



Rapid Assessment Protocol for sandstone headwater streams: a versatile and effective environmental assessment tool

Protocolo de Avaliação Rápida para riachos que drenam por formações areníticas: uma ferramenta de avaliação versátil e efetiva

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Abstract: Aim: In this study we validated a tool to assess and monitor streams ecosystems to subsidize future research, governmental surveillance and citizen science activities. Our primary objective was to (i) provide improvements and adaptations of the Rapid Assessment Protocol (RAP) proposed by Cionek et al. (2011) and provide a new RAP, and then (ii) evaluate the association among the RAP scores and limnological parameters. **Methods:** The RAP was adapted to streams draining through a sandstone geological formation, and the final validation process was conducted in 30 streams. We used linear models and correlation analysis to understand the association of the RAP scores with in-stream limnological and physical parameters (n=30) and nutrient concentrations in the water (n=9), respectively. Two parameters have been adjusted according to our professional's judgment which have provided feedback since 2011. **Results:** The RAP scores explained 29% of the variability of in-stream limnological and physical characteristics of the streams. Streams with higher RAP scores were those with higher dissolved oxygen and higher depths. Streams with lower RAP scores were those with higher widths, conductivity, and turbidity. Streams with higher orthophosphate and ammonium loads were those with the predominance of slow and shallow flow regimes, while streams with higher nitrate concentration were those with straight channels. Limnological and physical indicators showed the same tendency of ecosystems quality (degradation or preservation), and yet are complementary because they evaluate distinct features of the system. **Conclusions:** The RAP adapted for the Arenito Caiuá streams provide a good interpretation on the physical habitat features of streams and can be



used both as a single diagnostic and monitoring environmental tool or a complementary tool along with limnological and biotic parameters.

Keywords: sand bottom; physical habitat assessment; RAP; wadable stream; Atlantic Forest.

Resumo: Objetivo: Neste estudo validamos uma ferramenta para avaliar e monitorar riachos para subsidiar pesquisas, monitoramento para gestão e ciência cidadã. Nosso principal objetivo foi (i) proporcionar melhorias e adaptações do Protocolo de Avaliação Rápida (PAR) proposto por Cionek et al. (2011), criando uma nova versão do PAR e então (ii) avaliar a associação entre os escores do PAR e parâmetros limnológicos. **Métodos:** O PAR foi adaptado para riachos que drenam por formação geológica arenítica, e o processo de validação final foi realizado em 30 riachos. Utilizamos modelos lineares e análise de correlação para entender a associação dos escores de PAR com parâmetros limnológicos e físicos no riacho (n=30) e concentrações de nutrientes na água (n=9), respectivamente. Dois parâmetros foram ajustados de acordo com o julgamento de nossos profissionais, que fornecem feedback de aplicação desde 2011. **Resultados:** As pontuações do PAR explicaram 29% da variabilidade das características limnológicas e físicas dos riachos. Os riachos com pontuações mais altas foram aqueles com maior oxigênio dissolvido e profundidade. Os riachos com pontuações mais baixas foram aqueles com maiores larguras, condutividade e turbidez. Os riachos com maiores cargas de ortofosfato e amônio foram aqueles com predominância de regimes de fluxo lento e raso, enquanto os riachos com maior concentração de nitrato foram aqueles com canais retilíneos. Os indicadores limnológicos e físicos apresentam a mesma tendência de indicação de qualidade dos ecossistemas (degradação ou preservação), mas são complementares porque avaliam características distintas do sistema. **Conclusões:** O PAR adaptado para os riachos do Arenito Caiuá fornece uma boa interpretação sobre as características físicas do habitat dos riachos e pode ser usado tanto como uma ferramenta única de diagnóstico e monitoramento ambiental ou como uma ferramenta complementar juntamente com parâmetros limnológicos e bióticos.

Palavras-chave: substrato arenoso; avaliação física do habitat; PAR; riachos de pequena ordem; Mata Atlântica.

1. Introduction

Streams are dynamic systems, in which intense energy and matter are exchanged with the adjacent habitats, contributing to energy fluxes and the balance of the ecosystem (Lamberti et al., 2010). The stream's reliance on the adjacent terrestrial habitat has been extensively documented (Vannote et al., 1980; Magliozzi et al., 2018) and is reported as being primordial in preserving the quality of freshwaters. Studies have already reported that streams with pristine or highly preserved riparian vegetation hosts higher diversity of aquatic and terrestrial species and higher water quality compared with streams in which the riparian buffer is replaced by agricultural and/or urban uses (Cionek et al., 2021; Marques et al., 2021). For example, Marques et al. (2021) showed that Amazonian streams located in forested landscapes with riparian buffers have a positive impact on the energy flow of aquatic food webs because of the light and temperature buffer. Thus, understanding stream ecosystems integrated with the ecotone helps to build up the comprehension of their functioning.

The input of terrestrial organic matter from preserved riparian areas, such as branches and trunks are important to provide underwater habitat

complexity, creating distinct mesohabitats as well as different water velocity regimes (Cionek et al., 2011; Fiori et al., 2016). Additionally, the input of organisms (or living structures), such as leaves, fruits and small animals, from the riparian area are essential food resources to the complex aquatic biota food web (Cebrian & Lartigue, 2004; Cionek et al., 2021). Streams are habitat to small-bodied fish, amphibians, reptiles, invertebrates, algae, aquatic macrophytes, fungi and microorganisms (Moulton et al., 2004; Wulf & Pearson, 2017; Pazianoto et al., 2019), apart from being a nursery for some large-bodied fish species (Keller et al., 2019), and important water and food supply for terrestrial birds and mammals (Lees & Peres, 2008). Although allochthonous resources are important for maintaining stream ecosystem functioning, anthropogenic alteration has been undermining its resilience and disrupting its dynamics (Dala-Corte et al., 2020).

