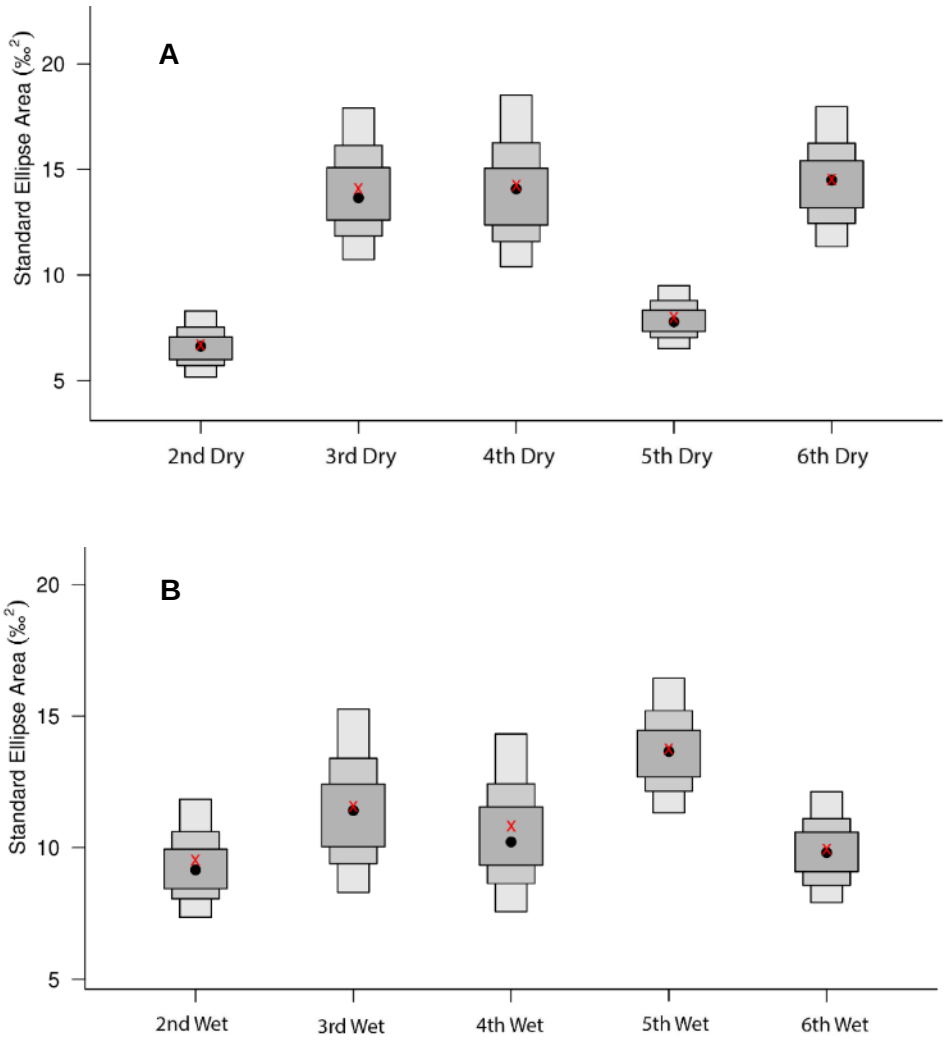

Figure 6-3: Box plots showing the position of the fish assemblages in the Tintinda River from 2nd-6th order sites for (A) dry and (B) wet surveys plotted with 95, 75, and 50% credibility intervals.

6.4.3 Isotopic indices and gradient patterns

Linear regressions between all isotopic index outputs (IRic, IDiv, IDis, IEve and IUni – **Table S1**) and environmental variables (**Table 6-1**) resulted in significant positive correlations for IDiv. The IDiv metric was positively correlated with river order and upstream catchment area in the dry season survey only, indicating an increased trophic diversity and specialisation with increased river size (**Table 6-4 -** P = <0.05). Details of the isotopic index outputs per site in each season can be found in **Table S1** in the supplementary material.

Table 6-4: Significant linear relationships linear between environmental variables and isotopic indices. IDiv = Isotopic Divergence, IPos = Isotopic Position.

Season	Index	Environmental measure	Slope	adjusted R^2	Pr (>|t|)	Relationship
Dry	IDiv	River Order	7.91	0.72	0.043	positive
Dry	IDiv	Log (catchment area km^2)	3.51	0.73	0.040	positive

6.4.4 Mixing models and basal carbon resource use

Based on the median values for consumers per site, the MixSIAR model predicted that POM had the largest contribution to consumer diet during both seasons at all sites along the river continuum with the exception of the 3rd order site in the dry season (**Figure 6-4A** and **B**). The POM resource component made up between 51.7 - 95.1% of basal carbon resource consumption at these sites over both seasons. Direct use of local riparian and aquatic vegetation resources was higher in 2nd and 3rd order headwaters in both seasons relative to the higher order (4th to 6th order) sites, suggesting that coarse local riparian material is a more important food web resource in small streams (**Figure 6-4A**). As the system increases in size (4th to 6th order), POM which is likely imported and processed material originating from upstream becomes more important as a basal carbon resource (**Figure 6-4B**). Whilst POM was the most consumed resource overall in both

seasons, local riparian and aquatic vegetation sources were relatively more important in the dry season (**Figure 6-4A**) and POM consumption increased marginally in the wet season.

