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The Caraça Sanctuary as an ecological reference area for water quality in the Upper Rio Doce basin

Santuário do Caraça como uma área de referência para qualidade de água na bacia do Alto Rio Doce

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Abstract: Aim: Our objective was to assess the extent to which reference sites within the Caraça Sanctuary protect surface water quality and physical habitat structure. We expected that outside the Sanctuary, human pressures—such as urbanization, untreated domestic sewage discharge, and riparian deforestation—would result in poorer water quality and altered physical habitat structure. We hypothesized that the Caraça Sanctuary maintains streams with high physical habitat integrity and water quality. **Methods:** Data were collected from 28 stream sites, including assessments of 22 physical habitat attributes and 12 water quality parameters. In addition, land use and land cover in the upstream microbasins were analyzed, and a rapid assessment protocol was applied at each site to evaluate riparian vegetation and physical habitat diversity. Principal Component Analysis (PCA) was used to identify the key variables explaining variation among sites and to distinguish those located within and outside the Sanctuary. Two PCA biplots were generated: one for the habitat structure data and another for the water quality data. **Results:** The first two PCA axes explained 50% of the variability in physical habitat structure and 48% in water quality, with the latter most affected at sites near urban and mining areas. **Conclusions:** The results indicate that the Caraça Sanctuary provides a valuable reference area for the upper Rio Doce basin.

Keywords: water quality; rapid assessment; physical habitat structure; Rio Doce.

Resumo: Objetivo: Nosso objetivo foi avaliar até que ponto os locais de referência dentro do Santuário do Caraça protegem a qualidade das águas superficiais e a estrutura física do habitat. Esperávamos que, fora do Santuário, as pressões humanas, como a urbanização, as descargas de esgoto doméstico sem tratamento e o desmatamento ribeirinho, resultariam em pior qualidade da água e alteração da estrutura física do habitat. Nossa hipótese é que o Santuário do Caraça mantém riachos com alta integridade física do habitat e qualidade da água. **Métodos:** Dados foram coletados em 28 riachos, incluindo avaliações de 22 atributos físicos do habitat e 12 parâmetros de qualidade da água. Além disso o uso e a cobertura do solo nas microbacias a montante, e um protocolo de avaliação rápida foi aplicado em cada local para avaliar a vegetação ripária e a diversidade física do habitat. A Análise de Componentes Principais (ACP) foi utilizada para identificar as variáveis mais importantes que explicam a variação entre os locais e para distinguir aquelas localizadas dentro e fora do Santuário. Dois biplots de ACP foram gerados: um para os dados de estrutura do habitat e outro para os dados de qualidade da água. **Resultados:** Os dois primeiros eixos da ACP explicaram 50% da variabilidade da estrutura física do habitat e 48% da qualidade da água, sendo esta última a mais afetada em locais próximos a áreas urbanas e de mineração. **Conclusões:** Os resultados indicam que o Santuário do Caraça oferece uma área de referência valiosa e útil para a bacia do alto Rio Doce.

Palavras-chave: qualidade da água; avaliação rápida; estrutura física do habitat; Rio Doce.

1. Introduction

The Caraça Sanctuary encompasses native vegetation, headwaters, rivers, and waterfalls, making it a key area within the Doce River basin for ecological research, the conservation of aquatic ecosystems, and the provision of ecosystem services (Zanin et al., 2024). The Província Brasileira da Congregação da Missão—a Catholic religious order of Vincentian Priests and Brothers—has diligently preserved the region for over 250 years, maintaining its near-pristine condition. This commitment includes a 30-year designation as a Private Natural Heritage Reserve (Minas Gerais, 2013; Brasil, 2013).

The Sanctuary offers a critical reference area for scientists and resource managers to quantify minimally disturbed benchmarks for the upper and middle Rio Doce basin. These benchmarks represent desired, expected, and historical ecological conditions within the basin. In the absence of such natural references, baseline conditions for water resources—and the biotic communities they support—can shift over time (Pauley, 1995). Consequently, empirical data on the ecological conditions of minimally disturbed sites in a region must be quantified and used to establish predictive benchmarks and realistic management objectives (Hughes et al., 1986).

Assessing the status of regional ecological water resources is essential for gaining a comprehensive understanding of freshwater environments and the relationship between water quality and natural environmental features. These assessments must be evaluated against accurate and precise water resource benchmarks to effectively monitor global change and to support targeted, practical frameworks for regional land-use planning. Water resource assessments serve five key purposes: (i) they characterize reference conditions for environmental evaluations within a river basin or ecoregion; (ii) they enable the assessment of human impacts on aquatic ecosystems; (iii) they provide a foundation for future biological monitoring programs; (iv) they support governmental surveillance of potential environmental risks; and (v) they offer opportunities for citizen science engagement (Capdevila et al., 2020; Cionek et al., 2024).