Land use alterations in the drainage basin induces numerous changes in aquatic ecosystems and modify instream dynamics, including the ecotone exchange area. A single change in the ecosystem may produce a cascading effect, enhancing the negative impacts over the physical habitat and the biological structure of streams. For example, the

removal of canopy vegetation at the expense of agriculture and/or urbanization enhances erosion and siltation of stream channels (Reis Oliveira et al., 2018), increasing the input of pesticide and sewer (Brovini et al., 2021). The absence of natural vegetation decreases the input of organic matter from the terrestrial area, such as fruits, insects, and leaf litter, impoverishing the food web structure (Casatti et al., 2009; Carvalho et al., 2019), disrupting biological interactions and, consequently, reducing species richness (Piscart et al., 2009, 2011) and changing the ecosystem structure (Englert et al., 2015; Pocewicz & Garcia, 2016). Therefore, tools for assessing anthropogenic alterations in stream ecosystems and its surrounding areas are fundamental to the preservation of these habitats.

Stream conservation involves knowing the system's characteristics to conduct specific conservation or recuperation actions, and the knowledge must be easily available to facilitate decision making. The pool of environmental assessment tools is wide, and can include biological, physical, and chemical indicators to provide a comprehensive diagnostic of freshwater systems (Barbour et al., 1999). Bioindicators are especially interesting since organisms' responses to the physical and chemical environment are known to be reliable (Karr, 1987; Ávila et al., 2018). One example is the Index of Biological Integrity (IBI) which is the evaluation of community level parameters in response to environmental quality (Gonino et al., 2020; Casatti & Ortigossa, 2021). Although considered a low-cost means of assessing the integrity of streams, IBI demands highly capacitated staff to conduct community and water sampling and further analysis. On the other hand, methodologies that rely on the evaluation of the physical habitat, such as Rapid Assessment Protocols (RAP), are more accessible and require less economic, logistic, and human resources, and provide a snapshot of the system integrity that can properly inform on the conservation status of the ecosystem.

Rapid Assessment Protocols have been used as tools to provide valuable information on the preservation status of stream ecosystems (Minatti-Ferreira & Beaumord, 2006; Cionek et al., 2011; Guimarães et al., 2017). The RAP provides a scale on environmental conditions based on easy access information from the stream channel and its surrounding area. It can be used as a physical habitat characterization, since the physical structure of a stream mostly determines the biodiversity and the type of organisms that will be found in a given

habitat (Barbour et al., 1999). The degradation of the physical habitat of streams is commonly observed as a consequence of land use alterations (Englert et al., 2015; Reis Oliveira et al., 2018; Taniwaki et al., 2019), thus the RAP may be used as a proxy to assess basin-level alteration. Considering the widely acknowledged importance of streams for providing ecosystem services (Palmer et al., 2014; Raitif et al., 2019) and that we currently entered the ecosystem restoration decade (UNEP, 2021), we are interested in providing a tool to assess and monitor streams ecosystems to subsidize future research, governmental surveillance, and citizen science activities. Our primary objective was to (i) provide improvements in the description of parameters to include new features to be observed and to clarify the parameters interpretation from the RAP proposed by Cionek et al. (2011), providing a new revised RAP, and then (ii) evaluate the association among the RAP scores and limnological parameters widely used for environmental quality assessment to validate the responses of the RAP as a proper management tool for rapid stream assessment. Since both sets of parameters (RAP and limnological parameters) are locally measured, and assuming that the RAP provides accurate rapid environmental assessment, we expect the RAP scores to be significantly associated with limnological parameters.

2. Material and methods

2.1. Study area

The studied region is located in Northwest Paraná State – South Brazil, delimited by rocks from Caiuá Sandstone Geological Formation (Figure 1). Mean temperature ranges from 18° to 25 °C and mean annual precipitation reaches 1.300 mm. This region is under the natural domain of the Semi-Deciduous Atlantic Forest (Campos et al., 2000). The study region has been subject to intense deforestation since the 1950's, with the predominance of agriculture and pasture farming. Nowadays forested areas are restricted to small, unevenly distributed fragments. The percentage of land use of sugar cane, pasture, forest or urban areas in each of the streams subbasin were assessed by means of SRTM images to create and Landsat 8 OLI images to verify the stream basis (Table S1). These metrics were not used in further analysis because our focus is on the local assessment. Instead, landscape metrics were important to provide the basis for our discussion, since local metrics are reflective of landscape level attributes (Barbour et al., 1999), and

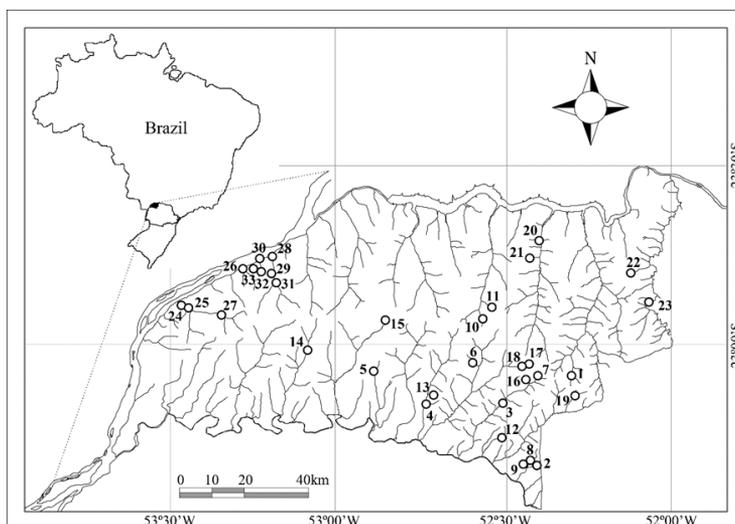


Figure 1. Spatial distribution of the sampling sites. Author: Jaime Luiz Lopes Pereira.

the different types of land use impose distinct effects over the local stream physical integrity (Figure 2).