The isoplots show an overlap between the standard deviations of the riparian and aquatic vegetation resource and the POM resource, indicating that POM is likely largely mostly derived from broken down riparian and aquatic plant material (**Figure 6-5**). Certain fish and macroinvertebrate consumers also appear to be feeding on resources that have very depleted carbon values (range of between -45 to -38 ^{13}C) and are not accounted for in the sampling or analyses of this study.

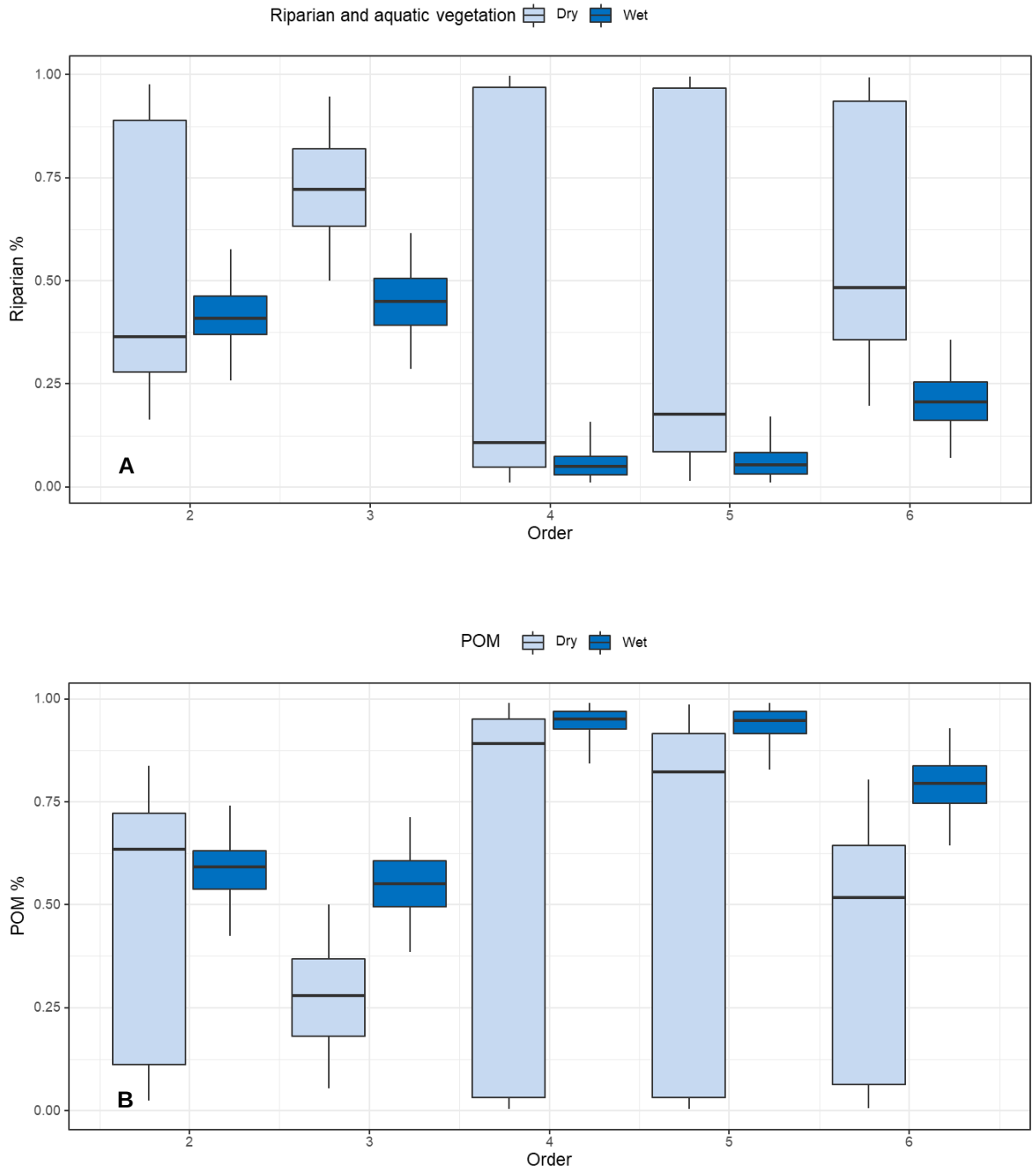

Figure 6-4: Estimated relative proportional contribution of two conspicuous food resources (A) Riparian and aquatic vegetation, (B) Particulate Organic Matter (POM) to fish and aquatic macroinvertebrate diets in the dry and wet season as shown by median values for resource contributions