Water resource availability is critical for directly or indirectly achieving nearly all of the United Nations' Sustainable Development Goals (SDGs). In Brazil, both public and private protected areas contribute to water security by ensuring a reliable supply for human use. The Brazilian National Water and Sanitation Agency (ANA) monitor the condition of aquatic resources using a Water Quality Index (WQI), which communicates the suitability of surface waters for various human activities, including boating, fishing, and swimming, through a multidimensional indicator comprising chemical, physical, and biological parameters. Water quality baselines also inform environmental assessments in highly disturbed river basins under significant urban and mining pressure, as they provide reference points across a gradient of environmental conditions. The Brazilian National System of Nature Conservation Units (Law 9,985/2000; Brasil, 2000) aims to protect and restore water resources while also promoting biodiversity conservation, regulating land use, and supporting sustainable development. To be considered safe for drinking, agriculture, or recreation, water must meet specific physicochemical and microbiological standards (CONAMA Resolution 357/2005; Brasil, 2005). In the state of Minas Gerais, water quality targets are defined by the Maximum Value Allowed (MVA), as established in ND COPAM/CERH-MG No. 8 of November 2022 (Minas Gerais, 2022).

The potential impact of research conducted in the Caraça Sanctuary on future policy and management decisions is substantial. This is because the Sanctuary can serve as a reference benchmark for long-term assessments of various anthropogenic stressors, including mining activities, tailings dam failures, urbanization, untreated domestic sewage discharges, agriculture, and global climate change. The most significant disturbance in the Rio Doce basin was the Fundão tailings dam disaster in 2015, which caused extensive damage to the ecological and cultural heritage, particularly to terrestrial and freshwater ecosystems, by releasing over 43 million m^3 of iron ore tailings polluting a 668 km stretch from the Rio Doce River basin to the Atlantic Ocean (Carmo et al., 2017). The deposition of mining waste across vast land areas and destroyed vegetation, wildlife habitats, and agricultural lands, while contaminating soils and hindering natural vegetation regeneration (Queiroz et al., 2018). The Doce River experienced a sharp decline in water quality due to the massive influx of tailings, resulting in potentially long-term ecological consequences.

Even prior to the disaster, the Iron Quadrangle region had undergone severe ecological degradation after centuries of mining activities, including deforestation, habitat fragmentation, and pollution (Salvador et al., 2022). As a result, the Doce River was already heavily impacted by sedimentation and contamination from mining and agricultural runoff, which had impaired water quality and aquatic biodiversity. When compounded by the Fundão disaster, these pre-existing environmental pressures created a complex array of ecological impacts, affecting terrestrial, freshwater, and marine ecosystems. These included the destruction of river channel and riparian habitats, severe water pollution, and widespread losses of both aquatic and terrestrial biodiversity (Sonter et al., 2014).

Riparian zones are critically important both intrinsically and for maintaining the biological composition and ecological processes of stream ecosystems (Gregory et al., 1991). Well-preserved riparian vegetation enhances physical habitat heterogeneity, through the presence of branches, trunks, and root structures, and plays a key role in reducing sediment erosion, siltation, and nutrient loading. In doing so, it buffers streams from pollutants and supports elevated levels of biodiversity. Additionally, leaf litter from riparian vegetation serves as a primary energy source for aquatic metabolism, particularly in shaded headwater streams. However, anthropogenic land-use changes, especially those associated with agricultural expansion and urbanization, frequently result in riparian deforestation. This deforestation increases the deposition of sand and fine sediments in stream channels, leading to habitat homogenization and triggering cascading effects that degrade stream physical structure and biological diversity (Allan, 2004).

Rapid Assessment Protocols (RAPs) require fewer economic, logistical, and human resources while still providing valuable information on the conservation status of stream ecosystems (Cionek et al., 2024). RAPs make it feasible to assess physical habitat conditions, riparian vegetation, environmental context, and water quality, and can effectively detect local anthropogenic alterations. In this study, we applied the Reference Condition Approach (Hughes et al., 1986) to compare minimally disturbed reference sites within the Caraça Sanctuary to more impacted sites located beyond its boundaries. To achieve this, we evaluated water quality, hydromorphology, riparian vegetation, and land use using RAPs (Feio et al., 2015).

Our aim was to evaluate the extent to which reference sites within the Caraça Sanctuary safeguard surface water quality and physical habitat structure. We expected that streams located outside the Sanctuary, subject to human pressures such as urbanization, untreated domestic sewage discharges, and riparian deforestation, would exhibit degraded water quality and altered physical habitat structure. We hypothesized that streams within the Caraça Sanctuary would maintain higher physical habitat integrity and water quality, whereas those outside the Sanctuary would show poorer conditions in both aspects.