2.2. Rapid Assessment Protocol adaptation based on expert opinions

The Rapid Assessment Protocol (RAP) proposed by Cionek et al., (2011) was based on the protocol proposed by Barbour et al. (1999), in which nine parameters that represents the physical habitat are evaluated by means of an explanation of their relevance to ecosystem and the way the parameters must be interpreted. Each parameter is divided in four categories of physical habitat quality, that contemplate a gradual decrease in the proportion of features that provides physical habitat complexity to the streams and is scored between 0 and 20 (Cionek et al., 2011). Scores from each parameter can be summed (up to 180) to provide a single physical quality assessment (Barbour et al., 1999). Once each parameter represents a specific habitat characteristic, scores can also be used separately according to the purposes of a given research question. When the RAP interpretation is based on the final summed score for quantitative purposes, the interpretation of physical characteristics influencing the results can be discussed separately to provide a more detailed comprehension of the specific features that are preserved or associated with the response. This is especially appropriate for streams with regular physical quality because some parameters can be scored higher (i.e.: underwater available substrate due to construction debris accumulation), while others can be scored lower (i.e.: the absence of riparian bank protection), and while the summed score is appropriate for modeling,

the interpretation of results require a more refined explanation. The present RAP has been thoroughly applied in stream environmental assessment for scientific purposes (Cionek et al., 2011, 2021; Cionek, 2016; Gonino et al., 2020; Pereira et al., 2021). Evaluator feedback was obtained along the years, as gaps were identified during the RAP application and interpretation about the environmental quality gradient. Feedback were provided whenever trained stream ecologists from the lab team encountered features in the streams (and surroundings) that were not properly described in the RAP sheets. Based on these feedback (i.e., the lack of a description about the proportion of grass on the stream margins), the description of parameters was further detailed. The original RAP sheet can be found in Cionek et al. (2011). The process was conducted based on professional judgement for habitat visual characterization, that although is inherently subjective, can be properly assessed by detailed habitat description.

2.3. RAP scores association to in-stream limnological and physical characteristics

The RAP evaluation has been coherent and efficient in providing an environmental quality assessment that closely relates to biological (Gonino et al., 2020) and streams functioning (Cionek et al., 2021) responses. However, to further validate the RAP suitability to provide complimentary and rapid environmental quality assessment, we tested its association with commonly used limnological parameters for environmental quality assessment. We sampled 40-meter reach, in 33 streams draining through the study region.



Figure 2. Characteristics of streams from Caiuá Sandstone region. Photos with green frame are representative of the most preserved streams. Photos with brown frame are representative of streams with grass dominated margins, turbid water with the absence of underwater structuring (i.e., trunks, leaf accumulation, pebbles), large soil misplacement and a very steep margin. Photos with red frame are representative of the most degraded streams, with steep margins, soil erosion, urban residues accumulating in a few trunks and turbid water due to domestic effluent inputs and proliferation of microorganisms. Underwater structuring is artificially provided by construction debris and domestic garbage. Additional pictures can be found in Figures S1 to S6, available in the Google Drive (2021).

We sampled streams draining through areas with forested, pasture, agriculture and urban in the watershed, that were representative of our study region (Table S1) and registered 8 in-stream limnological and physical in-stream parameters (Table S2). The selected limnological parameters were pH, electrical conductivity, water temperature, turbidity, and dissolved oxygen, measured with

portable probes. The physical variables were sampled in triplicate along the 40-meter reach and are provided as an average of stream width (m), depth (cm) and water velocity (m/s). Water samples were taken, in triplicates, from nine of those 33 streams, for the determination of concentrations of nitrate (NO_3^- ; Giné et al., 1980), ammonium (NH_4^+ ; Koroleff, 1976), and orthophosphate (PO_4^{3-} ;

Mackereth et al., 1978) (Table S3). Nutrient concentration values are provided as an average of three replicates for each stream site. Nutrient concentration determination was only conducted for nine streams.

The RAP was applied to the same 40-meter reach at each stream, by at least two evaluators. The evaluation process consists of averaging the observed features of the stream along the 40-m reach, and matching the observed physical habitat features to the description of the parameter in the RAP sheet. With a spreadsheet in hand with all parameter descriptions (Table 1), the evaluators scored the following parameters: underwater available cover, underwater habitat complexity, velocity/depth combinations, channel sinuosity, water level amplitude, channel integrity, bank stability (both margins), riparian bank protection (both margins) and vegetation conservation on the riparian zone (both margins). The scores of each parameter ranged from 0 (poor) to 20 (optimal) and the final stream reach score is provided by the sum of all parameters. If it fits the research purpose, the parameters scores can be used separately.