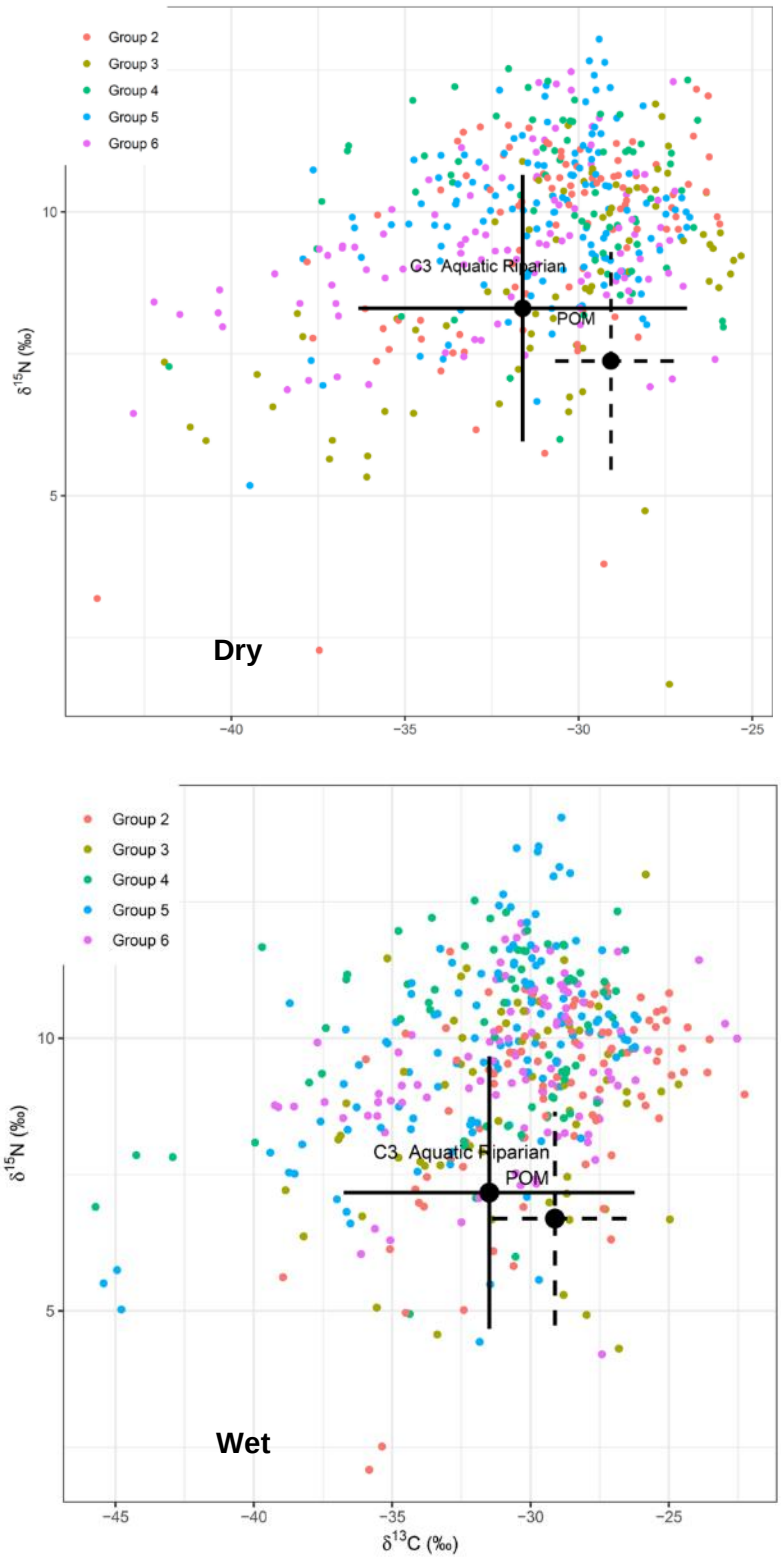

Figure 6-5: Isoplot of $\delta^{13}C$ and 15N stable isotope ratios of consumer assemblages (fish and aquatic macroinvertebrates) and two food resources (1) C3 riparian and aquatic vegetation and (2) POM from each site along the continuum of the Tintinda River for dry (n = 471 consumer tissue samples) and wet seasons (n = 452 consumer tissue samples). Group = Strahler Order. Values for consumers are adjusted for trophic fractionation.

6.5 Discussion

6.5.1 Trophic structure and community-wide metrics

Shifts in seasonal resource use by fish communities between small headwaters (2nd order) and other sites (3rd-6th order) along the continuum were observed. As our study area is situated in a wet tropical region with forested aquatic systems experiencing year-round flow, we expected habitat to be less constrained by seasonality, showing only a marginally increased trophic position in the wet season due to mobilisation of resources through the system (Junk and Wantzen, 2004; Abrantes et al., 2014; Humphries et al., 2014a). There was evidence for this in the 3rd to 6th order sites between the dry and wet season, where trophic position increased, and trophic redundancy plateaued. The lower trophic positions of local fish assemblages in the dry season suggest more frequent consumption of primary producers (plant and detrital material). Whereas expanding diversity of food intake in the wet season is likely due to increased availability of resources from a pulse in flows, mobilisation of resources and an increase in habitat availability (Zeug and Winemiller, 2008; Abrantes et al., 2014).

Cazzanelli et al. (2021) found this pattern in floodplain lakes of Mexico, where pulses in discharge in the wet season increased local runoff, mobilised POM, increased nutrients and microbial processing, and boosted productivity (Obermanna et al., 2009). Studies of Neotropical species, such as *Roeboides dayi,* show a dietary preference for aquatic invertebrates from drift in the wet season when this resource is abundant, moving their intake to fish scales during the dry season when there is more competition for this invertebrate resource due to habitat contraction (Peterson and Winemiller, 1997). The patterns observed in our study suggest that input from surrounding flooded forests and riparian fringes may have a significant seasonal effect on the trophic dynamics and stability of Afrotropical coastal systems, even in very small parts of the fluvial network.