2. Material and Methods

2.1. Study area

The Caraça Sanctuary Private Natural Heritage Reserve (RPPNSC) is located within the Iron Quadrangle, a region known for its high concentrations of gold, iron, manganese, aluminum, topaz, and other minerals. Situated in the southern portion of the Espinhaço Mountain Range, Brazil's largest mountain complex (Silveira et al., 2016), the Serra do Caraça forms the watershed division between the São Francisco and Doce River basins. The RPPNSC spans 10,187.89 hectares (20º05.914'S, 43º29.304'W) across the municipalities of Santa Bárbara, Catas Altas, Barão de Cocais, and Ouro Preto. Its landscape features a valley with a relatively flat and gently undulating floor, cut by the Caraça River, and is predominantly sedimentary in lithology. Elevations range from 750 m to 2,072 m above sea level, the latter marking the highest peak of the Espinhaço Range.

The reserve encompasses both the Atlantic Forest and Cerrado biomes experiences a dry season from April to September and a wet season from October to March. The climate is classified as Köppen's Cwa, with an annual average rainfall of 1,373 mm and a mean annual temperature of 19°C. Maximum temperatures rarely exceed 30°C, while minimum temperatures seldom fall below 0°C. Topography and rocky outcrops give rise to *Campos Rupestres* (rock outcrops) at higher elevations, with, while savannas, forests, and riparian forests occurring at lower elevations.

The RPPNSC is a prominent ecotourism destination, attracting thousands of visitors annually for its historical, religious, and biodiversity significance (Vasconcelos, 2022). Its large and mid-sized fauna includes the maned wolf (*Chrysocyon brachyurus*), tapir (*Tapirus terrestris*), and crab-eating fox (*Cerdocyon thous*), alongside with 372 bird species (Vasconcelos, 2022), 76 mammal species (Talamoni et al., 2014), 42 reptile species, and 57 amphibian species (Canelas & Bertoluci, 2007), many of which are endemic to the Atlantic Forest and *Campos Rupestres*. Evidence of gold mining in the region dates back to the early 1770s (Pedrosa, 2021), and artisanal gold mining activity was recorded in the 1980s near the municipality of Santana do Morro (site 6 of this study).

2.2. Sampling design

We selected 28 stream sampling sites within the Rio Doce basin: nine located in the RPPNSC, five outside the RPPNSC in the upper Rio Doce, and 14 in the middle Rio Doce (Figure 1).

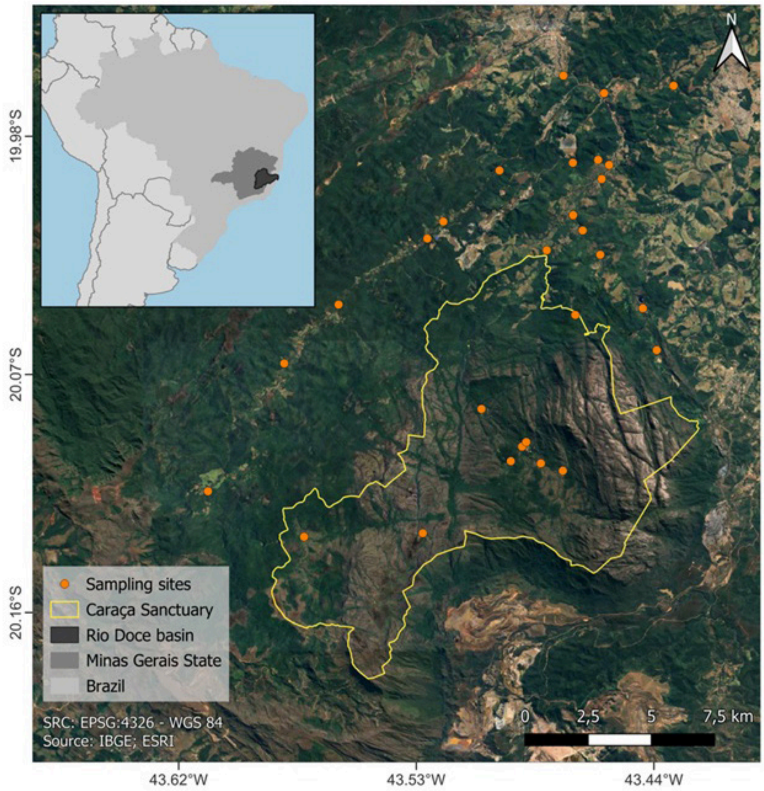

Figure 1. Locations of sampling sites in the Rio Doce basin (reference sites are located inside the Caraça Sanctuary).

Site selection was guided by their touristic relevance to Caraça visitors, uniqueness to the Caraça region, and accessibility. The nine RPPNSC sites were classified as least-disturbed reference sites based on abiotic and biotic criteria (Fernandes et al., 2022; Junqueira et al., 2018). The remaining 19 sites were subject to varying degrees and types of anthropogenic disturbances, including riparian vegetation removal, the introduction of non-native tree species, water abstraction, poor water quality, and human land uses along streambanks or within their drainage areas. The most degraded conditions were observed in streams draining small villages (populations up to 13,000), which were affected by solid waste discharge, bank modification, untreated sewage, riparian deforestation, sand extraction, high sediment loads, and channelization.

