

A test of the River Continuum Concept with copepod functional groups in an Amazonian river

Lone KVALHEIM^{1*}, Tânia QUARESMA², Raphael LIGEIRO³, Francieli F. BOMFIM³

¹ University of Oslo, Boks 1072 Blindern, NO-0316, Oslo, Norway

² Museu Paraense Emílio Goeldi, Programa de Pós Graduação em Biodiversidade, Av. Perimetral, 1901, Terra Firme, 66077-830, Belém - PA, Brazil

³ Universidade Federal do Pará, Programa de Pós Graduação em Ecologia, Rua Augusto Corrêa, 01, Guamá, 66075, Belém - PA, Brazil

* Corresponding author: lonkva12@icloud.com

ABSTRACT

The River Continuum Concept (RCC) proposes links between functional groups within assemblages with habitat and ecological characteristics of different river sizes. This theory is supported by extensive evidence from fish and macroinvertebrate assemblages, mainly in temperate environments. We tested this theory with functional groups of copepods, a crustacean zooplankton, in a tropical river. We sampled 10 locations in September 2022 along the Curuá River in the northern Amazon. The individuals collected were classified into three groups (Calanoida, Cyclopoida, and Harpacticoida), reflecting their functional roles in the ecosystem (filter-feeders, predator and detritivore, respectively). River size was positively related to Calanoida abundance, and Cyclopoida occurred along all the river sizes, as expected by the RCC due to habitat structure and resource availability. Other habitat metrics were unimportant in explaining the distribution of copepods. Our study advances the knowledge about copepod assembly in Amazonian rivers, showing that the river continuum concept explains to some extent the structure of this assemblage.

KEYWORDS: RCC, Cyclopoida, Harpacticoida, Calanoida, width gradient

Um teste do Conceito de Continuum Fluvial com grupos funcionais de Copépodes em um rio Amazônico

RESUMO

O Conceito de Continuidade Fluvial (no inglês representado pela sigla RCC) propõe ligações entre grupos funcionais de assembleias com características ecológicas e de habitat de rios de diferentes tamanhos. Essa teoria é amplamente sustentada na literatura em assembleias de peixes e macroinvertebrados, e principalmente em ambientes temperados. Testamos essa teoria com grupos funcionais de copépodes, um zooplâncton crustáceo, em um rio tropical. Coletamos amostras em 10 locais em setembro de 2022 ao longo do rio Curuá, no norte da Amazônia. Os indivíduos coletados foram classificados em três grupos (Calanoida, Cyclopoida e Harpacticoida), refletindo seus papéis funcionais no ecossistema (respectivamente, filtradores, predadores e detritívoros). O tamanho do rio apresentou correlação positiva com a abundância de Calanoida, e Cyclopoida ocorreu em rios de todos os tamanhos, como previsto pelo RCC, devido à estrutura do habitat e à disponibilidade de recursos. Outras métricas de habitat não foram relevantes para explicar a distribuição dos copépodes. Nosso estudo amplia o conhecimento sobre a composição de copépodes em rios amazônicos, demonstrando que o conceito de continuidade fluvial explica, em certa medida, a estrutura de comunidades.

PALAVRAS-CHAVE: RCC, Cyclopoida, Harpacticoida, Calanoida, gradiente de largura

The River Continuum Concept (RCC, Vannote *et al.* 1980; Doretto *et al.* 2020) proposes causal links between the dimensionality and kinetic energy of river systems and assemblage structure. The theory posits that the proportions of functional feeding groups expected at any position along the river system gradient are a consequence of the physical and ecological properties of the river, which change from headwaters to medium and large reaches (Vannote *et al.* 1980).

Headwaters are distinguished by the terrestrial vegetation over the stream channel, which provides shade and organic material to the aquatic ecosystem. The abundance of leaf detritus in headwaters results in many invertebrates employing shredder feeding strategies (Vannote *et al.* 1980; Curtis *et al.* 2018). A medium-sized river reach will be exposed to more light, as well as have a large amount of organic material transported downstream from the headwaters, making optimal (peak)

CITE AS: Kvalheim, L.; Quaresma, T.; Ligeiro, R.; Bomfim, F.F. 2026. A test of the River Continuum Concept with copepod functional groups in an Amazonian river. *Acta Amazonica* 56: e56bc25087.

conditions for primary productivity. RCC predicts that grazers will dominate here, as a consequence of higher primary productivity. A large river reach will contain mostly collector invertebrates, because primary productivity is lower and most available nutrients are small particles brought by the flow from upstream. Additionally, predators that prey on these invertebrates will maintain the same relative abundance with respect to their prey along the river gradient.