An opposing pattern in trophic position in 2nd order headwaters suggests that stream size in the region truly constrain habitat and resources, even in regions with high annual rainfall.

Abundance and variation of resources decreases in the dry season, increasing starvation and carnivory, thereby increasing the overall trophic position of fish assemblages (Wantzen et al., 2002). Low spatial heterogeneity, discharge, and channel widths combined with higher seasonal variation in headwater tributaries constrain resource availability more in small streams than in larger, more stable higher-order streams (Poff and Allan, 1995; Wantzen et al., 2002; Thorp et al., 2006; Pease et al., 2019, 2020). Higher dietary specialisation and a decrease in species dietary overlap has been recorded in the dry season for fishes in numerous studies in the Neotropics (e.g., Zaret and Rand, 1971; Winemiller and Pianka, 1990; Pease et al., 2019). A similar pattern is observed in our study where the dry season appears to constrict habitat and resources more severely in headwaters than in higher order sites along the continuum, which is also supported by the higher trophic redundancy in the headwaters in the dry vs. wet season.

Trophic diversity in freshwater consumers is expected to increase along a fluvial gradient due to increased resource diversity associated with habitat heterogeneity (Vannote et al., 1980; Thompson and Townsend, 2005; Thorp et al., 2006) and in environments which receive imports of resources from adjacent catchments (Polis et al., 1997). Indeed, in the present study, the headwaters had tighter assemblage breadths and higher trophic redundancy in fishes. This agrees with studies of trophic ecology in other tropical regions where fish trophic diversity increased with habitat size and associated resource (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., 2014a) we would expect to see this pattern more pronounced in the wet season.

Unexpectedly, assemblage-wide niche breadths of fish communities in small headwater systems were comparable to the downstream reaches in larger parts of the river in the wet season, suggesting that they are as trophically complex as larger systems. These results agree with results from Walsh et al (in review – **Chapter 3**) which reported uniquely high functional richness of lotic sites with small upstream catchments in comparison to much larger

systems along the river gradient. That study suggested that paleo-biogeographic factors may drive the high functional richness of the region, which preserve ancient trophic niche differentiation due to historic stability at longer geological timescales compared to other regions (Tedesco et al., 2005b). Walsh et al. (2022b) also showed that increased trophic diversity in Congo coastal basins was associated with higher habitat heterogeneity and that functional strategies were strongly driven by trophic traits. The results of the current study underscore the importance of season and hydrology in trophic strategies along a river continuum showing that seasonality may play a important role in driving diversification of resources by freshwater consumer assemblages (Pool et al., 2017; Cazzanelli et al., 2021).

6.5.2 Isotopic indices and gradient patterns

An increase in trophic specialisation in fish communities (IDiv) along the continuum was expected for both seasons as increased habitat volume and heterogeneity generally fosters greater diversity of resources and thus trophic diversity (Vannote et al., 1980; Pease et al., 2012b) resulting in a longer food chains and more trophic specialists (Power and Dietrich, 2002). However, we only saw a significant positive relationship between gradient and IDiv in the dry season. Low order headwaters with smaller habitats, lower hydrological discharge and more riparian canopy cover receive higher inputs of riparian detritus (Horwitz, 1978; Vannote et al., 1980). Consumers of small streams are often generalist feeders (Woodward and Hildrew, 2002; Pease et al., 2019) and these assemblages may have shorter food chains than in high order streams. In larger streams, increased spatial heterogeneity in general supports greater diversity of resources of consumers, including more specialists feeding on resources that are isotopically distinct (Thorp et al., 2006). Lowe-McConnell, (1987) suggested that in tropical rivers, food resources for fish are more limited in the dry season as habitat volume declines. This could perhaps concentrate food resources making specialised feeding more accessible to trophic specialists and increasing the strength of this relationship in the dry season.

6.5.3 Basal carbon resource use

We observed a significant use of both coarse (riparian and aquatic vegetation) and fine (POM) allochthonous resources in the food web, with relatively consistent patterns of resource use between seasons that conformed to the River Continuum Concept (dry season - Vannote et al., 1980) and the Flood Pulse Concept (wet season - Wantzen et al., 2002; Junk and Wantzen, 2004). The shift in basal carbon use noted between dry and wet season indicates that the River Wave concept was a good conceptual predictor of the patterns in basal resource use in the system (Humphries et al., 2014a) with relatively increased use of local coarse riparian and aquatic resources in the dry season along continuum in the in the dry season, and an increase in the use of POM in the wet season with the increased flow from the extended increased precipitation in the wet season.

That terrestrial inputs are frequently the most important basal resource in freshwater tropical food webs (Hoeinghaus et al., 2007a) was corroborated in our study. Riparian C3 plants were also the predominant energy source for fish in a study of the Congo River and tributaries by Soto et al. (2019). There was a large overlap of $\delta^{13}C$ signatures between riparian and aquatic plants and POM resources, which suggests that POM is derived from riparian sources, and is imported from upstream processed riparian material likely originating in the headwaters of the river (2nd and 3rd order systems in this study). Terrestrial riparian sources are important for the food web and are likely 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.

We did not collect sufficient samples of biofilm or algae to understand their contribution to the food web, as this resource was inconspicuous 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 limited production and growth of biofilm. The food web of our study did however consist of consumers that had very depleted

$\delta^{13}C$ values which were well below the $\delta^{13}C$ values of basal resources (riparian and POM) collected. Soto et al., (2019) also recorded this phenomenon in tributaries of the Congo River, speculating that the depleted carbon isotopic variability in some consumers could originate from the consumption of microalgae or methane-derived Carbon.