2.4. Data analysis

To verify the in-stream limnological and physical parameters that most contributed to the variability of stream quality and to summarize the environmental quality features within our study region, we applied a Principal Component Analysis (PCA) to our data. Data was standardized (i.e., observations were scaled to zero mean and unit variance) prior to the analysis using the *decostand* command, from *vegan* (Oksanen et al., 2019), in R software (R Core Team, 2020). The significance of the PCA axis was calculated based on the Brokenstick criterion, using the *PCAsignificance* command from BiodiversityR package (Kindt & Coe, 2005) in R.

To test if RAP results work as good predictors of limnological characteristics of the streams, and thus, provide suitable rapid environmental assessments, we applied a linear model to the data. To do so, we used the first principal component site scores (PC1) as our response variable, and the RAP score (i.e., sum of all parameter scores – the final result of the RAP application) as our predictor variable. Assumptions of normality and homoscedasticity were assessed by visual inspection of residual vs. fitted, normal Q-Q and residuals vs leverage plots (Zuur et al., 2009). Three influential observations were removed from the linear model analysis,

to attend the assumptions, and because they represented extremes in the environmental gradient. So, our final model was built with 30 streams.

We were also interested in understanding how well the RAP parameters would relate to nutrient concentration. This analysis was conducted in only nine streams from our data set, because we only had nutrient concentration for those streams (Table S3). Therefore, we ran a Pearson correlation analysis to assess if each of the nine RAP parameters were correlated to the three in-stream nutrient concentration (nitrate, ammonium, and orthophosphate). We used the *cor* command from the *stats* package in R (R Core Team, 2020).

3. Results

3.1. Rapid Assessment Protocol adaptation based on expert opinions

Two out of nine RAP parameters have been adjusted according to our professional judgement feedback. The ‘good’ quality status from the parameter “bank stability” stated: ‘*Margins present from 11% to 30% of erosion, with soil exposure due to the lack of preserved vegetation. Loss of soil masses that can be further colonized by terrestrial vegetation*’. It lacked a description of margins dominated by grasses, herbaceous or few small arboreous vegetation, commonly present in pasture-dominated riparian areas, that are not steep, yet are highly susceptible to erosion due to the lack of appropriate soil cohesion, since grasses possess short roots, and are constantly trampled. The proper description was also adjusted for regular and poor-quality status. Regular quality status stated: ‘*Erosion occurring in 31% to 65% of the stream reach, with root exposure and minimum vegetation occurrence. High susceptibility to heavy rain, with soil mass dislocation, preventing vegetation succession*’. Poor quality status stated: ‘*Reach with over 66% of eroded margins, clear signs of burial and flow interruption and absence of vegetation*’. All status now includes a more detailed explanation considering the presence of grasses, herbaceous and few small arboreous vegetation in the description (Table 1).

All quality status of the parameter “Riparian Bank Protection” lacked a description of natural vegetation structure to be visualized and did not consider the width of vegetation to be analyzed. Optimal quality status stated: ‘*Reach with over 90% of natural vegetation. No evidence of cultivated areas, pasture, or urban land use. Most plants can grow naturally*’. Good quality status stated: ‘*Reach with 70% to 89% of the riparian area with natural*

Table 1. Summary of parameters scored as part of the Rapid Assessment Protocol adapted to the study region.

Parameter	Significance	Quality status	Interpretation	Scoring
Underwater available substrate	Greater variety and/or proportion of potential substrates provide different food and fixation resources for organisms, favoring biodiversity and ecosystem functioning. In sandy bottom streams, the entry of substrates from adjacent land areas is essential for the provision of leaves, branches, trunks, and fruits.	Optimal	The site presents sand, deposition of organic material, aquatic vegetation, trunks, branches, and leaves accumulating underwater, providing bottom substrates in 76% to 100% of the evaluated site. Occasional occurrence of gravel can be detected	20-16
		Good	The site presents substrate for the aquatic fauna, such as sand, trunks, branches, and leaves accumulating underwater in 51% to 75% of the evaluated site. Occasional occurrence of aquatic vegetation and deposition of organic material can be detected.	15-11
		Regular	The site presents low occurrence or absence of organic material deposition or aquatic vegetation. The presence of sand prevails, with a few occurrences of trunks, branches, and leaves in 26% to 50% of the evaluated site	10-6
		Poor	The site presents sand dominated substrates. Water flow carries substrates and limits the establishment of submerged aquatic vegetation and reduces or buried organic material previously deposited. Less than 25% of the site presents trunks, branches and leaves.	5-0
Underwater habitat complexity	The variety of shapes, textures, sizes and the abundance of underwater structuring increase the availability of shelter against predation and provide places for reproduction and feeding. Underwater substrate contributes to the stabilization of the sediment and favors the occurrence of distinct flow regimes. Higher environmental heterogeneity contributes to the maintenance of higher biodiversity. In the case of sandy bottom streams, the input of structuring material from the terrestrial environment is essential for underwater heterogeneity	Optimal	The site presents aquatic vegetation, branches and leaves underwater, marginal vegetation leaning over the stream channel, presence of backwaters, small waterfalls and excavated banks distributed along 76% to 100% of the site as potential habitats.	20-16
		Good	The proportion of potential habitats is found in 51% to 75% of the site, with branches and leaves underwater, marginal vegetation leaning over the channel, small waterfalls. Minimal occurrence or absence of aquatic vegetation, excavated margins and large backwaters.	15-11
		Regular	The site presents 25% to 50% of potential habitats, with branches and leaves underwater, marginal vegetation leaning over the channel, small waterfalls. Minimal occurrence or absence of aquatic vegetation. Few or absence of backwaters for shelter and reproduction of aquatic communities	10-6
		Poor	Aquatic vegetation, backwaters, small waterfalls and marginal vegetation leaning over the channel are absent. Minimal occurrence of trunks, branches and leaves underwaters, in less than 25% of the site	5-0
Velocity Depth combinations	The variety of combinations of flow and depth favors the occurrence of organisms with different ecological requirements and increases biodiversity. It also contributes to the maintenance of a balanced sediment, particles and nutrients transport and deposition dynamics. In sandy bottom streams, the accumulation of branches, trunks and leaves contributes to the creation of small dams and differentiated flow regimes	Optimal	Occurrence of 4 types of regimes. Fast/shallow, fast/deep, slow/shallow; slow/deep	20-16
		Good	Occurrence of 3 types of regimes. Fast and shallow regimes must be detected	15-11
		Regular	Occurrence of 2 types of regimes. If fast and shallow regime is absent, scores must be lower.	10-6
		Poor	Predominance of only 1 type of regime. If the slow regimes predominate, scores must be lower	5-0
Channel sinuosity	Higher channel sinuosity provides higher availability of habitats such as backwaters and the main flow channel. It increases the streams' ability to retain flow fluctuations caused by heavy rains. Energy absorption by the bends protects the water body from excessive erosion and provides refuge for biota during peak flow events	Optimal	Occurrence of sharp and evident curves along the evaluated site	20-16
		Good	Channel sinuosity is not evident. Less sharpened and more distant curves can be observed	15-11
		Regular	The site presents a few soft and distant curves	10-6
		Poor	The site is straight. In case of man-made plumbing (with cement or other material), the scores must be lower	5-0