This theory was primarily based on assemblages of fish and aquatic invertebrates of temperate environments, and often has been supported in these groups (Brown *et al.* 2011; Heino and Peckarsky 2014). However, there is little evidence to support this theory for zooplankton assemblages and, more generally, for tropical rivers. Zooplankton are a vital component of freshwater environments, with important roles in ecological interactions and ecosystem functioning (Reid and Williamson 2010). A straightforward way to analyze their contribution to ecosystems is through the functional-group approach, which provides an overview of the diversity of niches in the habitat (Cadotte *et al.* 2011). Species richness alone may not be enough to detect the effect of habitat diversity on assemblage structure, while functional groups may be more closely tied to diversity and ecosystem function (Cadotte *et al.* 2011).

Freshwater zooplankton assemblages are dominated by Rotifera, Cladocera, and Copepoda, with the latter usually being the largest zooplankton in tropical and subtropical environments (Reid and Williamson 2010). Copepods are divided into three taxonomic groups that can also be considered functional groups: Calanoida, Cyclopoida, and Harpacticoida. These groups are easily distinguished by habitat, feeding habit, and morphology (Li *et al.* 2022). Calanoida have long antennae reaching down below their

bodies, slim body shape, and brush-like tail hairs (Dussart and Defaye 2001; Boxshall and Halsey 2004; Suárez-Morales and Gutiérrez-Aguirre 2020a). They are pelagic and feed mostly on algae (Marshall 1973; Suárez-Morales and Gutiérrez-Aguirre 2020a). Harpacticoida have very short antennae and one long tail hair (Dussart and Defaye 2001; Boxshall and Halsey 2004). They are benthic, living on the bottom sediments or near branches and macrophytes where they feed on small particles (Fleeger and Gee 1986; Suárez-Morales and Gutiérrez-Aguirre 2020a). Cyclopoida has medium-sized antennae that reach around the mid-body, a rounder body shape, and two tail hairs (Dussart and Defaye 2001; Boxshall and Halsey 2004; Suárez-Morales and Gutiérrez-Aguirre 2020a). This group is predatory, with a diverse diet that frequently consists of Rotifera and Protozoa (Adrian and Frost 1992; Suárez-Morales and Gutiérrez-Aguirre 2020b).

These functional groups are predicted to have different patterns of abundance along the river continuum. Benthic detritivore Harpacticoida should be more abundant in headwater regions, where substrate heterogeneity and benthic habitats dominate. In contrast, the planktonic filter-feeder, Calanoida, should be more abundant in the wider, downstream sections, where open-water conditions and increased primary productivity prevail. Predatory Cyclopoida should be relatively constant along the river, finding prey in all reaches. This pattern is expected because each group is adapted to distinct ecological niches shaped by longitudinal changes in hydrology, habitat structure, and resource availability, as predicted by the River Continuum Concept (Vannote *et al.*, 1980).

In this study, we tested the RCC expectations by examining the composition of functional groups of copepods along a river gradient in Amazonia. The study was conducted in the Curuá River (Figure 1), a tributary of the Anapu

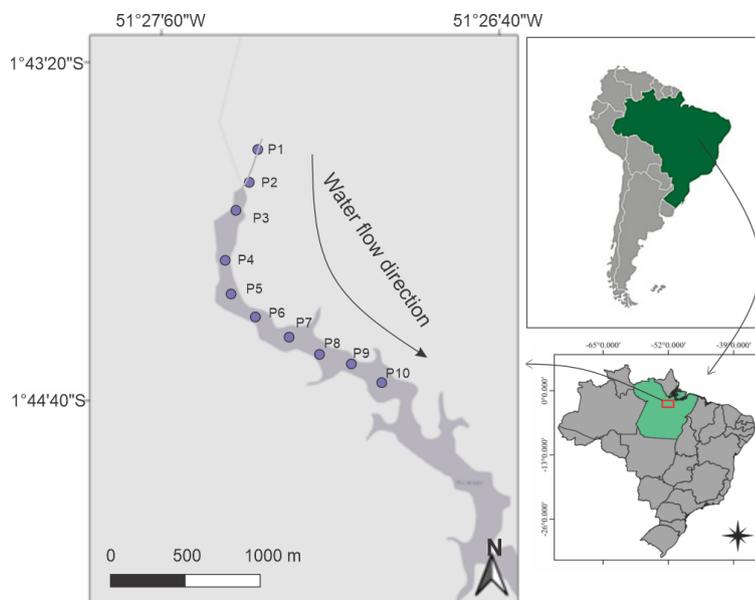


Figure 1. Map showing the location of the study site in South America, and the Curuá River with sampling points (from P1 to P10) in the Caxiuanã Forest.

River, located between the Tocantins River and the Xingu River in the eastern Amazon. The Curuá River drains the National Forest of Caxiuaná, a protected area located in the municipalities of Melgaço and Portel, State of Pará, Brazil (Lisboa *et al.* 2013).