In our study some aquatic invertebrates (Gomphidae, Libellulidae, Macromidae, Tipulidae and Pleidae and Atyidae families) and species of fish typically feeding on benthic insects, or living in swamps, were very depleted in ^{13}C (ranging between -45 to -38 ‰). In the dry season *Microctenopoma nanum* (Anabantidae), *Pelvicachromis subocellatus* (Cichlidae), *Petrocephalus microphthalmus* (Mormyridae), *Aphyosemion australe* (Nothobranchiidae) and *Channallabes apus* (Clariidae) showed very depleted signatures. These dry season specimens were predominantly captured from lateral swamp sections in the 6th order Tintinda River which has extensive swamp forest floodplain.

In the wet season after the system had experienced a pulse in hydrology, *M. nanum* (Anabantidae), *Neolebias ansorgii* (Distichodontidae), *Paramormyrops kingsleyae* (Mormyridae), *Marcusenius moori* (Mormyridae) and *Epiplatys ansorgii* (Nothobranchiidae) showed depleted ^{13}C values. These specimens were captured over a larger part of the lower continuum from 4th-6th order sites. The wet season specimens with depleted ^{13}C signatures were captured from both lateral swamps and the main stem river. This seasonal trend in depleted ^{13}C consumer signatures suggests that the lateral zone of the swamp forest is providing an additional basal resource potentially originating from rotting detritus within the swamps, thus expanding the base of the food web in the wet season when instream animals potential feed on methane-derived carbon. These depleted signatures were also observed in the Congo River study (Soto et al., 2019) suggesting that methane-derived carbon is an additional carbon resource more broadly in both large and small central African forested river basins. The reported isotopic signatures of methane-oxidizing bacteria were $\delta^{13}C = -47.5 \pm 2$ (Sessions et al., 1999; Whiticar, 1999). Specifically, oversaturation of dissolved greenhouse gases have been recorded in the Oubangui River in the Congo River basin, with highest levels

draining systems with riparian forest (Bouillon et al., 2014). Biogenic methane produced in anoxic detritus can enter the food web and support benthic and pelagic consumers (Deines et al., 2007; Sanseverino et al., 2012).

Our results indicate that although the Tintinda River appears to be primarily fuelled by forest allochthonous inputs of C3 vegetation, methane oxidizers could be a seasonally important source that is mobilised from anoxic detritus and lateral swamp zones in the wet season to augment basal carbon resources along the continuum of the river (see also Sepúlveda-Lozada et al., 2015; Soto et al., 2019; Cazzanelli et al., 2021).

6.6 Conclusion

We provide an insight into food web ecology and ecosystem functioning of an understudied Afrotropical freshwater region. We observed some conformity with the River Wave Concept (Humphries et al., 2014a), reflecting the importance of position and season in consumer trophic strategies along a river continuum, where seasonal variation in flow plays an important role in driving diversification of resources (Pool et al., 2017; Cazzanelli et al., 2021). We also corroborate studies in other tropical freshwaters which have elucidated the significance of riparian forest sources in directly supporting consumer assemblages (Wootton and Oemke, 1992; Pease et al., 2019) or through imported organic matter (Hoeinghaus et al., 2007a). Our current and previous work in the region (Walsh et al., 2022b; and Chapter 3 of this thesis), has shown that headwaters are distinctive in terms of trophic and functional diversity, but are also constrained by habitat size which makes them particularly vulnerable to anthropogenic impact. Larger parts of 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 cause resource homogenisation and reduction in available niches which have severe effects for a system which is primarily fuelled by riparian forest (Wilkinson et al., 2020).We also saw potential evidence that methanogenic pathways in lateral swamp forests may contribute to the freshwater food webs in larger parts of the river network in this region (see also Soto et al.,

2019; Cazzanelli et al., 2021) highlighting the importance of lateral swamp forest-river connectivity in subsidizing instream freshwater communities. Little research on inconspicuous carbon resources such as methane oxidising bacteria, and swamp forest-river carbon exchanges has been conducted to date, making it an important and relevant topic for future research and effective policy-making (Ramírez et al., 2008) based on the requirements of various international agendas such as the Convention on Biological Diversity's Aichi targets and the United Nations Framework Convention on Climate Change (Springgay et al., 2019; Lo et al., 2021).

6.7 Acknowledgments

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6.9 Supplementary material

Table S1: Isotopic indices calculated from scaled $\delta^{13}C$ and $\delta^{15}N$ data for fish assemblages per site in each season. IPos = Isotopic Position, IRic = Isotopic Richness, IDiv = Isotopic Divergence, IDis = Isotopic Dispersion, IEve = Isotopic Evenness, IUni = Isotopic Uniqueness.