All parameters are visually evaluated and scored from zero (poor condition) to 20 (optimal condition). The sum of all parameters scores is used as the stream local condition. The Portuguese version of the protocol can be assessed in Table S4 (Google Drive, 2021).

Table 1. Continued...

Parameter	Significance	Quality status	Interpretation	Scoring
Water level amplitude	Fluctuations in the water level affect the availability of substrates and shelter for the aquatic fauna and dictates sediment and nutrient transport through the stream. Constructions in cross and longitudinal sections of the streams alter the flow efficiency of the channel. Grounding, concreting and silting conditions cause loss of diversity and reduce the damping potential of flood events. Extended periods of drought reduce the amount of water available in the stream channel. High magnitude flow peaks carry organisms, nutrients and structures that promote environmental complexity.	Optimal	The water level is sufficient to include all available substrate underwater, suitable for colonization.	20-16
		Good	The water level fills more than 75% of the stream channel. Less than 25% of available substrate are exposed.	15-11
		Regular	Water level fills between 26% and 75% of the stream channel. Most of the available substrate are exposed.	10-6
		Poor	Very little water in the stream channel. Most of which is stagnant water in ponds.	5-0
Channel integrity	Channel integrity refers to changes in the structure of the stream channel, imposing restrictions on the survival of aquatic organisms and changing the hydrological dynamics of the streams. Dike formation, dredging, drainage, dams paving, and flow diversion are amongst some of the prejudicial alterations to aquatic ecosystem functioning.	Optimal	Absence or minimal occurrence of alterations such as pipelines, dredging, bridges, dikes, embankments, dams, concrete canalization of flow diversion. The stream follows a natural flow pattern.	20-16
		Good	Occurrence of older channel alterations such as bridges or dredging in up to 20% of the site, with no recent changes.	15-11
		Regular	Occurrence of dams, dikes, drainage or any of the aforementioned alterations that are recent, modifying from 21% to 50% of the natural course of the stream.	10-6
		Poor	Stream margins are covered with cement or supported by gabions. Alternatively, more than 51% of the stream channel is channeled, with flow disruption.	5-0
Bank stability	Stream banks comprise the area of soil immediately adjacent to the water body. Stable margins, with minimal occurrence of erosive processes favor the maintenance of the physical structure of the main channel and protect the biota. Unstable margins, in sandy soil regions, are prone to displacement of soil masses and high erosion, increasing siltation of streams. The evaluation must be performed on each margin separately and summed.	Optimal	Stream sections with minimal occurrence of erosive processes, with preserved and dense riparian vegetation supporting the soil. Up to 10% of the stream site presents small signs of erosion, as a natural process.	9-10
		Good	The site presents from 11% to 30% of the margins with sign of erosion, with soil exposure in sparse sections due to lack of preserved vegetation, colonization by grasses and herbaceous vegetation, roots exposed. Loss of soil masses that can be further colonized by terrestrial vegetation if the proper time is given.	6-8
		Regular	Erosive processes reach 31% to 65% of the site. Roots are exposed in the stream's margins, with the domain of grasses, herbaceous or small arboreous vegetation. Minimal occurrence of arboreous vegetation and higher susceptibility to the effects of heavy rain. Clear and abundant sections with soil masses displacement, limiting vegetation succession.	3-5
		Poor	More than 66% of the banks are eroded, with clear signs of burial of structures and interruption of water flow due to silting. Absence or minimal occurrence of vegetation in the margins. Reach dominated grasses, herbaceous or small arboreous vegetation.	2-0

All parameters are visually evaluated and scored from zero (poor condition) to 20 (optimal condition). The sum of all parameters scores is used as the stream local condition. The Portuguese version of the protocol can be assessed in Table S4 (Google Drive, 2021).

Table 1. Continued...