For each site, we recorded the geographic coordinates, elevation, percentage of native land cover, and percentage of urban land cover (Table 1). Land use and land cover were assessed within a 500-meter upstream buffer from each sampling site using Google Earth Engine imagery. We applied using object-oriented classification methods to categorize land use and cover types (Macedo et al., 2014; Madureira et al., 2024). Field sampling was carried out by 20 university students during a training course focused on the application of Rapid Assessment Protocols (RAPs) for evaluating water quality and physical habitat conditions.

2.3. Physical habitat index

The physical habitat index (Callisto et al., 2002; Supplementary Material 1) is an adaptation of the protocols originally developed by the Ohio Environmental Protection Agency (USEPA, 1987) and Hannaford et al. (1997), which have been validated for application in Brazilian headwaters. The RAP-Habitat protocol (Callisto et al., 2002) was applied in May 2024 to assess the physical

Table 1. Environmental variables in the 30 sampling sites.

Sites	Geographic Coordinates	Elevation (m)	Native land cover (%)	Urban cover (%)	Local site name
P1	20°6’23”S 43°28’28”W	1.249	100%	0%	Cascatinha
P2	20°6’13”S 43°28’58”W	1.237	100%	0%	Prainha
P3	20°05’50.2”S 43°29’23.7”W	1.221	100%	0%	ETE
P4	20°4’59”S 43°30’19”W	1.180	100%	0%	Taboões
P5	20°02’51”S 43°28’11”W	750	14%	86%	Buraco da Boiada
P6	20°01’22.97”S 43°28’49.92”W	729	14%	86%	Rio Caraça
P7	20°00’36”S 43°28’14”W	740	7.60%	92.40%	Santana do Morro
P8	20°00’35”S 43°28’14”W	740	7.47%	92.53%	Sumidouro
P9	19°59’46”S 43°27’35”W	731	0%	100%	Captação água Brumal
P10	19°59’27”S 43°27’25”W	720	15%	85%	Brumal
P11	19°57’39”S 43°25’57” W	724	81%	19%	Rio Santa Bárbara
P12	19°57’49.2”S 43°27’31.7”W	722	100%	0%	Barra Feliz início
P13	19°57’25.3”S 43°28’27.31”W	724	75%	25%	Rio São João
P14	19°57’39”S 43°25’57”	724	100%	0%	Barra Feliz fim
P15	20°7’48” S 43°31’39”W	1.450	100%	0%	Acima da Cachoeira do Campo de Fora
P16	20°07’53”S 43°34’21”W	1270	100%	0%	Capivari
P17	20°06’51”S 43°36’32”W	810	100%	0%	Rio Conceição cabeceira
P18	20°03’57”S 43°34’48”W	770	100%	0%	Conceição do Rio Acima
P19	20°02’3687”S 43°33’33.89” W	762	100%	0%	Galego
P20	20°01’0708”S 43°31’32,78”W	760	100%	0%	São Gonçalo
P21	20°00’44”S 43°31’11”W	750	100%	0%	Córrego do Sítio
P22	19°59’34.43”S 43°29’54.43”W	736	100%	0%	Córrego do Onça
P23	19°59’23.95”S 43°28’14,35”W	751	40%	60%	Barragem CDS II
P24	19°59’20”S 43°27’40”W	736	49%	51%	Ponte de Madeira
P25	20°03’39”S 43°26’20”W	760	100%	0%	Quebra-Ossos
P26	20°02’42”S 43°26’39”W	750	7%	93%	Bicame
P27	20°01’29”S 43°27’37”W	730	50%	50%	Pedreira Quebra-Ossos
P28	20°0’56”S 43°28’1”W	740	7%	93%	Sô Moço
P29	20°5’44”S 43°29’18” W	1.190	100%	0%	Tanque São Luis
P30	20°6’10” S 43°29’39”W	1.270	100%	0%	Tanque Grande

condition of streams and their riparian zones. This protocol is based on visual assessments and includes 22 parameters related to riparian zone use and occupation and observable water characteristics (e.g., riparian vegetation cover, presence of anthropogenic alterations, streambank erosion, and streambed siltation), each scored on a scale from 0 to 4. An additional 11 parameters evaluate flow conditions and substrate characteristics (e.g., streambed composition and water flow), each one scored from 0 to 5. These scoring systems follow the rationale of the original protocols. The results are synthesized into a final score reflecting the conservation status of each site: scores between 0–40 indicate degraded conditions, 41–60 indicate altered conditions, and scores above 60 are considered reference conditions (Callisto et al., 2002). These three categories were established following calibration and adaptation of the protocol to Brazilian headwater conditions. Additionally, we measured stream depth (m), wetted width (m), and flow velocity (m/s), each based on the average of three random measurements per site. Canopy cover was also assessed at each site using the free Canopy Capture® software, with results expressed as the average of three measurements taken along the stream channel during the same sampling events.