	IPos $\delta^{13}C$	IPos $\delta^{15}N$	IRic	IDiv	IDis	IEve	IUni
2nd Dry	-0.38	0.41	12.80	0.46	0.27	0.62	0.33
3rd Dry	-0.20	0.47	20.29	0.48	0.30	0.63	0.81
4th Dry	-1.69	0.19	14.25	0.70	0.23	0.61	0.46
5th Dry	-0.40	0.68	15.92	0.86	0.57	0.54	0.46
6th Dry	0.35	0.42	23.40	0.77	0.35	0.59	0.16
2nd Wet	-1.18	0.06	9.53	0.41	0.14	0.36	0.23
3rd Wet	-0.84	-0.32	16.16	0.68	0.33	0.59	0.39
4th Wet	-0.82	-0.70	32.22	0.73	0.21	0.53	0.16
5th Wet	0.13	0.79	15.88	0.63	0.52	0.57	0.53
6th Wet	0.46	0.15	13.77	0.70	0.53	0.56	0.65

Chapter 7: Synthesis and conclusion

This chapter presents a synthesis of the research presented in empirical chapters **two through six**. The rationale for embarking on these regional ecological studies is two-fold. Firstly, a sound understanding of freshwater community assembly is required to develop regional strategies and policies to effectively manage and conserve freshwater ecosystems and their associated biodiversity, and Afrotropical freshwater ecology is poorly understood in comparison to its temperate counterparts (Dudgeon, 2008; Lo et al., 2021) Secondly, Afrotropical forest and freshwater ecosystems are under increasing pressure from anthropogenic disturbance (Barlow et al., 2018; Curtis et al., 2018; Tickner et al., 2020), with a critical need for increased system specific knowledge in light of accelerated global change (Tickner et al., 2020).

Broadly, my objectives in attempts to mitigate this knowledge gap included the design and implementation of multiple field studies in an poorly explored area of the wet Afrotropics to: **(1)** understand the mechanisms of fish assemblage structure along the fluvial gradient of a large Afrotropical basin, and to test predictions of key lotic conceptual models developed in temperate zones using functional-trait and diversity indices (**chapters two and three)**, **(2)** to describe fish species new to science from speciose and taxonomically problematic families in this poorly explored region (**chapters four and five)**, and **(3)** to identify key basal carbon resources and drivers of fish assemblage trophic structure along a river gradient between seasons (**chapter six)**. In this final chapter, besides synthesizing the findings of my thesis, I will highlight implications that address some future research needs in west central Africa.

7.1 Contributions

7.1.1 Functional diversity

My study has contributed to the base knowledge of an understudied Afrotropical freshwater region at different levels of biological organisation from species (**chapters four & five**) to ecosystem (**chapters two, three & six**), and uncovered unique patterns of regional functional diversity. Understanding the mechanisms underlying freshwater community assembly allows

the prediction of response to environmental change (Bower and Winemiller, 2019c). Mechanisms of stream fish community assembly are thought to be largely driven by environmental filtering (Weiher et al., 2011; Mouchet et al., 2013) and niche partitioning (Montana et al., 2014), with opposing evidence for support of temperate lotic concepts such as the River Continuum Concept (RCC - Vannote et al., 1980) and Riverine Habitat Template (RHT - Townsend & Hildrew, 1994) in the tropics (Greathouse and Pringle, 2006; Pease et al., 2012b; Córdova-Tapia et al., 2018). I collected environmental and fish assemblage data along a fluvial gradient in a large basin in the Republic of the Congo and used functional-trait and functional diversity metrics to understand how fish assemblages are structured along this gradient (**chapter two**). I also used these data to explicitly test tenets of the RCC, RHT and stress-dominance hypothesis (sdh – Coyle et al., 2014) in **chapter three**.

I demonstrated partial support for environmental filtering (RHT and sdh), and found unique patterns of functional diversity along the fluvial gradient. I showed that fish assemblages in Afrotropical headwaters have different functional strategies than lowland assemblages (**chapter two**) and, importantly, that headwaters harbour substantial functional fish diversity in relation to upstream catchment size and position in the basin (**chapter three)**. My results also indicate that in addition to mechanisms of fish community structuring such as environmental filtering, paleoclimatic and biogeographic events in the Pleistocene may have played a part in structuring fish assemblages at higher elevations in the region (Tedesco et al., 2005b). Previously, freshwater fish assemblages have been used to explore expectations in relation to environmental gradients (Pease et al., 2012b; Oberdorff et al., 2019), habitat structure (Hoeinghaus et al., 2007b; Teresa and Casatti, 2012) and environmental filtering (Mouchet et al., 2013; Carvalho and Tejerina-Garro, 2015; Bower and Winemiller, 2019). Whilst Ibanez et al. (2007) explored fish species assemblages and trophic groups along a gradient in Gabon, **chapters two and three** represent the first use of functional methods in west Central Africa to explore fish community assembly and test temperate-based assumptions at a basin scale.

7.1.2 Taxonomic diversity

Accurate reporting of biodiversity is key for data-based conservation and the discovery and description of new species remain central tasks for advancing foundational understanding of biodiversity and conservation needs, particularly in poorly documented regions (Stiassny et al., 2021). Widespread and taxonomically under-studied species are often resolved as divergent lineages with previously unrecognised taxa, underscoring the underestimation of freshwater diversity in Africa (e.g Sonet et al., 2018; Arroyave et al., 2019; Liyandja et al., 2022).