Parameter	Significance	Quality status	Interpretation	Scoring
Riparian bank protection	Riparian vegetation contributes to the filtration of sediments and nutrient runoff. Riparian vegetation stabilizes the soil with its roots and protects the soil from the erosive action of rain and wind, through the formation of the organic layer of litter. The removal of the riparian vegetation to give place to agriculture, pasture and/or urban settlements exposes the terrestrial area to weathering and ecosystem function losses. The presence of lateral buffers (> 20 m) along the evaluation reach of 40 m are the most desirable condition for preservation. The evaluation must be performed on each margin separately and summed.	Optimal	More than 90% of the stream riparian area (site length of 40 m) is covered by natural vegetation, with arborous, shrubby, and herbaceous species, forming a multi-strata vegetation. No evidence of agriculture, pasture and/or urban land use in 20 meters lateral buffer. Plant species can grow naturally.	9-10
		Good	Riparian area covered by natural multi-strata vegetation from 70% to 89% of the stream site length (40 m). Minimal evidence of agriculture, pasture and/or urban land use in 20 meters lateral buffer. No representative discontinuities in riparian vegetation.	6-8
		Regular	Riparian area covered by few arboreous vegetation from 50% to 69% of the stream site length (40 m). Evident occurrence of occupation for agricultural, pasture and/or urban activities where natural vegetation is absent. Whenever urban land use occurs, scores are lower.	3-5
		Poor	Less than 50% of stream riparian area covered by any kind of natural vegetation. Large discontinuities or absence of arboreous vegetation. Dominated by herbaceous vegetation and grasses. 20-meter lateral buffer occupied by agriculture, pasture and/or urban land use. If urban use predominate, scores must be lowest.	2-0
Vegetation conservation on the riparian zone	Preserved riparian vegetation is represented by the occurrence of plants with different sizes, shapes and colors. Which include trees, shrubs, herbaceous, epiphytes. Most preserved areas are inhabited by native species. The increase in the presence of exotic species is indicative of deterioration. The evaluation must be performed on each margin separately and summed.	Optimal	Riparian vegetation is composed of native species in a good state of conservation. There are distinct vegetation strata, with diversity of sizes, shapes, and colors.	9-10
		Good	The riparian vegetation is composed not only by native species, but also exotic species, although in a good conservation status. There are distinct vegetation strata, with diversity of sizes, shapes and colors. Minimal evidence of anthropogenic impacts.	6-8
		Regular	Higher occurrence of exotic tree and shrub species, within a more homogenized landscape. Predominance of medium sized trees, within clearing spots. Evident anthropogenic impacts, with predominance of grasses and small trees	3-5
		Poor	Riparian vegetation is absent or with minimal coverage along the stream site. Few occurrences of medium sized trees. If the riparian area is completely replaced by impermeable surfaces such as in urban landscapes, the scores must be lowest.	2-0

All parameters are visually evaluated and scored from zero (poor condition) to 20 (optimal condition). The sum of all parameters scores is used as the stream local condition. The Portuguese version of the protocol can be assessed in Table S4 (Google Drive, 2021).

vegetation. Minimal evidence of cultivated areas, pasture, or urban land use. No large discontinuity in vegetation'. Regular quality status stated: 'Reach with 50 to 69% of riparian area covered with vegetation. Significant areas are occupied by agriculture, pasture, or urban land use. When urban predominates, scores are lower.'. Poor quality status stated: 'Reach with less than 50% of the riparian area with vegetation,

with large discontinuities or absence of vegetation'. All quality status of the Riparian Bank Protection were adjusted accordingly (Table 1).

The remaining parameters were appropriate and provided suitable visual assessments through our study region, nonetheless, their description was only improved for clarity of interpretation, to provide a new revised RAP sheet (Table 1).

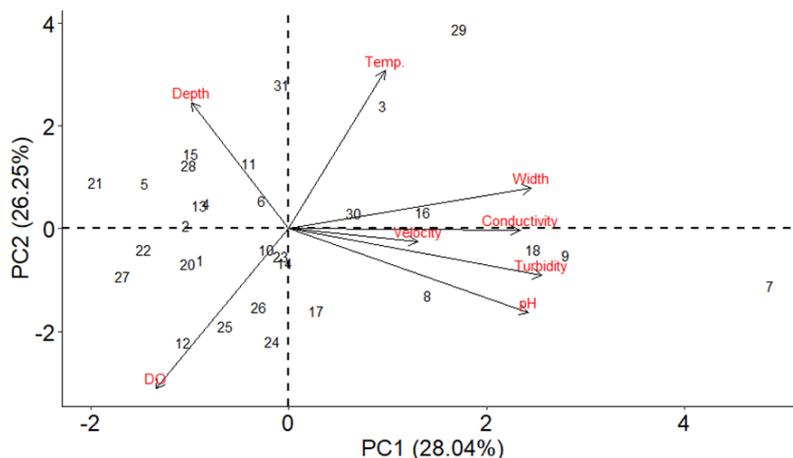


Figure 3. Scores distribution along the PCA axis with in-stream limnological and physical characteristics of 30 headwater streams. Numbers indicate each stream. DO = dissolved oxygen, Temp. = water temperature in °C.

3.2. In-stream limnological and physical variability

The PCA analysis showed that the headwater streams present low variability in the limnological and in-stream physical parameters (PC1% variance = 28.04; Figure 3). Streams with higher depths and dissolved oxygen concentration were separated from those with higher width, turbidity, conductivity, and pH (Figure 3).