2.4. Water quality

Field water quality variables were measured *in situ*, once at each stream site. They included temperature (°C), dissolved oxygen concentration (mg/L) and saturation (%) (YSI ProSolo model), pH and redox potential (mV) (Digimed DM-2P), turbidity (NTU; Digimed DM-TU), and electric conductivity (µS/cm), total dissolved solids (mg/L), and resistivity (KΩ/cm) (Digimed DM-3P). On the same occasions, 300 mL of water was collected in glass bottles for the determination of biochemical oxygen demand (BOD; mg/L), and 250 mL of water was collected in amber plastic bottles for later determinations of nutrients, ions, heavy metals, total phosphorus, and total nitrogen concentrations (mg/L) (APHA, 2012).

For the Total Phosphorus analysis, we used the ascorbic acid colorimetric method (4500-P B.5 APHA, 2012). For digestion, 50 mL of the sample was pipetted into an Erlenmeyer flask, and approximately 0.5 g of potassium persulfate was added. The sample was digested on a hot plate for 90 minutes at 250 °C, then increased again to 50 mL with ultra purified water, and the pH was corrected. The color reagent was added, and the samples were read on a V-5000 technical spectrophotometer at 880 nm.

Total nitrogen was determined using the HI93767A50 kit from Hanna Instruments, based on the chromotropic acid method, which requires digestion to convert all forms of Nitrogen to nitrate. Subsequent readings were taken on an HI83399 benchtop spectrophotometer.

Color was determined spectrophotometrically, with platinum-cobalt solutions standards. Chlorophyll was extracted using 90% acetone and also determined spectrophotometrically. Total waste was measured according to APHA-SMEWW.2540.

2.5. Water Quality Index

The Water Quality Index (WQI) was calculated for each stream site by the weighted product of several physical-chemical parameters, such as pH, turbidity, thermotolerant coliforms, BOD5, Nitrate, Total Phosphorus, Temperature, Total Solids, and Dissolved Oxygen, with their respective relative weights (*wi*). This index adopts values between 0 and 100, classifying water quality from “very bad” to “excellent”. Our results were compared to CONAMA Resolution 357/2005, a legal framework that establishes water quality standards for different classes of water bodies, to verify any divergences from Class 2 waters, which are designated for human consumption after conventional treatment, aquatic life protection, and recreational activities.

2.6. Ion chromatographic analysis

The anions (NO_2^-, NO_3^-, F^-, Cl^-, Br^-, PO_4^{3-} and SO_4^{2-}) and cations (Li^+, Na^+, $NH4^+$, K^+, Ca^{2+} and Mg^{2+}) were determined by ion chromatography (IC) using a Metrohm model ECOIC1, equipped with a self-healing suppressor system and conductivity detector. The anions were separated on a Metrosep A Supp 17 analytical column (250mm x 4.0mm), an eluent solution of 4mM Na_2CO_3 and 1mM $NaHCO_3$, and at a flow rate of 0.8 mL/min. Cations were separated on a Metrosep C 4 analytical column (250mm x 4.0mm), in an eluent solution of 2.7 mM oxalic acid dihydrate at a flow rate of 0.9 mL/min. Samples previously filtered in the field using a 0.45 µm syringe filter were inserted into the automatic sampler vials, and a volume of 60 µL was injected.

2.7. Metal analysis by ICP-MS

Analyses of aluminum (Al), arsenic (As), cadmium (Cd), lead (Pb), cobalt (Co), copper (Cu), chromium (Cr), iron (Fe), lithium (Li), manganese (Mn), mercury (Hg), nickel (Ni), silver (Ag), and zinc (Zn) were performed with an inductively coupled plasma mass spectrometer (ICP-MS)

(Agilent 7700 series, Agilent Technologies, Japan), in standard mode in a collision cell using He 99.995% (Air Liquide, Brazil) as collision gas. The nebulizer used was the Mira Mist type, nebulization chamber (double step) and quartz torch.

The samples filtered through a 0.45 μm syringe filter were transferred to a 10 mL Falcon tube, acidified with 20 μL of concentrated ultrapure nitric acid, and subsequently supplemented with 10 μL of the internal standard Rhodium. For the analytical curve, a multi-element intermediate solution of 1,000 μg/L was prepared from mono-element standard solutions of the respective elements. Then, dilutions were performed to prepare five standards at concentrations of 1, 5, 10, 50, 100, 250, and 500 μg/L. The samples followed by the analytical curve standards were injected using an automatic sampler.