Sampling took place in September 2022 at 10 sites, at least 200 m apart, along a 2.5 km section of the river, with a width gradient (Figure 1) from the narrower (site 1) to the wider (site 10) areas. A multimeter probe was used to measure pH, conductivity, and temperature at each sampling point. River width was measured using a measuring tape at the narrowest sites and satellite images at wider sections.

Copepods were sampled at approximately 30 cm depth in the river using a 10-liter bucket and a net with a 68 µm mesh, and 100 liters of water per sample, following Bomfim *et al.* (2024). After filtration, the animals were transferred to a Falcon tube and fixed in 4% formalin. In the laboratory, using a microscope, copepods were identified and counted in the three taxonomic orders (Reid 1985; Perbiche-Neves *et al.* 2015). The total number of individuals (abundance) in each group was counted in each sample. To test how the composition of copepod functional groups changed over the length of the studied part of the river, we performed a Permutational Multivariate Analysis of Variance (PERMANOVA) using the function ‘adonis2’ of the “vegan” package (Oksanen *et al.* 2019) in R (version 4.5.1). Abundance per group was the response variable, and river width was the predictor variable. The results were expressed in a bubble plot using ‘ggplot’ (Hamilton and Ferry, 2018) in R.

To analyze the physical-chemical variables (width, pH, conductivity, and temperature) driving the distribution of copepods groups, a redundancy analysis (RDA) was performed, using the function ‘rda’ of the “vegan” package in R.

A total of 617 Copepoda were found in the Curuá River samples, with Cyclopoida being the most abundant, followed by Calanoida (N = 11 individuals) and Harpacticoida (N = 7). The greatest abundance of copepods was observed in the larger reach of the river (greater width), totaling 503 individuals (Table 1). A significant change in the composition of copepod groups along the river was also observed (PERMANOVA $F_{(2,9)} = 5.59$, $p = 0.026$; Figure 2).

From the physical-chemical variables, only river width was an ecologically relevant gradient and was the only important variable for copepod distributions (RDA model: $F = 6.12$, $p = 0.026$, $r^2 = 36\%$; Figure 3).

The distribution of copepod taxonomic groups partially changed with river width according to the expected by the RCC. As expected, Calanoida (planktonic filter-feeders) were only found in the last half of the samples (but in very low abundances), and Cyclopoida (predators) were distributed along the all the river reaches. However, Harpacticoida (Benthic detritivore) did not follow the expected pattern under

Table 1. Physical-chemical variables (pH, Temp.: temperature, Cond.: conductivity, R. width: river width) and Copepoda absolute abundances (following parameters: Calan.: Calanoida, Harp.: Harpacticoida, and Cyclo.: Cyclopoida) at each sample point.

Site	pH	Temp.	Cond.	R. width	Calan.	Harp.	Cyclo.	Total
1	4.70	28.8	13.3	9.9	0	2	46	48
2	5.04	31.1	13.0	14	0	1	7	8
3	5.04	31.5	12.5	66	0	1	11	12
4	5.02	32.2	12.2	78	0	0	11	11
5	5.17	32.4	11.9	158	0	0	23	23
6	5.02	32.6	12.4	118	1	0	11	12
7	4.75	32.7	11.4	219	2	0	40	42
8	4.94	32.3	11.9	115	4	1	292	297
9	4.92	32.8	11.5	100	3	1	77	81
10	4.95	32.6	11.7	183	1	1	81	83

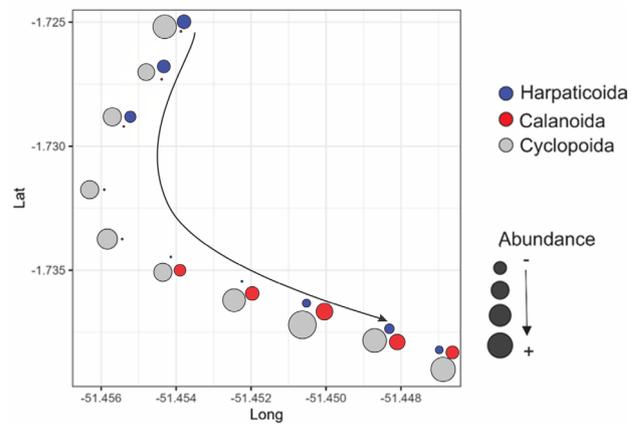


Figure 2. Copepod functional group abundance along the Curuá River. The bubble size reflects the abundance of individuals in each group.