3.3. RAP scores association with in-stream limnological and physical characteristics

The RAP scores variability among our data set was significantly related to the in-stream limnological and physical characteristics of the streams (RAP estimate = -0.0168, $t = -3.379$, $p = 0.002$, $R^2 = 0.29$; Table S5). The RAP score explained ~29% of the variability of in-stream limnological and physical characteristics of the streams (Figure 4). Streams with higher RAP scores (i.e., optimal environmental conditions) were those with higher dissolved oxygen concentration and higher average depths. Streams with lower RAP scores (i.e., poor environmental quality) were those with higher widths, conductivity, and turbidity.

Nutrient concentration was negatively correlated to Velocity and Depth combinations (RAP3) and to Channel Sinuosity (RAP4) (Table 2). Streams with higher orthophosphate and ammonium loads were those with the predominance of slow and shallow flow regimes (i.e., low RAP scores), while streams with higher nitrate concentration were those with straight channels (i.e., low RAP scores).

4. Discussion

The RAP scores were significantly correlated with limnological and in-stream physical parameters,

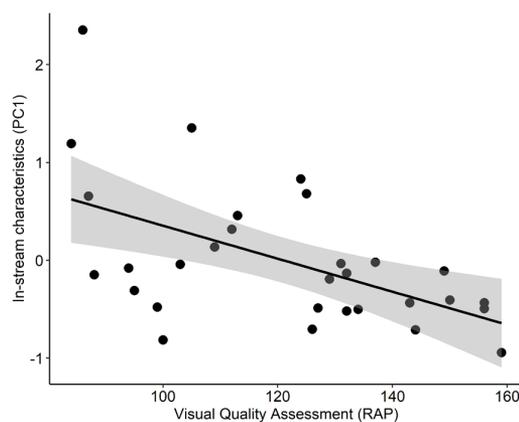


Figure 4. Linear relation between the RAP score sum (x axis) and in-stream characteristics summarized in the first principal component PC1 (y axis).

Table 2. Pearson correlation between each RAP parameter, RAP scores sum, and in-stream nutrient concentration, for the set of nine streams.

Parameters	PO ₄	NH ₄	NO ₃
RAP1	-0.43	-0.66	-0.23
RAP2	-0.15	-0.40	-0.10
RAP3	-0.69*	-0.86**	-0.26
RAP4	-0.52	-0.69*	-0.72*
RAP5	-0.40	-0.64	0.05
RAP6	-0.12	-0.21	-0.58
RAP7	-0.47	-0.65	-0.51
RAP8	-0.41	-0.52	-0.43
RAP9	-0.18	-0.17	-0.46
RAP _{sum}	-0.43	-0.63	-0.43

PO₄ = orthophosphate; NH₄ = ammonium; NO₃ = nitrate; RAP1 = Underwater substrate; RAP2 = Underwater habitat complexity; RAP3 = velocity/depth combinations; RAP4 = Channel sinuosity; RAP5 = Water level amplitude; RAP6 = Channel integrity; RAP7 = Bank stability; RAP8 = Riparian bank protection; RAP9 = Vegetation conservation on the riparian zone. * $p < 0.05$; ** $p < 0.01$.

which are recognized as good environmental quality descriptors (Yadav et al., 2019; Piffer et al., 2021). Contrasting results have also been reported in the literature in which RAP scores did not correlate well with physicochemical parameters (Machado et al., 2015). The versatility of the RAP relies on the fact that it can provide both a good interpretation about the stream conservation with a visual assessment as a single tool (i.e., for streams draining Arenito Caiuá Sandstone Formation), and as a complementary tool to limnological and in-stream characterization in describing the environment (as in Machado et al., 2015). Overall, visual assessments are recognized as good descriptors of the quality and availability of physical habitat to the aquatic fauna in small streams (Bentos et al., 2018); as well as to assessing, diagnosing, and monitoring environmental physical quality of preserved, degraded, and restored streams (Doll et al., 2016; Guimarães et al., 2017).

In this study, higher RAP scores were recorded in streams with higher dissolved oxygen concentration and depths, and low RAP scores were registered in wider streams with higher water conductivity and turbidity. This outcome is directly associated with the presence of riparian vegetation and its preservation status (Connolly et al., 2016; Chellaiah & Yule, 2018; Piffer et al., 2021). The input and accumulation of organic structure (i.e., branches and leaves) from the riparian area retain the water flow in some mesohabitats, creates distinct flow regimes (i.e., small waterfalls) that increase mechanical supply of oxygen and create complex meso-habitats such as riffles and pools with varying depths. On the other hand, streams without the protection of the riparian vegetation present the lower RAP scores because the lack of riparian cover favors erosion and siltation of the sandy soil. The margin erosion increases stream width and while carrying sediments into the water column, it enhances turbidity. In the absence of riparian cover, other activities take place, such as agriculture or urban settlements, which may increase the risk of inputs of fertilizers and sewers that can be detected with higher conductivity records (Ometo et al., 2000).