2.8. Data analyses

We conducted two separate Principal Component Analyses (PCAs) to reduce dimensionality, summarize the dataset, and identify spatial patterns. The first PCA evaluated the effects of the RAP-Habitat variables, while the second examined variation based on water quality variables measured both in situ and in the laboratory (Supplementary Material 2). All variables included in both PCAs were normalized to ensure comparability across different measurement scales, allowing each variable to contribute equally to the analysis and preventing bias due to differences in units or magnitudes. The analyses were performed in R (R Core Team, 2024) using the FactoMineR package (Le et al., 2008).

3. Results

For the RAP-Habitat PCA, the first two principal components explained 50% of the total variation in the dataset, with the first component accounting for 37.1% and the second for 12.9% (Figure 2). The variables most strongly correlated with components 1 and 2 included: oil on the water surface (cos^2 = 0.70), oil in the sediment (cos^2 = 0.70), channelization (cos^2 = 0.71), riparian vegetation width (cos^2 = 0.72), bank stabilization (cos^2 = 0.77), and sand deposition (cos^2 = 0.80). A complete list of variable contributions is provided in Figure S1 and Supplementary Material 3.

The PCA based on chemical data showed that the first two components explained 48.2% of the total variation, with the first axis accounting for 27.6% and the second for 20.6% (Figure 3). The variables most strongly correlated with axes 1 and 2 were: electrical conductivity (cos^2 = 0.71), total dissolved solids (cos^2 = 0.75), fecal coliforms (cos^2 = 0.80), total phosphorus (cos^2 = 0.80), and water turbidity (cos^2 = 0.81). A complete list of variable contributions is provided in Figure S2. Total phosphorus varied between 0.001 and 0.209 at P13 (Rio São João), whereas faecal coliforms were higher downstream the sewage treatment plant (4000 CFU/100ml) than in the Caraça Sanctuary preserved streams (0-80 CFU/100ml).

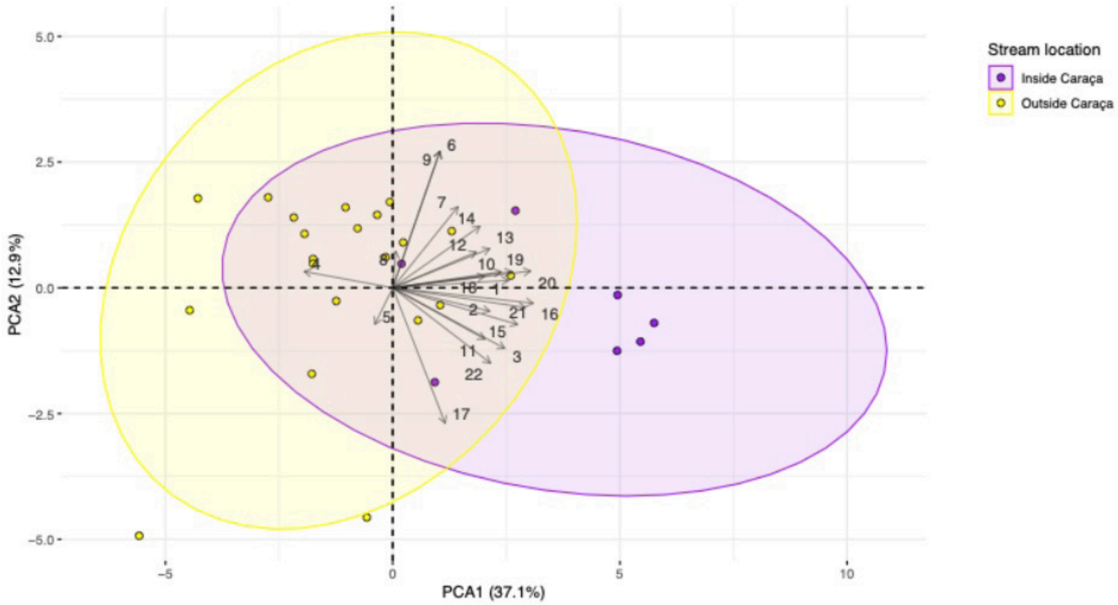

Figure 2. Principal Component Analysis on the RAP-Habitat variables.