Central African ichthyology is a “black box”, with considerable research gaps in terms of discovery and descriptions of new species and their habitat associations. Numerous taxonomically undescribed fish species were discovered during my field work to collect fish assemblage and trait data. As part of this research, I was able to describe two of these; diagnostically splitting a West African congener (*Thysochromis ansorgii*) from the newly discovered Congolese species, *Thysochormis emili* (**chapter four**). In **chapter five** I describe *Plataplochilus eliasi* which represents the first new description within the genus since 1981 and shows how the constrained habitat and distribution of this species (and likely many others in the genus) increases its vulnerability to potential impacts. *Plataplochilus,* like so many other genera in the region, is inadequately sampled and numerous new species are awaiting formal taxonomic description, many of which are highly range restricted such as *P. eliasi* (pers comm. L. Chirio).

7.1.3 Trophic ecology

Trophic diversity and seasonal resource use in freshwater consumer communities in the central African tropics are poorly understood. **Chapter six** contributes to a limited body of knowledge on the trophic ecology of the forested wet Afrotropics, a huge region considered by only one other study to date (Soto et al., 2019). I show the importance of stream order and

season in consumer trophic strategies along an Afrotropical river continuum, and highlight the significance of riparian forest derived sources in directly supporting consumer assemblages.

The isotopic analyses in **chapter six** corroborate work in **chapters two and three** and indicate that headwaters are distinctive in terms of trophic and functional diversity from larger rivers, as measures of trophic diversity using stable isotope values were unexpectedly high in the smaller headwater habitats sampled. The isotopic research in this empirical chapter suggests that headwaters are primarily supported at the base of the food web by direct riparian litter and are likely constrained by habitat size, which makes them particularly vulnerable to anthropogenic impact. Larger parts of the river appear to be supported more by processed carbon sources originating from riparian forests of the headwaters, indicating the cumulative significance of headwaters in the overall functionality of the fluvial network. Perhaps most interesting are the questions that arose in this final part of the thesis, which suggest that consumer assemblages are potentially supported to some degree by methane generated from rotting leaf litter in adjacent swamp forests, stressing the importance of lateral riparian-swamp-river connectivity for freshwater functionality.

7.2 Implications

The results of my research have shown that regional freshwater conservation strategies require site-specific considerations that **(1)** are scale dependent, **(2)** incorporate riparian forest cover into their strategies, and **(3)** consider the position in the landscape and fluvial continuum in their approach. Additionally, **(4)** it has demonstrated that that regional fish diversity is currently greatly underestimated.

In **chapter two** I showed that the spatial distribution of fish assemblage structure in the Lower Guinean forest originates mainly from environmental filtering which suggests that conservation approaches should focus on the preservation of habitat heterogeneity at suitable spatial scales, that are site dependent (López-Delgado et al., 2019b). Additionally, the distinction between the influence of turnover and nestedness on assemblage structure of freshwater

communities is important to ascertain, as these mechanisms require opposing conservation approaches (Wright and Reeves, 1992). For example, I found that fish assemblages in headwaters at higher elevations were nested, and conservation efforts prioritizing a smaller area of the functionally richest systems would likely be most effective. In contrast, communities displaying high turnover such as those in the lower coastal basin would require conservation strategies that incorporate larger spatial areas and greater system connectivity (Baselga, 2010).

Besides scale and spatially dependent considerations for freshwater conservation, the relatively high fish functional richness at high elevations (**chapter three**) suggests that both contemporary and historical phenomena have shaped the fish assemblages in the headwaters of the Chaillu Massif range. The contribution of tropical rainforest mountain streams to the regional functional diversity appears to be potentially significant, and the inclusion of elevated rainforest streams in conservation planning in inland Congo would likely maximise retention of functional richness in fish communities regionally.

The discovery of new (**chapter five**), and cryptic species from divergent lineages (**chapter four**) in the study area illustrates a paucity in regional fish surveys and studies, and a likely high number of unreported species. The region's biodiversity appears to be partially driven by vicariance events during the Last Glacial Maximum (**chapter three**) resulting in a large number of cryptic lineages/species. Future ichthyological studies will need to explore new genomic techniques, combined with geometric morphometric techniques to uncover divergent lineages and cryptic diversity, describe new species, and incorporate paleo-historical aspects of fish speciation and distributions. Based on a growing body of evidence (e.g., Arroyave et al., 2019; Walsh et al., 2019; Bragança et al., 2021; Liyandja et al., 2022), many of the "widespread" species in Lower Guinean basins have considerably more limited distributional ranges than previously thought, and are likely to be restricted to single river systems as is the case for *Plataplochilus eliasi* (**chapter five**). The implications of reduced distributional ranges could render many more species vulnerable to habitat impacts, increasing their threat status

(IUCN, 2022) and in some cases, potentially triggering Critical Habitat for biodiversity criteria on financial performance standards (e.g. IFC PS6 - IFC, 2019) for development projects. Triggering of Critical Habitat by infrastructural projects has major implications for how companies are expected to compensate for and mitigate their impacts, and in the context of further ecosystem degradation this is important information.