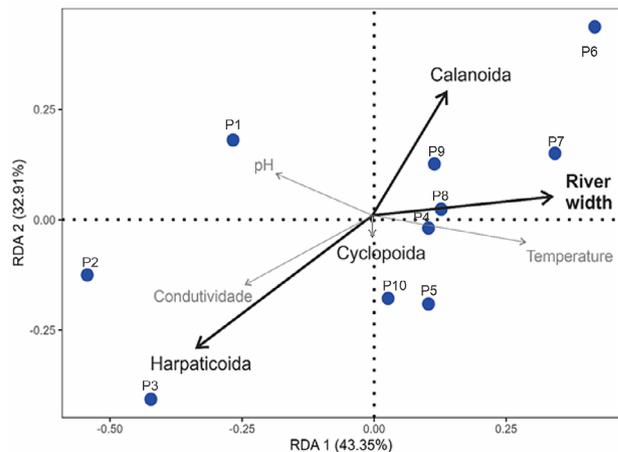


Figure 3. RDA plot shows the relationship between the physical-chemical variables and the Copepod functional groups. Grey arrows represent no significant relationships.

RCC, being found in very small numbers at the beginning and ending of the studied river section.

Cyclopoida predominated in all samples. The RCC explains the relatively even distribution of this group due to their predatory lifestyle. Carnivory is the primitive diet of the Cyclopoida, and they prey on a wide variety of items, including bacteria and small protozoa to other small copepods (Adrian and Frost 1992). This adaptability is why Cyclopoida can be found in all river widths as they change their diet depending on the available food resources (Suárez-Morales and Gutiérrez-Aguirre, 2020a). Calanoida are primarily filter feeders, mainly on algae, and are therefore directly dependent on primary productivity from pelagic areas (Lair, 2006; Suárez-Morales and Gutiérrez-Aguirre, 2020a). This is linked to the high incidence of sunlight that occurs in open areas where the river is wider and this is probably why they were only found in the final portions of the examined river section. The benthic Harpacticoida are dependent on materials from the riverbed, which is why they are found in low abundance in the water column (Marshall 1973; Fleeger and Gee 1986), as we found here.

In general, copepods were uncommon, as is typical in rivers (Allan and Castillo, 2007; Lair, 2006; Reid, 1987). We sampled along 2.5 kilometers and found only a few individuals. The Curuá River is a typical Amazonian blackwater river, characterized by acidic water, high temperature, and low productivity (Lisboa *et al.* 2013). All these variables directly affect Copepod growth and development (Lair, 2006; Suárez-Morales and Gutiérrez-Aguirre 2020a). Although we did not observe a significant relationship between the functional groups and pH, temperature and conductivity, these factors are typically important to Copepods (Amaral *et al.* 2022). The reason we did not observe a any response of the studied assemblage to these factors may be due to their low variance along the river gradient studied. Variation in river width was much more pronounced than in limnological variables. Other small-scale studies found responses of plankton to RCC and environmental variables (Barbosa *et al.*, 1999; Rocha *et al.*, 2021). Therefore, additional research on this subject in a black-water river will require more exhaustive sampling and increasing the river width gradient.

Our results suggest that copepods may follow some predictions of the RCC in Amazon waters, providing new insights into copepod ecology in these riverine systems. Zooplankton ecology requires further study in Amazonian environments, particularly in streams and rivers, such as the Curuá River, which has unique physical and chemical water characteristics. Finally, future studies should identify copepods across developmental stages, incorporate species-level resolution, include seasonal data and greater sampling effort, and include nutrient and chlorophyll measurements to enhance our understanding of the ecological roles of zooplankton in the River Continuum Concept (RCC).

ACKNOWLEDGMENTS

We would like to thank our institutions, the University of Oslo and *Museu Paraense Emilio Goeldi*, and the course Tropical Ecology for letting us get into the rain forest and collect samples so far away. RL would also like to thank the *Conselho Nacional de Desenvolvimento Científico e Tecnológico* (CNPq) for a research productivity fellowship (Grant # 312531/2021-4). FFB thanks *Fundação de Amparo a Pesquisa do Estado do Pará* (FAPESPA - process: 2022/1437669). Special thanks to Jean Ortega for field and theoretical guidance. This research was supported by the *Consórcio de Pesquisa em Biodiversidade Brasil – Noruega* (BRC).

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RECEIVED: 25/04/2025

ACCEPTED: 19/11/2025

ASSOCIATE EDITOR: David Santana 

DATA AVAILABILITY: The data that support the findings of this study were published in this article.

AUTHOR CONTRIBUTION: Lone Kvalheim - formal analysis, data curation, investigation, methodology, visualization, writing – original draft, writing – review & editing; Tânia Quaresma - formal analysis, data curation, investigation, methodology, visualization, writing – original draft; Raphael Ligeiro - conceptualization, investigation, supervision, visualization, writing – original draft, writing – review & editing; Francieli F. Bomfim - formal analysis, data curation, investigation, methodology, resources, supervision, visualization, writing – original draft, writing – review & editing.



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