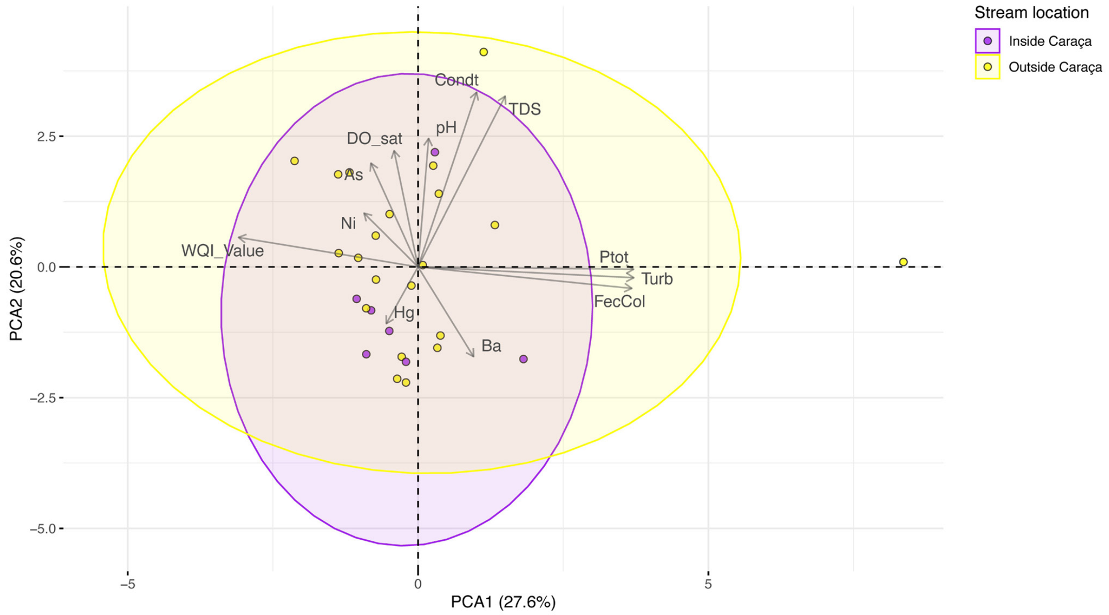

Figure 3. Principal Component Analysis based on water quality data. As: Arsenium, Ba: Barium, Hg: Mercury, Ni: Nickel, FecCol: Fecal Coliforms, Condt: Electrical conductivity (uS/cm), DO_sat: Dissolved Oxygen (% saturation), pH: Potential of Hydrogen, Ptot: Total Phosphorus (VMP 0.03 mg/L), TDS: Total Dissolved Solids (ppm), Turb: Turbidity (NTU), WQI_Value: Water Quality Index.

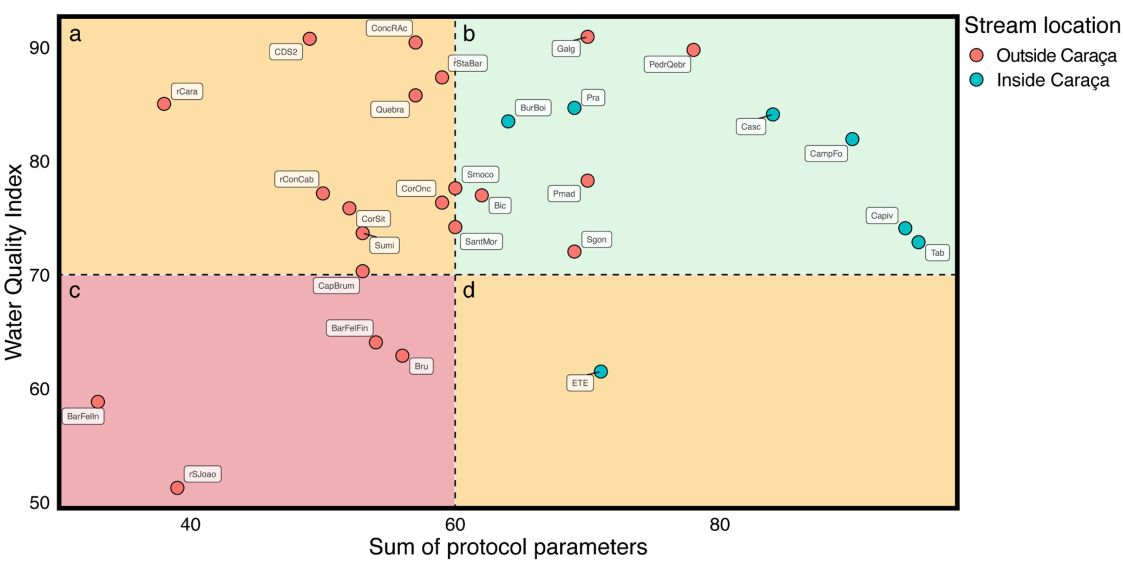

Figure 4. Patterns in RAP-Habitat and water quality index scores of the 28 stream sites: (a) top-left (orange): poor physical habitat but good water quality; (b) top-right (green): the best environmental scenario (good physical habitat and good water quality); (c) bottom-left (pink): poor physical habitat and poor water quality; (d) bottom-right (yellow): good physical habitat but poor water quality.

The ellipses and centroids for the RAP-Habitat data indicated a significant difference between Caraça sites and those outside the sanctuary (Figure 2); however, that was not the case for the water quality data (Figure 3). Given the driving variables listed for Figure 2, the non-Caraça sites were likely driven by their locations near roads or in urban areas.