Key implications of **chapter six** relate to the importance of seasonality and the maintenance of lateral connectivity of forests to rivers for the preservation of freshwater trophic interactions and overall ecosystem health within the region. The limited understanding of trophic ecology and the riparian-river nexus (Lo et al., 2021) has resulted in a lack of policies and conservation approaches that explicitly include riparian areas in freshwater conservation (Luke et al., 2019b) and environmental safeguards for the financing of large infrastructure projects. Referring to the International Finance Corporation's Performance Standard 6 (IFC PS6) and General Guidance Note 6 (IFC GN6) on the delineation of Ecologically Appropriate Areas of Analysis (EAAA), EAAA boundaries need to account for the "distribution of species or ecosystems and the ecological patterns, processes, features, and functions that are necessary for maintaining them" (IFC, 2019). Though the PS6 specifies that ecological studies should incorporate processes and functions into the assessment of priority biodiversity, the general lack of understanding of the connectivity and functionality of lateral zones of the rivers in this region (i.e., riparian and swamp forest) often means that they are disregarded in the holistic consideration of instream biota and their ecological requirements. Defining EAAAs for biodiversity assessment is dependent on the biodiversity values present and the ecological processes that maintain them including connectivity of lateral and longitudinal river habitat, as these habitats support numerous important functions for biodiversity maintenance.

A literature debate continues as to which sources of basal carbon are key resources for tropical systems (e.g., Jepsen and Winemiller, 2007; Reis et al., 2020; Roussel et al., 2021). Yet, it is clear from this research that regional riparian forests primarily fuel freshwater consumers and that attempts to manage and conserve freshwater diversity should thus

incorporate the river-riparian connection. If riparian areas are not considered and managed, then reduction of allochthonous inputs to regional freshwaters may cause resource homogenisation and a reduction in available niches, which would have cascading effects for a system which is primarily fuelled by riparian forest (Wilkinson et al., 2020). In addition to riparian forest-river connectivity, lateral swamp forest-river connectivity could also provide a significant additional basal resource that subsidizes the river food webs in the region, and maintenance of the processes in these swamp areas may be important to the overall health of central African systems even in small swamp forests connected to streams in mid reaches of rivers.

7.3 Research needs/gaps identified

This doctoral thesis provides the some of the first comprehensive data on functional and trophic ecology in the Lower Guinean ichthyofaunal province. However, much work remains to be done to document biodiversity and untangle the processes shaping and maintaining freshwater communities in the region.

First and obviously, from a taxonomic view point more collections are required from most parts of Lower Guinea i.e., that well documented collections with associated habitat information that are deposited in museums that curate, manage, and coordinate loans for research, and provide data accessibility via internet resources. Increasingly, collections arriving at museums result from biodiversity surveys undertaken as part of required environmental impact assessments, which are generally well resourced and undertaken in geographically remote areas. It is critical that these are authoritatively identified, well-curated, and made publicly available as a resource for future investigators. International finance institutions such as the World Bank, IFC, European Bank for Reconstruction and Development, African Development Bank etc. should incorporate specific requirements into their respective performance standards for biodiversity that include obtaining correct permits for collection, export, and archiving of specimens and data into publicly accessible museum collections with associated websites for data dissemination.

Future research applying similar functional methods, thereby allowing comparison between regions, should be employed in other large basins in Lower Guinea to determine if the patterns noted in this study are observable across the larger region. If similar patterns are recorded in other basins along fluvial gradients encompassing high elevations and coastal areas, then undoubtedly new regional lotic concepts would need to be considered in conservation planning. Future studies should also explicitly test the Pleistocene refugia hypothesis using data on quaternary climate instability, hypothesised Pleistocene refugia, and areas which were likely under water due to sea level incursion. More systematic research including new genomic approaches such as ultra-conserved elements (UCEs), target capture sequencing (TCS), and whole genome sequencing (WGS) will undoubtedly help to uncover paleo-historical aspects of species diversity and distribution, which would in turn assist in motivating these areas for inclusion in protected areas (PAs), World Heritage Sites and Key Biodiversity Areas (KBAs).

The functional changes noted in this thesis considered elevational gradient changes and not a stream order gradient between the upper and lower sites in a large basin. This study largely considered changes in smaller to medium sized streams and rivers across an elevational gradient. The results might have shown a stronger pattern of functional diversity changes in stream order instead of elevational gradient were considered and suggest that future studies more explicitly consider functional changes in Afrotropical systems in relation to stream order and discharge.

This work also presents the first seasonal study of food webs using stable isotopes in a Lower Guinean basin. Given the importance of rivers and fisheries in supporting human populations in this tropical area, and the significance of terrestrial forest resources for aquatic ecosystems as shown in this study, more focus is needed on riparian buffer requirements (particularly in the case of agriculture and mining) and delineation of appropriate upstream and sub-basin scale areas for inclusion in freshwater PAs, biodiversity offsets, conservation areas etc. To date most riparian policy in Africa has been gleaned from other continents or regions, and only two west-central African countries have buffer policy incorporated into national laws but these

are vague and general as to requirements and delineation (Gabon and Democratic Republic of the Congo - Luke et al., 2019).

Depleted carbon signatures in the isotopic analyses undertaken hint at methane being important for food webs in central Africa. Including methods in food web research to incorporate inconspicuous carbon resources such as methane oxidising bacteria, and swamp forest-river carbon exchanges are urgently needed. Based on the requirements of various international agendas such as the Convention on Biological Diversity's Aichi targets and the United Nations Framework Convention on Climate Change (Springgay et al., 2019; Lo et al., 2021), this is an important topic for effective policymaking (Ramírez et al., 2008). Therefore it is recommended that future studies should consider the incorporation of additional tracers such a δ^2H as per Soto et al. (2019) to better distinguish critical resources for freshwater consumer communities. This information could also feed into more comprehensive requirements for conservation and management of swamp and peatland buffers.

7.4 References

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