The RAP parameters that were most related to nutrient concentrations were those that described the velocity and depth combinations, and channel sinuosity. More homogeneous and rectilinear stream channels were those with higher nitrate concentration. Streams with the predominance of slow and shallow flow regimes were also those with higher orthophosphate and ammonium concentration in the water. Streams that drain into landscapes without riparian vegetation protection

are usually channelized or develop more straight and shallow channels due to silting (Hanna et al., 2020). These systems are also subjected to higher input of sewer or fertilizers (Jankowski et al., 2021). Urban and agriculture land use has been long acknowledged to contribute to nitrate and phosphorus pollution of streams (Olareswaju et al., 2009), and for some tropical system it does not matter if the whole watershed or riparian scales were considered (Tromboni & Dodds, 2017), nutrients will eventually reach the streams. Such outcomes are of particular risk for communities without alternative sources of potable water (Olareswaju et al., 2009). The association between the physical habitat evaluation provided by the RAP and the nutrient concentration detected in the streams is representative of the multiple physical and chemical impacts of stream degradation in their drainage basin, and should be interpreted together, rather than substitutive, to provide more accurate comprehension of the system.

The application of the RAP in urban streams without major structural changes such as canalization, can provide intermediary physical habitat quality scores, and it should be interpreted with caution. Because urban streams may receive construction waste inputs (i.e., bricks, ties, and ceramics), they can present underwater complexity and distinct flow regimes. Some of the urban streams may also present marginally preserved riparian cover that, even if not well preserved, can provide some underwater habitat heterogeneity, and enhance the scale of physical quality provided by the RAP. For example, macroinvertebrate communities were found to be more diverse on anthropogenic litter than on rocks, with community composition variation from the natural substrates in temperate streams (Wilson et al., 2021). However, the input of domestic residuals (i.e., plastic bottles, plastic bags, soda cans) can imprison aquatic fauna, produce microplastic, and are easily transported downstream during spates. These characteristics should be acknowledged in the RAP evaluation, accompanied by interpretations of the surrounding environment and water quality parameters. That is because some of the most conspicuous impacts to such streams are mostly related to sewer and surface runoff from the cities, that are properly measured with limnological parameters.

The improvements made in the RAP parameters description represents a long-time effort (>10 years) with the application and validation of this protocol in the region it was developed for. The RAP suitably to provide complementary and widespread physical environmental assessments of streams draining through the sandstone geological formation has been

acknowledge in studies where the identification of a degradation gradient was trustworthy conducted with the RAP and predictably reflected the ecosystem functioning and biological community structure responses in the streams (Gonino et al., 2020; Cionek et al., 2021; Pereira et al., 2021). Streams with higher natural underwater habitat complexity registered with the RAP scores, where also those with higher leaf litter breakdown rates, that were 10 times higher in streams with better physical habitat quality than those with low physical habitat quality (Cionek et al., 2021). The fish-based Index of Biotic Integrity developed for the same region, was positively correlated to the RAP scores, and the authors states that the habitat quality profile provided by the RAP can improve the ability to interpret biological responses to environmental degradation in the studied streams (Gonino et al., 2020). As an overall pattern, RAP developed for different regions have all been successfully applied in integrated studies including multiple aquatic organisms (Winger et al., 2005), microorganisms (Kieling-Rubio et al., 2015), invertebrates, vertebrates, and plants (Cooke & Zack, 2009) and proved very useful.

The study region presents a rather homogenized landscape, with the streams' sub-basins dominated by agriculture and pasture (see Table S1). As a result, streams are subjected to historically similar impacts and alterations, which ends up producing a set of similar physical habitat conditions in our sample set. Gonino et al. (2020) also identified a wider amplitude of fish community structure responses in intermediary degraded streams and attributed it to biological resilience of the fish community. Despite that, the RAP assessment was able to effectively identify in-stream and local features of streams with sufficiently distinct environmental characteristics (Gonino et al., 2020). This outcome reflects the importance of RAPs for stream diagnostics and monitoring whenever financial support is restricted or as a complementary and comparable tool for physical habitat assessments along with a limnological and biological set of responses. Physical habitat attributes are acknowledged as important drivers of species diversity and composition, and ecosystem functioning in streams due to differences in species ecological requirements for food, spawning sites and refuge (Vyas et al., 2013; Keller et al., 2019), which in turn are important to maintain stream functioning (Dudgeon, 2008; Kim & Choi, 2019).

In conclusion, the RAP adapted in this study has been reliably providing a diagnostic of the physical habitat condition of streams. It assisted with the

description of the quality status of aquatic habitats, and by explaining biological responses to habitat characteristics in local scales (Cionek et al., 2011; Cionek, 2016; Gonino et al., 2020; Cionek et al., 2021; Pereira et al., 2021). We know very little about the conservation status of headwater streams in Brazil, and the application of low-cost, user-friendly tools can increase the potential for diagnostics of a wide range of streams within a region and assist with management and future decision making (Bjorkland et al., 2001; Ward et al., 2003). As this environmental assessment tool depends upon a visual assessment, evaluators should be previously trained about the application procedures (Barbour et al., 1999; Cionek et al., 2011). More accurate evaluations are obtained with the average RAP scores from at least three evaluators. However, when necessary, the RAP scores outcome from one well trained evaluator are also admissible. The use of RAP is widespread and the vast majority of them are based on similar sets of parameters, which makes them relatively simple to understand and compare across larger spatial scales (Barbour et al., 1999; Minatti-Ferreira & Beaumord, 2006; Guimarães et al., 2017). The use of RAP can be further extended to educational and citizen science initiatives (Callisto et al., 2011). This can aid in enhancing environmental consciousness and give people power to understand and help with environmental management of its surroundings with a simple and widespread monitoring tool.

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Data availability

Additional research material analyzed in the research (Figures S1 to S6 and Tables S1 to S5) can be accessed in: <https://drive.google.com/drive/folders/1Tsy0KpD0FeMaEGBJhWjuj-1RJK4jzIQ?usp=sharing>

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