Based on the combination of both scores (WQI and Protocol) from the physical habitat and water quality indices, 11 sites were deemed to be in good condition, located in the upper right quadrant of Figure 4. Four sites were classified in poor condition, positioned in the lower left quadrant. A single site exhibited good physical habitat condition but poor water quality (lower right quadrant), and nine sites had good water quality but degraded physical habitat (upper left quadrant).

4. Discussion

Our hypothesis that the Caraça Sanctuary preserves streams with good physical habitat condition and water quality was largely supported. Six of the seven headwater sites within the Sanctuary exhibited both high water quality and well-preserved physical habitat. However, water quality had declined in one stream, likely due to urbanization, untreated sewage discharges, and riparian deforestation (Figure 4). Our second hypothesis, that streams outside the Sanctuary would exhibit poor physical habitat condition and water quality, was only partially supported. Five of the 21 external sites displayed both good water quality and physical habitat condition, suggesting their potential to serve as additional reference sites (Figure 4).

The RAP-Habitat and Water Quality PCA results identified the most influential variables separating sites: oil in water and sediment, sand deposition, channelization, bank stabilization, riparian vegetation loss, as well as elevated conductivity, total dissolved solids, fecal coliforms, total phosphorus, and turbidity (Figures 2 and 3). These factors were likely driven by traffic on dirt roads, urban channelization, and the reduction of riparian vegetation in urban and roadside areas. Previous studies of Cerrado streams have also linked high levels of catchment and local disturbances to poor macroinvertebrate assemblage conditions (Martins et al., 2021). In a broader regional study, Silva et al. (2018) identified agricultural land use, fine sediment input, and turbidity as key stressors on macroinvertebrate communities. However, unlike our targeted site selection, these earlier studies used probabilistic sampling designs.

We identified four site groups, ranging from those with both good physical habitat and water quality to those with poor conditions in both metrics (Figure 4). Notably, nine sites showed good water quality index scores but poor RAP-Habitat scores, underscoring the importance of assessing physical habitat alongside water quality (Karr & Dudley, 1981; Kaufmann et al., 2022). Conversely, one site within the Caraça Sanctuary, although maintaining good physical habitat condition, was located downstream from a sewage treatment plant and exhibited impaired water quality that still requires improvement.

4.1. Environmental education training

Our sampling was carried out during a field course on the application of Rapid Assessment Protocols (RAPs) for water quality and physical habitat (Callisto et al., 2002), designed for undergraduate and graduate students. A total of 20 students participated in the field training and sampling activities. All participants visited the sampling sites and received instruction in the fundamental principles of water quality and physical habitat assessment. Based on RAP scores, the students classified stream sites as "Reference" (scores between 80 and 96), "Altered" (scores between 45 and 55), or "Degraded" (scores below 40). By visiting reference sites within the Caraça Sanctuary, participants gained both a deeper understanding of the physical and chemical characteristics of reference conditions in the upper Doce River basin and a heightened sense of environmental responsibility. Similar RAP training efforts have been conducted with local visitors, riverine residents, and staff from the state environmental protection agency in Gandarela National Park (Callisto et al., 2023) and in the headwaters of the Rio das Velhas (Callisto et al., 2021). These initiatives have proven effective in translating teaching and learning into field-based practice, demonstrating the utility of RAPs as low-cost, rapid, and effective tools for assessing stream conditions in both academic and community-based contexts (Callisto et al., 2011).

5. Future Recommendations

Our rapid assessment results support the conclusion that the Caraça Sanctuary provides multiple reference sites for assessing stream condition within the Rio Doce basin. Future studies should build on these findings by linking abiotic indicators to biotic responses, such as macroinvertebrate and fish assemblage patterns. The PCA and quadrant analyses suggest that both physical habitat condition and water quality may limit stream biota, highlighting the need for river rehabilitation efforts to prioritize the protection and restoration of riparian habitats. Our RAP-based training of university students demonstrated the effectiveness of experiential learning and should be expanded to include broader environmental education initiatives within the Caraça region. This includes engaging local schoolteachers and students and integrating RAP-based abiotic assessments with biomonitoring programs (França et al., 2019). In addition, it is essential to assess the social impacts of protected areas and their influence on local quality of life, in order to identify strategies that strengthen human–nature connections and promote long-term conservation (Jones et al., 2017; Golgher et al., 2023). Finally, our results indicate that physical habitat and water quality conditions are closely linked to rapid urbanization outside the Caraça Sanctuary. We therefore recommend implementing integrated watershed management strategies across multiple spatial scales in the upper Rio Doce basin, including the creation and expansion of large protected areas, such as the Caraça Sanctuary, and the revegetation of riparian zones.

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Data availability

All research data analyzed in the research is available in the Dataverse of Acta Limnologica Brasiliensia on SciELO Data. Access is free. It can be accessed in https://doi.org/10.48331/SCIELODATA.2VR33X.

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